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March 16, 2011

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NPDES PERMIT NO. CA0082295
WDR ORDER #R5-2007-0170
TOXICITY IDENTIFICATION EVALUATION

Dear Mr. Harvey,

As required by the above referenced Order, Chevron respectfully submits the enclosed document entitled "Toxicity Identification Evaluation for Chevron USA Inc and Cawelo Water District Produced Water Reclamation Project, Kern River Station 36, NPDES No. CA0082295. This TIE was initiated in compliance with NPDES Section VI.C.2.b. "Special Studies, Chronic Whole Effluent Toxicity".

If you have any questions regarding this submittal, please contact me at (661) 654-7122.

Sincerely,

Jim Waldron

cc: w/o enclosure

David Ansolabehere, Manager, Cawelo Water District
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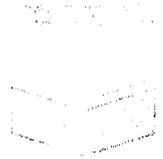
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March 10, 2011

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WDR ORDER 03-2007-010
NPDES PERMIT NO. CA000338
TOXICITY IDENTIFICATION EVALUATION

Dear Mr. Harvey:

As required by the above referenced Order, Chevron respectfully submits the enclosed document entitled, "Toxicity Identification Evaluation for Chevron USA Inc and Casajo Water District Product Water Restoration Project, Kern River Station SE NPDES No. CA000338. This TIE was initiated in compliance with NPDES Section VI.C.2.b. "Special Studies, Chronic Whole Effluent Toxicity."

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March 10, 2011



**Toxicity Identification Evaluation for Chevron USA Inc and
Cawelo Water District Produced Water Reclamation Project,
Kern River Station 36
NPDES No. CA0082295**

February 24, 2011

1 of 2

**CHEVRON
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**Toxicity Identification Evaluation for Chevron USA Inc and Cawelo Water District
Produced Water Reclamation Project, Kern River Station 36
NPDES No. CA0082295**

Prepared By:

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February 24, 2011

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Table of Contents

1	EXECUTIVE SUMMARY	1
2	INTRODUCTION	3
3	INITIAL DATA AND INFORMATION ACQUISITION	4
3.1	Background on Produced Water Toxicity	5
3.2	Chevron Kern River Oilfield Produced Water.....	6
3.2.1	Kern River Area Station 36 Treatment Process	8
3.2.2	Station 36 Treatment Facility – General Housekeeping.....	8
3.3	Treatment Chemicals used at Kern River Station 36 Treatment Facility .	9
3.4	NPDES Toxicity Testing	10
4	TIE BACKGROUND AND APPROACH	12
5	MATERIALS AND METHODS	15
5.1	Sampling Sites.....	15
5.2	Sample Handling and Receipt.....	17
5.3	Toxicity Testing Protocols	17
5.3.1	Survival and Reproduction Toxicity Testing with <i>Ceriodaphnia dubia</i>	17
5.3.2	Survival and Growth Toxicity Testing with Larval Fathead Minnows.....	18
5.3.3	TIE Testing Procedures	19
5.3.4	Reference Toxicity Testing.....	19
6	RESULTS.....	20
6.1	Results of the “Initial Assessment” Toxicity Tests.....	20
6.1.1	Effects of Chevron/Cawelo Effluent (Inlet to Reservoir B).....	20
6.1.2	Valley Waste Discharge	22
6.1.3	Splitter Box and EFF-002 (discharge from Reservoir “B”).....	22
6.1.4	Pre-Poso Creek (Irrigation Canal Top of Hill, EFF-003).....	24
6.2	TIE Results	24
6.2.1	Phase I TIE results for <i>Ceriodaphnia dubia</i>	25
6.2.2	Phase I TIE Results for Fathead Minnows	25
6.3	Phase II TIE.....	29
6.3.1	Results for Toxicity Recovery in the Initial Phase II TIE Evaluation .	29
6.3.2	Results for Toxicity Recovery in Step 2 of the Phase 2 TIE Evaluation (Sequential Elutions)	31
6.4	Chemical Analysis of the Toxic C18 SPE Eluate Fractions.....	33

6.4.1	Characteristics of the Compounds Detected at the Highest Concentrations in the C18 Eluates.....	36
7	TOXICITY REDUCTION EVALUATION.....	38
7.1	Granular Activated Carbon (GAC)	41
7.2	Peroxide (H ₂ O ₂).....	42
7.3	Ozonation.....	42
7.4	Biological.....	42
7.5	Membrane Treatment.....	43
8	CONCLUSIONS AND RECOMMENDATIONS.....	44
9	REFERENCES.....	45
Appendix A.....		48
A.1 Description of TIE Treatment Methods.....		48
A.1.1 TIE Treatment Method Blanks.....		48
A.1.2 Baseline Testing.....		48
A.1.3 pH Adjustment Treatments.....		48
A.1.4 Graduated pH Adjustment Treatment.....		48
A.1.5 Centrifugation Treatment.....		49
A.1.6 Filtration Treatment.....		49
A.1.7 C18 Solid Phase Extraction (SPE) Treatment.....		49
A.1.8 Aeration Treatment.....		50
A.1.9 Aeration Washdown Treatment.....		50
A.1.10 Piperonyl Butoxide (PBO) Treatment.....		50
A.1.11 Humic Acid Treatment.....		50
A.2 Phase II TIE Testing Procedures - Toxicity Recovery in the C18 SPE Eluate.....		51
A.2.1 Initial Evaluation of Toxicity Recovery in the C18 SPE Eluate.....		52
A.2.2 Evaluation of Toxicity Recovery by Sequential C18 Elutions.....		53
ATTACHMENTS.....		54

TABLES

Table 3-2.	List of treatment chemicals used at Kern River Station 36 treatment facility.....	9
Table 3-3.	List of treatment Chemicals used in the oilfield operations or in Surge tanks, clarifier, or floatation units in Kern River Station 36 treatment facility.....	9

Table 3-4. Active ingredients found in treatment chemicals used at Kern River Station 36 Treatment Plant (Table 3-3 above).	10
Table 6-1. Summary of effects of Chevron/Cawelo effluent (EFF-003) on <i>Ceriodaphnia dubia</i> and fathead minnow prior to the initiation of the TIE/TRE.....	20
Table 6-2. Summary of chronic toxicity tests <i>Ceriodaphnia dubia</i> and fathead minnow conducted using waters collected from EFF-001 (Inlet to Reservoir "B") as part of the TIE/TRE investigation.....	21
Table 6-3. Summary of chronic toxicity tests for <i>Ceriodaphnia dubia</i> and fathead minnow conducted using waters collected from Valley Waste discharge into Reservoir "B". Water collected as part of the TIE/TRE investigation.	22
Table 6-4. Summary of chronic toxicity tests for <i>Ceriodaphnia dubia</i> and fathead minnow conducted using waters collected wetland splitter box (coalesced waters from Reservoir "B").	23
Table 6-5. Summary of chronic toxicity tests for <i>Ceriodaphnia dubia</i> and fathead minnow conducted using waters collected from EFF-002 ("Outlet to Canals").	23
Table 6-6. Summary of chronic toxicity tests for <i>Ceriodaphnia dubia</i> and fathead minnow conducted using waters collected from EFF-003 [" Pre-Poso Creek" (irrigation canal at top of hill)].	24
Table 6-7. Phase I TIE Results for the <i>Ceriodaphnia dubia</i> Reproduction Response	27
Table 6-10. Recovery of C18 column eluate toxicity to fathead minnow survival (columns from the Phase I TIE of the September 21, 2009 sample). Attachment 8	30
Table 6-11. Recovery of C18 column eluate toxicity to fathead minnow growth (columns from the Phase I TIE of the September 21, 2009 sample). (Attachment 8)	30
Table 6-12. Recovery of C18 column eluate toxicity to fathead minnow survival and growth (columns from the Phase I TIE of the January 2010 sample). (Attachment 9) ..	31
Table 6-13. Recovery of Toxicity to <i>Ceriodaphnia dubia</i> and fathead minnow using Sequential C18 SPE elutions (50%, 75%, 80%, 85%, 90%, 95% and 100% methanol). C18 SPE treatment conducted on sample collected on 9/21/09. (Attachment 9).....	32
Table 6-14. Results of chemical analysis on Phase II TIE C18 Eluate fractions for effluent samples collected on September 9, 2009 and Jan 11, 2010 (units = mg/L).	34
Table 7-1. Summary of Best Available Techniques for treatment of Produced Water.	39
Table 7-2. Effects of GAC treatment on the toxicity of Reservoir B waters to fathead minnows (sample collected September 3, 2010). (Attachment 5)	41

Table 7-3. Effects of GAC treatment on the toxicity of C18 eluates from Reservoir B waters to fathead minnows (effluent sample collected January 11, 2010). (Attachment 9).....	41
Table 7-4. Effects of GAC treatment on the toxicity of Valley Waste effluent to Reservoir B to fathead minnows (sample collected September 3, 2010). (Attachment 5).....	42
Table 7-4. Summary of toxicity removal in Produced water treated by demonstration constructed wetland. Samples collected January 11, 2010. (Attachment 6).....	43

FIGURES

Figure 3-1. Site location map showing effluent monitoring locations (NPDES permit CA0082295).	4
Figure 3-2. Kern River field produced water system schematic and reservoir for produced water for Cawelo Water District (source: Produced Water Reuse at the Kern River Oil Field, Southwest Hydrology, November/December 2005 p. 26-27).	7
Figure 3-3. Process Flow Diagram of the Kern River Produced Water Treatment System. The treated produced water is reused for cogeneration, steamflood operations and irrigation.	8
Figure 4-1: Phase I Confirmation Toxicity Identification Evaluation (TIE) Treatment Procedures.....	13
Figure 4-2. Phase II Toxicity Identification Evaluation Treatment Procedures.	14
Figure 5-1. NPDES and TIE sampling locations near Reservoir B.	15
Figure 5-2. TIE toxicity assessment sample locations at Reservoir B and CWD distribution canal.	16
Figure A-1. Phase II Toxicity Identification Evaluation Treatment Procedures	52

ATTACHMENTS

- Attachment 1 - Chronic Toxicity Testing and Toxicity Identification Evaluation (TIE) of the Chevron/Cawelo Water District Effluent. Samples Collected April 21, 2009. Pacific EcoRisk. May 2009.
- Attachment 2 - NPDES Compliance Chronic Toxicity Testing of Chevron/Cawelo Water District "Inlet to Reservoir B" Effluent. Sample collected August 31, 2009. Pacific Ecorisk. October 2009.

Attachment 3 - NPDES Compliance Chronic Toxicity Testing of Chevron/Cawelo Water District "Inlet to Reservoir B" Effluent. Sample collected September 21, 2009. Pacific Ecorisk. October 2009.

Attachment 4 - Chronic Toxicity Testing of the Chevron/Cawelo Water District "Inlet to reservoir B" Effluent. Samples collected January 11, 2010. Pacific EcoRisk. May 2010.

Attachment 5 - Chronic Toxicity Testing of the Chevron/Cawelo Water District "Inlet to reservoir B" and "Valley Waste" Effluents. Samples collected September 3, 2010. Pacific EcoRisk. October 2010.

Attachment 6 - Chronic Toxicity Testing of Chevron/Cawelo Water District Nearby Water Samples. Samples collected January 11, 2010. Pacific Ecorisk. May 2010.

Attachment 7 - Chronic Toxicity Testing of the Splitter Box and Pre-Poso Creek Effluents. Samples Collected September 21, 2009. Pacific EcoRisk. October 2009.

Attachment 8 - Chronic Toxicity Testing and Toxicity Identification Evaluation (TIE) of the Chevron/Cawelo Water District Effluent. Samples collected September 21, 2009 and January 11, 2010. Pacific EcoRisk. May 2010.

Attachment 9 - Chronic Toxicity Testing of the Chevron/Cawelo Water District "Inlet to Reservoir B" C18 Eluate: Assessment of GAC Treatment and Chemical Analysis of the C18 Eluate. Pacific Ecorisk. December 2010.

1 EXECUTIVE SUMMARY

A Toxicity Identification Evaluation and Toxicity Reduction Evaluation (TIE/TRE) investigation was initiated in April 2009 in response to recurring toxicity observed to *Ceriodaphnia dubia* reproduction and fathead minnow survival following exposure to waters collected from EFF-003 of the Produced Water Reclamation Project, Kern River Area Station 36, Kern River Oil Field, Kern County, CA (NPDES No. CA0082295). The results of the data acquisition and initial toxicity assessment indicated that the source of the toxicity was the treated produced water effluent entering Reservoir B from both the Chevron operated Kern River Area Station 36 and from the Valley Waste Disposal Company (Kern Front Oil Field). Phase I TIE investigations were completed using treated produced water entering Reservoir B collected from three different sampling dates: April 21, 2009, September 21, 2009, and January 11, 2010. Results of these Phase I TIE investigations concluded that the C18 SPE column was the only TIE treatment that effectively removed the toxicity to both *C. dubia* and the fathead minnow. The following key observations were made during the Phase I TIE's conducted:

- There was significant removal of survival toxicity by the filtration treatments, which suggests that some fraction of the toxicants present were associated with particulates.
- There was complete removal of any residual toxicity (i.e., toxicity remaining after the filtration treatment) by the C18 SPE treatment, indicating that non-polar organics were a cause of the observed toxicity.
- The toxicity was pH-labile, with toxicity increasing as pH decreased to pH6, and toxicity decreasing as pH increased to pH8. This is suggestive of a weakly acidic toxicant that becomes less polar as the pH decreases and more polar as the pH increases.

After evaluating the results of the Phase I TIE Investigations, a Phase II TIE investigation (that included chemical analysis) was conducted using eluate collected from the C18 SPE columns that had been used in the Phase I TIE treatments. In all cases, the toxicity was able to be recovered in the C18 column eluates, although the magnitude of the observed toxicity was less than the initial toxicity tests or Phase I baseline toxicity tests. To attempt to determine the causative agent of the recovered toxicity, samples of the eluates at different methanol concentrations were analyzed for constituents typical of petroleum operations (volatile organic compounds, naphthenic acids, naphthalenes, phenolics, alkanes and amines). Results of these analyses indicated that the constituents measured at the highest concentrations were naturally occurring compounds commonly found in oilfield produced waters. These included:

- Naphthenic acids, naturally occurring linear and cyclic carboxylic compounds associated with the acidic fraction of petroleum and recognized as a common cause of toxicity in petroleum effluents;
- Phenols, which can occur naturally in petroleum and will partition into produced water depending on the molecular weight and which are known to impact the reproduction and growth of fathead minnows; and
- Volatile organic acids that include benzene, ethylbenzene, toluene and xylene (BTEX) that can be toxic to aquatic organisms although they typically degrade rapidly.

Based on the results of the Phase I and Phase II TIE, a TRE investigation was initiated. A review of available treatment alternatives and the current treatment process indicated that a

polishing step or tertiary treatment alternative would be most suitable for the treating the Kern River produced waters. Treatment methods considered in the initial evaluation included physical, chemical, biological and membrane treatment technologies. Based on feasibility related to volume of water to be treated, long term effectiveness and proven implementation ability at full scale operations, and generated waste and cost, there are no known technologies that can be readily implemented to treat Kern River Produced waters. However, two technologies were selected for further evaluation: granular activated carbon (GAC) and constructed wetland treatment systems. Initial bench top and pilot scale studies conducted as part of this TRE indicate that both of these technologies can effectively remove the observed toxicity to *C. dubia* and fathead minnows. Chevron plans to conduct a feasibility evaluation for treating the Kern River Produced waters as well as explore other options for decreasing toxicity in surface waters while maintaining the Kern River produced waters as a source of irrigation water for growers in the Cawelo Water District (CWD).

2 INTRODUCTION

In April 2009, Chevron U.S.A. Inc and Cawelo Water District (termed the site) initiated a Toxicity Identification Evaluation (TIE) and Toxicity Reduction Evaluation (TRE) on waters collected from the Produced Water Reclamation Project, Kern River Area Station 36, Kern River Oil Field, Kern County, CA (NPDES No. CA0082295). This TIE/TRE was initiated in compliance with NPDES permit Section VI.C.2.b. Special Studies, Chronic Whole Effluent Toxicity. As defined in the TRE Work Plan submitted in September 2008, if a pattern of toxicity is observed during the routine and accelerated monitoring, the site will begin to implement the initial tiers of the TIE/TRE work plan which includes:

- Step 1 of TRE: Data acquisition and facility information. This step is to be initiated as part of the accelerated monitoring program. Under this step, the site will investigate process and treatment chemicals currently in use, review historical effluent toxicity and compliance data and review operations, performance and maintenance data to determine potential sources of toxicity and identify corrective measures for reducing toxicity.
- Step 2 of TRE: Toxicity identification evaluation (TIE). The chemical fractions that have the greatest potential to cause toxicity in oilfield produced waters have been previously identified from the literature, historical investigations, and research on produced waters. Based on this information, a targeted TIE is to be implemented to target chemical fractions related to hydrocarbons, treatment chemicals (surfactants), oxidizable compounds, and particulate bound constituents. If the targeted TIE fails to identify the class of compound generating the toxicity, a traditional TIE that considers an expanded set of treatments will be implemented.

After the class of chemical has been identified, steps 3 – 5 of the TRE process will be completed:

- Step 3 identifies potential sources of toxicity once chemical has been identified.
- Step 4 identifies treatment alternatives for decreasing toxicity.
- Step 5 is the implementation of the best treatment alternative and follow-up monitoring.

To date, the site has completed TRE Steps 1, 2, and 3, and is in the process of identifying feasible treatment alternatives (Step 4). This report will provide details and the results of the toxicity bioassays and TIEs conducted during the completion of Steps 1 through 3, and provides a summary of the alternatives being investigated and preliminary data as part of Step 4. The toxicity experiments were initiated in April 2009 and have continued through November 2010.

3 INITIAL DATA AND INFORMATION ACQUISITION

Monitoring Location EFF-003 is located at the outfall of the Cawelo Water District (CWD) distribution canal into Poso Creek. The discharge from this canal is made up of water from several sources, including treated oilfield produced water from the Chevron Kern River Oil Field, treated produced water from Valley Waste Disposal Company (Kern Front Oil Field), and surface and groundwater from other water sources managed by CWD. These waters are comingled into a single water source in CWD's Reservoir "B" which is located at the head of the CWD Distribution Canal (Figure 3-1). The Distribution System is approximately 43 miles long, with 5.3 miles of lined canal and approximately 38 miles of pipeline ranging from 15 to 60 inches in diameter. . CWD uses the distribution system to supply irrigation water to growers in the North Kern Hydrologic Area. Typically CWD discharges water into Poso Creek only in the winter months or when there is no or insufficient surface water in Poso Creek to extend past the CWD downstream boundary (. In the winter months (October to March), when irrigation water demand is low, CWD discharges excess blended water from the canal to Poso Creek for the intentional recharge of groundwater.

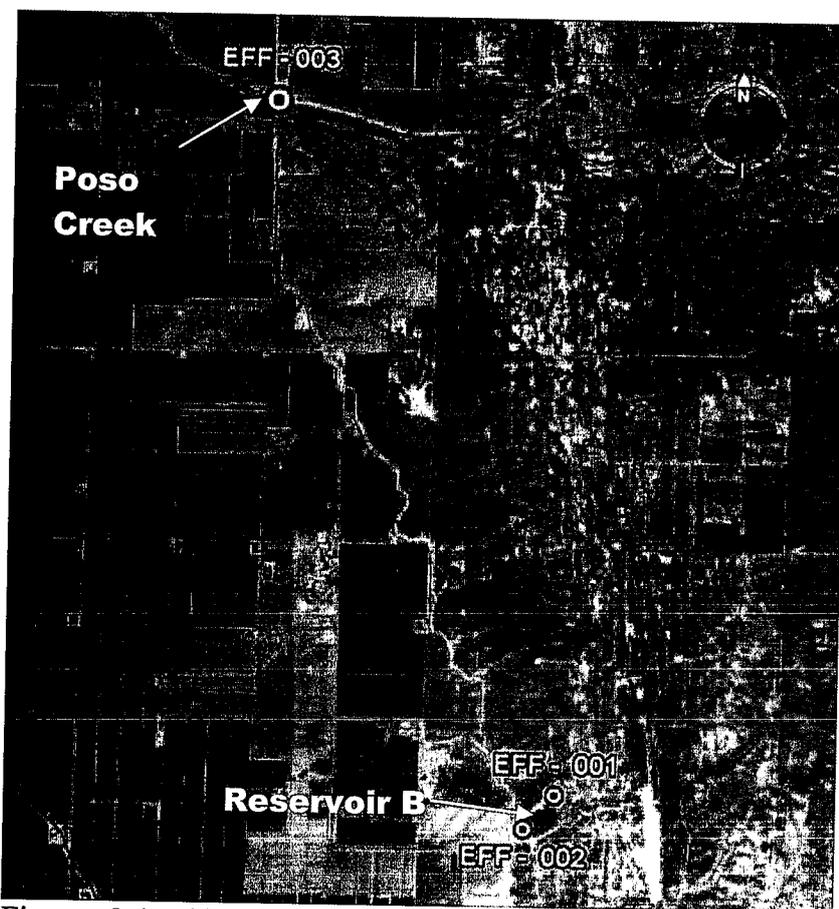


Figure 3-1. Site location map showing effluent monitoring locations (NPDES permit CA0082295).

The volume of treated produced water that enters Reservoir B remains fairly constant throughout the year with the volume of Chevron treated produced water averaging 30 ft³/sec (20 mgd) and the volume of treated produced water from Valley Waste averaging 10 ft³/sec (6.4 mgd). However, the total volume of water that is discharged from Reservoir "B" to the distribution canal fluctuates over the year based on irrigation water demand and the volume of surface and groundwater from other sources (managed by CWD) that are blended with the treated produced water in Reservoir "B". During the winter months (October to March) when the demand for irrigation water is low, treated produced water from Chevron and Valley Waste can make up almost 100% of the total flow entering the distribution canal. In the summer months, treated produced water may make up only 25% of the total volume entering the distribution canal. The percentages of treated produced water to CWD surface and ground water fluctuate between these extremes over the course of the year. Table 3-1 presents the volume/time for the dates water was collected for the TIE/TRE testing.

Table 3-1. Volume of water and percentages of total flow for treated produced water and CWD waters entering the distribution canal from Reservoir "B".

Source of Water	April 21, 2009		September 21, 2009		January 11, 2010	
Chevron Treated Produced water	30 ft ³ /sec	40 % total flow	31 ft ³ /sec	25 % total flow	30 ft ³ /sec	75 % total flow
Valley Waste Treated produced water	10 ft ³ /sec	13 % total flow	10 ft ³ /sec	8 % total flow	10 ft ³ /sec	25 % total flow
CWD surface water from other sources	35 ft ³ /sec	46 % total flow	81 ft ³ /sec	66 % total flow	0 ft ³ /sec	0 % total flow

Because treated produced water is a significant source of water to Reservoir "B" and ultimately to monitoring point EFF-003, and Chevron is the primary source of treated produced water into Reservoir B, the Chevron produced water was investigated under Tier I of the TRE, *Information and Data Acquisition*.

3.1 Background on Produced Water Toxicity

The characteristics of produced water can vary significantly depending on geological formation, oil production operation and oil field age. Similarly, the toxicity associated with produced waters will vary as a result of the produced water characteristics. TIE treatment (sometimes termed 'fractionation') techniques have been successfully demonstrated on a variety of produced waters and have found that no single fraction was consistently toxic among produced waters from different sources (Sauer et al, 1997). Using different TIE treatment techniques, the following parameters have been identified as the causative agents to the toxicity of various produced waters: hydrogen sulfide, hydrocarbons, ammonia, salinity, acidic organic compounds, basic organic compounds and abnormal major ion concentrations.

By reviewing what is known about the Kern River field, several potential sources of toxicity could be readily eliminated. One of these sources was abnormal major ion concentrations. Ion imbalance has been identified as the source of the toxicity in many produced waters (Tietge et al, 1997) and is often associated with produced waters that have high salinities and high total dissolved solids (TDS). However, the Kern River produced water is low in minerals (TDS typically less than 1000 mg/L) with low salinity. Therefore, it is unlikely the observed toxicity was related to ion imbalance. Hydrogen sulfide could be removed from consideration, based on knowledge of the field and treatment information. Using this knowledge of the Kern River field and data presented in the scientific literature and professional experience, this TIE focused on potential toxicity related to hydrocarbons, ammonia, organic compounds and production chemicals.

3.2 Chevron Kern River Oilfield Produced Water

Chevron recovers approximately 80,000 barrels per day (bpd) of crude oil from the Kern River Oil Field. For every barrel of oil that is extracted, approximately 9 barrels of water are produced. The oil and produced water from the field is collected and routed to the Kern River Area Station 36 treatment facility (Figure 3-2). Following the removal of oil, the produced water is treated using mechanical separation, sedimentation, air floatation (Wemco units) and filtration (walnut hull vessels) (Figure 3-3). The Station 36 treatment facility has the capacity to process up to 37.8 million gallons per day (mgd).

Following treatment, Chevron reclaims approximately half of the produced water to generate new steam to enhance oil production and for other in-field uses. The remaining treated produced water, approximately 58 acre-feet per day, is piped via an 8.5 mile coated steel pipeline to the CWD Reservoir "B" for agricultural reuse (Figure 3-2). Prior to discharge to Reservoir B, treated produced water enters a concrete and PVC lined polishing pond (adjacent to Reservoir B) for final treatment.

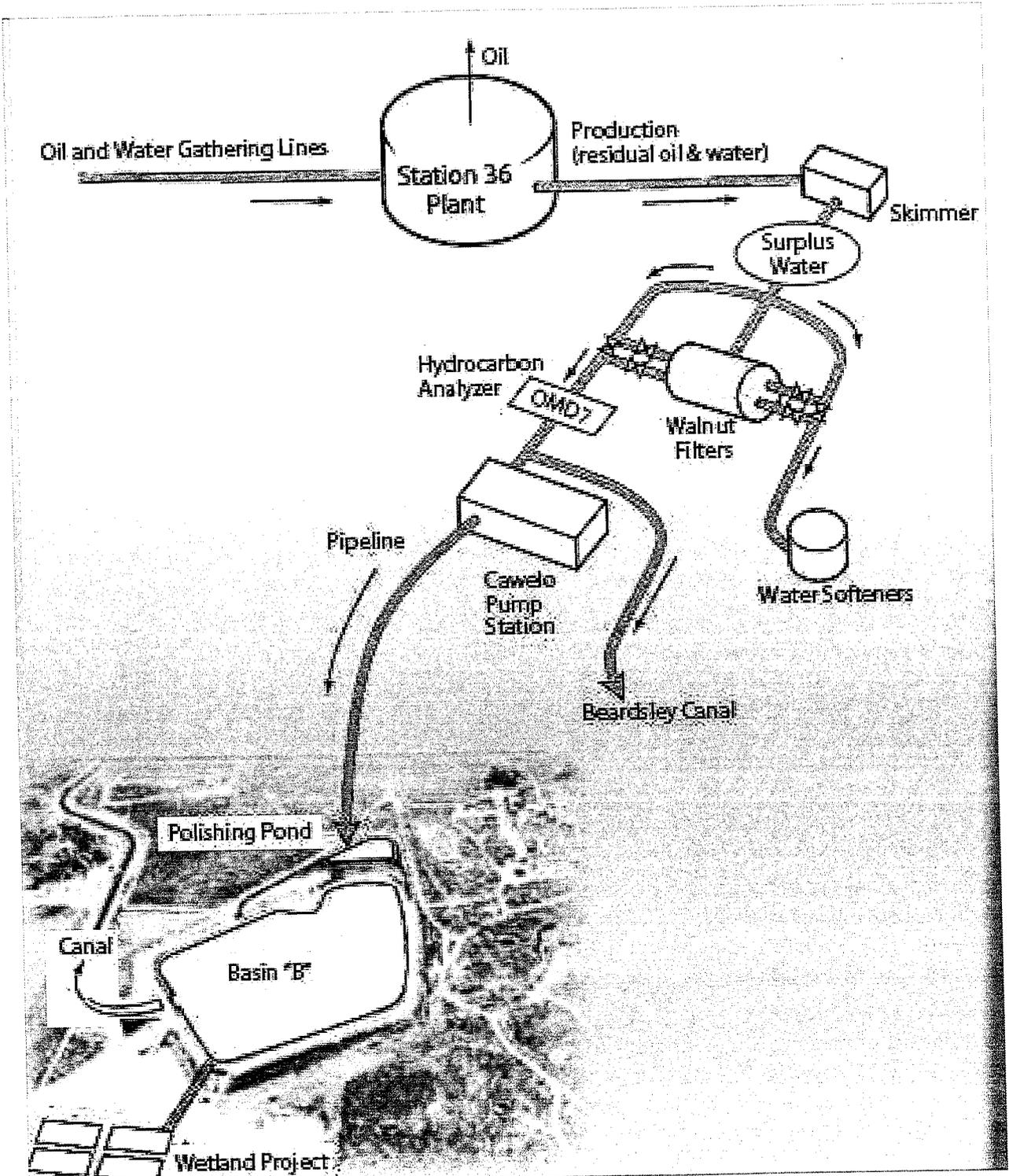


Figure 3-2. Kern River field produced water system schematic and reservoir for produced water for Cawelo Water District (source: Produced Water Reuse at the Kern River Oil Field, Southwest Hydrology, November/December 2005 p. 26-27).

3.2.1 Kern River Area Station 36 Treatment Process

Kern oil field produced water is treated in a multi-step process using gravity, chemicals and filtration (Figure 3-3). The oil/water mixture enters from the production header (No. 1). The initial oil is separated gravimetrically in surge and clarifier tanks (No. 2 and 3). The produced water then flows to the floatation (Wemco) units where any additional oil is removed (No. 4). After floatation, the flow is split and approximately half the water is retained for re-use by Chevron. The remaining ~50% is treated using walnut shell filters to remove additional oil and fine particles and then piped through an 8.5 mile, coated steel pipeline to a polishing pond (adjacent to Reservoir "B") for final treatment. Treated produced water then flows from the polishing pond into the CWD Reservoir "B" for agricultural reuse.

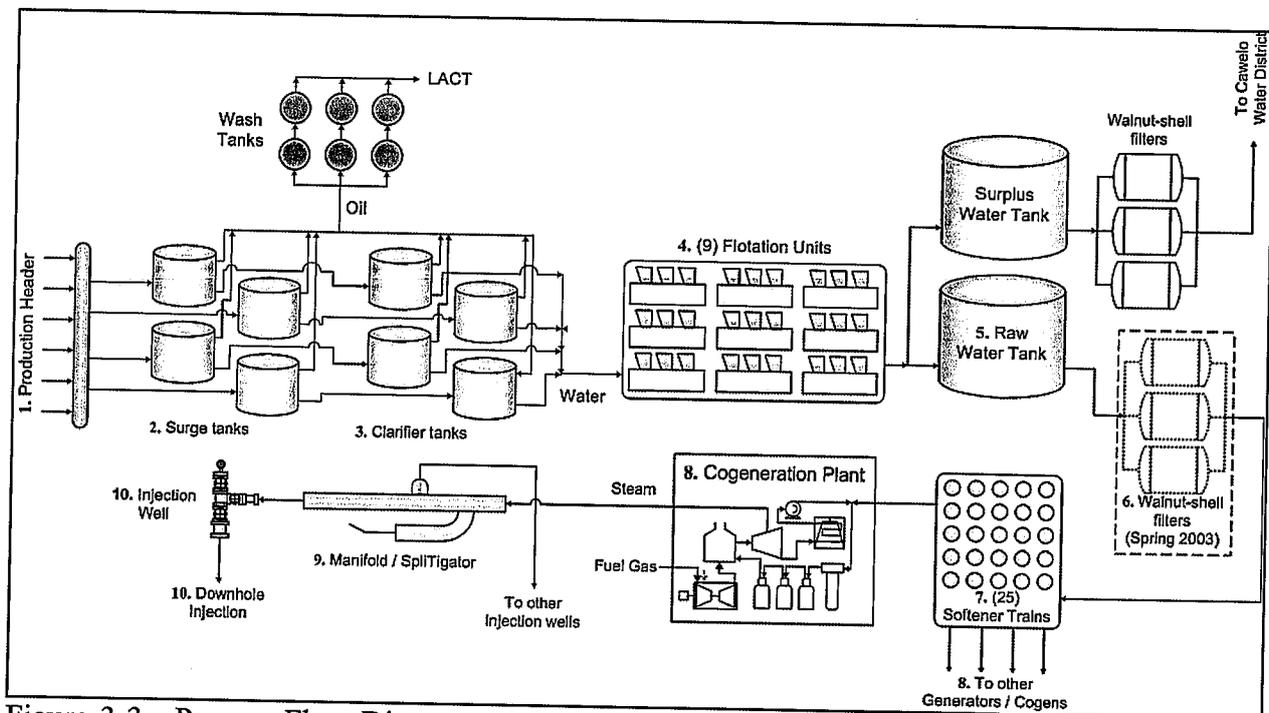


Figure 3-3. Process Flow Diagram of the Kern River Produced Water Treatment System. The treated produced water is reused for cogeneration, steamflood operations and irrigation.

3.2.2 Station 36 Treatment Facility – General Housekeeping

Each piece of equipment used in the Kern River Station 36 treatment facility undergoes routine monitoring and maintenance procedures. These monitoring and maintenance procedures include a step-wise set of tasks with a specific description of the task and instruction for completing the task. The procedures also include system diagrams, spill or other applicable emergency procedures, references to applicable Material Safety Data Sheets (MSDS) and personal protective equipment (PPE) necessary to complete the task. Operators undergo a training program prior to operating and a maintenance record is kept for each piece of equipment.

Chevron and its chemical suppliers routinely undergo an internal self initiated "Best Practice Evaluation" for the treatment plant operations and process chemicals. During this evaluation, potential issues or concerns related to the treatment process are identified and best practices procedures are defined and shared with other Chevron operations. At the same time, treatment

chemicals and their effectiveness and potential hazards are reviewed and new options are evaluated.

3.3 Treatment Chemicals used at Kern River Station 36 Treatment Facility

As with most water treatment facilities, a variety of treatment chemicals are used during the Station 36 treatment processes, many of which are proprietary. Table 3-2 provides a list of treatment chemicals used in the Kern River Station 36 treatment facility. Although all of these chemicals are used at the treatment facility, only the chemicals listed in Table 3-3 have the potential to be present in the treated produced waters going to Reservoir "B". The chemicals listed in Table 3-3 are either used in the oil field as part of operations or are used in the initial treatment processes [surge tanks, clarifier, or floatation units (Number 1 – 6 for process flow diagram Figure 3-3)] prior to the pipeline to Reservoir "B". The other chemicals are used later in the treatment process as part of the Chevron water reuse for cogeneration and steamflood operations.

Table 3-2. List of treatment chemicals used at Kern River Station 36 treatment facility.

- RBW-301X reverse breaker
- FLW-163 Wemco polymer
- WAW-400 surfactant
- CRW-10 corrosion inhibitor
- OSW-5200 oxygen scavenger
- CLW-3075 detergent
- CLO-64 detergent
- BPB 59480
- CRW 132 corrosion inhibitor
- DMO7051 – emulsion breaker
- PAW4 – cold oil treatment
- DF091 – antifoamer
- BPR45120 - antifoamer

Table 3-3. List of treatment Chemicals used in the oilfield operations or in Surge tanks, clarifier, or floatation units in Kern River Station 36 treatment facility.

Product	Application	Treatment Applied to:	Chemical Site Location
CRW10	Corrosion Inhibitor	Water	Station 36 Lease Water Site
WAW400	EOA Solution Wemcos (Wetting Agent)	Water	Station 36 By Surge Tank North of Surge Tank 2
DMO7051	Demulsifier	Oil	Field
PAW4	Cold Oil Treatment	Oil	Field
DF091	Antifoamer	Oil	Field
RBW301X	Reverse Breaker	Water	Field
FLW163	Polymer	Water	Station 36 Wemcos

Given the proprietary nature of many of the treatment chemicals, little is known about the aquatic toxicity of these compounds. To the extent possible, MSDS sheets, available chemical specific Environmental Assessment Sheets and EcoTox Reports, and the physical/chemical properties of these of these treatment chemicals and their active ingredients (Table 3-4) were evaluated for potential aquatic toxicity. For most of the active ingredients identified, aquatic toxicity is not well defined in the scientific literature and therefore toxicity profiles do not exist beyond what is available from the manufacturer. However, given the aquatic toxicity information that is readily available (e.g., LC50 data [Lethal Concentrations determined to cause mortality in 50% of the population] and other toxicity benchmark data) and the estimated concentrations and percent by weight in the treatment chemicals, it does not appear that any of these chemicals are the sole causative agent of the observed aquatic toxicity. In addition, it should be noted that the produced water undergoes treatment after these chemicals are added (walnut shell filters at a minimum) and a significant amount of elapsed time and atmospheric exposure (collectively termed 'weathering') occurs prior to the water reaching EFF-003. Ultimately it is possible that components of the treatment chemicals are contributing to the observed toxicity. However, it is unlikely they are the sole source of observed toxicity.

Table 3-4. Active ingredients found in treatment chemicals used at Kern River Station 36 Treatment Plant (Table 3-3 above).

- 1,2,4- trimethylbenzene
- 1,2,3-trimethylbenzene
- 1,3,5-trimethylbenzene
- Xylene
- ethylbenzene
- ethylene glycol
- light aromatic naptha
- acetic acid
- methanol
- Isopropanol

3.4 **NPDES Toxicity Testing**

As defined in NPDES permit CA0082295, acute and chronic toxicity tests are conducted following standard EPA procedures:

- Methods for Measuring the Acute Toxicity of Effluent and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition, EPA-821-R-02-012, October 2002;
- Short term methods for estimating chronic toxicity of Effluents in Receiving Waters to Freshwater Organisms, Fourth Edition, EPA-821-R-02-013; and
- Technical Support Document for Water Quality-based Toxics Control, EPA 505/2-90-001, March 1991.

Acute Toxicity Testing – The acute toxicity test consists of a 96-hr survival test with fathead minnows. As per the NPDES permit, the compliance limits for acute toxicity testing are:

- Survival of the fathead minnows in 96-hour bioassays of 100% effluent shall be no less than:
 - i. 70% for any one bioassay; and

- ii. 90% for the median of any three consecutive bioassays.

Chronic Toxicity Testing – The chronic toxicity testing includes short-term chronic toxicity tests using three species:

- A 96-hr algal growth test with the green alga *Selenastrum capricornutum*;
- A 6-8 day survival & reproduction test with the freshwater crustacean *Ceriodaphnia dubia*;
- A 7-day survival & growth test with the larval life stage of the freshwater fish *Pimephales promelas* (the fathead minnow).

There is currently no numerical “limit” for chronic toxicity tests (although the permit can be reopened to include a chronic toxicity limit). Instead, there is a “monitoring trigger” of >1 TUc (where TUc = $100/\text{‘No Observed Effect Concentration’}$ [NOEC], where the NOEC is the highest tested effluent concentration at which no statistically significant reduction in test response [e.g., survival, growth, or reproduction] at any effluent concentration relative to the Control treatment is observed). If and when any statistically significant reduction in test response at any effluent concentration is observed, accelerated monitoring is triggered. Accelerated monitoring consists of four chronic toxicity tests every two weeks using the test species that exhibited toxicity. If no toxicity is observed in the four accelerated tests, then chronic toxicity testing will return to the routine schedule as defined by the permit. If a pattern of toxicity is observed (i.e., toxicity exceeds the monitoring trigger more than 20 percent of the time), the site will initiate a TRE (CA0082295 Section IV.C.2).

Results of the March 2, 2009, NPDES chronic toxicity testing demonstrated significant reductions in *C. dubia* reproduction and complete mortality of fathead minnows exposed to the Chevron/Cawelo Water District effluent (EFF-003). As a result of the observed toxicity, accelerated monitoring was initiated and the effluent was resampled on March 13, 2009. The results of the accelerated monitoring also showed significant toxicity with:

- Significant reductions in *C. dubia* reproduction (reproduction NOEC was 50% effluent resulting in 2.0 TUc (where TUc = $100/\text{NOEC}$), and
- Significant reductions in fathead minnow survival (survival NOEC was 12.5% effluent, resulting in 8.0 TUc (where TUc = $100/\text{NOEC}$).

Accelerated monitoring indicated continued significant chronic toxicity. As a result, the TIE/TRE investigation was initiated using sample(s) collected on April 21, 2009.

4 TIE BACKGROUND AND APPROACH

The TIE/TRE workplan used by the Site is modeled after USEPA TIE/TRE methodology:

- Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Characterization Procedures, Second Edition, EPA 600/6-91/005F, Feb. 1991;
- Toxicity Identification Evaluation: Characterization of Chronically Toxic Effluents, Phase I, EPA 600/6-91/005F, May 1992;
- Methods for Aquatic Toxicity Identification Evaluations: Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA-600/R-92/080. U.S.EPA, Environmental Research Laboratory, Duluth, MN;
- Toxicity Reduction Evaluation Guidance for Municipal Wastewater Treatment Plants, EPA/883B-99/002, August 1999; and
- Generalized Methodology for Conducting Industrial TREs, EPA/600/2-88-070.

Based upon knowledge of the chemistry of this produced water and previous produced water toxicity investigations, the site elected to conduct a targeted TIE that did not include all of the traditional TIE treatments, but instead targeted the fraction of compounds that have historically demonstrated toxicity in produced waters (Proposed Work Plan, September 2008) as well as some potential toxicants of recent regional concern (e.g., selected pesticides).

The initial targeted TIE was performed on an effluent sample collected April 21, 2009, and included 4 treatments for testing with *C. dubia* survival & reproduction and fathead minnow survival & growth:

- Baseline;
- Centrifugation;
- C18 Solid Phase Extraction (SPE) treatment;
- Aeration; and
- Piperonyl butoxide (PBO).

A second “confirmation” Phase I and Phase II TIE were performed on an effluent sample collected September 21, 2009. Based on the results of the previous TIE, additional toxicity testing performed during the interim, and professional experience, the 2nd TIE toxicity testing focused on the *C. dubia* reproduction and fathead minnow survival & growth responses, and the TIE treatments were modified to include:

- Baseline;
- pH3 and pH11 adjustments;
- Filtration at the ambient pH, plus filtration at pH3 and at pH11;
- C18SPE treatment at the ambient pH, plus C18 SPE at pH3 and at pH11;
- Aeration at the ambient pH, plus aeration at pH3 and at pH11;
 - Aeration “wash down”; and
- Humic acid addition.

The third and final Phase I and Phase II TIEs were performed on an effluent sample collected January 11, 2010. Based on the results of the previous two TIEs, additional toxicity testing, and

professional experience, the 3rd TIE toxicity testing focused on fathead minnow survival, and the TIE treatments were modified to include:

- Baseline;
- Graduated pH testing at pH6, pH7, and pH8;
- pH3 and pH11 adjustments;
- Filtration at the ambient pH, plus filtration at pH3 and at pH11; and
- C18 SPE treatment at the ambient pH, plus C18 SPE at pH3 and at pH11.

Figures 4-1 and 4-2 outline the steps of the Phase I and Phase II TIE process. Appendix A provides a brief description of the nature of the TIE treatments included in the TIEs of the April 2009, September 2009, and Jan 2010 effluent samples.

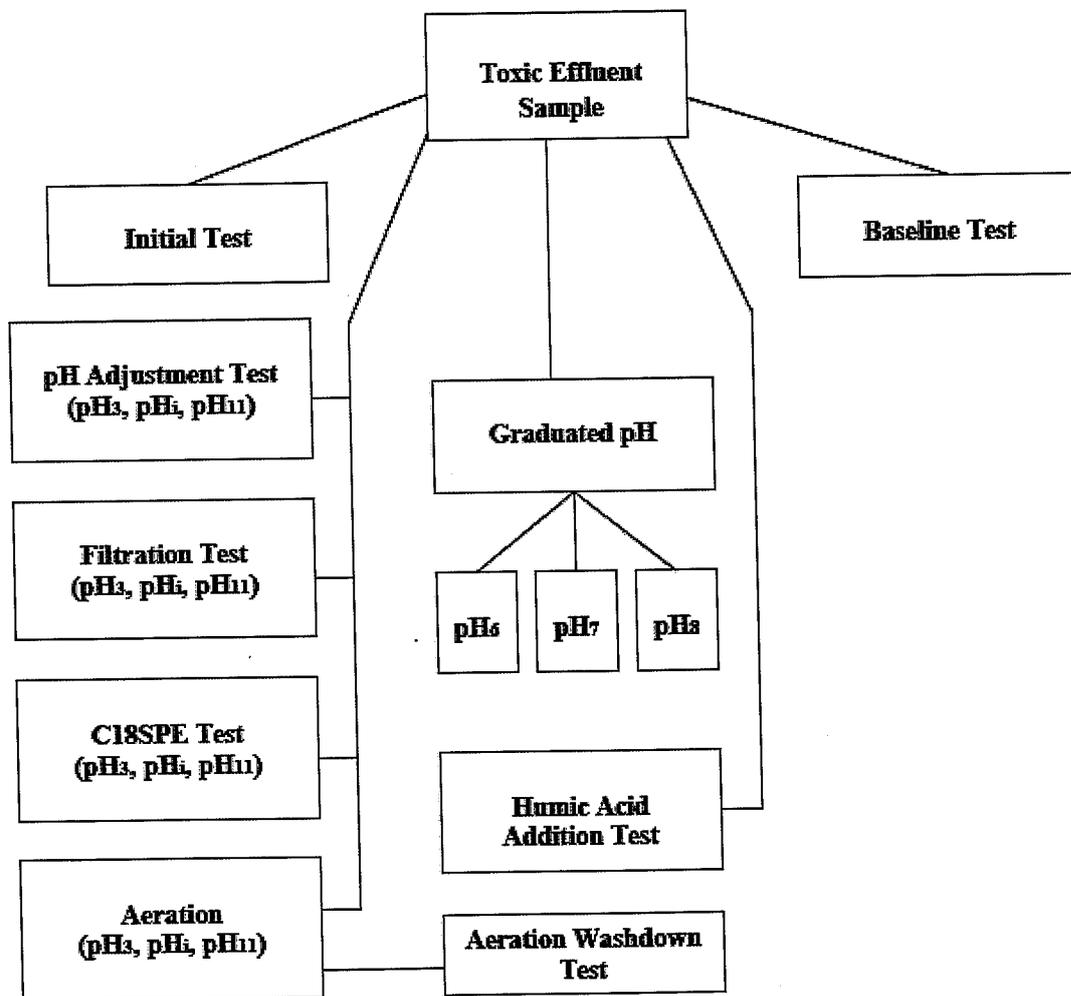


Figure 4-1: Phase I Confirmation Toxicity Identification Evaluation (TIE) Treatment Procedures

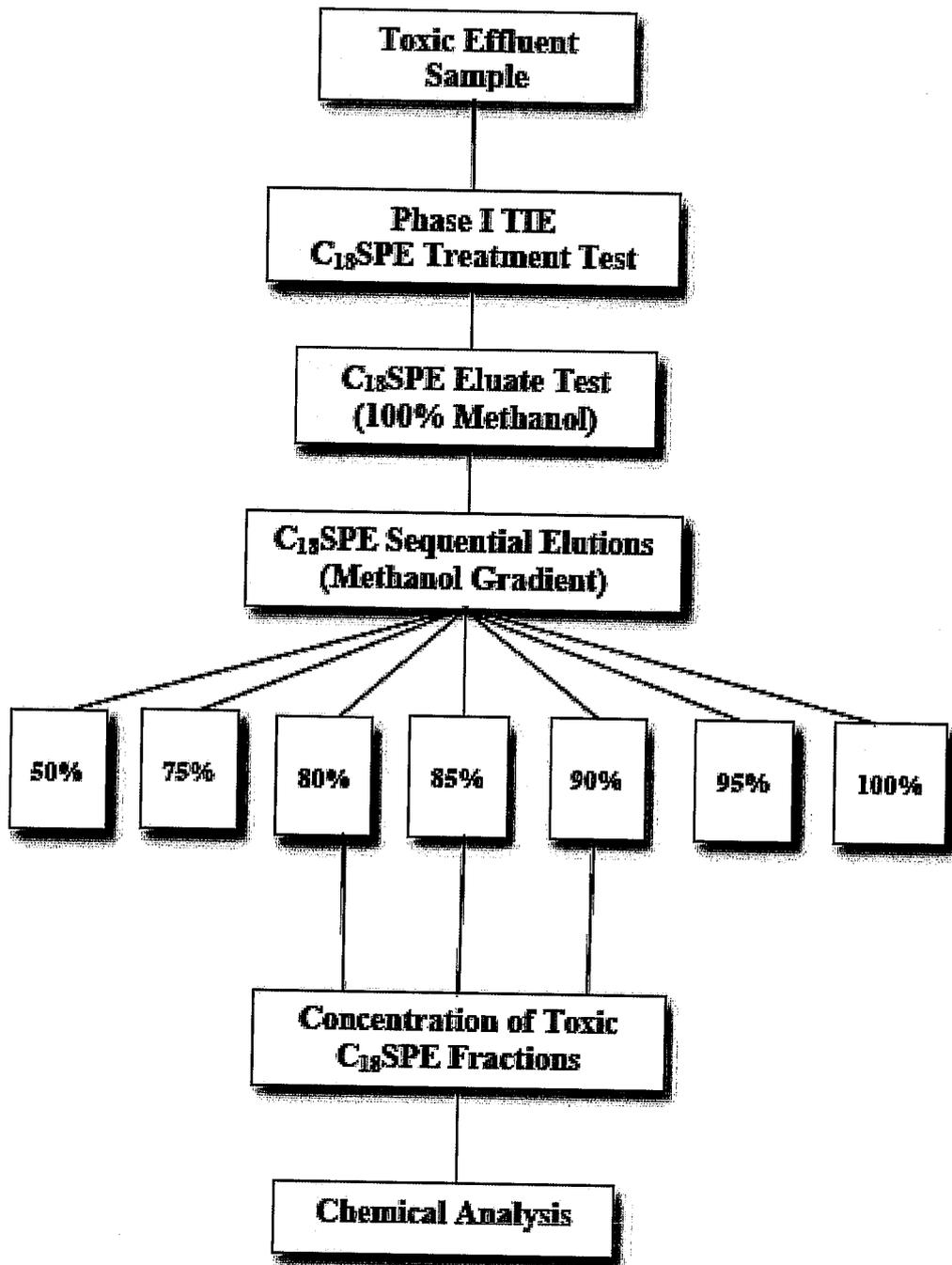


Figure 4-2. Phase II Toxicity Identification Evaluation Treatment Procedures

5 MATERIALS AND METHODS

5.1 Sampling Sites

As described in Section 3.4, the TIE was initiated as a result of repeated observed toxicity to *C. dubia* reproduction and fathead minnow survival in samples collected from the Chevron/ Cawelo effluent (EFF-003 - the outfall from the distribution canal to Poso Creek). Therefore, sample collection sites for the initial toxicity assessment (Step 1 of TRE) were selected to try to identify the primary source of the observed toxicity. As described in Section 3, there are several sources of water into Reservoir B and the CWD distribution canal. In order to identify if a specific effluent stream into Reservoir B was the source of the toxicity, or if surface runoff from the land surrounding the distribution canal was contributing to the toxicity, five sample locations were selected for toxicity assessment:

- Inlet to Reservoir B – treated produced water entering Reservoir B (EFF 001);
- Valley Waste – discharge point into Reservoir B;
- Outlet to Canal – combined flow from Reservoir B [located at discharge to CWD distribution canal (EFF-002)];
- Splitter Box - the combined flow from Reservoir B (located at the entrance to the demonstration wetland); and
- Top of Hill - the irrigation canal prior to discharge into Poso Creek (termed irrigation canal “top of hill”) (located at EFF-003).

Figure 5-1 and 5-2 show the locations of the TRE “source” sampling sites. Table 5-1 shows the sample dates and type of toxicity test for each sample location.

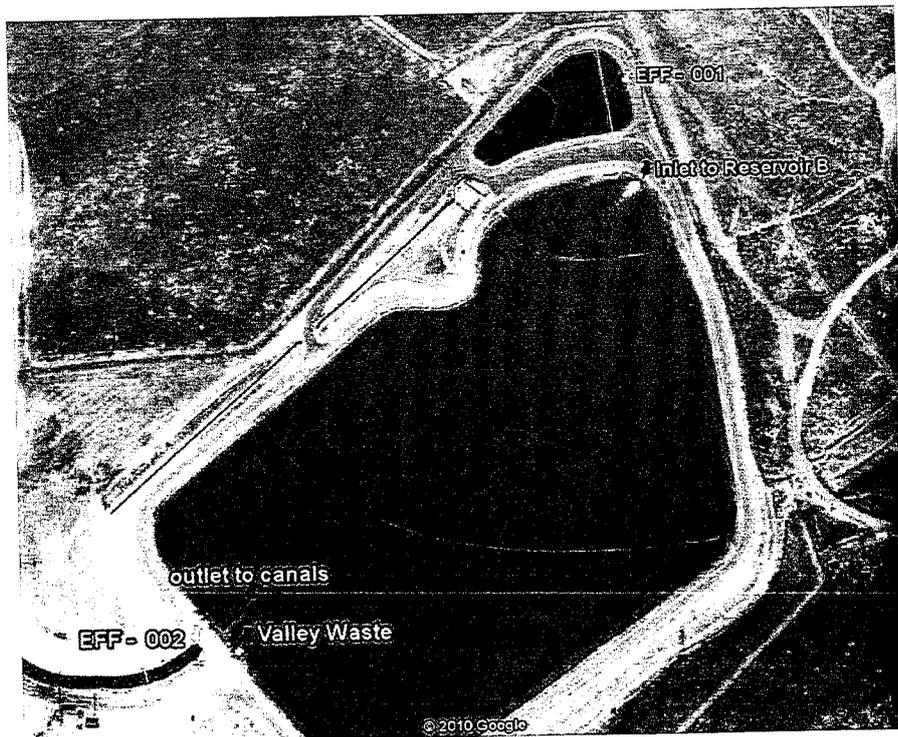


Figure 5-1. NPDES and TIE sampling locations near Reservoir B.

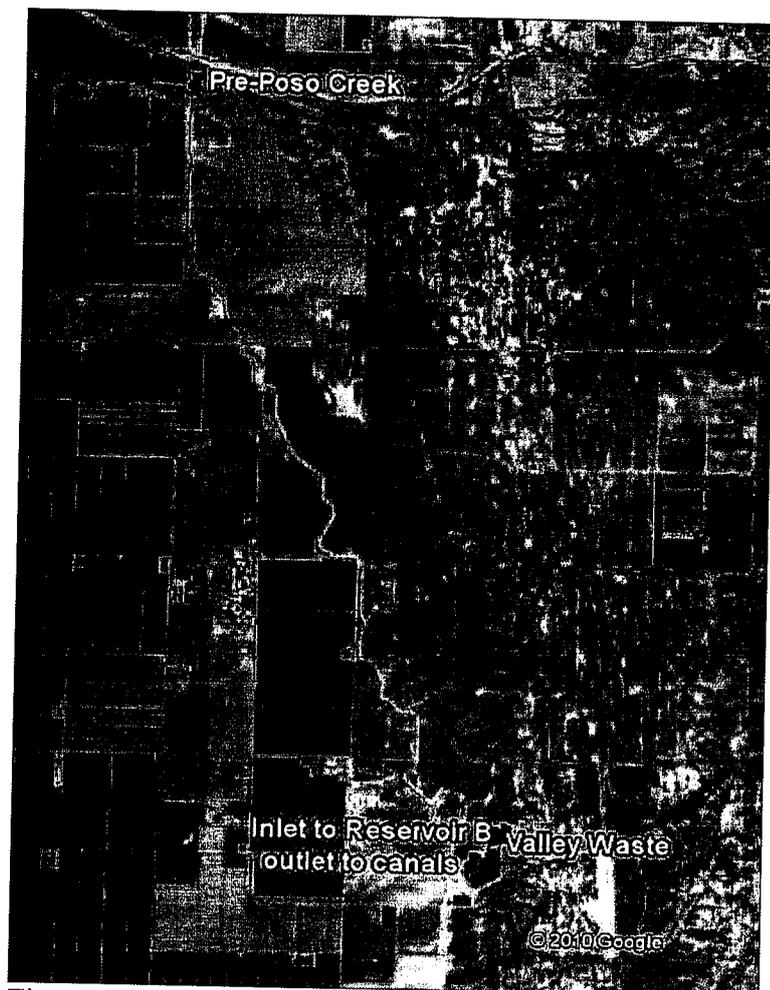


Figure 5-2. TIE toxicity assessment sample locations at Reservoir B and CWD distribution canal.

Table 5-1. Sample dates and type of toxicity test for Chevron/CWD TIE investigation

Sample Location	Sample Date	Type of Test	Dilution Series
Inlet to Reservoir "B"	April 21, 2009	Chronic toxicity / TIE	Full dilution
	August 31, 2009	Chronic toxicity	Full dilution
	September 21, 2009	Chronic toxicity / TIE	Full dilution
	January 11, 2010	Chronic toxicity / TIE	Full dilution
Valley Waste	April 21, 2009	Chronic toxicity	100% screen
	January 11, 2009	Chronic toxicity	Full dilution
Outlet to Canal (EFF-002)	January 11, 2010	Chronic toxicity	Full dilution
Splitter Box	April 21, 2009	Chronic toxicity	100% screen
	August 31, 2009	Chronic toxicity	Full dilution
	September 21, 2009	Chronic toxicity	Full dilution
Pre-Poso Creek (EFF-003)	April 21, 2009	Chronic toxicity	100% screen
	September 21, 2009	Chronic toxicity	Full dilution

5.2 Sample Handling and Receipt

Water samples were collected by Precision Analytical staff and transported by courier on ice and under chain-of-custody (COC) to the Pacific Ecorisk (PER) laboratory in Fairfield, CA for toxicity testing. Samples were collected as grab samples in appropriately cleaned 5 gallon sample containers. For the initial toxicity tests, 5 gallons of water were collected from each site. When needed for potential TIE testing, an additional 25 gallons were collected from the Reservoir B sample location. In the initial toxicity assessment tests of samples collected in April 2009, water was collected from Lerdo Canal for dilution water. However, due to pathogen-related mortalities this water caused in the fish, it was decided to only use laboratory waters for dilution and control treatment purposes for all testing (this is consistent with the NPDES permit). Upon receipt at PER, aliquots of each sample were collected for analysis of initial water quality characteristics, with the remainder of the samples being stored at 0-6°C except when being used to prepare test solutions. COC records for the collection and delivery of each sample are provided in Appendix A of each PER report. Individual PER reports are included as attachments 1 through 9 of this report.

5.3 Toxicity Testing Protocols

5.3.1 Survival and Reproduction Toxicity Testing with *Ceriodaphnia dubia*

The short-term chronic *C. dubia* test consists of exposing individual females to effluent for the length of time it takes for the Lab Control treatment females to produce 3 broods (typically 6-8 days), after which effects on survival and reproduction are evaluated. The specific procedures used in this test are described below.

The Lab Water Control treatment for these tests consisted of a mixture of Type 1 lab water (reverse-osmosis, de-ionized water) with a commercial spring water (Perrier®). The Lab Water and the effluent samples were used to prepare test solutions at the 12.5, 25, 50, 75 and 100% effluent concentrations for the full dilution tests. For the 100% screen, water samples were tested at the 100% concentration only. A lab water control was also tested. For each treatment, ~150 mL of test solution was amended with the alga *Selenastrum capricornutum* and Yeast-Cerophyll®-Trout Food (YCT) to provide food for the test organisms. "New" water quality characteristics (pH, D.O., and conductivity) were measured on these food-amended test solutions prior to use in these tests. Fresh test solutions and a "new" set of replicate cups were prepared and characterized daily, as before.

For routine chronic toxicity testing there were 10 replicates for each test treatment, each replicate consisting of 15 mL of test solution in a 30-mL plastic cup. These "3-brood" tests were initiated by allocating one neonate (<24 hrs old) *C. dubia*, obtained from ongoing laboratory cultures, into each replicate. The replicate cups were placed into a temperature-controlled room at 25°C, under cool-white fluorescent lighting on a 16L:8D photoperiod.

Each test replicate cup was examined every other day, with surviving "original" individual organisms being transferred to the corresponding new cup containing fresh test solution. The contents of each remaining "old" replicate cup were carefully examined, and the number of neonate offspring produced by each original organism was determined, after which "old" water

quality characteristics (pH, D.O., and conductivity) were measured for the old media from one randomly-selected replicate at each treatment.

After it was determined that 60% of the *C. dubia* in the Lab Water Control treatment had produced their third brood of offspring, the accompanying tests were terminated. The resulting survival and reproduction (number of offspring) data were analyzed to evaluate any impairment(s) caused by the effluent; all statistical analyses were performed using the CETIS® statistical software.

5.3.2 Survival and Growth Toxicity Testing with Larval Fathead Minnows

The chronic fathead minnow test consists of exposing larval fish to effluent for 7 days, after which effects on survival and growth are evaluated. The specific procedures used in this test are described below.

The Lab Water Control treatment for these tests consisted of US EPA synthetic moderately-hard water. The Lab Water and the effluent sample were used to prepare daily test solutions at the 12.5, 25, 50, 75 and 100% effluent concentrations for the full dilution tests. For the 100% screen, water samples were tested at the 100% concentration only. "New" water quality characteristics (pH, D.O., and conductivity) were measured on these test solutions prior to use in this testing.

There were 4 replicates at each test treatment, each replicate consisting of 400 mL of test media in a 600-mL glass beaker. These tests were initiated by randomly allocating 10 larval fathead minnows (<48 hrs old) into each replicate. The replicate beakers were placed in a temperature controlled room at 25°C, under cool-white fluorescent lighting on a 16L:8D photoperiod. The test fish were fed brine shrimp *nauplii* daily.

Each replicate was examined daily, with any dead animals, uneaten food, wastes, and other detritus being removed. The number of live fish in each replicate was determined and then approximately 80% of the test media in each beaker was carefully poured out and replaced with fresh test solution. "Old" water quality characteristics (pH, D.O., and conductivity) were measured on the old test water that had been discarded from one randomly-selected replicate at each treatment.

After 7 days exposure, the number of live fish in each replicate beaker was recorded. The fish from each replicate were then carefully euthanized in methanol, rinsed in de-ionized water, and transferred to a pre-dried and pre-tared weighing pan. These fish were then dried at 100°C for >24 hrs and re-weighed to determine the total weight of fish in each replicate; the total weight was then divided by the initial number of fish per replicate (n=10) to determine the "biomass value". The resulting survival and growth ("biomass value") data were analyzed to evaluate any impairment(s) caused by the effluent; all statistical analyses were performed using the CETIS® statistical software.

5.3.3 TIE Testing Procedures

For the TIE investigation, the same test procedures as described above were used for both the *C. dubia* and fathead minnow with the following modifications:

- For *C. dubia* TIE testing, 5 replicates were used for each test treatment, each replicate consisting of 15 mL of test solution in a 30-mL plastic cup. The Lab Water, the effluent sample and TIE treated effluent samples were used to prepare test solutions at the 50% and 100% effluent concentrations.
- For the fathead minnow TIE testing, there were 2-3 replicates at each test treatment, each replicate consisting of 400 mL of test media in a 600-mL glass beaker. The Lab Water, the effluent sample and the TIE treated effluent samples were used to prepare daily test solutions at the 50% and 100% effluent concentrations.

5.3.4 Reference Toxicity Testing

In order to assess the sensitivity of the *C. dubia* and larval fathead minnows to toxic stress, reference toxicant testing was performed. The reference toxicant tests were performed similarly to the effluent tests, except that test solutions consisted of "Lab Control" media spiked with NaCl at appropriate ranges of concentrations for each species. The resulting test response data were analyzed to determine key dose-response point estimates (e.g. EC50); all statistical analysis were made using CETIS[®] statistical software. These response endpoints were then compared to the 'typical response' ranges established by the mean ± 2 SD of the point estimates generated by the 20 most recent previous reference toxicant tests performed by PER.

6 RESULTS

Waters were collected from multiple sources for “initial assessment” toxicity tests to determine potential toxicity for waters that discharged in to Reservoir B. The full toxicity test reports prepared by PER are included in Attachments 1 through 6 for each toxicity test and TIE test conducted. Table 6-1 to 6-5 below provides a summary of each toxicity test conducted as part of the TIE/TRE investigation. Table 6-6 and 6-7 provides a summary of the TIE treatment results for *C. dubia* and fathead minnow, respectively.

6.1 Results of the “Initial Assessment” Toxicity Tests

6.1.1 Effects of Chevron/Cawelo Effluent (Inlet to Reservoir B)

Significant toxicity was observed for *C. dubia* reproduction and fathead minnow survival and growth in multiple 2008 and Spring 2009 NPDES toxicity tests of the EFF-003 samples (discharge to Poso Creek).

Table 6-1. Summary of effects of Chevron/Cawelo effluent (EFF-003) on *Ceriodaphnia dubia* and fathead minnow prior to the initiation of the TIE/TRE.

Sample Collection Date	Effluent Test Treatment	<i>Ceriodaphnia dubia</i>		Fathead Minnows	
		Mean % Survival	Reproduction ^a	Mean % Survival	Mean fish biomass
December 1, 2008	Lab Control	100	25.7	100	0.64
	100%	100	6.5*	7.5*	0.02
December 3, 2008	Lab Control	100	25.2	100	0.85
	100%	100	0*	0*	-
March 2, 2009	Lab Control	100	24.7	100	0.43
	100%	100	19.4*	0*	-

*Significantly less than the lab control treatment response at $p < 0.05$.

Similar toxicity patterns were observed when testing the Chevron treated produced water discharged into Reservoir B (Table 6-2). *C. dubia* survival was not impaired during these tests; however, significant toxicity to *C. dubia* reproduction was observed with the NOEC's ranging from 25% effluent in the April 2009 test to 75% effluent in the August 2009 and January 2010 events. This suggests that the source of *C. dubia* toxicity in the treated produced water may fluctuate over time.

Significant toxicity to fish survival and growth was observed in each of the tests using water from EFF-001 “Inlet to Reservoir B” (NOECs for survival ranging from 12.5% to 25% water, NOECs for growth ranging from <12.5% to 12.5% water)(Table 6-2). A comparison of the NOECs from each sample suggests that the source of the fathead toxicity in the treated produced water is fairly constant over time.

Table 6-2. Summary of chronic toxicity tests *Ceriodaphnia dubia* and fathead minnow conducted using waters collected from EFF-001 (Inlet to Reservoir "B") as part of the TIE/TRE investigation.

Sample Collection Date	Effluent Test Treatment	<i>Ceriodaphnia dubia</i>		Fathead Minnows	
		Mean % Survival	Reproduction ^a	Mean % Survival	Mean fish biomass
April 21, 2009 (Attachment 1)	Lab Control	100	25.4	100	0.48
	Receiving Water Control	100	28.7	55	0.30
	12.5%	100	26.8	67.5	0.23
	25%	100	23.6	22.5	0.05*
	50%	100	15.9*	0*	-
	75%	100	8.2*	0*	-
	100%	100	1.9*	0*	-
August 31, 2009 (Attachment 2)	Lab Control	80	15.1	97.5	0.45
	12.5%	100	24.1	90	0.34*
	25%	80	21.3	80*	0.27*
	50%	100	19.7	40*	0.09*
	75%	90	10.0	0*	-
	100%	90	1.8*	0*	-
September 21, 2009 (Attachment 3)	Lab Control	90	21.1	100	0.48
	12.5%	90	20.8	67.5	0.23
	25%	100	22.5	47.5	0.05*
	50%	100	16.7	0*	-
	75%	90	7.8*	0*	-
	100%	100	3.6*	0*	-
January 11, 2010 (Attachment 4)	Lab Control	100	28.2	90	0.31
	12.5%	90	32.1	90	0.30
	25%	100	35.2	75	0.19*
	50%	100	34.4	7.5*	0.01*
	75%	100	30.1	0*	-
	100%	100	19.6*	0*	-
September 3, 2010 (Attachment 5)	Lab Control	NA	NA	100	0.38
	12.5%	NA	NA	92.5	0.32*
	25%	NA	NA	37.5	0.10*
	50%	NA	NA	0*	-
	75%	NA	NA	0*	-
	100%	NA	NA	0*	-

NA = Not analyzed

* = The response at this test treatment was significantly less than the lab Control treatment response at $p < 0.05$.

a - *C. dubia* reproduction is assessed as the mean # of offspring produced per surviving female.

6.1.2 Valley Waste Discharge

The results of the toxicity tests conducted using Valley Waste treated produced water discharged into Reservoir ‘B’ are similar to the results observed when testing the Chevron treated produced water (Table 6-3). Significant reproductive effects were observed for *C. dubia* (NOEC = 25% effluent), with no impairment of survival. A comparison of results from the January 2010 event shows that treated produced water collected from Chevron during this event had less toxicity (reproductive NOEC = 75% water) than was observed from Valley waste for the same date (NOEC = 25%). However, during the April 2009 event, Chevron produced water produced a reproduction NOEC of 25% water. This suggests that a similar source of toxicity to *C. dubia* may be present in both produced waters and may fluctuate over time.

Results of the fathead minnow toxicity tests indicate significant impacts to both survival and growth (survival NOEC = 25% water and growth NOEC = 12.5% water). These results are very similar to what was observed in the Chevron treated produced waters and suggest a similar source of toxicity.

Table 6-3. Summary of chronic toxicity tests for *Ceriodaphnia dubia* and fathead minnow conducted using waters collected from Valley Waste discharge into Reservoir ‘B’. Water collected as part of the TIE/TRE investigation.

Date	Effluent Test Treatment	<i>Ceriodaphnia dubia</i>		Fathead Minnows	
		% Survival	Reproduction ^a	% Survival	Mean fish biomass
April 21, 2009 (Attachment 1)	Lab Control	80	15.8	97.5	0.48
	100%	90	2.2*	0*	-
January 11, 2010 (Attachment 6)	Lab Control	100	31.8	92.5	0.29
	12.5%	100	35.9	95	0.25
	25%	100	30.9	92.5	0.24*
	50%	100	16.5*	44.4*	0.10*
	75%	100	2.5*	10*	0.01
	100%	100	0.4*	0*	-
September 3, 2010 (Attachment 5)	Lab Control	NA	NA	100	0.41
	12.5%	NA	NA	87.5	0.33*
	25%	NA	NA	62.5*	0.22*
	50%	NA	NA	10*	0.03*
	75%	NA	NA	0*	-
	100%	NA	NA	0*	-

NA = Not analyzed

* = The response at this test treatment was significantly less than the lab Control treatment response at $p < 0.05$.

a - *C. dubia* reproduction is assessed as the mean # of offspring produced per surviving female.

6.1.3 Splitter Box and EFF-002 (discharge from Reservoir ‘B’)

As part of the TIE/TRE investigation, water was collected from the splitter box of the constructed wetland pilot project (located adjacent to Reservoir ‘B’). This water was collected in September 2009 to represent the coalesced water discharged from Reservoir ‘B’ (Table 6-4).

In January 2011, the discharge from Reservoir "B" to the distribution canal (EFF-002) was collected to represent the coalesced waters (Table 6-5). In both cases, the results of the toxicity tests for *C. dubia* and fathead minnows were similar to the results observed from the treated produced waters from Chevron and Valley Waste. This would be expected in the January 2011 sample because the total flow from Reservoir "B" was comprised of treated produced water. However, this also demonstrates that the causative agents for toxicity observed from the produced waters are not additive or synergistic.

As might be expected, less toxicity was observed during the September 2009 sampling event where waters from other CWD sources were blended into Reservoir "B". During the September 2009 event, the *C. dubia* reproduction NOEC increased to 75% water and the fathead survival and growth NOECs increased to 50% water.

Table 6-4. Summary of chronic toxicity tests for *Ceriodaphnia dubia* and fathead minnow conducted using waters collected wetland splitter box (coalesced waters from Reservoir "B").

Sample Collection Date	Effluent Test Treatment	<i>Ceriodaphnia dubia</i>		Fathead Minnows	
		Mean % Survival	Reproduction ^a	Mean % Survival	Mean Fish Biomass
September 21, 2009 (Attachment 7)	Lab control	100	25.4	100	0.39
	12.5%	100	26.2	97.5	0.36
	25%	100	24.7	92.5	0.36
	50%	100	25.4	92.5	0.33
	75%	100	22.7	60*	0.19*
	100%	90	18.5*	27.5*	0.06*
January 11, 2010 (Attachment 6)	Lab control	100	33.5	90	0.30
	100%	100	21.7*	0*	-

*= The response at this test treatment was significantly less than the lab Control treatment response at $p < 0.05$.
a - *C. dubia* reproduction is assessed as the mean # of offspring produced per surviving female.

Table 6-5. Summary of chronic toxicity tests for *Ceriodaphnia dubia* and fathead minnow conducted using waters collected from EFF-002 ("Outlet to Canals").

Sample Collection Date	Effluent Test Treatment	<i>Ceriodaphnia dubia</i>		Fathead Minnows	
		Mean % Survival	Reproduction ^a	Mean % Survival	Mean Fish Biomass
January 11, 2010 (Attachment 6)	Lab control	100	36.5	100	0.33
	12.5%	100	39.5	100	0.31
	25%	100	41.0	90	0.25*
	50%	90	35.5	12.5*	0.03*
	75%	100	32.7	0*	-
	100%	100	20.3*	0*	-

*= The response at this test treatment was significantly less than the lab Control treatment response at $p < 0.05$.
a - *C. dubia* reproduction is assessed as the mean # of offspring produced per surviving female.

6.1.4 Pre-Poso Creek (Irrigation Canal Top of Hill, EFF-003)

The results of the water collected from the discharge to Poso Creek (irrigation canal at top of hill, EFF-003) (Table 6-6) are similar to the results observed from the discharge from Reservoir "B" (Table 6-5). This indicates that the causative agent of the toxicity is not being removed or significantly altered as the water flows down the distribution canal (approximately 5.3 miles exposed to ambient conditions and 38 miles in a pipeline).

Table 6-6. Summary of chronic toxicity tests for *Ceriodaphnia dubia* and fathead minnow conducted using waters collected from EFF-003 ["Pre-Poso Creek" (irrigation canal at top of hill)].

Sample Collection Date	Effluent Test Treatment	<i>Ceriodaphnia dubia</i>		Fathead Minnows	
		Mean % Survival	Reproduction ^a	Mean % Survival	Mean Fish Biomass
April 21, 2009 (Attachment 1)	Lab Control	80	15.8	97.5	0.48
	100%	90	4.1*	0*	-
September 21, 2009 (Attachment 7)	Lab control	100	23.2	92.5	0.34
	12.5%	100	25.9	96.9	0.34
	25%	100	29.0	90.0	0.33
	50%	100	26.2	87.5	0.26*
	75%	100	27.7	75.0	0.16*
	100%	90	21.8	40.0*	0.06*
January 11, 2010 (Attachment 6)	Lab Control	100	36.2	100	0.32
	12.5%	100	37.4	97.5	0.32
	25%	90	42.0	85	0.25*
	50%	100	38.6	37.5*	0.01*
	75%	100	34.6	0*	-
	100%	90	20.4*	0*	-

* = The response at this test treatment was significantly less than the lab Control treatment response at p<0.05.

a - *C. dubia* reproduction is assessed as the mean # of offspring produced per surviving female.

Several conclusions can be made based on the results of the Baseline toxicity tests that were conducted as part of the TIE/TRE:

1. Based on the observed NOEC's for both *C. dubia* and fathead minnows, the magnitude of toxic effects may fluctuate slightly over time but it appears that the causative agent(s) are found in the produced waters from both Chevron and Valley Waste.
2. It appears that the causative agent(s) are not readily removed by the treatment processes currently in place at the Chevron or Valley Waste facilities; and
3. The causative agent(s) are not readily degraded over time or from exposure to ambient conditions.

6.2 TIE Results

Based on the results of the toxicity tests conducted as part of the NPDES testing and TRE assessment, it was decided that the Chevron treated produced waters [Inlet to Reservoir "B" -

EFF-001], would be used for conducting the TIE investigation. Table 6-7 and 6-8 present summaries of the TIE Phase I TIE results for *C. dubia* and fathead minnows, respectively.

6.2.1 Phase I TIE results for *Ceriodaphnia dubia*

Phase I TIE's for *C. dubia* toxicity were conducted on waters from the Inlet to Reservoir B (EFF-001) using water samples collected on April 21, 2009 and September 21, 2009 (Results presented in Table 6-7). For both tests, no toxicity was observed to *C. dubia* survival in the untreated effluent, which was consistent with the "initial assessment" toxicity tests. The reductions in reproduction in the Baseline treatments (untreated effluent) were also consistent with the initial assessment tests confirming that toxicity was persistent and present at the time of the TIEs. The key findings of these TIEs follow:

- Reproductive toxicity was removed by the C18 SPE treatments at all pH adjustments indicating that non-polar organics were a cause of the observed toxicity.
- There was a slight increase in toxicity at the 100 µg/L PBO treatment which is suggestive of a contaminant that would normally be detoxified by the Cytochrome P-450 enzyme system. Pyrethroid pesticides are an example of a contaminant that might elicit this type of result.
- There was partial removal of toxicity by the pH_i (and pH₃) filtration treatments, which suggests that some fraction of the toxicants present had a high affinity for sorption to particulates or that may have had an affinity for sorption to the filter membrane; furthermore, this affinity for sorption was pH-dependent, increasing as pH decreased.
- There was significant removal of toxicity by the 20 mg/L and 40 mg/L humic acid treatments, with greater removal relative to the method blank being exhibited at the 40 mg/L treatment, indicating that contaminants amenable to sorption to dissolved organic carbon were a primary cause of the observed toxicity.

These test results are indicative of one or more organic contaminants that have a strong affinity for sorption to particulates and dissolved organic carbon. These results also indicate that the toxicant(s) are pH-labile. The PBO test results suggest the presence of a slightly-toxic contaminant that is detoxified by the Cyp450 enzyme system; while pyrethroid pesticides are typically linked to these PBO test results, other contaminants (e.g., polymers and some petroleum hydrocarbons) may also exhibit the same TIE effects.

6.2.2 Phase I TIE Results for Fathead Minnows

Phase I TIE's were conducted on Inlet to Reservoir B (EFF-001) effluent samples collected April 21, 2009, September 21, 2009, and January 11, 2010 (Results of these TIEs are presented in Table 6-8). In all cases, there were significant reductions in survival in the Baseline (untreated effluent) treatments, confirming that this toxicity was persistent and present at the time of the TIEs. These results were also consistent with the "initial" fathead minnow toxicity tests.

The key findings of these TIEs follow:

- There was significant removal of survival toxicity by the filtration treatments, which suggests that some fraction of the toxicants present were associated with particulates or had sorbed to the filtration membrane.

- There was complete removal of any residual toxicity (i.e., toxicity remaining after the filtration treatment) by the C18 SPE treatment, indicating that non-polar organics were a cause of the observed toxicity.
- There was pH-labile toxicity, with toxicity increasing as pH decreased to pH6, and toxicity decreasing as pH increased to pH8. This is suggestive of a weakly acidic toxicant that becomes less polar as the pH decreases and more polar as the pH increases. This type of pattern would be consistent with naphthenic acids as a cause of toxicity.
- In contrast to the *C. dubia* TIE results, there was no significant removal of toxicity by the humic acids treatments, which suggests that there may be different toxicants causing the toxicity to these two different species, with the *C. dubia* toxicants having a greater affinity for sorption.

Table 6-7. Phase I TIE Results for the *Ceriodaphnia dubia* Reproduction Response

TIE Treatment	April 21, 2009 - retest ^a (Attachment I)			September 21, 2009 (Attachment 8)		
	# of Neonates per Female		Was Toxicity Removed?	# of Neonates per Female		Was Toxicity Removed?
	Lab Control	100% Effluent		Lab Control	100% Effluent	
Baseline	24.8	7.8	na	37.7	8.7	na
centrifugation	24.2	9.2	No	-	-	-
pH3	-	-	-	28.0	4.4	No
pH11	-	-	-	31.8	12.8	No
pHi filtration	-	-	-	34.6	15.8	Partial removal
pH3 filtration	-	-	-	32.3	8.5	inconclusive
pH11 filtration	-	-	-	28.0	4.8	No
pHi C18 SPE	25.2	20.2	YES	a	38.2	Yes
pH3 C18 SPE	-	-	-	34.0	31.2	Yes
pH11 C18 SPE	-	-	-	31.2	24.4	Yes
pHi Aeration	23.0	8.8	No	28.2	11.7	No
pH3 aeration	-	-	-	29.3	b	Could not be determined
pH11 aeration	-	-	-	29.0	7.5	No
pH11 aeration washdown	-	-	-	39.0	44.2	Toxicity not recovered
PBO 25 µg/L	24.6	9.8	No	43.3	12.5	No
PBO 100 µg/L	20.8	2.8	Toxicity increased	40.0	0.0	Toxicity increased
Humic acid 20 mg/L	-	-	-	36.0	23.7	Yes
Humic acid 40 mg/L	-	-	-	29.3	29.0	Yes

a - Due to poor reproduction in the first TIE attempted, the TIE test was repeated. Results in the re-test were similar to what was observed in the initial TIE attempt.

na - not applicable.

Table 6-8. Phase I TIE for the Fathead Minnow Survival Response

TIE Treatment	April 21, 2009 – retest (Attachment 1)				September 21, 2009 (Attachment 8)				January 11, 2009 (Attachment 8)			
	Mean % Survival		Was Toxicity Removed?	100% Effluent	Mean % Survival		Was Toxicity Removed?	100% Effluent	Mean % Survival		Was Toxicity Removed?	100% Effluent
	Lab Control				Lab Control				Lab Control			
Baseline	90	0	na	90	0	na	100	13.3	100	na	na	
centrifugation	100	0	No	-	-	-	-	-	-	-	-	
pH3	-	-	-	85	0	No effect	100	26.7	100	Slight removal	-	
pH11	-	-	-	90	0	No effect	100	20	100	Slight removal	-	
pHi filtration	-	-	-	90	0	No	100	73.3	100	Significant removal	-	
pH3 filtration	-	-	-	95	55	Significant removal	100	93.3	100	Yes	-	
pH11 filtration	-	-	-	90	0	No	100	86.7	100	Yes	-	
pHi C18 SPE	70a	90	Yes	95	90	Yes	100	100	100	Residual toxicity removed	-	
pH3 C18 SPE	-	-	-	85	90	Yes	66.7	80	100	Toxicity removed by precursor	-	
pH9 C18 SPE	-	-	-	85	85	Yes	100	100	100	Residual toxicity removed	-	
pHi Aeration	100	0	No	100	0	No	-	-	-	-	-	
pH3 aeration	-	-	-	80	0	Increase in toxicity	-	-	-	-	-	
pH11 aeration	-	-	-	80	0	No	-	-	-	-	-	
pH11 aeration washdown	-	-	-	75	95	Toxicity not recovered	-	-	-	-	-	
Humic acid 20 mg/L	-	-	-	95	5	No	-	-	-	-	-	
Humic acid 40 mg/L	-	-	-	70	5	No	-	-	-	-	-	

a - PRM associated with dead fish.
 “-” - not tested during this TIE investigation.

6.3 Phase II TIE

The goal of the Phase II TIE is to identify specific contaminants responsible for the effluent toxicity (Figure 6-1). Based on the consistent removal of toxicity observed following C18 SPE treatment, the Phase II TIE was targeted toward identification of contaminants adsorbed to the C18 columns. As part of the Phase I TIE, the C18 columns were frozen immediately following their use in treating the effluent. For the Phase II TIE, a subset of these frozen columns were removed from the freezer and thawed to room temperature. The C18 columns were then eluted and the elutriate was tested for toxicity. If the toxicity could be recovered in the eluate and was similar in magnitude to the Baseline (untreated effluent), then the eluate underwent further chemical analysis to attempt to identify the specific contaminant(s) responsible for the toxicity.

As the first step in the Phase II TIEs, the C18 columns were eluted using 100% methanol. The elutriate was then diluted up to the 1X effluent concentrations and tested for toxicity. For the Phase II TIE of the September 2009 sample, toxicity tests were conducted on both *C. dubia* and fathead minnows using elutriate reconstituted to the initial 50% (=0.5X) and 100% (=1X) effluent concentrations. The Phase II TIE of the January 2010 sample was performed using only the fathead minnow. Preparation of method blanks and Baseline treatments accompanied the tests of the C18 eluates.

6.3.1 Results for Toxicity Recovery in the Initial Phase II TIE Evaluation

For both test species, significant toxicity was observed in the "New" Baseline (untreated effluent) tests, indicating that the toxicity that had been observed in the initial testing of the effluent was still present. However, in both cases the magnitude of the observed toxicity was less than that observed in the initial toxicity test and in the Phase I TIE; this reduction in the magnitude of the toxicity suggests that:

1. the contaminant(s) in the effluent may have become more strongly bound to particulates and/or the effluent sample container during the interim sample storage period; and/or
2. the contaminant(s) in the effluent may have undergone some degradation during the interim sample storage period.

6.3.1.1 Initial Phase II TIE Results for Toxicity to *Ceriodaphnia dubia*

Significant recovery of reproductive toxicity was recovered in the C18 eluate from the columns that had removed the toxicity in testing of the September 2009 C18 SPE effluent sample (Table 6-9). However, the magnitude of the toxicity that was recovered was less than that which had been removed in the Phase I TIE; this reduction in the magnitude of the toxicity recovery likely reflects incomplete desorption of the bound contaminants by 100% methanol (note that methanol is a much weaker solvent than other compounds such as methylene chloride).

Table 6-9. Recovery of C18 column eluate toxicity to *Ceriodaphnia dubia* reproduction (columns from the Phase I TIE of the September 21, 2009 effluent sample). **Attachment 8.**

TIE Treatment	Mean number of offspring per surviving Female ^a			Was Toxicity Recovered?
	Control/blank	50% effluent	100% effluent	
“New Baseline” ^b	27.0	26.6	16.4	No
“Old Baseline” ^c	37.7	34.3	8.7	No
100% C18 Eluate	21.0	22.8	14.5	Yes

a - In order to evaluate the effects of the effluent on the *C. dubia* reproduction response without any interfering effects of variability in the survival response, mean reproduction responses were limited to surviving organisms.

b - This was a new test of the effluent sample that had been collected on 9/21/09 and stored.

c - This was the Baseline test that was performed in the Phase I TIE of the 9/21/09 sample.

6.3.1.2 Initial Phase II TIE Results for Toxicity to Fathead Minnows

Significant recovery of survival and growth toxicity was observed in the C18 eluate from the columns that had removed the toxicity in testing of the September 2009 C18 SPE effluent sample (Table 6-10 and Table 6-11, respectively). As was observed in the *C. dubia* toxicity tests, the magnitude of the toxicity that was recovered was less than that which had been removed in the Phase I TIE; this reduction in the magnitude of the toxicity recovery likely reflects incomplete desorption of the bound contaminants by 100% methanol (note that methanol is a much weaker solvent than other compounds such as methylene chloride).

Table 6-10. Recovery of C18 column eluate toxicity to fathead minnow survival (columns from the Phase I TIE of the September 21, 2009 sample). **Attachment 8.**

TIE Treatment	Mean % Survival			Was Toxicity Recovered?
	Control/blank	50% effluent	100% effluent	
“New Baseline” ^a	80	86.7	36.7	Na
“Old Baseline” ^b	90	60	0	Na
100% C18 Eluate	100	73.3	6.7	Yes

a - This was a new test of the effluent sample that had been collected on 9/21/09 and stored.

b - This was the Baseline test that had been performed in the Phase I TIE of the 9/21/09 sample.

Table 6-11. Recovery of C18 column eluate toxicity to fathead minnow growth (columns from the Phase I TIE of the September 21, 2009 sample). **(Attachment 8)**

TIE Treatment	Mean Dry Weight (mg)			Was Toxicity Recovered?
	Control/blank	50% effluent	100% effluent	
“New Baseline” ^a	0.40	0.30	0.14	Na
“Old Baseline” ^b	0.27	0.19	0	Na
100% C18 Eluate	0.40	0.28	0.09	Yes

a - This was a new test of the effluent sample that had been collected on 9/21/09 and stored.

b - This was the Baseline test that had been performed in the Phase I TIE of the 9/21/09 sample.

To confirm the results of the Phase II TIE, C18 eluate from the January 11, 2010 effluent sample were also tested for toxicity to fathead minnows (Table 6-12). The results for the January 2010

effluent sample are comparable to those for the September 2009 effluent sample: toxicity was recovered.

Table 6-12. Recovery of C18 column eluate toxicity to fathead minnow survival and growth (columns from the Phase I TIE of the January 2010 sample). (Attachment 9)

TIE Treatment	Control/blank		100% effluent		Was Toxicity Recovered?
	Survival	Growth	Survival	Growth	
100% C18 Eluate	100%	0.32	5%	0.01	Yes

6.3.2 Results for Toxicity Recovery in Step 2 of the Phase 2 TIE Evaluation (Sequential Elutions)

As described in Section 4.2.2, the C18 columns that had been used to treat the 9/21/09 effluent sample were sequentially eluted with seven methanol concentrations (50, 75, 80, 85, 90, 95, and 100%) and the eluates were tested to determine if the toxicity that had been removed from the effluent samples by the C18 columns could be recovered in the C18 column eluate fractions. For both the *C. dubia* (survival and reproduction) and the fathead minnow (survival) there was significant recovery of toxicity at the 80%, 85%, and 90% methanol eluate fractions (Table 6.13).

Table 6-13. Recovery of Toxicity to *Ceriodaphnia dubia* and fathead minnow using Sequential C18 SPE elutions (50%, 75%, 80%, 85%, 90%, 95% and 100% methanol). C18 SPE treatment conducted on 9/21/09. (Attachment 9)

TIE Treatment	<i>Ceriodaphnia dubia</i>											Fathead Minnow			
	Mean % survival					Mean # offspring/surviving female						Mean % Survival			
	Control/blank	1X	2X	4X	Recover Toxicity ?	Control/blank	1X	2X	4X	Recover Toxicity?	Control/blank	1X	2X	4X	Recover Toxicity?
Lab Water Control	100	--	--	--	--	21.0	--	--	--	--	100	--	--	--	--
50% Methanol	100	80	100	100	no	21.6	27.3	25.4	24.6	no	100	90	90	90	no
75% Methanol	100	100	100	100	no	18.8	29.0	31.4	170	Slight	80	80	30	25	partial
80% Methanol	60	100	100	20	YES	23.0	24.6	23.2	3.0	YES	90	30	5	0	YES
85% Methanol	100	100	80	40	YES	19.8	25.8	8.8	0.0	YES	90	20	0	0	YES
90% Methanol	60	80	75	40	YES	19.0	21.0	19.7	8.5	YES	100	100	15	0	YES
95% Methanol	80	100	80	100	no	19.3	22.0	18.3	12.0	partial	80	100	80	60	slight
100% Methanol	100	80	100	80	no	15.4	25.0	22.2	13.0	partial	70	60	100	70	no

6.4 Chemical Analysis of the Toxic C18 SPE Eluate Fractions

Produced water composition varies from one field to another, within a field and during the lifespan of a field. Therefore, the potential contributors to produced water toxicity may vary over time making identification of the causative agent difficult. Produced water naturally contains a wide variety of dissolved organic compounds (oil and soluble oil products) characteristic of the reservoir and geologic formation from which the water is produced (OGP, 2005). In addition, oil field additives and other naturally occurring substances and elements (i.e. dissolved inorganic salts and insoluble oil droplets) may be present in the produced waters. To assist in determining the compounds that may be contributing to the observed toxicity, the 80%, 85%, and 90% methanol concentrations of the September 21, 2009 C18 eluates, and the 100% methanol eluate of the January 11, 2010 C18 columns (and their corresponding blanks) were shipped on ice to Dr. Cliff Lange at Auburn University for chemical analysis. The analysis targeted chemicals that are typical constituents of petroleum refinery operations (e.g., volatile organic compounds, naphthenic acids, naphthalenes, phenolics, alkanes, and amines).

For the analysis, samples were extracted using methylene chloride and concentrated to twenty times the initial concentration using a rotary evaporator. Naphthenic acids were analyzed using BF3/Methanol derivatization followed by GC-FID analysis. Phenols, alkanes, and aromatics were analyzed using EPA Method 625. The amounts of phenolic compounds, alkanes, naphthenic acids, and surfactants were determined by gas chromatography. The results of these analyses are summarized in Table 6-14, below. Results are reported in Pacific EcoRisk reports Attachment 8 and Attachment 9.

The results of the chemical analysis indicate that three classes of compounds (Volatile Organics, Phenolics, and Naphthenic Acids) were found in the highest concentrations in the samples tested. It should be noted that there are hundreds of potential compounds found in each of these classes of chemicals and for these analyses, only a subset of compounds were analyzed for. Furthermore, it should be noted that in the Phase II TIE C18 elutions, methanol was used as the solvent (due to the fact that when the eluate is reconstituted to the 1X concentration with Control water, the residual amount of methanol present is below toxicity thresholds). A stronger solvent such as methylene chloride, hexane, etc., would likely result in greater desorption of these compounds from the C18 columns than did methanol, which would have resulted in even higher recovered concentrations.

Table 6-14. Results of chemical analysis on Phase II TIE C18 Eluate fractions for effluent samples collected on September 9, 2009 and Jan 11, 2010 (units = mg/L).

Sample Collection Date	Methanol Fractions				Dichloromethane Fractions			
	Sept. 9, 2009		Jan. 11, 2010		Sept. 9, 2009		Jan. 11, 2010	
Analytes	90%	85%	80%	100%	90%	85%	80%	100%
VOAs								
1,2,4-trimethylbenzene	0.09	0.07	0.07	0.13	0.01	0.01	0.00	0.01
1-ethyl-2-methyl benzene	0.14	0.11	0.10	0.11	0.01	0.01	0.01	0.03
1,3-diethyl benzene	0.07	0.05	0.05	0.16	0.00	0.00	0.00	0.01
1-methyl-3-propyl benzene	0.12	0.10	0.09	0.18	0.00	0.00	0.00	0.02
1-methyl-3-(1-methylethyl)-benzene	0.08	0.07	0.07	0.09	0.01	0.00	0.00	0.01
1,2-diethyl benzene	0.22	0.21	0.19	0.14	0.01	0.01	0.01	0.04
benzene	0.70	0.72	0.65	0.23	0.00	0.00	0.00	0.01
toluene	0.56	0.47	0.43	0.59	0.01	0.01	0.01	0.03
p-xylene	0.77	0.62	0.56	0.41	0.01	0.01	0.01	0.02
ethylbenzene	0.56	0.43	0.39	0.32	0.01	0.00	0.00	0.03
1-methyl-2-propyl benzene	0.90	0.74	0.69	0.67	0.01	0.01	0.01	0.01
1,3,5-trimethyl benzene	0.10	0.08	0.07	0.17	0.01	0.01	0.01	0.00
1,2-dimethylbenzene	0.89	0.92	0.85	0.69	0.02	0.01	0.01	0.03
1,3-dimethylbenzene	0.74	0.63	0.58	0.55	0.01	0.01	0.00	0.03
Total VOAs	5.94	5.22	4.79	4.44	0.12	0.09	0.07	0.28
Phenolics								
Phenol	2.55	2.17	2.05	1.92	0.03	0.02	0.02	0.12
2-methyl phenol	1.70	1.34	1.23	1.34	0.01	0.01	0.01	0.07
3-methyl phenol	2.00	1.53	1.39	1.08	0.02	0.01	0.01	0.06
3,4-dimethyl phenol	1.37	1.13	1.03	0.73	0.01	0.01	0.01	0.06
3-ethyl phenol	0.38	0.29	0.26	0.33	0.00	0.00	0.00	0.02
Aniline	0.51	0.39	0.35	0.41	0.00	0.00	0.00	0.05
Total Phenolics	8.51	6.85	6.31	5.81	0.07	0.05	0.05	0.38
Napthalenes								
1-methyl-napthalene	0.09	0.07	0.07	0.11	0.01	0.01	0.00	0.00
2-methyl-napthalene	0.14	0.11	0.10	0.13	0.00	0.01	0.01	0.00
1,5-dimethyl-napthalene	0.17	0.18	0.16	0.11	0.00	0.00	0.00	0.00
1,7-dimethyl-napthalene	0.06	0.05	0.04	0.03	0.00	0.00	0.00	0.00
Napthalene	0.36	0.28	0.25	0.21	0.00	0.00	0.00	0.01
Total Napthalenes	0.82	0.69	0.62	0.59	0.01	0.02	0.01	0.01

Sample Collection Date	Methanol Fractions			Methanol Blank Fractions				
	Sept. 21, 2009		Jan. 11, 2010	Sample Collection Date		Sept. 21, 2009		
Analytes	90%	85%	Analytes	90%	85%	Analytes	90%	85%
NAPHTHENIC ACIDS								
Cyclohexanecarboxylic acid	0.61	0.51	0.48	0.97	0.00	0.00	0.00	0.04
Methyl-pentyl cyclohexanecarboxylic acid	0.48	0.41	0.39	0.61	0.00	0.00	0.00	0.06
Methyl,pentylcyclopentanecarboxylic acid	0.59	0.45	0.41	0.80	0.01	0.00	0.00	0.03
Heptylcyclohexanecarboxylic acid	0.78	0.59	0.54	0.75	0.01	0.00	0.00	0.09
Cyclopentanecarboxylic acid	0.96	0.75	0.64	1.13	0.01	0.01	0.00	0.05
Diethylcyclopentanecarboxylic acid	0.84	0.72	0.66	0.76	0.01	0.00	0.00	0.04
Total Naphthenic Acids	4.26	3.43	3.12	5.02	0.04	0.01	0.00	0.31
ALKANES								
3-methy-1-pentene	0.49	0.37	0.34	0.56	0.01	0.01	0.01	0.07
Decane	0.62	0.47	0.43	0.70	0.03	0.02	0.02	0.04
2,7-dimethyl octane	0.26	0.25	0.22	0.52	0.01	0.01	0.00	0.05
4-methyl-nonane	0.30	0.28	0.27	0.56	0.01	0.01	0.01	0.09
2,6-dimethyloctane	0.25	0.24	0.22	0.13	0.01	0.01	0.01	0.01
3-ethyl-2methyl-heptane	0.26	0.25	0.24	0.21	0.01	0.01	0.01	0.00
undecane	0.28	0.27	0.25	0.37	0.01	0.01	0.01	0.00
dodecane	0.22	0.22	0.21	0.34	0.01	0.01	0.00	0.02
tridecane	0.34	0.33	0.33	0.48	0.01	0.01	0.01	0.03
tetradecane	0.16	0.14	0.13	0.23	0.01	0.01	0.01	0.02
Pentadecane	0.19	0.18	0.18	0.13	0.01	0.01	0.01	0.04
hexadecane	0.34	0.32	0.28	0.22	0.01	0.01	0.02	0.03
heptadecane	0.11	0.10	0.10	0.08	0.00	0.01	0.01	0.01
octadecane	0.05	0.05	0.04	0.05	0.00	0.00	0.00	0.01
nonadecane	0.04	0.03	0.03	0.06	0.00	0.00	0.00	0.03
eicosane	0.06	0.06	0.06	0.08	0.00	0.00	0.00	0.00
heneicosane	0.04	0.04	0.03	0.03	0.00	0.00	0.00	0.00
docosane	0.06	0.06	0.05	0.02	0.01	0.01	0.01	0.01
octacosane	0.14	0.13	0.12	0.02	0.01	0.01	0.01	0.00
dotriacontane	0.06	0.05	0.05	0.04	0.02	0.02	0.02	0.00
tetracontane	0.11	0.11	0.10	0.06	0.02	0.01	0.01	0.01
Total Alkanes	4.38	3.95	3.68	4.89	0.2	0.19	0.18	0.47
AMINES								
diethanolamine	0.34	0.30	0.22	0.15	nd	nd	nd	0.02
methylamine	0.27	0.22	0.19	0.34	nd	nd	nd	0.02
ethyl amine	0.17	0.16	0.15	0.26	nd	nd	nd	0.03
Ethanol amine	0.14	0.11	0.10	0.11	nd	nd	nd	0.01
Triazene	0.00	0.00	0.00	0.00	nd	nd	nd	0.00
Methyl diethanol amine	0.11	0.10	0.08	0.19	nd	nd	nd	0.02
thylenediamine	0.07	0.06	0.04	0.28	nd	nd	nd	0.06
Total Amines	1.1	0.95	0.78	1.33	nd	nd	nd	0.16

6.4.1 Characteristics of the Compounds Detected at the Highest Concentrations in the C18 Eluates

The following 3 sub-sections provide a summary of characteristics and known toxicity information for each of the chemical classes that were found at the highest concentrations in the C18 methanol elutions (Volatile Organics, Phenolics, and Naphthenic Acids).

6.4.1.1 Volatile Organic Acids (VOAs)

As part of the chemical analyses, benzene, ethylbenzene, toluene, and p-xylene (BTEX) and a variety of associated benzene compounds were analyzed for a total of fourteen VOAs. BTEX was selected because these compounds naturally occur in crude oil and essentially occur in all produced waters. However, the concentrations of BTEX compounds vary significantly from type of field and location (OGP, 2005). BTEX are low molecular weight mono-aromatic hydrocarbons that are moderately soluble in water, highly volatile and which biodegrade rapidly in the water column. Once released to the surface waters, BTEX can volatilize (evaporate), dissolve, attach to soil or other particulates or degrade biologically. Primary mechanisms of BTEX toxicity include non-polar narcosis (membrane disruption) and alterations in the permeability of cells. However, due to the rapid loss of these compounds from water (through volatilization) exposure to aquatic organisms is expected to be very low (OGP, 2005).

6.4.1.2 Phenolics

Phenols and phenolic substances are aromatic hydroxy compounds classified as monohydric, dihydric, or polyhydric (with three or more hydroxyl groups), depending on the number of hydroxyl groups attached to the aromatic benzene ring (Saha et al., 1999). Alkylated phenols occur naturally in petroleum and will partition into produced water dependant on their molecular weight (OGP, 2005). Phenols are extremely water soluble with low vapor pressure. Models have shown that when phenol is released to water, >99% partitions to water (Mackay and AEL 1996). Photooxidation, oxidation, and microbial degradation are expected to be the major fate processes of phenols in the aquatic environment (CCME, 1999); sorption and volatilization are not significant removal mechanisms. The toxicity of phenolic compounds varies widely with the organism tested, dissolved oxygen content, and water temperature (Alabaster and Lloyd 1982). Mechanisms of phenol toxicity include affects on the metabolism (Holmberg et al. 1972), survival and growth (DeGraeve et al. 1980; Holcombe et al. 1982; Saha et al., 1999) and reproductive impacts of fish (Dauble et al. 1983; Mukherjee et al. 1990, 1991). The LC50 values for fathead minnows (*Pimephales promelas*) exposed to phenol range from 8.3 mg/L (48 hr LC50) (Phipps et al. 1981) to 68 mg/L (96 hr LC50) (DeGraeve et al. 1980).

6.4.1.3 Naphthenic Acids

Naphthenic acids are a diverse group of saturated aliphatic and alicyclic carboxylic acids. Naphthenic acids are natural constituents of petroleum and crude oils and are present at different concentrations depending on the source of oil (Clemente and Federak, 2005). Naphthenic acids form a complex group of compounds in the environment that are non-volatile, chemically stable, and tend to act as surfactants due to the presence of hydrophobic alkyl groups and a hydrophilic carboxylic moiety (McMartin et al., 2004, Clemente and Federak, 2005, Frank et al, 2008). Currently, the specific naphthenic compounds that are the most toxic and/or corrosive have not been conclusively identified. It is believed that a probable primary mode of action for acute

toxicity of naphthenic acids is narcosis, also known as membrane disruption (McMartin et al., 2004, Frank et al., 2008). Toxicity does not always correlate directly with the concentration of naphthenic acids. However, it is well-established, that naphthenic acids are the primary group of compounds contributing to fish toxicity observed in produced waters (Dorn, 1995, McMartin et al, 2004).

7 TOXICITY REDUCTION EVALUATION

Results of the TIE and chemical analysis of the target waste streams (treated produced water from Kern River Station 36 and Valley Waste) support other produced water investigations in identifying naphthenic acids, BTEX and phenols as the classes of chemicals most likely contributing to the observed toxicity to *C. dubia* and fathead minnows. There have been numerous studies and reviews on treatment technologies for oilfield produced waters. From the literature, potential technologies that target naphthenic acids, BTEX and phenols could be narrowed down to physical treatments (adsorption technologies); chemical treatments (ozone and peroxide); biological treatment (constructed wetlands); and membrane treatment (reverse osmosis). Table 7-1 provides a summary of the technologies evaluated as well as advantages and disadvantages with each. Using the literature data evaluated in Table 7-1, a desktop feasibility assessment was conducted for the Kern River Station 36 produced water. Results of this initial desktop evaluation indicated that a polishing step alternative was the best treatment option for this produced water based on the existing treatment facility and magnitude of toxicity. This finding is consistent with other studies that found that technologies to reduce or remove soluble aromatic species are best applied as a final polishing or tertiary treatment after the removal of the dispersed oil phase (OGP, 2002)

Several treatment options are currently under consideration by Chevron. An in-depth evaluation to assess the probability of long term success, capital and operational economics, ease of implementation, generated waste product and disposal and regulatory perspectives needs to be completed. A key consideration of the treatment option evaluation will be its capacity to cost-effectively meet the volume and variability in the produced water, as well as potentially more stringent regulations.

Chevron is still in the early phases on these evaluations and the data are insufficient to allow selection of a final treatment options. However, two treatment options under consideration are activated carbon and constructed treatments wetlands. As part of the TRE investigation, bench-top feasibility studies were performed to ascertain if activated carbon could decrease toxicity in the effluent from Reservoir B to levels that would ensure compliance with the permit mandated effluent toxicity limit. In addition, Chevron has conducted previous pilot studies to assess the potential for using a constructed treatment system as a polishing step. Currently, a 2 acre, multi-cell demonstration wetland is in operation at the head of the CWD canal. This wetland routinely receives a portion of the discharge from Reservoir B. The following sections summarize the treatment technologies considered and the results of the benchscale and pilot studies conducted on the Inlet to Reservoir B produced water.

Table 7-1. Summary of Best Available Techniques for treatment of Produced Water.

	Treatment Alternative	Advantages	Disadvantages	Citations:
Physical Treatment	Activated Carbon	<ul style="list-style-type: none"> - Technology has been successfully evaluated and installed by multiple operators including Chevron - Process can effectively remove BTEX, naphthenic acids and phenols - Regenerative technique 	<ul style="list-style-type: none"> - Solid waste stream generated - Potential clogging - Regeneration issues – may need replacement of carbon - Performance may be affected by temperature and pH, concentration of contaminants - Technical feasibility can be screened at benchscale but must be verified through pilot and actual field conditions - Can be energy intensive (high cost and maintenance) 	<ul style="list-style-type: none"> - OGP, 2002 - Fakhru'l-Razi et al., 2009 - Wong et al., 1996
	Zeolite	<ul style="list-style-type: none"> - Process can effectively remove BTEX - Regenerative technique 	<ul style="list-style-type: none"> - Not proven at full scale over time - Not proven for removal of naphthenic acids and phenols - Performance may be affected by temperature, Ph, concentration - Regeneration is critical to success 	<ul style="list-style-type: none"> - Ranck et al., 2004 - Fakhru'l-Razi et al., 2009
Chemical treatment	H ₂ O ₂ (Peroxide)	<ul style="list-style-type: none"> - Breakdown byproduct is water and oxygen 	<ul style="list-style-type: none"> - Literature indicate mixed results - More effective when combined with other technologies (photocatalysts) - Safety Issues - Short shelf life 	<ul style="list-style-type: none"> - Bessa et al., 2001
	Ozonation	<ul style="list-style-type: none"> - Benchscale studies show effective removal of BTEX and naphthenic acids 	<ul style="list-style-type: none"> - Not proven for removal of phenols - Likely cost prohibitive at full 	<ul style="list-style-type: none"> - Scott et al., 2008 - Fakhru'l-Razi et al., 2009

Treatment Alternative	Advantages	Disadvantages	Citations:
Biological	<ul style="list-style-type: none"> - Constructed Treatment Wetland 	<ul style="list-style-type: none"> - scale due to volume of water and cost to implement - Benchscale testing indicated not as effective in removal of toxicity as other methods - Energy intensive (high cost and maintenance) - Consumption of chemicals for treatment - Energy intensive technology (GHG emissions) 	<ul style="list-style-type: none"> - Fakhru'l-Razi et al., 2009 - Murray-Gulde et al., 2003
Membrane	<ul style="list-style-type: none"> - Pilot studies demonstrate effective removal of water soluble organics and toxicity - Typically cost effective - Low operation and maintenance effort and cost - Produces no additional waste stream - Dependant on type of membrane and pretreatment can remove the majority of aromatics (BTEX, naphthalenes and PAHs) found in produced water 	<ul style="list-style-type: none"> - Not proven in full scale produced water treatment - High capital expense - Requires a large amount of land - High cost of treatment - May use toxic chemicals for treatment - Produces secondary wastewater that must be managed - Unproven in oilfield type installations - Most useful as a polishing step - May require a lot of space for full scale operation 	<ul style="list-style-type: none"> - Fakhru'l-Razi et al., 2009 - OGP, 2002

7.1 Granular Activated Carbon (GAC)

There have been numerous studies using activated carbon to remove contaminants from petroleum wastestreams. Results of these studies indicate the granular activated carbon (GAC) is effective in removing soluble organic compounds from wastewater. As part of the TRE investigation, benchscale treatment studies were conducted using GAC to treat effluent collected from Inlet to Reservoir B, Valley Waste, and eluate from C18 columns used to treat effluent from Reservoir B as part of the TIE. Results of these benchscale studies indicate that survival of fathead minnow increased significantly following treatment by GAC compared to the untreated waters (Tables 7-2 to 7-4). The benchscale investigations demonstrate that GAC treatment could effectively remove toxicity observed in Kern River produced waters. Therefore this treatment option will be investigated further.

Table 7-2. Effects of GAC treatment on the toxicity of Reservoir B waters to fathead minnows (sample collected September 3, 2010). (Attachment 5)

Sample Collection Date	Effluent Treatment	Inlet to Reservoir B Effluent		GAC-Treated Reservoir B Effluent	
		Mean % Survival	Mean Biomass Value	Mean % Survival	Mean Biomass Value
September 3, 2010	GAC Treatment Blank	NA	NA	100	0.40
	Lab Control	100	0.38	97.5	0.36
	12.5%	92.5	0.32*	97.5	0.35
	25%	37.5*	0.10*	100	0.36
	50%	0*	-	97.5	0.34
	75%	0*	-	97.5	0.33
	100%	0*	-	95	0.34

* The response at this test treatment was significantly less than the lab control treatment response as $P < 0.05$.

NA - treatment not evaluated

- response not evaluated due to significant mortality

Table 7-3. Effects of GAC treatment on the toxicity of C18 eluates from Reservoir B waters to fathead minnows (effluent sample collected January 11, 2010). (Attachment 9)

TRE Treatment	Mean % Survival			
Sample Collection Date	Control/blank	Eluate Control	100% Untreated Effluent Eluate	GAC-Treated 100% Effluent Eluate
January 11, 2010	100	100	5	95

Table 7-4. Effects of GAC treatment on the toxicity of Valley Waste effluent to Reservoir B to fathead minnows (sample collected September 3, 2010). (Attachment 5)

Sample Collection Date	Effluent Treatment	Valley Waste Effluent		GAC-Treated Valley Waste Effluent	
		Mean % Survival	Mean Burdens	Mean % Survival	Mean Burdens
September 3, 2010	GAC Treatment Blank	NA	NA	100	0.40
	Lab Control	100	0.41	100	0.43
	12.5%	87.5	0.33*	97.5	0.38
	25%	62.5*	0.22*	100	0.38
	50%	10*	0.03*	100	0.38
	75%	0*	-	100	0.40
	100%	0*	-	100	0.38

* The response at this test treatment was significantly less than the lab control treatment response as $P < 0.05$.

NA - treatment not evaluated.

- response not evaluated due to significant mortality.

7.2 Peroxide (H₂O₂)

Peroxide and other chemical oxidants can remove the organic compounds suspected of causing toxicity. Peroxide is typically one of the preferred chemical oxidants because the breakdown byproducts are water and oxygen. Other chemical oxidants may leave residual compounds that have adverse side effects. Transport and use of peroxide and other chemical oxidants may have safety issues to consider (road accidents, chemical exposure, chemical burns, etc.). Peroxide tends to breakdown quickly and does not have a long storage life. Based on safety issues and shelf life, peroxide is not a preferred choice.

7.3 Ozonation

Ozonation of the produced water was considered during the feasibility evaluation. Studies on the ozonation of produced water demonstrate that it can be effective in removing BTEX and naphthenic acids. However, results from other investigators have found that it is not as effective as other alternatives considered. In addition, there is limited information on its effectiveness over time. It is also more energy intensive than other technologies and typically not cost effective when operated at a large scale. Energy intensive technologies tend to generate more green house gas (GHG) emissions than low-energy technologies. Therefore, ozonation is not considered a feasible treatment alternative at this time.

7.4 Biological

In biological treatment, microorganisms convert dissolved organics into CO₂ and water. The dominant mechanism of hydrocarbon removal is biodegradation, adsorption, and occlusion of particles. Biological treatment alternatives include aerobic and anaerobic treatments (e.g., activated sludge, trickling filters, sequence batch reactors (SBRs), lagoons and treatment

wetlands). Based on the character of this produced water (very low carbon content [measured as BOD]), it was determined that treatment wetlands would have the lowest operating cost and be the least labor intensive of the biological technologies available. It would also be the easiest and most effective to introduce as a polishing step. Studies have indicated that surface flow treatment wetlands and reed beds can effectively remove hydrocarbons, other water soluble organics, and toxicity. At the Kern River facility, adjacent to Reservoir B, a demonstration treatment wetland was constructed in 2003. As part of this TRE, effluent from Reservoir B was treated using the demonstration wetland. Results of this study indicate that toxicity to fish decreased to 80% survival following treatment with the demonstration wetland vs. 0% survival (complete mortality) in the produced water prior to treatment, and there was no observed toxicity to *C. dubia* in the wetland-treated effluent. The TRE investigation demonstrates that a treatment wetland can effectively remove toxicity observed in the Kern River produced waters. Therefore, this treatment option will be investigated further.

Table 7-4. Summary of toxicity removal in Produced water treated by demonstration constructed wetland. Samples collected January 11, 2010. (Attachment 6)

Sample Collection Date	Effluent Treatment	<i>Ceriodaphnia dubia</i>		Fathead Minnows	
		Mean % Survival	Reproduction ^a	Mean % Survival	Mean Biomass Value
January 11, 2010	Lab Water Control	100	33.5	90	0.30
	Splitter Box Inlet	100	21.7*	0*	-
	Wetland Effluent	100	33.1	80	0.24*

a - *C. dubia* reproduction is assessed as the mean # of offspring produced per surviving female.

7.5 Membrane Treatment

Membrane treatment systems such as microfiltration, nanofiltration and reverse osmosis have been used effectively to remove the majority of aromatic hydrocarbons from oilfield produced water. However, efficacy is dependent on the type of membrane used, this technology typically requires a pretreatment step, and use in full-scale oilfield operations is unproven (OGP, 2002, Fakhru'l-Razi et. al., 2009). Therefore, membrane treatment was abandoned as a feasible treatment alternative at this time.

8 CONCLUSIONS AND RECOMMENDATIONS

The results of the TIE data acquisition and initial toxicity assessment indicated that the source of the observed aquatic toxicity was the treated produced water effluent entering Reservoir B from both the Chevron operated Kern River Area Station 36 and from the Valley Waste Disposal Company (Kern Front Oil Field). Results of the Phase I TIE investigations concluded that the C18 SPE column was the only TIE treatment that effectively removed the observed toxicity to both *C. dubia* and the fathead minnow during the three sampling events. The following key observations were made during the Phase I TIE's conducted:

- The toxicity was pH-labile, with toxicity increasing as pH decreased to pH6, and toxicity decreasing as pH increased to pH8. This is suggestive of a weakly acidic toxicant that becomes less polar as the pH decreases and more polar as the pH increases.
- There was significant removal of survival toxicity by the filtration treatments, which suggests that some fraction of the toxicants present were associated with particulates.
- There was complete removal of any residual toxicity (i.e., toxicity remaining after the filtration treatment) by the C18 SPE treatment, indicating that non-polar organics were a cause of the observed toxicity.

The Phase II TIE investigation confirmed that the toxicity removed by the C18 columns could be recovered and that it was pH influenced with toxicity decreasing as pH increased to pH8. This is suggestive of a weakly acidic toxicant that becomes less polar as the pH decreases and more polar as the pH increases. Chemical analysis of the elutriate recovered from the C18 column confirmed the TIE conclusions, and indicated that the constituents measured at the highest concentrations in the produced water were naphthenic acids, phenols, and volatile organic acids.

A desktop TRE evaluation was conducted to evaluate tertiary or polishing step treatment options for the Kern River produced waters that included granular activated carbon (GAC), peroxide, ozonation, constructed treatment wetlands and reverse osmosis. Based on feasibility related to volume of water to be treated, long term effectiveness and proven implementation ability at full scale operations, generated waste and cost, there are no known technologies that can be readily implemented to treat Kern River Produced waters. Initial bench top and pilot scale studies conducted as part of this TRE indicate that GAC and constructed wetland treatment systems technologies can effectively remove the observed toxicity to *C. dubia* and fathead minnows. Chevron plans to conduct a feasibility evaluation for treating the Kern River Produced waters as well as explore other options for decreasing toxicity in surface waters while maintaining the Kern River produced waters as a source of irrigation water for growers in the CWD.

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APPENDIX A

A.1 Description of TIE Treatment Methods

A.1.1 TIE Treatment Method Blanks

As part of the TIE process, a method blank is utilized for each TIE treatment to determine whether any of the treatment procedures contribute any artifactual toxicity to the manipulated sample. The treatment method blanks for this test consisted of aliquots of the Lab Control water (for each species) that were subjected to each of the test treatments discussed below.

A.1.2 Baseline Testing

The Baseline toxicity test is performed concurrently with the TIE treatment tests, and consists of a test of the untreated effluent sample to assess toxicity at the time of the performance of the TIE, and to serve as a reference benchmark against which toxicity removal by the other TIE treatments can be assessed. The physical chemical nature of the compound(s) responsible for the observed toxicity can be determined by the pattern of toxicity removal by the TIE treatments relative to the Baseline test. Baseline testing was conducted during each TIE.

A.1.3 pH Adjustment Treatments

Separate aliquots of the effluent sample were adjusted to pH3 and pH11, pH manipulations that can affect the solubility, polarity, volatility, stability and speciation of potentially toxic compound(s). The sample pH was decreased to pH3 or increased to pH11 by adding reagent grade HCl or NaOH, respectively, to the test sample. An aliquot of each pH-adjusted effluent sample was immediately poured off and set aside for assessment of the pH adjustment treatment itself, with the remainder of each sample being allowed to sit for 1 hr until used in subsequent filtration, C18 SPE, and aeration treatment manipulations. At the end of the day, all pH-manipulated samples were readjusted to the initial Baseline pH (pHi) of the sample. The pH-adjusted effluent samples and all appropriate method blanks were then tested to determine if changes in effluent toxicity had occurred as a result of the pH-adjustment manipulation. Treatments for pH adjustment were only performed as part of the January 2010 confirmation TIE.

A.1.4 Graduated pH Adjustment Treatment

The graduated pH tests are performed to determine whether effluent toxicity is caused by compounds whose toxicity is pH-dependent. For example ammonia, which is common in many effluents, is generally much less toxic in its ionized form (NH_4^+ , the dominant form at lower pH levels) relative to its un-ionized form (NH_3 , the dominant form at higher pH levels). In addition, pH differences can also affect metal toxicity through changes in solubility and speciation. The effluent sample pH is adjusted to pH6, pH7, and pH8 by adding reagent grade HCl and/or NaOH to the test sample until the pH reading is + 0.1 pH units of the target pH. Throughout the day, all samples are readjusted to the target pH. The pH-adjusted effluent solutions and method blanks

are then tested to determine if changes in effluent toxicity occurred as a result of the increase or decrease in pH relative to the Baseline (initial) conditions. Graduated pH adjustment was only performed as part of the TIE of the January 2010 effluent sample.

A.1.5 Centrifugation Treatment

Centrifugation of the effluent sample can affect sample toxicity through the removal of toxicants associated with suspended particulates. An aliquot of the effluent sample was centrifuged at 4500g for 30 minutes; a sub sample of supernatant was set aside for direct testing, with the remaining supernatant being used in C18 SPE treatments. The centrifuged effluent test solution was then tested to determine if changes in effluent toxicity had occurred as a result of the centrifugation. A method blank was prepared in an identical fashion. Centrifugation was only performed as part of the TIE of the April 2009 effluent sample.

A.1.6 Filtration Treatment

Filtration of the effluent sample can affect sample toxicity through the removal of toxicants associated with suspended particulates or other filterable material. In addition, some contaminants can sorb to the filter membrane. This treatment also determines the effects of pH adjustment in combination with filtration: by filtering pH-adjusted aliquots of effluent, compounds typically in solution at pH_i but which are insoluble or associated with particles to a greater extent at more extreme pH's are removed. Aliquots of effluent and method blank samples at pH₃, pH_i, and pH₁₁ were filtered through either a 0.45 µm or 1 µm filter membrane. At the end of the day, all pH-manipulated samples were re-adjusted back to pH_i. The manipulated effluent samples and all appropriate method blanks were then tested to determine if changes in effluent toxicity had occurred as a result of filtration. Filtration treatments were only performed as part of the TIEs of the September 2009 and January 2010 effluent samples.

A.1.7 C18 Solid Phase Extraction (SPE) Treatment

The C18 SPE test is used to identify effluent toxicity that is due to compounds that are removed or sorbed onto chromatographic resin (i.e., C18 columns) specific for non-polar organic compounds. This treatment also determines the effects of pH adjustment and centrifugation/filtration in combination with C18 SPE extraction: at pH₃ and pH₉, organic bases and acids, respectively, can be made more or less polar by shifting the equilibrium between the ionized vs. un-ionized species, affecting their affinity for the C18 sorbant. Prior to passage over the C18 SPE column, the preliminary aliquots of filtered pH₁₁ effluent sample and method blank were re-adjusted to pH₉ (C18 column degradation will occur at >pH₉). Appropriate aliquots of pH₃, pH_i, and pH₉ effluent sample at were passed over C18 columns. The first 25 mL of solution that passed through each column was discarded, after which the remaining C18 SPE treated samples were collected. At the end of the day, all pH-manipulated samples were re-adjusted back to pH_i. The manipulated effluent samples and all appropriate method blanks were then tested to determine if changes in effluent toxicity had occurred as a result of C18 SPE treatment.

Upon completion of the Phase I TIE C18 SPE treatments, the C18 columns were frozen for potential follow-up Phase II TIE work.

A.1.8 Aeration Treatment

This TIE treatment is designed to determine the extent of effluent toxicity that can be attributed to volatile, sublutable, or oxidizable compounds. This treatment also determines the effects of pH adjustment in combination with aeration (some compounds can be removed or oxidized more easily under acidic or basic conditions). Aliquots of pH3, pHi, and pH11 effluent were aerated in graduated cylinders under a ventilation hood for 1 hr. After this aeration period, the aerated effluent samples were carefully siphoned off into glass beakers to ensure that any compounds deposited on the aeration glassware via sublation (e.g., foam) were not introduced back into the sample. At the end of the day, all pH-manipulated samples were re-adjusted back to pHi. The aeration-treated effluent samples and all appropriate method blanks were then tested to determine if changes in effluent toxicity had occurred as a result of aeration. Aeration treatment was only performed for the TIEs of the April 2009 and the September 2009 effluent samples.

A.1.9 Aeration Washdown Treatment

This treatment is intended to determine if compounds isolated during the aeration treatment can be used to recover toxicity. While the aeration procedure is underway, it was noted that the pH 11 aeration treatment had the most foam and deposits on the glass graduated cylinder. After the effluent was siphoned out of the cylinder, the cylinder was rinsed with control water to remove any compounds on the walls, and the rinsate was then diluted back up to the 1X sample volume with control water. The aeration-washdown media was then tested to determine if any toxicity that might have been removed by the aeration treatment could be recovered in the washdown media. The aeration washdown treatment was only performed as part of the TIE of the September 2009 effluent sample.

A.1.10 Piperonyl Butoxide (PBO) Treatment

The PBO treatment is used to identify contaminants whose toxicity is mediated by the Cytochrome P-450 (Cyp450) enzyme system. PBO inactivates this enzyme system, so that the toxicity of contaminants whose toxicity would have been removed by Cyp450 is increased (e.g. pyrethroid pesticides, etc), whereas the toxicity of contaminants whose toxicity would have been increased by Cyp450 is reduced (e.g., OP pesticides [such as chlorpyrifos], etc.). To prepare the PBO treatments, aliquots of the effluent were spiked with PBO at concentrations of 25 µg/L and 100 µg/L. The PBO-treated solutions and method blanks were then tested to determine if changes in effluent toxicity occurred as a result of the PBO addition. PBO treatment was only performed for the TIE of the April 2009 effluent sample.

A.1.11 Humic Acid Treatment

This treatment is designed to characterize effluent toxicity caused by materials that will sorb to dissolved organic carbon. The addition of humic acid to the sample can produce nontoxic complexes (via chelation or sorption) with potentially toxic compounds. Aliquots of the effluent were spiked with humic acid at two test concentrations: 20 mg/L and 40 mg/L. After mechanical mixing for 1 hr, the samples were stored in the dark at 4°C until used for test initiation the following day. The treated effluent samples and corresponding method blanks were then tested to

determine if changes in effluent toxicity had occurred as a result of humic acid addition. Humic acid treatment was only performed for the TIE of the September 2010 effluent sample.

A.2 Phase II TIE Testing Procedures - Toxicity Recovery in the C18 SPE Eluate

Following the analysis of the Phase I TIEs, Phase II TIEs were conducted using samples collected in September 2009 and January 2010. The goal of the Phase II TIE is to identify specific contaminants responsible for effluent toxicity. Based on the results of the Phase I TIE testing and previous experience, the Phase II TIEs were targeted towards identification of contaminants adsorbed to the C18 SPE columns that had removed significant amounts of the toxicity present in the effluent samples.

Upon completion of the Phase I TIE C18 SPE treatment, the C18 columns had been frozen for potential follow-up Phase II TIE work. A sub-set of these frozen columns was removed from the freezer and thawed out to room temperature. The C18 columns were then eluted and the eluate was tested for recovery of the initially-observed toxicity (Figure 4-2). If the toxicity could be recovered in the eluate and was of sufficient magnitude, then the eluate underwent further chemical analysis to attempt to identify the specific contaminant(s) responsible for the toxicity.

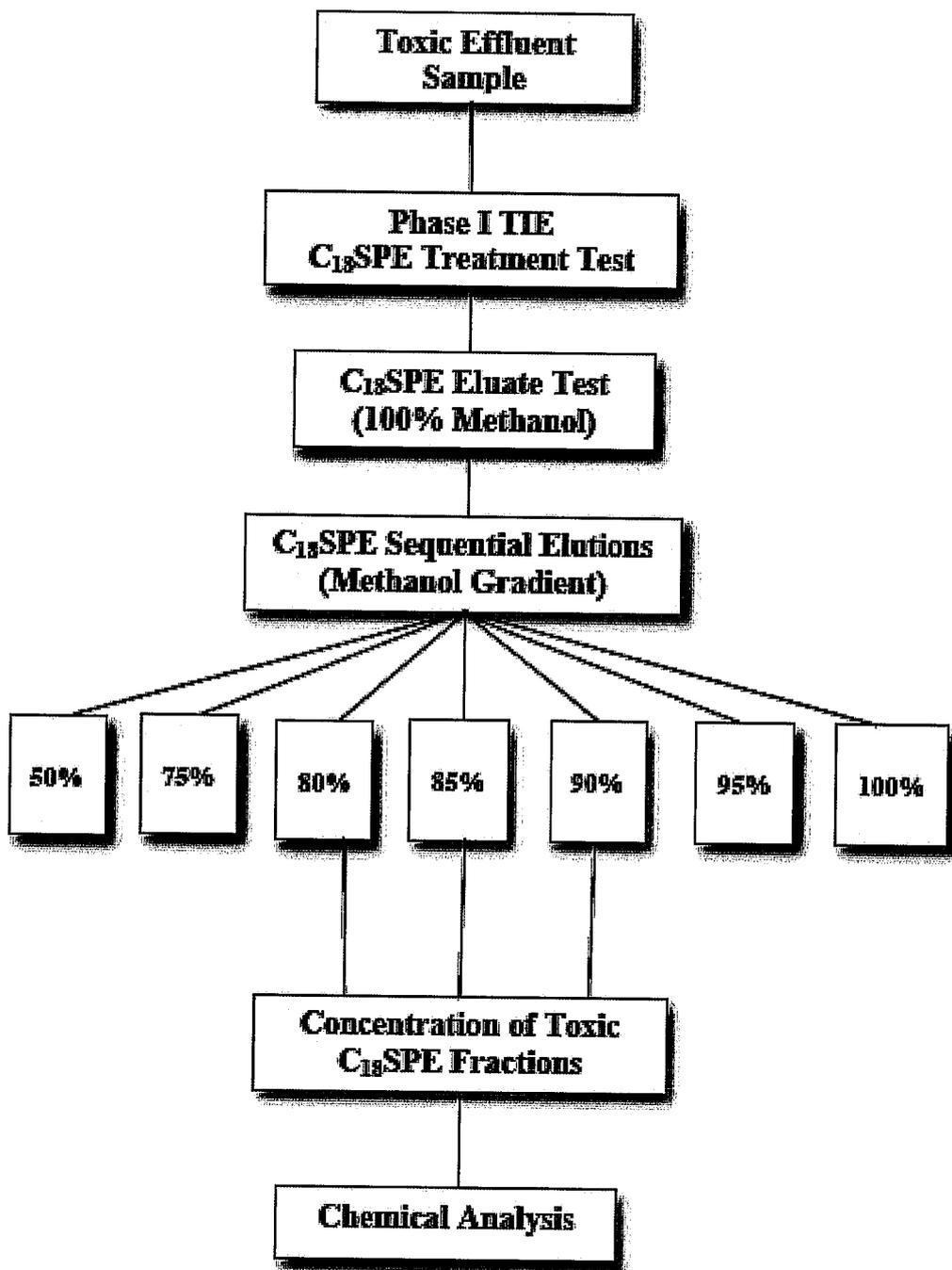


Figure A-1. Phase II Toxicity Identification Evaluation Treatment Procedures

A.2.1 Initial Evaluation of Toxicity Recovery in the C18 SPE Eluate

The C18 columns were eluted with 100% methanol and the eluate was collected and diluted back up to the 1X effluent concentration for toxicity testing. Method blank columns were similarly eluted. *C. dubia* and fathead minnows were tested at the 100% (= 1X) effluent concentration.

Aliquots of the original untreated effluent sample were also tested to provide a Baseline treatment.

A.2.2 Evaluation of Toxicity Recovery by Sequential C18 Elutions

Because there are a large number of organic compounds present in typical refinery and/or municipal wastewater, the Phase II TIE process is intended to separate the toxic components from the non-toxic components, thus simplifying, or "cleaning up", the sample matrix and allowing for the identification of the compound(s) responsible for toxicity in the effluent. The Phase II TIE procedures included an initial step to see if toxicity could be recovered and a second step (Step 2) that included sequential elution of C18 SPE columns over a methanol gradient of 50-100% methanol, identification of toxic eluate fractions, compositing and backconcentration of toxic fractions and re-elution into 100% methanol in preparation for subsequent chemical analyses (e.g., Gas Chromatography/Mass Spectrometry (GC/MS)).

Step 2 of the Phase II TIE of the September 2009 effluent sample - A set of the remaining frozen C18 columns were thawed to room temperature and eluted sequentially with 2 mL of each of seven methanol concentrations (50, 75, 80, 85, 90, 95, and 100%). The eluate of each methanol concentration was diluted in control water to make stock solutions of 4X effluent concentration and used for testing on *C. dubia* and fathead minnows at the 1X, 2X, and 4X concentrations. Sequential elutions were similarly performed on the method blank columns. Aliquots of the original untreated effluent sample were also tested to provide a Baseline treatment.

The eluates at each methanol concentration remaining after preparing the toxicity testing solutions were kept refrigerated for the duration of the tests. After recovery of toxicity was observed in the 80%, 85%, and 90% eluate solutions, these three eluates and their corresponding blanks were shipped on ice to Dr. Cliff Lange at Auburn University for chemical analysis of selected volatile organic compounds, naphthenic acids, naphthalenes, phenolics, alkanes, and amines.

Step 2 of the Phase II TIE of the January 2010 effluent sample - As before, frozen C18 columns were thawed, eluted with 100% methanol, the eluate was collected and diluted back up to the 1X effluent concentration for fathead minnow toxicity testing. Method blank columns were similarly eluted and tested. Aliquots of the 1X eluate were shipped on ice to Dr. Cliff Lange at Auburn University for chemical analysis.

ATTACHMENTS

Attachment 1 - Chronic Toxicity Testing and Toxicity Identification Evaluation (TIE) of the Chevron/Cawelo Water District Effluent. Samples Collected April 21, 2009. Pacific EcoRisk. May 2009.

Attachment 2 – NPDES Compliance Chronic Toxicity Testing of Chevron/Cawelo Water District “Inlet to Reservoir B’ Effluent. Sample collected August 31, 2009. Pacific Ecorisk. October 2009.

Attachment 3 – NPDES Compliance Chronic Toxicity Testing of Chevron/Cawelo Water District “Inlet to Reservoir B’ Effluent. Sample collected September 21, 2009. Pacific Ecorisk. October 2009.

Attachment 4 – Chronic Toxicity Testing of the Chevron/Cawelo Water District ‘Inlet to reservoir B” Effluent. Samples collected January 11, 2010. Pacific EcoRisk. May 2010.

Attachment 5 – Chronic Toxicity Testing of the Chevron/Cawelo Water District ‘Inlet to reservoir B” and “Valley Waste” Effluents. Samples collected September 3, 2010. Pacific EcoRisk. October 2010.

Attachment 6 – Chronic Toxicity Testing of Chevron/Cawelo Water District Nearby Water Samples. Samples collected January 11, 2010. Pacific Ecorisk. May 2010.

Attachment 7 - Chronic Toxicity Testing of the Splitter Box and Pre-Poso Creek Effluents. Samples Collected September 21, 2009. Pacific EcoRisk. October 2009.

Attachment 8 – Chronic Toxicity Testing and Toxicity Identification Evaluation (TIE) of the Chevron/Cawelo Water District Effluent. Samples collected September 21, 2009 and January 11, 2010. Pacific EcoRisk. May 2010.

Attachment 9 – Chronic Toxicity Testing of the Chevron/Cawelo Water District “Inlet to Reservoir B” C18 Eluate: Assessment of GAC Treatment and Chemical Analysis of the C18 Eluate. Pacific Ecorisk. December 2010.