American Petroleum Institute

STEP Strategies for Today's Environmental Partnership

Summary of Produced Water Toxicity Identification Evaluation Research

Health and Environmental Sciences Department
Publication Number 4641
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API PUBLICATION NUMBER 4641

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EXECUTIVE SUMMARY

In response to a U.S. EPA mandate under the Clean Water Act, wastewater discharge permittees subject to National Pollutant Discharge Elimination System (NPDES) regulations may be required to perform effluent toxicity reduction evaluations (TRE) of wastewater discharges. Before a TRE can be carried out, a toxicity identification evaluation (TIE) must be conducted. TIEs consist of physically or chemically subdividing an effluent into various fractions and determining the toxicity (acute or chronic) of each fraction. By relating the change in toxicity to the particular fraction isolated from the whole effluent, a cause-and-effect relationship can be hypothesized. U.S. EPA Region 10 has recently proposed that operators detecting chronic produced water (PW) toxicity above permit limits be required to initiate a TIE to determine the cause of toxicity and a TRE to evaluate effectiveness of pollution control or in-plant modifications towards attaining compliance (60 FR 48814).

The American Petroleum Institute (API) sponsored a series of studies applying TIE procedures to oil and gas produced water (PW). The purpose of this research was to evaluate the efficacy of TIE procedures (fractionation and toxicity test designs) in determining the potential toxicants in PW effluents. Standard EPA and new PW-specific fractionation schemes were used on a variety of PW samples (14) of varying salinities. Samples were contributed by oil and gas production inland facilities in Wyoming, coastal sites in Texas and California, and coastal and offshore sites in Louisiana. Acute (24-h and 48-h LC50s) toxicity tests were conducted on whole PW and fractions. For low-salinity (<3 parts per thousand [ppt]) PW samples, the freshwater species, *Ceriodaphnia dubia* (daphnia) and *Pimephales promelas* (fathead minnow) were used in the toxicity tests. For high-salinity (>3 ppt) PW samples, the marine species, *Mysidopsis bahia* (mysid) and *Cyprinodon variegatus* (sheepshead minnow) were used as the test species. A toxicity test procedure using the sea urchin, *Arbacia punctulata*, was also performed on some samples.

Factors influencing PW TIEs were investigated. These include the effect of salinity in selecting fractionation manipulations, the effect of toxicity test replication (i.e., reproducibility) in distinguishing changes in toxicities between whole PW and its fractions, and the suitability of different test species in PW TIEs. From this work a number of important lessons were learned which yield suggestions for improving the conduct of produced water toxicity identification evaluations.

Components, or fractions, contributing to toxicity differed for each PW with no specific fraction being consistently toxic. For most produced water samples, toxicity attributed to any one fraction represented only a part of the toxicity of the whole sample. However, no more than two fraction types were identified as potential toxicants in any sample. Potential toxicants identified during this study, besides salinity, included the following:

- Acidic and basic organic compound class fractions;
- Particulates removed by filtration at pH11;
- Ionic imbalance or excess (e.g., excess calcium or potassium);
- Ammonia;
- Hydrocarbons;
- Hydrogen sulfide;
- Material removed by pH change; and
- Volatile compounds.

Section 1
INTRODUCTION

The toxicity of produced water (PW) that may be discharged into the offshore waters of the Western Gulf of Mexico is limited by a National Pollutant Discharge Elimination System (NPDES) permit (58 FR 63964-63986). This permit requires operators to determine the threshold concentration for toxicity in a 7-day toxicity test conducted according to an EPA protocol and to show that the threshold concentration is higher than the predicted concentration 100 m from the discharge point. Operators with effluents failing to meet the toxicity limit can either cease discharging, modify the outfalls to increase dispersion, or treat the effluent to reduce its toxicity. In an effort to improve understanding of the origin of toxicity in PW from U.S. facilities, the American Petroleum Institute (API) has sponsored research involving application of toxicity identification evaluation (TIE) procedures to a wide range of PW effluents. This report summarizes the results and lessons learned from API's TIE research program.

Produced waters can exhibit both acute and chronic toxicity in laboratory tests (Neff, 1987). Traditionally, sources of toxicity in effluents have been identified by performing a series of chemical analyses in order to generate an inventory of potential toxicants. The toxicity of the individual components is then evaluated, typically using information in the scientific literature. There are several limitations associated with this approach: 1) chemical analyses may not identify all potential toxicants; 2) toxicants may behave differently in combination than they do individually (i.e., additive, synergistic, and antagonistic effects are not considered); and 3) the toxicity of all components may not be well understood. These limitations become magnified when considering PWs, which are complex mixtures of organic and inorganic constituents.

This toxicity-based approach consists of subdividing the test effluent into various fractions and experimentally determining the toxicity of each fraction. This division reflects the need to select toxicity testing protocols and species as a function of the salinity of the effluent and receiving waters. By relating any reduction of toxicity to the class of chemicals that was removed from the sample, it is theoretically possible to determine a cause-and-effect

relationship. This procedure forms the basis of what EPA describes as a toxicity identification evaluation or TIE (Burkhard and Ankley, 1989; Mount and Anderson-Carnahan, 1989).

There are two major types of fractionation schemes in TIEs: reduction type and isolation type. The reduction type involves removing a particular class of compounds by some physical manipulation, and then conducting toxicity tests on the remaining effluent. The isolation type involves isolating a particular class of compounds by some manipulation, and then conducting toxicity tests on the isolated fraction. Most of the fractionations involve performing a manipulation at different pHs in which the pH of the whole effluent is adjusted to acidic or basic conditions before performing the manipulations. The state of organic and inorganic compounds (ionic or un-ionized form) is influenced by the pH of the solution and the equilibrium constant (K_a) of the compound. When the pH of the solution is equal to the pK_a ($-\log_{10}K_a$) of the compound, there are equal amounts of ionic and un-ionized forms (for monoprotic compounds). For acidic compounds, if the pH > pK_a, then the ionic form predominates; if the pH < pK_a, then the un-ionized form is predominate. The opposite relationship is observed for basic compounds.

ORGANIZATION OF THE STUDY

This study involved TIEs of 14 PW samples collected from oil and gas production inland facilities in Wyoming, coastal sites in Texas and California, and coastal and offshore sites in Louisiana. Both onshore and offshore facilities were sampled. This diversity in PW samples was intended to evaluate the efficacy of the TIE manipulations to support determinations of potential toxicants in a representative range of PW types. The study was conducted in three phases (Table 1-1). The methods used in the latter phases evolved based on experience gained during the previous phases.

Table 1-1. Summary of Produced Water Toxicity Research Studies

	Study Area 1 **Low-TDS Produced Water**	**Study Area 2** **High-TDS Produced Water**
Phase 1 Experiments	• one gas PW sample from coastal Texas and two oil PW samples from Wyoming • ten fractionation/adjustment procedures • toxicity tests with two freshwater species	• two oil PW samples from coastal California and offshore Louisiana • nine fractionation/ adjustment procedures (EDTA chelation for low-TDS samples only) • toxicity tests with two seawater species
Phase 2 Experiments	• two oil PW samples from coastal and offshore Louisiana[a] • nine fractionation/adjustment procedures (solid-phase extraction (SPE) dropped) • toxicity tests with two freshwater species	• two gas PW samples from coastal and offshore Louisiana and two oil PW samples from coastal California and coastal Louisiana[b] • eight fractionation/ adjustment procedures (solid-phase extraction [SPE] dropped) • toxicity tests with two seawater species; *Mysidopsis bahia* substituted for *Arbacia punctulata*
Phase 3 Experiments	• Low-TDS PW samples not tested	• two oil, two gas, and one gas/oil PW samples from offshore Louisiana production facilities • eight fractionation/adjustment procedures (EDTA chelation dropped) • developed NCE/ACE/BCE fractionation procedures • toxicity tests with two seawater species in triplicate (two samples) and single seawater species in duplicate (three samples) • salinity tolerance of *Mysidopsis bahia* tested

[a]Because one "Low-TDS" PW sample had high salinity (90 ppt), results evaluated from single sample.
[b]Because one PW sample had low toxicity, results evaluated from three samples.

Phase 1

The EPA TIE procedures do not address effluents with high salinities. The objectives of the first phase (Phase 1) were to: 1) evaluate the use of standard EPA TIE methods on representative high- and low-salinity PW; 2) to test the newly developed fractionation procedures; and 3) to test the performance of alternative toxicity test organisms in TIEs on PWs with high salinities. Three low-salinity (<3 ppt) PWs were analyzed by EPA TIE procedures. Two higher-salinity (>35 ppt) PWs were analyzed by TIE procedures modified for use on high-salinity effluents. The modifications included new extraction procedures that isolated neutral, acidic, and basic organic compounds (NCE, ACE, and BCE fractionations) for direct toxicity testing. The invertebrates *Mysidopsis bahia* and *Arbacia punctulata*, and the fish *Cyprinodon variegatus* were evaluated as toxicity test species for high-salinity PWs.

Phase 2

Based on the results of Phase 1, additional TIEs were performed with the fractionations and toxicity test organisms that appeared to be best suited for PW TIEs. Toxicity tests using *Arbacia* were dropped and extraction fractionations (NCE, ACE, and BCE) were substituted for the solid-phase extraction (SPE) fractionation in the EPA procedure. One low-salinity sample was analyzed using the freshwater TIE procedures, and four higher-salinity PW samples were analyzed by the modified procedures.

Phase 3

Additional TIEs were performed on five high-salinity PWs to further document the toxicity characteristics of PWs and to evaluate aspects of the testing procedure. *Mysidopsis* was used as the toxicity test species for all fractions. *Cyprindon* was also used as the test species for whole PWs only. The variability of the toxicity tests was evaluated by conducting the tests with duplicate or triplicate samples at each concentration. The salinity tolerance of *Mysidopsis* was evaluated to help interpret toxicity test results for PW with very high salinity. The chemical oxygen demand (COD) of the PWs was tested in this phase after it was observed that dissolved oxygen concentrations decreased in test media for some PW samples.

This report summarizes the objectives, methods, and results of this research on the toxicity of PW. Section 2 of this report summarizes the fractionation procedures and toxicity tests used

and developed during the three phases of research. The method summaries in Section 2 are supplemented by a set of standard operating procedures (SOPs) for the new methods that were developed (Appendices A through C); established methods are summarized and cited in the text of Section 2. The primary implications of these experimental results are discussed in Section 3 with conclusions and lessons learned resulting from this research presented in Section 4. A series of tables and supporting text summarizing important experimental conditions, observations, and results for the three sets of experiments are included in Appendix D.

Section 2
METHODS

This section summarizes the TIE procedures employed in this study. These procedures are modifications of EPA methods (Norberg-King *et al.*, 1991; Burgess *et al.*, 1993). The specific standard operating procedures (SOPs) used for sample collection, fractionation, and toxicity testing of PWs are presented in Appendices A through C.

SAMPLING

Toxicity identification evaluations were conducted on 14 PW samples with salinity concentrations ranging from <3 parts per thousand (ppt) to >100 ppt. In selecting samples, a mix of samples from gas and oil production operations in different areas of the United States at various salinity ranges was desired. Background information on the 14 samples used for the study is summarized in Table 2-1.

Sixteen liters (four 4-L bottles) of PW were collected from participating facilities using the sampling kit provided and following SOP API-TIEPW3 (Appendix C). Samples were taken at a discharge point immediately downstream from the last conventional water-treating vessel. The bottles were filled slowly from the bottom in order to completely remove any airspace, and minimize loss of volatile compounds. The samples were transported from the field in coolers packed in ice and shipped to the TIE laboratory for next-day delivery.

INITIAL PROCESSING

On the day of receipt (Day 1), a whole sample was tested for toxicity and fractionated for further toxicity testing the next day (Day 2). On Day 1, the contents of the four 4-L bottles were combined in a 20-L polycarbonate container and gently mixed for 30 seconds. Each container was tightly capped and the sample aliquoted from the container by positive pressure decanting, to minimize agitation. After mixing, sample aliquots underwent initial toxicity testing and preliminary chemical analysis. This initial processing followed the procedures in Norberg-King *et al.* (1991).

Table 2-1. Types of Produced Water Samples Tested

Sample ID	Origin	Salinity (ppt)	Type of Production	Production Additives
Phase 1 - Low TDS				
PWF-WY1	Wyoming	<3	oil	EB,CI,REB,SI,GT
PWF-TX1	Coastal Texas	<3	gas	C
PWF-WY2	Wyoming	<3	oil	CI CB
Phase 1- High TDS				
PWS-LA3	Offshore/Louisiana	43	oil	EB,REB,SI,GTC
PWS-CA1	Coastal California	41	oil	EB,CI,REB,SI,C, F,GTC
Phase 2 - Low TDS				
PWF-LA5	Coastal Louisiana	<3	oil	CI
PWF-LA4[a]	Offshore Louisiana	90	oil	None
Phase 2 - High TDS				
PWS-CA2	Coastal California	3	oil	EB,CIW,REB,F, SI,AC,CI
PWS-LA6	Offshore Louisiana	>100	gas	EB,PCC,GTC
PWS-LA7	Coastal Louisiana	59	gas	CI
PWS-LA8[b]	Coastal Louisiana	82	oil	EB,CIW,F,PCC
Phase 3 - High TDS				
PWS-LA11	Louisiana	5	gas	GTC
PWS-LA13	Louisiana	6	gas/oil	CI,GTC,WC
PWS-LA9	Louisiana	98	oil	EB,C,F,SI
PWS-LA10	Louisiana	>100	gas	B,AC,GTC,WC
PWS-LA12	Louisiana	>100	oil	EB,WC

[a]Results not evaluated because PW salinity was too high for tests with freshwater species.
[b]Results not evaluated because PW toxicity was too low.
EB = Emulsion Breaker
CI = Corrosion Inhibitor (oil soluble)
REB = Reverse Emulsion Breaker
SI = Scale Inhibitor
PCC = Paraffin Control Chemical
GTC = Gas Treating Chemical
C = Coagulant
F = Flocculent
AC = Acidizing Chemicals
WC = Water Clarifier
B = Bactericide

Initial (Range Finding) Toxicity Tests

Depending on whether the PW was considered a low-salinity or high-salinity sample, freshwater or marine species methods were used in the initial toxicity tests (U.S. EPA, 1993). The freshwater species used were the invertebrate, *Ceriodaphnia dubia*, and the fish species, *Pimephales promelas*, and the marine species used were the invertebrates, *Mysidopsis bahia* and *Arbacia punctulata*, and fish species, *Cyprinodon variegatus*. The test species were selected based on their sensitivity and availability and on the fact that they have been widely used for the evaluation of a variety of toxicants. Toxicity test conditions for each species are summarized in Table 2-2 for the freshwater species and Tables 2-3 and 2-4 for the marine species. The dissolved oxygen (DO) concentration, pH, and temperature were measured for each concentration during the toxicity tests at times 0 h, 24 h, and 48 h to monitor water quality. The results of the Day 1 initial tests were used to determine the concentration range for the fraction toxicity tests on Day 2.

Chemical Properties Measurements

Each PW sample was analyzed for pH (Method 423; APHA 1985), alkalinity (APHA Method 403), salinity (APHA Method 210A), hardness (APHA Method 314B), dissolved oxygen (APHA Method 421F), ammonia (APHA Method 417), oil and grease content (EPA Methods 413.1 [Infrared] and 413.2 [Gravimetry]), and iron (EPA Methods 3010, 6010).

Chemical analyses in the high-salinity PWs included determining of the major cations/anions: sodium (EPA Methods 3010, 6010), potassium (EPA Methods 3010, 6010), calcium (EPA Methods 3010, 6010), magnesium (EPA Methods 3010, 6010), chloride (EPA Method 325.1), sulfate (EPA Method 300.0), and bicarbonate (APHA Method 403).

Chemical oxygen demand (APHA Method 410.4) was determined for the Phase 3 samples.

Table 2-2. Summary of Test Conditions for Acute Toxicity Tests Using the Freshwater Species Fathead Minnow, *Pimephales promelas*, and Daphnid, *Ceriodaphnia dubia*

	Species	
Condition	*Pimephales promelas* (Fathead Minnow)	*Ceriodaphnia dubia* (Daphnid)
Reference:	U.S.EPA, 1985	U.S.EPA, 1985
Test Type:	Static	Static
Test Duration:	24 hr	24 hr
Dilution Water:	Well or surface water	Pond water
Age/Size of Test Organisms:	2 to 24 hr old	2 to 24 hr old
Temperature:	20 ± 1°C	20 ± 1°C
Photoperiod:	16 hr light/8 hr dark	16 hr light/8 hr dark
Light Intensity:	Ambient laboratory lighting	Ambient laboratory lighting
Number of Concentrations:	5 plus a control	5 plus a control
Number of Replicates:	1	1
Number of Organisms/Replicate:	5	5
Test Vessel Water Volume:	100 mL	20 mL
Loading Rate:	5 animals per 100 mL	5 animals per 20 mL
Feeding:	Live newly-hatched brine shrimp	Yeast, trout chow, cerophyl and/or fresh water algae
Aeration:	None	None
Effects measured:	Death	Death

Table 2-3. Summary of Test Conditions for Acute Toxicity Tests Using the Seawater Species *Cyprinodon variegatus* and *Mysidopsis bahia*.

Species:	*Cyprinodon variegatus*	*Mysidopsis bahia*
Reference:	U.S. EPA, 1985	U.S. EPA, 1985
Test Type:	Static	Static
Test Duration:	48 h	48 h
Dilution Water:	Seawater (20 ppt)	Seawater (20 ppt)
Age of Test Organisms:	2 to 24 h old	2 to 24 h old
Temperature:	20 ± 1°C	20 ± 1°C
Photoperiod:	16 h light/8 h dark	16 h light/8 h dark
Light Intensity:	Ambient laboratory lighting	Ambient laboratory lighting
Number of Concentrations:	5 plus a control	5 plus a control
Number of Replicates:	2-3	2-3
Number of Organisms/Replicate:	5	5
Test Vessel Water Volume:	100 mL	20 mL
Loading Rate:	5 animals per 100 mL	5 animals per 20 mL
Feeding	Live, newly-hatched brine shrimp	Live, newly-hatched brine shrimp
Aeration	None	None
Effects Measured	Death	Death

Table 2-4. Summary of Test Conditions for Acute Toxicity Tests Using the Sea Urchin, *Arbacia punctulata*

Reference:	U.S. EPA, 1987
Test Type:	Static
Test Duration:	1 hr sperm exposure followed by 90-minute exposure of combined sperm and eggs
Dilution Water:	Natural seawater adjusted to 30 ppt
Age/Size of Test Organisms:	Less than 1 hr old
Temperature:	20 ± 1°C
Photoperiod:	Continuous light
Light Intensity:	Ambient laboratory lighting
Number of Concentrations:	5 plus a control
Number of Replicates:	1
Number of Organisms/Replicate:	5,000,000 sperm and 2,000 eggs
Test Vessel Water Volume:	5 mL
Loading Rate:	Not applicable
Feeding:	None
Aeration:	None
Effects Measured:	Fertilization success

FRACTIONATION AND TOXICITY TESTING

Fractionation procedures were carried out after aliquots were taken for initial toxicity tests and chemical analyses. Up to ten major physical/chemical fractionation procedures were conducted on each PW sample. The fractionation types and objectives are presented in Table 2-5. Figures 2-1 and 2-2 present flowcharts for the fractionation procedures. Detailed procedures for sample preparation are presented in SOPs API-TIEPW1 for low-salinity samples (Appendix A) and API-TIEPW2 for high-salinity samples (Appendix B).

The pH Adjustment, Aeration, Filtration, SPE, and NCE/ACE/BCE fractionations involve adjustment to different pHs: pH*i* (*initial* pH of the PW as received), acidic pH, and basic pH. These pH adjustments were made prior to manipulations, using HCl and/or NaOH solutions.

For many organic and inorganic compounds, the pH of the solution influences the ionic (ionized or un-ionized) form of the compound.

Depending on the dissociation constant (K_a) of the compound, an adjustment of pH may cause a change in the compound speciation and permit isolation or removal of the target compound group(s) by a particular physical manipulation (e.g., filtration).

Table 2-5. Fractionation and Physical Adjustments Performed on the Produced Waters (pH*i* denotes the *initial* pH of the PW as received)

Fractionation Type	Fractionation Objectives
pH Adjustment at pHs 3 and 11	Determination of the effect of pH adjustment in fractionations; provides a "blank" for pH-adjusted fractionations, addresses issue of increased ionic strength and chemical reversibility.
Aeration with N_2 at pHs *i*, 3, and 11	Removal of volatile compounds under effluent pH, acidic, and basic conditions without presence of oxygen.
Aeration with air at pHs *i*, 3, and 11	Removal of volatile and oxidizable compounds under effluent pH, acidic, and basic conditions.
Filtration at pHs *i*, 3, and 11	Removal of particulates (with a filter) under effluent pH, acidic, and basic conditions.
Acidic Organic Compound Extraction (ACE) at pH~2	Isolation of acidic organic compounds.
Basic Organic Compound Extraction (BCE) at pH~12	Isolation of basic organic compounds.
Neutral Organic Compound Extraction (NCE) at pH = *i*	Isolation of neutral organic compounds.
Solid-phase Extraction (SPE) at pHs *i*, 3, and 9	Removal of semivolatile non-polar organic compounds and metal chelates, removal of some acidic and basic organic compounds with limited pH adjustment.
Graduated pH Test at pHs 6, 7, and 8	Determination of the possible presence of ammonia.
EDTA chelation (low-salinity effluent only)	Removal of cationic metals.

The Graduated pH Test and the EDTA Chelation are performed on whole PW on Day-2 as part of the fraction toxicity tests. The chelation with EDTA to bind metal cations should only be used with low-salinity PW because major cations in high-salinity PW may interfere with the intended chelation of potentially toxic metals. Procedures are provided in SOP API-TIEPW1 (Appendix A). The EDTA Chelation fractionation method requires the results of the whole PW (Day-1) test to estimate the amount of EDTA to be added to the effluent.

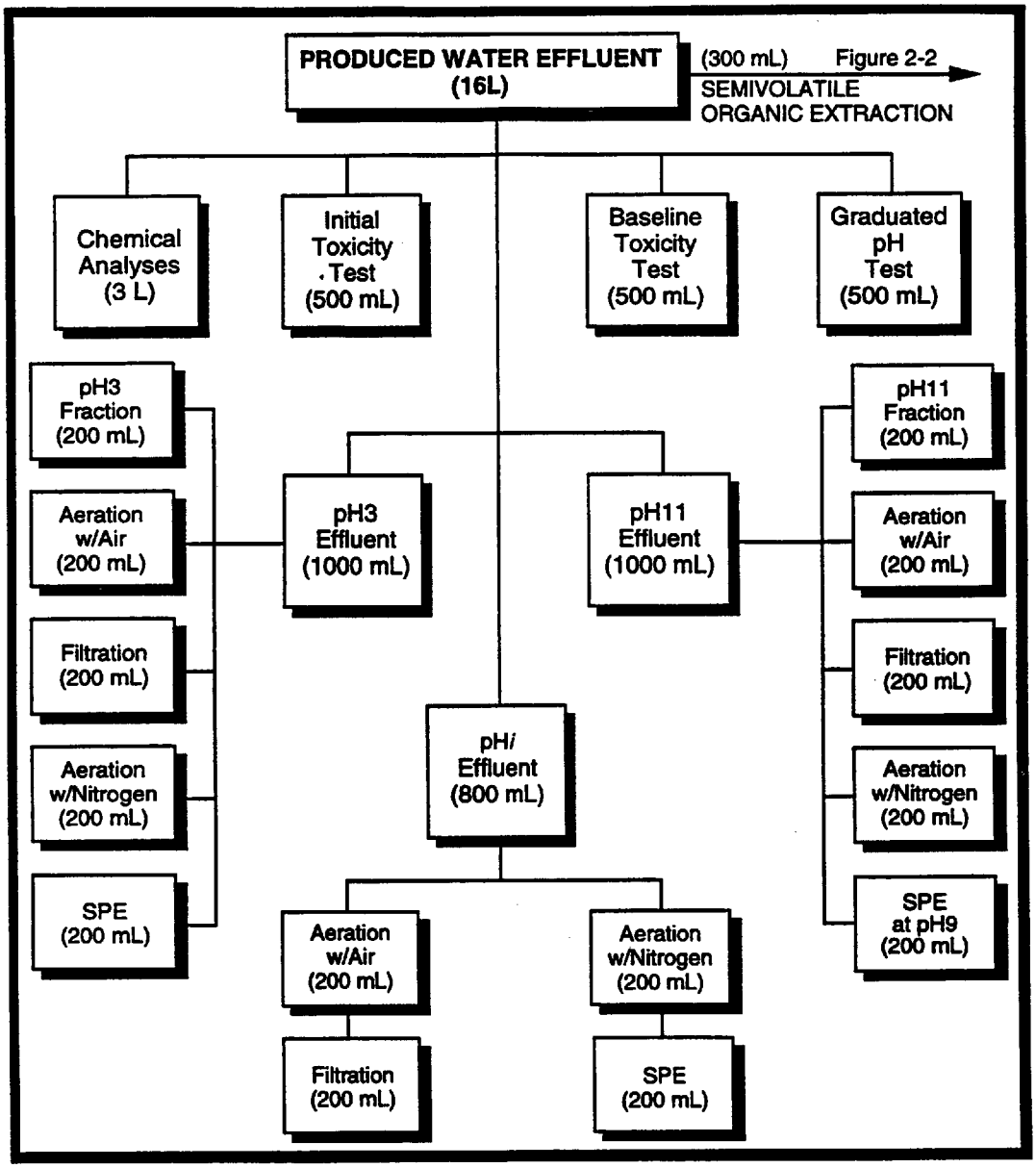

Figure 2-1. Flow diagram of the physical adjustments performed on the produced water samples. (pH*i* denotes the *initial* pH of the PW as received)

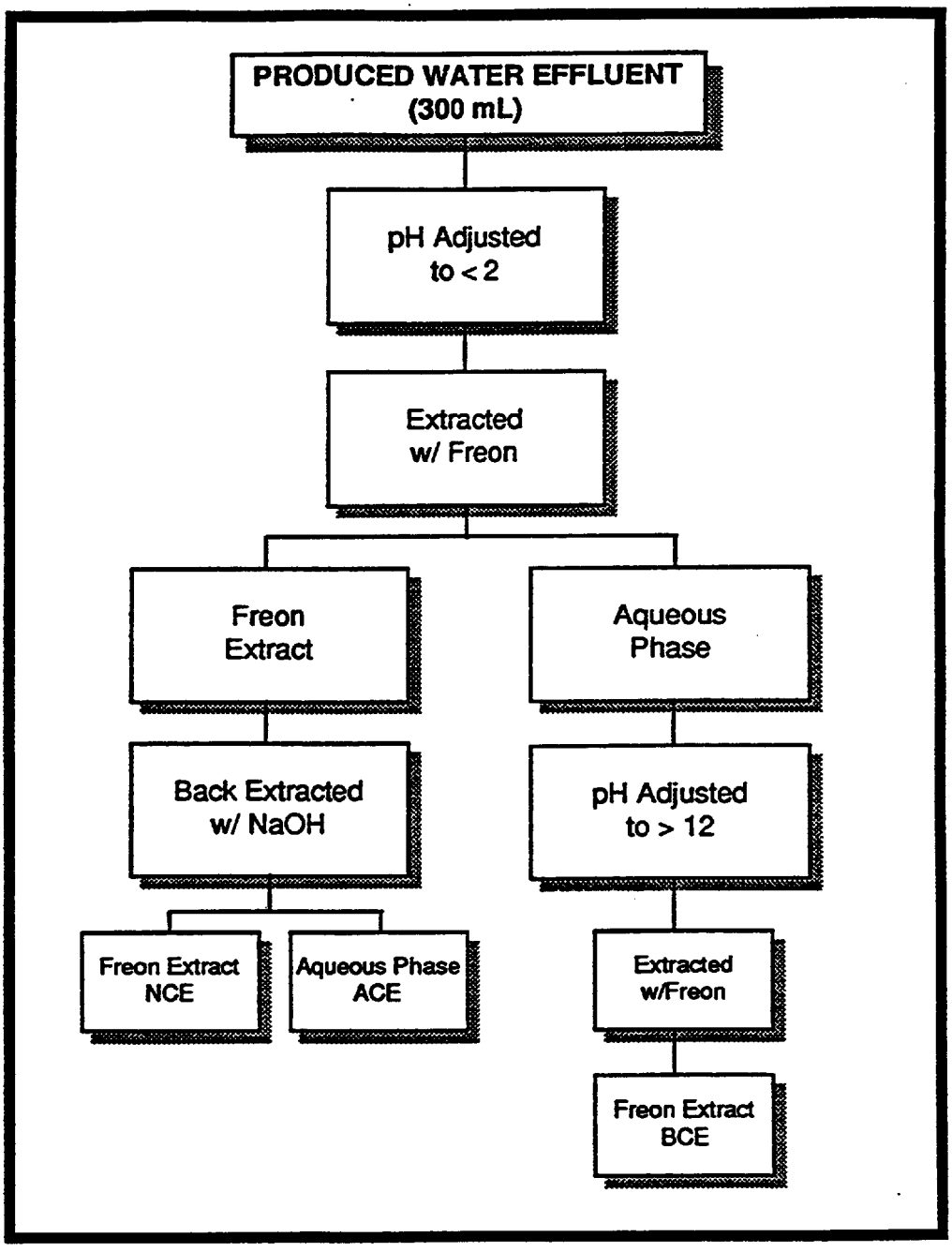

Figure 2-2. The semivolatile organic compound fractionation procedure (ACE/BCE/NCE) performed on the produced water samples.

The Neutral Organic Compound Extraction, Acidic Organic Compound Extraction, and Basic Organic Compound Extraction (NCE/ACE/BCE) fractionations, which isolate the neutral, acidic and basic semivolatile organic compounds respectively from the PW, were specifically developed for this study. This manipulation uses a wide range of pHs, from <3 to >9, which increases the percent conversion of the semivolatile organic compounds to their un-ionized, extractable form, thereby enabling a more complete isolation of the desired compound groups from the saline matrix. NCE/BCE/ACE fractionation procedures are provided in the SOPs API-TIEPW1 (Appendix A) and API-TIEPW2 (Appendix B).

For the reducing fractionations (e.g., filtration), the sample fractions used for toxicity testing were pH-adjusted back to the original pH (pH*i*) at the end of Day 1. Isolated fractions (e.g., NCE) used for toxicity testing were added to dilution water at a concentration comparable to that of the existing PW. All fractions of the PW samples and the whole PW were stored overnight in a refrigerator at approximately 4 °C (± 2 °C) for toxicity testing on Day 2.

On Day 2, after pH adjustment of the fractionation solutions (other than the pH*i* fraction), toxicity tests were conducted on whole PW (baseline) and on each sample fraction and sample blank. Sample blanks were generated and run in conjunction with each fractionation procedure, using dilution water adjusted to the appropriate pH. For the sample blank, toxicity tests were conducted at the highest concentration of the fraction solution. Control sample toxicity tests were performed with each set of fractions. For each test, an LC50 was calculated from the number of animals that died at each concentration at 24 and 48 hours using the binomial/nonlinear interpolation method (U.S. EPA, 1993).

For low-salinity PWs, the EDTA Chelation fraction was prepared from whole PW. Also, for all PWs, the Graduated pH Test fraction was prepared, and both fractions were submitted for toxicity testing. In Phase 3, pH buffers (Rausina *et al.*, 1992) were used in the Graduated pH Test.

ACE/BCE/NCE FRACTIONATION METHOD VALIDATION

To determine the efficiency of the ACE/BCE/NCE fractionation method in isolating acidic, basic, and neutral organic compounds from the PW samples, a method validation study was

performed. The ACE/BCE/NCE fractionation method validation involved spiking triplicate reagent water samples with a suite of acidic, basic, and neutral organic compounds (50 μg of each compound). The selection of compounds for spiking was intended to represent compound classes that could be 1) found in PW and 2) contribute toxicity. The acidic and neutral spike compounds included the EPA priority pollutant matrix spike analytes (i.e., phenol, chlorophenols, chlorobenzenes, PAHs, and nitro-compounds). The basic spike compounds were comprised of nitrogen heterocyclic compounds (i.e., quinolines, carbazole, and azafluorene).

The spiked reagent water samples were processed through the same acid/base/neutral extraction procedure as the PW samples for the toxicity tests (Figure 2-2). The one exception was the ACE extract, which was acidified and further extracted with freon to isolate the acidic compounds in an organic solvent suitable for instrumental analysis. The resulting ACE/BCE/NCE solvent extracts were concentrated to 1 mL, and analyzed by full-scan gas chromatography/mass spectrometry (GC/MS) to determine the recovery of the spiked analytes in each of the respective fractions. The analyte recoveries were calculated versus the surrogate compounds added to each sample prior to extraction. The recovery of the surrogate compounds were determined versus internal standards added to each extract prior to instrumental analysis.

Section 3
DISCUSSION OF RESULTS

The TIE approach to identifying components of PW contributing to toxicity was performed on a representative range of PWs of different production origin and chemical and physical properties. Produced water samples were collected from 14 major oil and gas production sites: inland facilities in Wyoming; coastal sites in Texas and California; and coastal and offshore sites in Louisiana. The chemical and physical characteristics varied widely. Salinities of these PWs ranged from fresh (less than 3 ppt) to highly saline (over 100 ppt). Each PW had received different types or combinations of production additives. The results of the three phases of this research are summarized in Appendix D. In this section, the key aspects of these results are discussed.

The differences in PW origin and chemical characteristics were manifested in the different constituents that were found to cause toxicity. Fractions showing differences in toxicity compared to whole PW are summarized in Table 3-1. Determining toxicity differences between whole PW and fractions was primarily based on the judgement of the research investigators. Because single replicate toxicity testing was performed on most PW samples, statistical significance could not be established (95 percent confidence intervals were usually large). The use of duplicate toxicity tests later in the study reduced the effect of variability on interpreting differences in toxicity between samples.

Causes of PW toxicity (possible toxicants) were hypothesized for some samples based on 1) toxicological results, 2) observations made during sample manipulations, and 3) chemical properties of the PW. This approach was consistent with the objective of this TIE research, which was to evaluate the efficacy of the manipulations (fractionations) in determining potential toxicants in PW. Specific identification of the toxicant(s) would have required implementation of a Phase 2 component of a TIE (Mount and Anderson-Carnahan, 1989).

Table 3-1. Summary Results of Produced Water TIE (pH*i* denotes the *initial* pH of the PW as received)

Produced Water (Salinity)	Fraction(s) Showing Toxicity Difference	Other Observation	Possible Toxicant
Freshwater Species TIE			
PWF-WY1 (<3 ppt)	All fractions at pH*i* and pH11	Rotten egg odor	Hydrogen sulfide
PWF-WY2 (<3 ppt)	None	Very low toxicity; Day-1 Toxicity completely lost by Day-2 Slight rotten egg odor	Unknown
PWF-TX1 (<3 ppt)	NCE All pH fractions for filtration and SPE	Oil sheen Oil/grease ~100 mg/L	Hydrocarbons
PWF-LA5 (<3 ppt)	Filtration pH11 (particulates)	Relatively high iron - 50 mg/L Toxicity lost by Day-2	Unknown
Marine Species TIE			
PWS-LA3 (42 ppt)	None	Very low toxicity Day-1 Toxicity completely lost by Day-2	
PWS-LA6 (230 ppt)	Graduated pH (ammonia)	PW LC50 near salinity tolerance of test species. DO decreased in test media. Extremely high salinity. Ionic imbalance (calcium). Ammonia - 162 mg/L	Ammonia Salinity
PWS-LA 7 (59 ppt)	None	Loss of toxicity Day-2	Unknown
PWS-CA1 (21 ppt)	All acidic and basic fractions of aeration, filtration, and SPE (not pH*i*)	DO concentrations low (<2 mg/L) for PW and pH*i* fractions at LC50 value. Normal DO (>6 mg/L) for basic and acidic fractions	Related to COD removed at pH3 and pH11
PWS-CA2 (4 ppt)	ACE	ACE toxicity near pH toxicity	Acidic organic compounds
PWS-LA9 (100 ppt)	ACE	ACE only slight toxicity contribution Low DO concentrations in test media for all fractions COD=3500 mg/L	Unknown
PWS-LA10 (112 ppt)	NCE Filtration - all pH (especially pH11)	Low oil/grease - 9 mg/L Ammonia - 100 mg/L NCE only slight toxicity contribution Many additives including bactericide	Unknown

Table 3-1. Summary Results of Produced Water TIE (continued)

Produced Water (Salinity)	Fraction(s) Showing Toxicity Difference	Other Observation	Possible Toxicant
PWS-LA11 (5 ppt)	BCE	Rotten egg odor BCE toxicity near PW toxicity	Basic organic compounds
PWS-LA12 (171 ppt)	None (Very minor NCE and Filtration pHi)	High salinity Very low DO concentrations (<2 mg/L) in test media at LC 50 value. High COD=8400 mg/L	Unknown
PWS-LA13 (7 ppt)	Filtration pH11	Highest PW toxicity Precipitate formed at pH11 Filtration pH11 only slight toxicity contribution Irregular ion composition compared to other PW High K^+ - 490mg/L High alkalinity	Irregular major ion composition

The discussion of key results of this research focuses on the following topics:

- potential PW toxicants;
- effects of salinity/ionic imbalances;
- effects of dissolved oxygen;
- isolation of acid/base/neutral organic compounds;
- particulates associated with pH change;
- suitability of test species;
- toxicity test reproducibility;
- experimental limitations for PW samples.

POTENTIAL PW TOXICANTS

Whole PW toxicities (Initial 24-h LC50s) ranged from approximately 5.8 to 22 percent for the low-salinity samples using the freshwater *Ceriodaphnia dubia* test species and 1.7 to 17 percent for the higher-salinity samples using the marine species *Mysidopsis bahia*. Most PW LC50s were greater than 5 percent. PWS-LA13 had the highest toxicity (i.e., lowest LC50) of all the PWs.

The potential causes of toxicities in the four low-salinity PW samples from Wyoming, Texas, and Louisiana (Table 3-1) were either 1) not identifiable using TIE manipulations because of the very low toxicity of the PW or 2) readily identifiable from the chemical characterization and observations noted during the TIE manipulations. For example, samples PWF-WY2 and PWF-LA5 had low toxicities without any identifiable contributions to toxicity from any TIE fractions (except possibly volatile compounds). Conversely, the major causes of toxicity in samples PWF-WY1 and PWF-TX1 were clearly indicated from the TIE manipulations to be hydrogen sulfide and hydrocarbons, respectively.

The potential causes of toxicity in the higher-salinity samples (5 ppt to greater than 100 ppt) appeared to be more variable (Table 3-1). Often, the best predictors for causes of toxicity were limited to identifying the fraction(s) contributing to effluent toxicity (e.g., ACE, filtration [filtered residue] at pH11). Interpretation of results was complicated by factors that were not differentiated explicitly by the fraction manipulations, such as effects of salinity and ionic imbalances, and in some PWs, chemical oxygen demand.

EFFECTS OF SALINITY/IONIC IMBALANCES

Salinity played an important role in assessing causes of toxicity in the high-salinity samples: PWS-LA6, PWS-LA9, PWS-LA10, and PWS-LA12 (near or above 100 ppt). Salinity was a factor contributing directly to the toxicity of the sample or restricting the range of fraction concentrations that can be used in the "reduced" fraction tests. The isolation procedures for organic compounds (i.e., ACE, NCE, and BCE) were very useful in identifying potential toxicant fractions that would have been lost if the fraction had to be diluted for the toxicity tests.

Because the salinity tolerance for the marine species *Mysidopsis bahia* is 35 ppt (estimated no-observed-effect concentration), high-salinity samples required dilution from 10 to 15 percent to avoid the effect of salinity-stress toxicity. In the PWS-LA6 and PWS-LA9 samples, the LC50s were near the mysid salinity tolerance indicating that the toxicity of the PW was principally from the ionic strength of the effluent. The other high-salinity PWs tested had LC50s lower than the salinity tolerance limits of the mysids after dilution.

Ionic strengths outside the tolerance range of the test species are known to cause mortality from the effect of salinity-stress toxicity. Recently it has been shown that ionic imbalances in freshwater tests can cause mortality to the test organism (Mount *et al.*, 1993; GRI, 1994). Individual ions or combination of ions that exceed the tolerance of the organisms can act as a toxicant. This effect of ionic imbalance on toxicity tests with marine species was not investigated as part of this research.

Although salinity varied among high-salinity PW samples, ionic composition (e.g., sodium, potassium, etc.) was generally consistent (Table 3-2). Irrespective of the salinity, the chloride ion concentration was consistently 60 percent (± 5 percent) of the total major ions in the sample. The sodium ion was 35 percent (± 3 percent) of the total ions and the ratio of potassium and calcium (K/Ca) was 1:9. Exceptions were predominately in the K/Ca ratio for samples PWS-LA3, PWS-CA1, PWS-CA2, and PWS-LA13 where ratios ranged from 1:1 to 1:5. Only PWS-LA13 had sodium ion at 17 percent.

Because the effect of ionic imbalance would not be revealed by the TIE fractionation procedures performed in this study, the ion composition of the PWs was compared to that of the dilution water (20 ppt) used in the toxicity tests. Dilution water contains the ion composition that the test species would naturally experience in the environment, and by normalizing the ion composition to chloride, ionic imbalances (excess or deficiency) in the PW can be demonstrated. The imbalances in ion composition will provide information on the potential for the effect of ionic imbalance contributing to the overall toxicity of the effluent.

Results of the ionic imbalance calculations are presented in Table 3-2 for all samples in which ionic compositions were determined. In almost all PW samples, salinity and sodium ion concentrations were very comparable to those of the dilution water. Calcium was generally in excess whereas magnesium and sulfate ions were deficient. Except for PWS-CA2 and PWS-LA13, which contained excess amounts of potassium, potassium was also deficient.

Table 3-2. Relative Composition of Dilution Water and the Saline Produced Waters Normalized to the Chloride Ion[1]

	Salinity	Sodium	Potassium	Calcium	Magnesium	Sulfate
Dilution Water[2]	1.81	0.56	0.021	0.021	0.07	0.14
PWS-LA6	1.7(0)	0.63(0)	0.002(-)	0.022(0)	0.006(-)	0.002(-)
PWS-LA7	1.6(0)	0.58(0)	0.004(-)	0.029(0)	0.006(-)	0.000(-)
PWS-CA1	1.7(0)	0.62(0)	0.004(-)	0.020(0)	0.004(-)	0.002(-)
PWS-CA2	1.8(0)	0.63(0)	0.045(+)	0.091(+)	0.003(-)	0.005(-)
PWS-LA9	1.6(0)	0.55(0)	0.005(-)	0.044(+)	0.011(-)	0.000(-)
PWS-LA10	1.6(0)	0.55(0)	0.004(-)	0.052(+)	0.017(-)	0.000(-)
PWS-LA11	1.8(0)	0.60(0)	0.008(-)	0.076(+)	0.007(-)	0.007(-)
PWS-LA12	1.6(0)	0.52(0)	0.003(-)	0.029(0)	0.006(-)	0.000(-)
PWS-LA13	1.6(0)	0.27(-)	0.11(+)	0.10(+)	0.008(-)	0.002(-)

[1] PWS-LA3 was not included because of ion composition analytical measurement error; chloride ion data provided in Table D-4
[2] Based on ion composition of dilution water (seawater composition)

Note: (0) = Nearly the same as dilution water
(-) = Deficient compared to dilution water
(+) = Excess compared to dilution water

EFFECTS OF DISSOLVED OXYGEN

In many PW samples, the dissolved oxygen (DO) concentration in the test media of whole and fractionated PW toxicity tests decreased during the test (summarized in Table 3-3). This decrease in DO concentration correlated with the chemical oxygen demand (COD) concentration of the PW. For those samples in which COD was determined, a reduction in DO concentration in the toxicity tests was observed for PW samples with COD concentrations greater than 2300 mg/L. Generally, the higher the COD value, the more drastic the change (decrease) in DO in the test media. Also, the change in DO concentrations was generally not affected by TIE manipulations (including air sparging). In samples PWS-CA1 and PWS-LA12, the test media DO may have been low enough (<2 mg/L) from an unknown oxygen demand material to have influenced the toxicity of the PW.

ISOLATION OF ACID/BASE/NEUTRAL ORGANIC COMPOUNDS

As part of this PW study, a method of isolating semivolatile organic constituents from PW for toxicity testing was developed by extracting sequentially with a solvent at different effluent pHs. Neutral, acidic, and basic compound extractable (NCE, ACE, and BCE) fractions were generated by this method. The organic constituents that would be represented in these fractions were determined in a validation experiment.

Table 3-3. Relationship of Chemical Oxygen Demand of Produced Water and Dissolved Oxygen Concentration in the Toxicity Tests (pH*i* denotes the *initial* pH of the PW as received)

Produced Water (Salinity)	Test Species	24-hr LC50 (percent)	COD (mg/L)	Dissolved Oxygen Observations
PWF-WY1	*Ceriodaphnia*	6-26	NA	DO concentrations were within normal range (6-8 mg/L) for all fractions.
PWF-WY2		22	NA	
PWF-TX1		8-11	NA	
PWF-LA5		8	NA	
PWS-LA3 (42 ppt)	*Arbacia*	17-100	NA	No DO measurements with *Arbacia.*
PWS-LA6 (233 ppt)	*Mysidopsis*	6	NA	DO concentrations decreased in all fractions reaching 3-4 mg/L at 25% dilution.
PWS-LA7 (50 ppt)	*Mysidopsis*	5-34	NA	DO concentractions decreased in all fractions reaching 3-4 mg/L at 25% dilution.
PWS-CA1 (21 ppt)	*Cyprinodon*	32-41	NA	DO concentrations decreased in PW and pH*i* fractions only, reaching <3 mg/L at 40% dilution.
PWS-CA2	*Mysidopsis*	7-22	NA	DO concentrations were within normal range (6-8 mg/L) for all fractions.
PWS-LA9 (100 ppt)	*Mysidopsis*	11-15	3,500	DO concentrations decreased in all fractions starting at the lowest tested concentration (3% dilution), reaching 3 to 4 mg/L at 10% dilution. Consistent at both 24 hr and 48 hr.

Table 3-3. Relationship of Chemical Oxygen Demand of Produced Water and Dissolved Oxygen Concentration in the Toxicity Tests (Continued)

Produced Water (Salinity)	Test Species	24-hr LC50 (percent)	COD (mg/L)	Dissolved Oxygen Observations
PWS-LA 10 (112 ppt)	*Mysidopsis*	8	2,100	DO concentrations were within normal range (6-8 mg/L) for all fractions at 24 hr and 48 hr.
PWS-LA11 (5 ppt)	*Mysidopsis*	15-16	2,300	At 24 hr, DO concentrations were within normal range for all fractions. At 48 hr, DO concentrations decreased in all fractions, starting at the lowest tested concentration (5% dilution), reaching 4-5 mg/L at 10% dilution.
PWS-LA12 (171 ppt)	*Mysidopsis*	2-6	8,400	At 24 hr, DO concentrations decreased in all fractions to <4 mg/L at 5% dilution and <1 mg/L at 10% dilution. At 48 hr, DO concentrations rebounded at all dilutions to levels in the normal range (6-8) mg/L).
PWS-LA13 (7 ppt)	*Mysidopsis*	1.7-1.9	1,000	DO concentrations were within normal range for all fractions at 24 hr and 48 hr.

The results of the validation experiment are summarized in Table 3-4 as mean percent recoveries of the acidic, basic, and neutral compounds spiked into triplicate water samples. The mean percent recoveries of the spiked analytes were generally within the expected range (50-134 percent) for the different types of compounds. The exceptions were phenol, 4-nitrophenol, and the surrogate phenol-d_6; 4-nitrophenol was not recovered in any of the fractions, while only traces of phenol-d_6 and phenol were recovered in the acid fraction. The phenol, phenol-d_6, and 4-nitrophenol results are not surprising since these highly polar compounds are difficult to extract from water with a non-polar solvent such as freon.

Table 3-4. Summary of Recoveries of Spiked Analytes from the ACE/BCE/NCE Validation Experiment

Fraction/Analytes	Mean % Recovery (n = 3)
Neutral Fraction	
Analytes	
1,4-Dichlorobenzene	95
1,2,4-Trichlorobenzene	101
N-Nitroso-di-n-propylamine	83
2,4-Dinitrotoluene	98
Acenaphthene	99
Pyrene	111
Carbazole	108
Surrogates	
1,2-Dichlorobenzene-d_4	71
Nitrobenzene-d_5	85
P-Terphenyl-d14	77
Carbazole-d_8	76
Acid Fraction	
Analytes	
Phenol	14
2-Chlorophenol	97
4-Chloro-3-methylphenol	53
4-Nitrophenol	Not Detected
Surrogates	
Phenol-d_6	<1
2-Chlorophenol-d_4	50
Base Fraction	
Analytes	
Quinoline	114
Isoquinoline	104
4-Azafluorene	134
5,6-Benzo(f)quinoline	124
Surrogates	
Quinoline-d_7	5

The remaining spiked analytes generally partitioned into the expected fractions; the chlorophenols were recovered in the acid fraction, the nitrogen heterocyclic compounds partitioned into the base fraction, and the PAH and chlorobenzenes were recovered in the neutral fraction. The exceptions were some of the weak basic compounds (carbazole, n-nitroso-di-n-propylamine, and 2,4-dinitrotoluene) which were found to partition into the

neutral fraction. Overall, the validation experiment served to identify the classes of compounds which partitioned into the three different fractions, allowing confirmation of the potential types of organic toxicants associated with fraction toxicity test results.

As indicated in Table 3-1, toxicity was often found to reside in any one of these fractions. The fractions represent general classes of organic constituents (and possibly other constituents extractable with organic solvent at neutral, acidic, and basic pHs). The following list, which includes some compounds that are not likely to be in PW, identifies representative compound types that may be found in these fractions:

- ACE (acidic organic compounds) - phenols, chloro- and bromophenols (including pentachlorophenol), and fatty and naphthenic acids
- BCE (basic organic compounds) - nitrogen-polycyclic aromatic compounds (N-PACs) that include the quinolines and azafluorenes (not carbazoles)
- NCE (neutral organic compounds) - hydrocarbons (including PAHs), chlorobenzenes, nitrosoamines, nitrobenzenes, carbazoles

PARTICULATES ASSOCIATED WITH pH CHANGE

In many samples, toxicity was associated with particulate material collected on filter paper after adjusting the pH (acidic, basic, and neutral). Identification of specific toxicants in particulate fractions would of course require detailed chemical analysis; however, based on the general behavior of organic and inorganic compounds in pH adjusted solutions, some inference of possible constituent types can be made. Hydrocarbons in the form of emulsified oil can be collected by filtration at any pH. Basic pH causes precipitation of certain trace metals (magnesium, iron, manganese) which can coprecipitate other metals from solution. Particulates of a variety of compound types have been observed to form with temperature and oxygen changes. Also, some organic surfactants precipitate at pH changes.

SUITABILITY OF TEST SPECIES

Because of the wide range of salinities in PW, either freshwater or marine species were used in the toxicity test. At the time this study was initiated, only procedures with freshwater

species, *Ceriodaphnia dubia* and *Pimephalas promelas*, were published by EPA for TIE studies because most effluents of interest to EPA are low in salinity. In high-salinity PWs, freshwater toxicity tests would be inappropriate. As a result, toxicity test procedures using marine species, *Mysidopsis bahia* and *Cyprinodon variegatus* were evaluated. Another sensitive toxicity test using the sea urchin *Arbacia punctulata* was also evaluated.

From the TIEs of this study, the sensitivities of the vertebrate species *(Pimephalas and Cyprinodon)* to PW were generally lower (i.e., higher LC50s) than those of the invertebrate species *(Ceriodaphnia, Mysidopsis, and Arbacia).* The fish specie *(Pimephalas)* was determined to be more sensitive than the invertebrate specie *(Ceriodaphnia)* only with the PW sample (low-salinity) containing the notable odor of hydrogen sulfide. The sea urchin *Arbacia* test was perhaps slightly more sensitive to PW toxicants than the other tests, but toxicity results were more variable. In PWs where the fish or *Arbacia* seemed to be more sensitive, the invertebrate species were also able to detect toxicity differences indicated by the other species.

In PW TIEs, the fresh and marine invertebrate species, *Ceriodaphnia* and *Mysidopsis*, would be the species of choice because they responded to PW toxicants with minimum variability and sufficient sensitivity compared to the other species.

TOXICITY TEST REPRODUCIBILITY

An experimental design without replication in the test assay provides no measure of reproducibility. The primary concern was that experimental variability could make evaluating the subtleties in toxicity changes in sample fractionations difficult. The extent to which LC50 values would be influenced from experimental variability would of course depend on the degree of variability (precision) and the range of test concentrations in the toxicity test. After performing the early series of investigations using single-replication tests, the reproducibility of the TIE toxicity tests was evaluated using *Mysidopsis*. Replicate analyses were performed on whole and fraction PW samples, in triplicate for two PW samples and in duplicate for three PW samples. Part of the evaluation considered value obtained in documenting experimental variability versus incremental test cost.

In replicated trials, survival of test organisms ranged as much as 40 to 100 percent for a replicate analysis at a particular test concentration, especially for tests at concentrations near the LC50 value of the sample. It was not uncommon for organism survival between replicates to differ by 40 percent (e.g., 1st replicate - 20 percent survival; 2nd replicate - 60 percent survival). The results of the replicated analyses suggested that conducting single replication tests, while adequate for screening, could produce unacceptable uncertainty in distinguishing subtle changes in toxicity between whole PW and its fractions. Although more costly than single replicate toxicity testing, duplicate tests are recommended for Phase I TIEs. However, triplicate tests were considered to provide little incremental value beyond duplicate tests for the associated increase in cost.

EXPERIMENTAL LIMITATIONS

Limitations of PW TIEs are principally related to the effects of salinity. Salinity not only affects the toxicity of PW directly, but also affects the types of manipulations that can be employed in the TIE. For high-salinity PWs, the sample requires dilution to bring the salinity within the tolerance range of the test organisms. *Mysidopsis* show salinity stress at approximately 40 ppt. For fraction manipulations in which the salt of the PW remains with the fraction tested (e.g., SPE, aeration, filtration), dilution of the test media is required which also dilutes the potential toxicants. Since many PWs are not highly toxic, detecting the contribution of particular fractions to the toxicity of samples would be difficult if dilution of the sample is necessary. Also, some of the freshwater fractionation methods are not applicable because of salinity. The EDTA Chelation method, which detects the toxicity contribution from trace metals in freshwater effluents, is ineffective at salinities above 3 ppt because many of the major cations in saline water interfere with the chelation process. In addition, buffering of test media, which is usually needed in the Graduated pH Test (ammonia toxicity determination), is difficult with high-salinity effluents.

To reduce the effect of salinity in PW TIEs, manipulations that isolate particular fractions from the saline sample for direct toxicity testing are required. In this TIE study, a method that isolated organic constituents (NCE, ACE, BCE) from high-salinity PW was developed and found to be effective in providing information on potential toxicants that would have been lost if non-isolating methods were used (e.g., SPE manipulation).

Section 4
LESSONS LEARNED

In this TIE research program, standard EPA fractionation schemes and newly developed PW-specific fractionation schemes were applied on a variety of samples of different salinities. The EPA fractionation schemes used in this study were the reduction type (i.e., aeration, filtration, SPE), and the effluent-specific schemes were the isolation type (NCE, ACE, and BCE).

During the course of this research, a number of experimental design changes were implemented to address the different objectives of the study. Initially, the viability of the mysid and sea urchin toxicity tests in high-salinity PW TIEs was investigated, as was the necessity for conducting both vertebrate and invertebrate species toxicity tests. Thus, in the early phases of the research, different test species were alternatively used on selected PW samples. Also in the early stages, new fractionation procedures for isolating organic material from high-salinity PW were evaluated and side-by-side results with the SPE method (organic material reduction scheme) were compared. Later into the research, toxicity test reproducibility was investigated. In the early phases, toxicity tests were performed without replication because many different parameters in the PW TIE needed to be compared and evaluated. Replication was built into the experimental design for the mysid toxicity tests performed on the last five PW samples.

LESSONS LEARNED

The results of these investigations and other observations made during this research provided insight on the classes of potential toxicants that may be expected in PW and on the types of procedures required to successfully conduct PW TIEs.

Potential PW Toxicants

The components (or fractions) contributing to PW toxicity were different for each PW with no single fraction being consistently toxic. No more than two fraction types were identified

as potential toxicants in any one produced water sample. Potential toxicants, besides salinity, were as follows:

- NCE, ACE, and BCE fractions
- Particulate matter removed by filtration at pH11
- Ionic imbalance or excess (e.g., excess calcium or potassium)
- Ammonia
- Hydrocarbons
- Hydrogen sulfide
- Material removed by pH change to acid or base
- Volatile compounds

Rarely was the cause of toxicity attributed to components removed by aeration (air or nitrogen). In many PWs for which toxicities were low (high LC50s), toxicities decreased from Day-1 to Day-2. The cause of this loss was not evident from the TIE procedures, except to indicate that the potential toxicant was a volatile component or one that precipitated from solution after the sample containers were opened on Day-1. In the Graduated pH test for ammonia, maintaining the pHs constant at 6.0, 7.0, and 8.0 was difficult and required the use of pH buffers. The salinity tolerance range of the *Mysidopsis* was 5 to 35 ppt.

High-Salinity Effects

In PWs with salinity above the tolerance threshold of the test species (35 ppt for mysids), salinity becomes an important influence in the TIE. The sample has to be diluted (with test dilution water) to insure that the salinity of the test media is within the species threshold limits. As a result, potential toxicants are also diluted making identification of the toxicant difficult. Also, dissolved solids content in PW does not generally have the same ionic composition as the test medium, which further stresses test organisms. Most PWs are deficient in some ions (magnesium, potassium, and sulfate) or in excess (calcium). The effects of ion imbalance from high-salinity PW on test species are not, however, as well known as those from low-salinity (fresh) PWs (Mount *et al.*, 1993).

In some of the high-salinity PWs, detecting the contribution of the organic fractions to the toxicity of the samples would not have been possible if the fractions were not isolated from

solution and tested without dilution of the test media. As a result, the NCE, ACE, and BCE fractionations provided information on potential toxicants that would have been lost if SPE methods were used.

Toxicity Testing

The fresh and marine invertebrate species, *Ceriodaphnia* and *Mysidopsis*, were the species of choice in PW TIEs. They were generally more sensitive to toxicants than the vertebrate species that were tested (*Pimephales* and *Cyprinodon*), and responded to toxicants with minimum variability and sufficient sensitivity to permit toxicity differentiation. The *Arbacia* test was found to be more variable than the *Mysidopsis*.

In replicated toxicity tests with mysids, survival of test organisms ranged as much as 40 to 100 percent for a replicate analysis at a particular test concentration, especially for tests at concentrations near the LC50 value of the sample. It was not uncommon for organism survival between replicates to differ by 40 percent (e.g., 1st replicate - 20 percent survival; 2nd replicate - 60 percent survival). The results of the replicated analyses suggested that conducting single replication tests, while adequate for screening, could produce unacceptable uncertainty in distinguishing subtle changes in toxicity between whole PW and its fractions. Although more costly than single replicate toxicity testing, duplicate tests are recommended for Phase I TIEs. However, triplicate tests were considered to provide little incremental value beyond duplicate tests for the associated increase in cost.

The extent to which DO concentrations decreased in the test media of the toxicity tests seemed to be directly related to the chemical oxygen demand (COD) of the sample. The cause of the oxygen demand was not, however, isolated by the fractionations. Most often, DO concentration decreased, it was irrespective of the fractionation manipulation.

SUGGESTIONS FOR CARRYING OUT PRODUCED WATER TIEs

The following suggestions derived from this research have been divided into the major phases in performing a TIE.

PW Characterization

Whole PWs should be analyzed for the chemical parameters measured in this study:

- Water quality--salinity (APHA Method 210A), pH (APHA Method 423), alkalinity (APHA Method 403), hardness (APHA Method 314B), dissolved oxygen (APHA Method 421F)
- Chemical oxygen demand (APHA Method 410.4)
- Ammonia (APHA Method 417)
- Oil and grease content (EPA Methods 413.1 [Infrared] or 413.2 [Gravimetry])
- Major cations/anions--sodium, potassium, calcium, and magnesium (EPA Methods 3010, 6010), chloride (EPA Method 325.1), sulfate (EPA Method 300.0), and bicarbonate (APHA Method 403)
- Iron (EPA Methods 3010, 6010)

Fractionation

In deciding on fractionation schemes to use in the TIE, consider procedures in addition to the standard EPA techniques (Norberg-King *et al.*, 1991) that will address the type and particular toxicological problems specific for the PW being evaluated. Initial toxicity testing of whole PW (range finding) and fractionation manipulations should start on the day of sample delivery. Fractionation schemes suggested for initial toxicological characterization of PWs are the following (the functions of each of these manipulations are provided in Table 2-5):

- Simple pH Adjustments at pH3 and 11
- Aeration with N_2 at pHs *i*, 3, and 11 and/or
  Aeration with air at pHs *i*, 3, and 11
- Filtration at pHs *i*, 3, and 11
- NCE, ACE, and BCE
- Graduated pH Test at pH6, 7, and 8
- Solid-Phase Extraction (SPE) at pHs *i*, 3, and 9

SPE methods for organic toxicant determination can be used for low-salinity PWs that do not require significant dilution in performing the toxicity tests. However, the SPE method is limited in its usefulness because the effective pH range is only between 3 and 9 (pHs outside this range cause degradation of the solid-phase adsorbent). This pH range limits the types of organic compounds that can be removed.

For PW fractionation schemes, the API SOPs (API-TIEPW1 for low-salinity PWs, and API-TIEPW2 for high-salinity PWs) provide effective procedures for reduction and isolation manipulations. The EPA documents (Norberg-King *et al.*, 1991; Burgess *et al.*, 1993) describe the toxicity test procedures for both freshwater and marine species. It is important to observe and document the behavior of the PW (precipitation, color change, etc.) during the fractionation procedures; these observations may provide information regarding toxicants.

To reduce the number of toxicity tests conducted, consider performing only one of the aeration (air or nitrogen) fractionations. The EDTA method for presence of trace metals as a toxicant is only useful in PW with salinity less than 3 ppt because the EDTA can complex with ions in highly saline PW.

Toxicity Testing

In performing Phase-I TIEs, the invertebrate test species are suggested. Use *Ceriodaphnia* for PWs with salinity less than 3 ppt, and *Mysidopsis* for PWs with salinity greater than 3 ppt. These invertebrate species are generally more sensitive then their vertebrate counterpart; the tests are less variable than other invertebrate tests (e.g., sea urchin fertilization); and they are amongst the most common species tested in effluent testing with an extensive historical database.

Acute toxicity studies (24-hr, 48-hr, or 96-hr LC50s or EC50s) are suggested for initial evaluations, using the procedures developed by EPA (U.S. EPA, 1993). Long-term chronic studies are more difficult and expensive, especially for saline PWs requiring marine species (e.g., feeding mysids is difficult for long-term studies). Chronic studies may be required when acute toxicities are low, or the potential toxicants are only detected by chronic studies. There are EPA methods for chronic freshwater TIE studies (Norberg-King *et al.*, 1991).

In the initial screening of PW for potential toxicants, single-replicate toxicity testing is acceptable. For more definitive identification of toxicant fractions, duplicate toxicity testing is recommended.

Accurately measure the DO concentration of the test medium periodically during the toxicity test, and aerate appropriately to maintain the oxygen level in the normal range (6 to 9 mg/L). In the Graduated pH test (for ammonia), pHs of 6, 7, and 8 need to be accurately maintained during the entire exposure period to successfully conduct the test. Buffers are recommended for maintaining constant pH (Rausina *et al.*, 1992; Mount and Mount, 1992).

Section 5
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APPENDIX A

STANDARD OPERATING PROCEDURE

API-TIEPW1

SOP No.: API-TIEPW1
Date Initiated: January 1, 1991
Revision No.: 00
Revision Date:
Page 1 of 11

CHEMICAL FRACTIONATION OF LOW TOTAL DISSOLVED SOLIDS PRODUCED WATER FOR TOXICITY IDENTIFICATION EVALUATIONS

1.0 Purpose

This standard operating procedure (SOP) presents a series of fractionation procedures used to isolate classes of compounds or properties of low total dissolved solids (< 3 parts per thousand) produced water for toxicity testing. This physical/chemical fractionation task is part of a toxicity identification evaluation (TIE) to identify the major toxicants of produced water samples. The fractions can be used for either acute or chronic toxicity tests. The required volume of produced water will depend on the types of toxicity tests performed.

The sample fractionations for the low-TDS produced water in this SOP include:

1. Simple pH Adjustment
2. Filtration
3. Aeration using air or nitrogen gas
4. Solid phase extraction (SPE)
5. Acidic/Basic/and Neutral Compound Extraction (ACE, BCE, and NCE)
6. EDTA Chelation
7. Graduated pH Test

For most of the sample fractionations of a TIE, the pH of the sample is adjusted to either acidic or basic conditions and the pH adjusted samples subjected to a number of manipulations such as aeration and filtration. After the manipulation, the pH of the pH-adjusted samples is restored to its original pH and submitted for toxicity testing.

All preparation of sample fractions except the Graduated pH Test is performed on the day the sample arrives into the laboratory (day 1). Usually, this preparation of sample fractions requires one full day. The day after the fractions are prepared (day 2), the fractions are made available for toxicity testing. The Graduated pH Test is performed on day 2.

2.0 References

Mount, D.I. and L. Anderson-Carnahan. 1988. Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Identification Procedures, USEPA Environmental Research Laboratory, Duluth, MN EPA/600/388/035.

3.0 Preparation

3.1 Supplies and Equipment

The following supplies and equipment are needed to perform the various TIE fractionations:

- Burettes (6) - For acid and base titration
- 1000-mL Buchner funnels
- Glass funnels
- 250-mL, 500-mL, and 1000-mL Beakers
- 25-mL, 50-mL, 250-mL, and 500-mL Graduated cylinders
- 250-mL Separatory funnels
- Glass frits
- 1.0-um Glass fiber filters
- Solid Phase Extraction (SPE) apparatus including SPE C_{18} column, vacuum manifold, and pump
- Ring stands and clamps
- Magnetic stir plates and bars
- pH meter
- Pre-cleaned polyethylene jars (125-mL)

3.2 Solvents and Reagents

- 6N Hydrochloric Acid (HCL)
- 1.2N Hydrochloric Acid (HCL)
- 0.12N Hydrochloric Acid (HCL)
- 0.012N Hydrochloric Acid (HCL)
- 1.0N Sodium Hydroxide (NaOH)
- 0.1N Sodium Hydroxide (NaOH)
- 0.01N Sodium Hydroxide (NaOH)

- Sodium Sulfate (Na_2SO_4) - baked at 400 °C and stored in a clean, air tight, glass container
- Pesticide Grade Freon
- Pesticide Grade Acetone
- Pesticide Grade Methanol
- Disodium Ethylenediamine Tetraacetate (EDTA)
- Zero Grade Air
- VHP Grade Nitrogen Gas

3.3 Fractionation Water

- Dilution Water - same water used in diluting effluent for toxicity tests
- High Purity Water - usually distilled or deionized water

4.0 Fractionation Procedures

4.1 Sample Preparation

Produced water samples are expected to arrive into the laboratory one day after collection in the field in coolers containing ice. Approximately 16 liters of produced water are required to conduct the fractionations for use in two acute toxicity tests.

All samples and fractions are to be stored at 4°C.

4.1.1 Sample Mixing and Subsampling

1. Combine the sample (effluent) from the sample containers into a 20-liter polycarbonate (or similar) container.
2. Gently mix the sample for 30 seconds.
3. Place a stopper with appropriate tubing on the 20-L container that will allow the sample to be subsampled by applying positive pressure to the container headspace.
4. Subsample 1 liter of sample for each chemical analysis and initial toxicity testing.

4.1.2 pH Adjusting

1. Measure and record the initial pH of the produced water sample (pH*i*), pH of the dilution water (pHo), and pH of the high purity water (pHd).
2. Measure 800 mL of effluent into each of two 1000-mL beakers and 800 mL of dilution water into each of two 1000-mL beakers. Also, measure 800 mL of high purity water (DI water) into each of two 1000-mL beakers.
3. Adjust one beaker of the effluent sample, the dilution water, and the DI water to pH3.
4. Adjust the other beaker of the effluent sample, the dilution water, and the DI water to pH11.
5. Record the volumes and normalities of HCl and NaOH used.

4.2 Simple pH Adjustment

This fractionation determines the effect of adjusting the pH of the effluent to pHs 3 and 11.

1. Subsample 70 mL of each of the pH3 effluent, pH3 dilution water, pH3 DI water, pH11 effluent, pH11 dilution water, pH11 DI water in 100-mL beakers.
2. At the end of the working day, adjust the pH3 and pH11 effluent subsamples to pH*i* and the pH3 and pH11 dilution water subsamples to pHo, and pH3 and pH11 DI water subsamples to pHd.
3. Record the volumes and normalities of HCl and NaOH used.

4.3 Filtration

At different pHs, the effluent is filtered to determine the effect of particulates on the toxicity of the effluent. The filtrate from these filtration fractionations are also used in other fractionations (e.g., SPE).

4.3.1 Filter Preparation

1. Set up three Buchner funnels lined with 1.0-µm glass fiber filters on top of three 500-mL Erlenmeyer flasks.
2. Through the first funnel, filter 100 mL of pH3 DI water and discard the filtrate. This filter is used to filter the pH3 dilution water and the pH3 effluent.

3. Repeat this procedure for the second funnel using pHd DI water. This filter is used to filter the pHo dilution water and the pH*i* effluent.
4. Repeat step 2 for the third funnel using pH11 DI water. This filter is used to filter the pH11 dilution water and the pH11 effluent.

4.3.2 Sample Filtration

1. Through the pH3 filter, filter 440 mL of pH3 dilution water. Subsample 40 mL of this filtrate for day 2 toxicity tests. Set aside the remaining 400 mL for use in Solid Phase Extraction (SPE) fractionation. Through this same filter, filter 470 mL of pH3 effluent. Subsample 70 mL of the filtered effluent for day 2 toxicity tests. Set aside the remaining 400 mL for SPE fractionation.
2. Repeat step 1 using the pH*i* filter and the pHo dilution water and the pH*i* effluent.
3. Repeat step 1 using the pH11 filter and the pH11 dilution water and the pH11 effluent.
4. At the end of the working day, readjust the pH3 and pH11 effluent subsamples to pH*i* effluent and the pH3 and pH11 dilution water subsamples to pHo dilution water. Record the normalities and volumes of any HCL and NaOH added.

4.4 Aeration

At different pHs, the effluent is aerated with either air or nitrogen gas to determine the contribution of the volatile components to the toxicity of the effluent.

4.4.1 Aeration with Air

1. Subsample 70 mL of the pH*i*, pH3, and pH11 effluent and the pHo, pH3, and pH11 dilution water in each of six 100-mL graduated cylinders.
2. Place a tube with a glass frit at the end and bubble air through each of the 6 samples at approximately 10 mL/min for 1 hour.
3. Check the pH of the pH3 and pH11 samples every 5 minutes for the first 30 minutes and at 10 minute intervals thereafter, until the end of the hour.
4. Adjust the pH at each time interval when necessary to keep the samples at pH3 or pH11.

5. Record the normalities and volumes of HCL or NaOH added.
6. Record the pH of all 6 samples at the end of the hour.
7. Transfer each aerated/air subsample to a pre-cleaned polyethylene container for day 2 toxicity tests.
8. If present, remove all residue from the inside of the graduated cylinder and retain for possible toxicity tests.
9. At the end of the working day, readjust the pH3 and pH11 aeration/air subsamples to pH*i* and the pH3 and pH11 dilution water aeration/air subsamples to pHo. Record the normalities and volumes of any HCL and NaOH added.

4.4.2 Aeration with Nitrogen

1. Repeat all steps in Section 4.4.1 substituting nitrogen gas in place of air.

4.5 Acidic/Basic/ and Neutral Compound Extraction

This fractionation isolates from the effluent the acidic, basic, and neutral semivolatile organic compounds of the effluent.

4.5.1. Isolation of the Neutral Compound Fraction

1. Acidify 200 mL of effluent to pH<2 with 6N HCl in a 250-mL separatory funnel.
2. Extract the pH-adjusted effluent three times with 10 mL of Freon. Combine the extracts in a 125-mL erlenmeyer flask. Save the acidic aqueous phase for Section 4.5.2.
3. Back extract the Freon extract three times with 4 mL of 1.0 N NaOH. Save the aqueous phase extract for Section 4.5.3.
4. Dry the Freon extract over approximately 10 grams sodium sulfate.
5. Concentrate the Freon extract to 100 µL under a stream of nitrogen gas.
6. Displace the Freon with acetone and concentrate by nitrogen gas evaporation to a final volume of 100 µL.
7. Dilute the extract to 200 mL with pHo dilution water. This extract is called the Neutral Compound Extraction (NCE) effluent.
8. Store for day 2 toxicity tests at 4°C.

4.5.2 Isolation of the Basic Compound Fraction

1. Adjust the pH of the extracted effluent from step 2 of Section 4.5.1 to pH>12 with 1.0 N NaOH.
2. Extract the previously extracted, pH adjusted effluent three times with 10 mL of Freon. Combine the extracts in a 125-mL Erlenmeyer flask.
3. Dry the Freon extract over approximately 10 grams of sodium sulfate.
4. Concentrate the Freon extract to 100 µL by nitrogen gas evaporation.
5. Displace the Freon with acetone, and concentrate by nitrogen gas evaporation to a final volume of 100 µL.
6. Dilute the extract to 200 mL with pHo dilution water. This is called the Basic Compound Extraction (BCE) effluent.
7. Store for day 2 toxicity tests at 4°C.

4.5.3 Isolation of Acidic Compound Fraction

1. Dilute the aqueous NaOH extract from Section 4.5.1, Step 3 to 200 mL with pHo dilution water. If necessary adjust the pH of the diluted aqueous extract to pHo. This is called the Acidic Compound Extraction (ACE) effluent.
2. Store for day 2 toxicity tests at 4°C.

4.5.4 Blanks

1. Follow all steps in Sections 4.5.1, 4.5.2, and 4.5.3 to a dilution water sample. Call the fractions NCE blank, BCE blank, and ACE blank.

4.5.5 pH Readjustment

1. At the end of the working day, readjust the NCE, BCE, and ACE effluent fraction to pH*i* and NCE, BCE, and ACE blanks to pHo.
2. Record the normalities and volumes of HCl and NaOH used.

4.6 Solid Phase Extraction (SPE)

With the use of a C18 bonded solid phase, organic compounds are removed at different pHs. At each pH, two fractions are collected.

4.6.1 Solid Phase Column Preparation

1. Assemble a solid phase extraction apparatus with three C18 solid phase columns.
2. Prepare each column by drawing (by vacuum) 25 mL of methanol through the column followed by 25 mL of DI water. Do not let the sorbent go dry.
3. Designate a column for pH*i*, pH3, and pH9 samples.

4.6.2 Sample Extraction

1. Adjust the pH of the pH11 filtered dilution water and the pH11 filtered effluent to pH9. (See Section 4.3 for filtered samples).
2. Through each column draw 200 mL of the appropriate pH of filtered dilution water. Collect all 200 mL. Aliquot 10 mL for day 2 toxicity tests.
3. Without letting the sorbent go dry between additions draw 200 mL of the appropriate pH filtered effluent (See Section 4.3). Collect 30-mL samples after 25 mL have been drawn through and another 30-mL after a total of 150 mL have been drawn through the column.
4. At the end of the working day, readjust the pH of the pH3 and pH9 dilution water SPE samples to pHo dilution water. Readjust both pH3 and both pH9 SPE effluent samples to pH*i* effluent. Record the normalities and volumes of HCL and NaOH added.

4.7 EDTA Chelation

The EDTA chelation test is used to determine the extent of toxicity caused by cationic metals. Increasing amounts of the chelating agent EDTA are added to aliquots of the effluent to remove the metals that may contribute to toxicity of the effluent. Because of the effect of salinity and hardness on the effectiveness of EDTA, unreacted EDTA is itself toxic.

4.7.1 Determination of EDTA Standard Solution Concentration

1. Determine the LC50 of the effluent (use initial toxicity test results).
2. By using the hardness determination method (APHA,1980), record the number of mL (X) of a standard solution of EDTA needed to titrate the hardness (end point) of a 4X-LC50 concentration of effluent, of known volume. Record the

molarity of the EDTA solution (Y) and the volume of the effluent (Z) used to determine the hardness of the 4X-LC50 concentration of effluent.

3. A 0.2 mL addition of a standard solution of EDTA to a 10 mL effluent volume provides the EDTA desired at the midrange of EDTA additions. The volume of effluent required for toxicity testing depends on the type of toxicity test (assume 10 mL). The new molarity of the EDTA solution (M) used for the toxicity tests is calculated from the following formula:

$$M = (X \text{ mL} \times 10 \text{ mL} \times Y) / (0.2 \text{ mL} \times Z \text{ mL}).$$

4.7.2 Preparation of EDTA Solution

1. To determine the amount, in grams, of EDTA (E) to add to make a 100 mL standard EDTA solution, use the following formula:

$$E(g) = (A \times M) / 10$$

Where: A = 372.3 g (Molecular weight of Na_2EDTA is 372.3).

2. Add the amount EDTA (E) to 100 mL of distilled water.

4.8.3 EDTA Standard Solution Addition to Effluent

1. Depending on the number of toxicity tests to be conducted, prepare 6 to 11 aliquots (10 mL) of 4X-LC50 concentration of effluent. One aliquot is for the Control, the other 5 to 10 aliquots are used for EDTA addition.

2. For a 5 aliquot test, add 1.0 mL of EDTA standard solution (M molarity) to the first 10 mL aliquot, 0.6 mL to the second aliquot, 0.2 mL to the third, 0.05 mL to the fourth, and 0.12 mL to the fifth.

3. To Control aliquot, do not add any EDTA.

4. Submit aliquots from Steps 2 and 3 for toxicity tests.

4.8 Graduated pH Test

This test is performed to determine whether effluent toxicity may be attributed to the presence of ammonia in the produced water. A small volume or large volume pH stabilization procedure may be followed. This test is performed on day 2.

4.8.1 Small Volume Procedure

If the dissolved oxygen content of the effluent is adequate, this small volume pH stabilization procedure should be tried.

1. Dilute a volume of the effluent to the 24-hour initial LC50 concentration using dilution water to a final volume of 120 mL.
2. Subsample a 40 mL portion of this solution.
3. Adjust the subsample to pH7 being careful to titrate a minimum of HCL (0.12 N or 0.012 N) and NaOH (0.1 N and 0.01 N).
4. Transfer approximately half of this volume to a one ounce disposable test chamber.
5. Add five test organisms.
6. Transfer the remaining volume of pH7 effluent to the test chamber until the level of effluent rises just above the chamber.
7. Seal the test chamber with parafilm taking care to seal out any air without losing any organisms.
8. Repeat Steps 1-7 using 33 mL aliquots of effluent adjusted to pH6 and pH8, respectively.
9. Check the pHs of all the solutions and adjust if necessary, every hour during the first few hours, and as needed for the duration of the test.
10. Record pH drift and dissolved oxygen concentration.

4.8.2 Large Volume Procedure

If the above method does not hold pH or the dissolved oxygen of the effluent is too low, the following large volume pH stabilization method will be used.

1. Dilute a volume of the effluent to the 24-hour initial LC50 concentration with dilution water to a final volume of 1500 mL.
2. Subsample 500 mL of this solution in a 600-mL beaker.
3. Adjust the subsample to pH7 being careful to titrate a minimum of HCL (1.2 N and 0.12 N) and NaOH (1.0 N and 0.1 N).
4. Suspend a 2-inch high stainless steel mesh cylinder with a "Petri dish type," water-tight bottom in the 500-mL test solution.

5. Transfer the test organisms to these cages.
6. Repeat Steps 1-5 for solutions of pH6 and pH8, respectively.
7. Check the pHs of all the solutions and adjust if necessary, every hour during the first few hours, and as needed for the duration of the test.
8. Record pH drift and dissolved oxygen concentration.

4.9 Day 2 pH Adjustment of Fraction Solutions

On the day after the fractionations are performed, the pH of the fraction solutions, which are stored at 4°C, is checked and, if necessary, adjusted to the appropriate pH. After adjustment, the fraction solutions are ready for toxicity tests.

1. Check pH of all fraction solutions with pH meter.
2. If necessary, readjust the pH of the effluent fraction solutions to pH*i* with either HCl or NaOH.
3. If necessary, readjust the pH of the dilution water and blank solutions to pHo.
4. Record the normalities and volumes of HCl and NaOH added.

APPENDIX B

STANDARD OPERATING PROCEDURE

API-TIEPW2

SOP No.: API-TIEPW2
Date Initiated: January 1, 1991
Revision No.: 00
Revision Date:
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CHEMICAL FRACTIONATION OF HIGH TOTAL DISSOLVED SOLIDS PRODUCED WATER FOR TOXICITY IDENTIFICATION EVALUATIONS

1.0 Purpose

This standard operating procedure (SOP) presents a series of fractionation procedures used to isolate classes of compounds or properties of high total dissolved solids (> 20 parts per thousand) produced water for toxicity testing. This physical/chemical fractionation task is part of a toxicity identification evaluation (TIE) to identify the major toxicants of produced water samples. The fractions can be used for either acute or chronic toxicity tests. The required volume of produced water will depend on the types of toxicity tests performed.

The sample fractionations for the high-TDS produced water in this SOP include:

1. Simple pH Adjustment
2. Filtration
3. Aeration using air or nitrogen gas
4. Solid phase extraction (SPE)
5. Acidic/Basic/and Neutral Compound Extraction (ACE, BCE, and NCE)
6. Graduated pH Test

For most of the sample fractionations of a TIE, the pH of the sample is adjusted to either acidic or basic conditions and the pH adjusted samples subjected to a number of manipulations such as aeration and filtration. After the manipulation, the pH of the pH-adjusted samples is restored to its original pH and submitted for toxicity testing.

All preparation of sample fractions except the Graduated pH Test is performed on the day the sample arrives into the laboratory (day 1). Usually, this preparation of sample fractions requires one full day. The day after the fractions are prepared (day 2), the fractions are made available for toxicity testing. The Graduated pH Test is performed on day 2.

2.0 References

Mount, D.I. and L. Anderson-Carnahan. 1988. Methods for Aquatic Toxicity Identification Evaluations: Phase I Toxicity Identification Procedures, USEPA Environmental Research Laboratory, Duluth, MN EPA/600/388/035.

3.0 Preparation

3.1 Supplies and Equipment

The following supplies and equipment are needed to perform the various TIE fractionations:

- Burettes (6) - For acid and base titration
- 1000-mL Buchner funnels
- Glass funnels
- 250-mL, 500-mL, and 1000-mL Beakers
- 25-mL, 50-mL, 250-mL, and 500-mL Graduated cylinders
- 250-mL Separatory funnels
- Glass frits
- 1.0-um Glass fiber filters
- Solid Phase Extraction (SPE) apparatus including SPE C_{18} columns, vacuum manifold and pump
- Ring stands and clamps
- Magnetic stir plates and bars
- pH meter
- Pre-cleaned polyethylene jars (125-mL)

3.2 Solvents and Reagents

- 6N Hydrochloric Acid (HCL)
- 1.2N Hydrochloric Acid (HCL)
- 0.12N Hydrochloric Acid (HCL)
- 0.012N Hydrochloric Acid (HCL)
- 1.0N Sodium Hydroxide (NaOH)
- 0.1N Sodium Hydroxide (NaOH)
- 0.01N Sodium Hydroxide (NaOH)

- Sodium Sulfate (Na_2SO_4) - baked at 400°C and stored in a clean, air tight, glass container
- Pesticide Grade Freon
- Pesticide Grade Acetone
- Pesticide Grade Methanol
- Zero Grade Air
- VHP Grade Nitrogen Gas

3.3 Fractionation Water

- Dilution Water - same water used in diluting effluent for toxicity tests
- High Purity Water - usually distilled or deionized water

4.0 Fractionation Procedures

4.1 Sample Preparation

Produced water samples are expected to arrive into the laboratory one day after collection in the field in coolers containing ice. Approximately 16 liters of produced water are required to conduct the fractionations for use in two acute toxicity tests. All samples and fractions are to be stored at 4°C.

4.1.1 Sample Mixing and Subsampling

1. Combine the sample (effluent) from the sample containers into a 20-liter polycarbonate (or similar) container.
2. Gently mix the sample for 30 sec.
3. Place a stopper with appropriate tubing on the 20-L container that will allow the sample to be subsampled by applying positive pressure to the container headspace.
4. Subsample 1 liter of sample for each chemical analysis and initial toxicity testing.

4.1.2 pH Adjusting

1. Measure and record the initial pH of the produced water sample (pHi), pH of the dilution water (pHo), and pH of the high purity water (pHd).

2. Measure 800 mL of effluent into each of two 1000-mL beakers and 800 mL of dilution water into each of two 1000-mL beakers. Also, measure 800 mL of high purity water (DI water) into each of two 1000-mL beakers.
3. Adjust one beaker of the effluent sample, the dilution water, and the DI water to pH3.
4. Adjust the other beaker of the effluent sample, the dilution water, and the DI water to pH11.
5. Record the volumes and normalities of HCl and NaOH used.

4.2 Simple pH Adjustment

This fractionation determines the effect of adjusting the pH of the effluent to pHs 3 and 11.

1. Subsample 70 mL of each of the pH3 effluent, pH3 dilution water, pH3 DI water, pH11 effluent, pH11 dilution water, pH11 DI water in 100-mL beakers.
2. At the end of the working day, adjust the pH3 and pH11 effluent subsamples to pH*i* and the pH3 and pH11 dilution water subsamples to pHo, and pH3 and pH11 DI water subsamples to pHd.
3. Record the volumes and normalities of HCl and NaOH used.

4.3 Filtration

At different pHs, the effluent is filtered to determine the effect of particulates on the toxicity of the effluent. The filtrate from these filtration fractionations are also used in other fractionations (e.g., SPE).

4.3.1 Filter Preparation

1. Set up three Buchner funnels lined with 1.0-µm glass fiber filters on top of three 500-mL Erlenmeyer flasks.
2. Through the first funnel, filter 100 mL of pH3 DI water and discard the filtrate. This filter is used to filter the pH3 dilution water and the pH3 effluent.
3. Repeat this procedure for the second funnel using pHd DI water. This filter is used to filter the pHo dilution water and the pH*i* effluent.
4. Repeat step 2 for the third funnel using pH11 DI water. This filter is used to filter the pH11 dilution water and the pH11 effluent.

4.3.2 Sample Filtration

1. Through the pH3 filter, filter 440 mL of pH3 dilution water. Subsample 40 mL of this filtrate for day 2 toxicity tests. Set aside the remaining 400 mL for use in Solid Phase Extraction (SPE) fractionation. Through this same filter, filter 470 mL of pH3 effluent. Subsample 70 mL of the filtered effluent for day 2 toxicity tests. Set aside the remaining 400 mL for SPE fractionation.
2. Repeat step 1 using the pH*i* filter and the pHo dilution water and the pH*i* effluent.
3. Repeat step 1 using the pH11 filter and the pH11 dilution water and the pH11 effluent.
4. At the end of the working day, readjust the pH3 and pH11 effluent subsamples to pH*i* effluent and the pH3 and pH11 dilution water subsamples to pHo dilution water. Record the normalities and volumes of any HCL and NaOH added.

4.4 Aeration

At different pHs, the effluent is aerated with either air or nitrogen gas to determine the contribution of the volatile components to the toxicity of the effluent.

4.4.1 Aeration with Air

1. Subsample 70 mL of the pH*i*, pH3, and pH11 effluent and the pHo, pH3, and pH11 dilution water in each of six 100-mL graduated cylinders.
2. Place a tube with a glass frit at the end and bubble air through each of the 6 samples at approximately 10 mL/min for 1 hour.
3. Check the pH of the pH3 and pH11 samples every 5 minutes for the first 30 minutes and at 10 minute intervals thereafter, until the end of the hour.
4. Adjust the pH at each time interval when necessary to keep the samples at pH3 or pH11.
5. Record the normalities and volumes of HCL or NaOH added.
6. Record the pH of all 6 samples at the end of the hour.
7. Transfer each aerated/air subsample to a pre-cleaned polyethylene container for day 2 toxicity tests.

8. If present, remove all residue from the inside of the graduated cylinder and retain for possible toxicity tests.

9. At the end of the working day, readjust the pH3 and pH11 aeration/air subsamples to pH*i* and the pH3 and pH11 dilution water aeration/air subsamples to pHo. Record the normalities and volumes of any HCL and NaOH added.

4.4.2 Aeration with Nitrogen

1. Repeat all steps in Section 4.4.1 substituting nitrogen gas in place of air.

4.5 Acidic/Basic/ and Neutral Compound Extraction

This fractionation isolates from the effluent the acidic, basic, and neutral semivolatile organic compounds of the effluent. This extraction fractionation removes the effect of salinity on the toxicity tests.

4.5.1. Isolation of the Neutral Compound Fraction

1. Acidify 200 mL of effluent to pH<2 with 6N HCl in a 250-mL separatory funnel.

2. Extract the pH-adjusted effluent three times with 10 mL of Freon. Combine the extracts in a 125-mL Erlenmeyer flask. Save the acidic aqueous phase for Section 4.5.2.

3. Back extract the Freon extract three times with 4 mL of 1.0 N NaOH. Save the aqueous phase extract for Section 4.5.3.

4. Dry the Freon extract over approximately 10 grams sodium sulfate.

5. Concentrate the Freon extract to 100 µL under a stream of nitrogen gas.

6. Displace the Freon with acetone and concentrate by nitrogen gas evaporation to a final volume of 100 µL.

7. Dilute the extract to 200 mL with pHo dilution water. This extract is called the Neutral Compound Extraction (NCE) effluent.

8. Store for day 2 toxicity tests at 4°C.

4.5.2 Isolation of the Basic Compound Fraction

1. Adjust the pH of the extracted effluent from step 2 of Section 4.5.1 to pH>12 with 1.0 N NaOH.
2. Extract the previously extracted, pH adjusted effluent three times with 10 mL of Freon. Combine the extracts in a 125-mL erlenmeyer flask.
3. Dry the Freon extract over approximately 10 grams of sodium sulfate.
4. Concentrate the Freon extract to 100 µL by nitrogen gas evaporation.
5. Displace the Freon with acetone, and concentrate by nitrogen gas evaporation to a final volume of 100 µL.
6. Dilute the extract to 200 mL with pHo dilution water. This is called the Basic Compound Extraction (BCE) effluent.
7. Store for day 2 toxicity tests at 4°C.

4.5.3 Isolation of Acidic Compound Fraction

1. Dilute the aqueous NaOH extract from Section 4.5.1, Step 3 to 200 mL with pHo dilution water. If necessary adjust the pH of the diluted aqueous extract to pHo. This is called the Acidic Compound Extraction (ACE) effluent.
2. Store for day 2 toxicity tests at 4°C.

4.5.4 Blanks

1. Follow all steps in Sections 4.5.1, 4.5.2, and 4.5.3 to a dilution water sample. Call the fractions NCE blank, BCE blank, and ACE blank.

4.5.5 pH Readjustment

1. At the end of the working day, readjust the NCE, BCE, and ACE effluent fraction to pH*i* and NCE, BCE, and ACE blanks to pHo.
2. Record the normalities and volumes of HCl and NaOH used.

4.6 Solid Phase Extraction (SPE)

With the use of a C18 bonded solid phase, organic compounds are removed at different pHs. At each pH, two fractions are collected.

4.6.1 Solid Phase Column Preparation

1. Assemble a solid phase extraction apparatus with three C18 solid phase columns.
2. Prepare each column by drawing (by vacuum) 25 mL of methanol through the column followed by 25 mL of DI water. Do not let the sorbent go dry.
3. Designate a column for pH*i*, pH3, and pH9 samples.

4.6.2 Sample Extraction

1. Adjust the pH of the pH11 filtered dilution water and the pH11 filtered effluent to pH9. (See Section 4.3 for filtered samples).
2. Through each column draw 200 mL of the appropriate pH of filtered dilution water. Collect all 200 mL. Aliquot 10 mL for day 2 toxicity tests.
3. Without letting the sorbent go dry between additions draw 200 mL of the appropriate pH filtered effluent (See Section 4.3). Collect 30-mL samples after 25 mL have been drawn through and another 30-mL after a total of 150 mL have been drawn through the column.
4. At the end of the working day, readjust the pH of the pH3 and pH9 dilution water SPE samples to pHo dilution water. Readjust both pH3 and both pH9 SPE effluent samples to pH*i* effluent. Record the normalities and volumes of HCL and NaOH added.

4.7 Graduated pH Test

This test is performed to determine whether effluent toxicity may be attributed to the presence of ammonia in the produced water. A small volume or large volume pH stabilization procedure may be followed. This test is performed on day 2.

4.7.1 Small Volume Procedure.

If the dissolved oxygen content of the effluent is adequate, this small volume pH stabilization procedure should be tried.

1. Dilute a volume of the effluent to the 24-hour initial LC50 concentration using dilution water to a final volume of 120 mL.
2. Subsample a 40 mL portion of this solution.

3. Adjust the subsample to pH7 being careful to titrate a minimum of HCL (0.12 N or 0.012 N) and NaOH (0.1 N and 0.01 N).
4. Transfer approximately half of this volume to a one ounce disposable test chamber.
5. Add five test organisms.
6. Transfer the remaining volume of pH7 effluent to the test chamber until the level of effluent rises just above the chamber.
7. Seal the test chamber with parafilm taking care to seal out any air without losing any organisms.
8. Repeat Steps 1-7 using 33 mL aliquots of effluent adjusted to pH6 and pH8, respectively.
9. Check the pHs of all the solutions and adjust if necessary, every hour during the first few hours, and as needed for the duration of the test.
10. Record pH drift and dissolved oxygen concentration.

4.7.2 Large Volume Procedure.

If the above method does not hold pH or the dissolved oxygen of the effluent is too low, the following large volume pH stabilization method will be used.

1. Dilute a volume of the effluent to the 24-hour initial LC50 concentration with dilution water to a final volume of 1500 mL.
2. Subsample 500 mL of this solution in a 600-mL beaker.
3. Adjust the subsample to pH7 being careful to titrate a minimum of HCL (1.2 N and 0.12 N) and NaOH (1.0 N and 0.1 N).
4. Suspend a 2-inch high stainless steel mesh cylinder with a "Petri dish type," water-tight bottom in the 500-mL test solution.
5. Transfer the test organisms to these cages.
6. Repeat Steps 1-5 for solutions of pH6 and pH8, respectively.
7. Check the pHs of all the solutions and adjust if necessary, every hour during the first few hours, and as needed for the duration of the test.
8. Record pH drift and dissolved oxygen concentration.

4.8 Day 2 pH Adjustment of Fraction Solutions

On the day after the fractionations are performed, the pH of the fraction solutions, which are stored at 4°C, is checked and, if necessary, adjusted to the appropriate pH. After adjustment, the fraction solutions are ready for toxicity tests.

1. Check pH of all fraction solutions with pH meter.
2. If necessary, readjust the pH of the effluent fraction solutions to pH*i* with either HCl or NaOH.
3. If necessary, readjust the pH of the dilution water and blank solutions to pHo with either HCl or NaOH.
4. Record the normalities and volumes of HCl and NaOH added.

APPENDIX C

STANDARD OPERATING PROCEDURE

API-TIEPW3

SOP No.: API-TIEPW3
Date Initiated: May 14, 1992
Revision No.: 00
Revision Date:
Page 1 of 2

SAMPLING PROCEDURES FOR COLLECTING PRODUCED WATER OR REFINERY EFFLUENTS

1.0 Purpose

This Standard Operating Procedure (SOP) will be used for the collection of produced water and/or refinery effluent samples for toxicity testing in conjunction with TIE Studies.

2.0 Application

This procedure applies to produced water or refinery effluent sample collection, packing, and shipping to the testing laboratory. The following sampling instructions should be followed carefully.

3.0 References

None

4.0 Associated SOPs

None

5.0 Procedure

5.1 Sampling Kit

5.1.1 Use only the sampling kit supplied by the laboratory to collect the samples. The kit will contain four 4-liter bottles to collect 16 liters of aqueous effluent sample. There is also an ice chest type container in which to pack the bottles for return shipment.

5.2 Data Sheet/Chain of Custody

5.2.1 The data sheet, enclosed with sampling kit, must be completed. One copy will accompany the samples.

5.2.2 A chain of custody sheet, enclosed with the sampling kit, should be completed and the white and yellow sheets sent along with the samples. The pink should be retained for the facility files.

5.3 Sampling Steps

5.3.1 The sample is to be taken at a sampling point immediately downstream of the last conventional water treating vessel (skim tank, Vortoil, filters, or floatation cell, etc.) or other designated location.

5.3.2 Flush the line thoroughly for several minutes before sampling.

5.3.3 Fill each bottle completely. With a clean nipple or a piece of stainless steel tubing attached to the sampling point and extending to the bottom of the bottle, fill each bottle from the bottom in order to completely remove any airspace. The bottle should be filled slowly in order to minimize turbulence and the loss of volatile components of the water. An amount of water equivalent to one volume of the bottle should be flushed through the bottle before collecting the sample.

5.3.4 The sample is to be taken near the beginning of the week (Monday, no later than Tuesday) and shipped on the same day collection. The sample should be stored refrigerated (4°C) prior to shipment. The sample must arrive at the testing laboratory the day after collection. The sample is not to be taken within three (3) days of the start of production or after a batch chemical treatment.

5.4 Packing and Shipping

5.4.1 After the sample has been taken, the bottles are to be wrapped in bubble wrap and packed in ice in the shipping container provided. The sample is then shipped by overnight express service to the testing laboratory.

6.0 Proficiency Measure

None

7.0 Safety

Sampling procedures should be performed within the health and safety guidelines of the participating facility.

APPENDIX D

SUMMARY OF RESEARCH DATA

Appendix D
SUMMARY OF RESEARCH DATA

A large volume of data and information has been generated throughout the course of API's PW TIE research. This appendix summarizes the results in a series of tables. Relevant observations recorded by chemists performing the fractionations are provided in Table D-1. Chemical characteristics of the whole PW samples are provided in Tables D-2 through D-4. The results of the toxicity tests are summarized in Tables D-5 through D-9.

EXPERIMENTAL OBSERVATIONS

Observation of color, odor, organic phases (e.g., sheens), and precipitate formation at different pH values can provide clues as to the cause(s) of toxicity. The specific implications of these observations to the fraction toxicities are discussed in Section 4 of this report. In general, odor characterized as "rotten egg" indicates a sulfide-containing source (H_2S). Surface sheens indicate a petroleum residue. Colors are primarily associated with the inorganic mineral and/or clay content of the PW sample. Partial precipitation of inorganic compounds and/or complexes is indicated by cloudiness resulting from a pH change or other manipulation. The formation of solid precipitates indicates the presence of ionic species that form insoluble compounds at the respective pH values.

Table D-1. Experimental Observations During Produced Water Sample Fractionations (pH*i* denotes *initial* pH of the PW as received)

Produced Water Sample	Initial Observations	Fractionation Observations	Day 2 Fraction Observations
Phase 1 Low Salinity PWF-WY1	Strong "rotten egg" odor; light surface sheen	pH Adjustment: pH3 - cloudy Air Aeration: pH*i* - cloudy	Whole effluents cloudy, color change to green
PWF-TX1	Sweet "oily" odor; slight "rotten egg" odor; light surface sheen	pH Adjustment: pH 11 - cloudy, yellow to rust, rust ppt Aeration: pH11 - ppt settled leaving clear effluent ph11, pH*i* - color change BCE: Extract slightly green; oil odor	pH3 all fractions: yellowish color pH11 all fractions: light brown color
PWF-WY2	Strong "rotten egg" odor: light surface sheen	pH Adjustment: pH11 - cloudy	pH11 Aeration: both exhibit "oily" surface ring; pH11 Adjustment: "rotten egg" odor
Phase 1 High Salinity PWS-LA3	Sweet "oily" odor; cloudy, yellow tint	pH Adjustment: pH11 - clear to burnt orange Filtration: pH3 and pH11 - cloudy SPE: pH9 - rusty ppt in cartridge	All fractions: orange coloration
PWS-CA1	Sweet "oily" odor	pH Adjustment: pH11 - cloudy	All fractions: orange coloration
Phase 2 Low Salinity PWF-LA5	Sweet "oily" odor; murky yellow/brown	pH Adjustment: pH11 - green ppt pH3 - yellow/orange color change	All fractions: orange ppt Whole effluent: color change to rust
Phase 2 High Salinity PWS-LA6	No observations	pH Adjustment: pH11 - dark green to cloudy white ppt	All fractions: orange ppt
PWS-CA2	Dark brown	No observations	All fractions: clear with a brownish tint

Table D-1. Experimental Observations During Produced Water Sample Fractionations (Continued)

Produced Water Sample	Initial Observations	Fractionation Observations	Day 2 Fraction Observations
PWS-LA7	No observations	No observations	All fractions except pH11 filtered: light brown ppt
Phase 3 High Salinity PWS-LA11	Colorless; strong "rotten egg" odor	Suspended ppt in pH11 fractions; range in color: effluent-black; N_2Aeration - green; Air/Aeration - brown/green; black ppt on filter paper. ppt in pH3 fractions were all yellow	Non-filtered pH11 fractions had brown suspended ppt and no odor. pH3 fractions all had brown suspended ppt with "rotten egg" odor
PWS-LA13	Dark yellowish tint; no odor	Yellow/brown ppt in all fractions; pH11 blank was cloudy with white flocculent	Same as Day 1
PWS-LA9	Yellowish tint; no odor	Effluent turned slightly orange with flocculent after adding NaOH; non-filtered pH11 blank had white flocculent	All non-filtered fractions had a light brown suspended ppt
PWS-LA10	Orangish tint; faint smell of oil; light surface sheen	All non-filtered fractions were cloudy brown with ppt; non-filtered pH11 blanks had white flocculent	Same as Day 1, also pH3 filtered fraction was slightly brown and cloudy
PWS-LA12	Brownish tint; faint smell of oil; light surface sheen	All fractions were cloudy, light brown, except filtration; pH11 was clear; non-filtered pH11 blanks had white flocculent; NCE fraction contained oil droplets and had a surface sheen	Same as Day 1, except that blanks were all clear; BCE and NCE fractions had an oily odor

Table D-2. Chemical Properties of Phase 1 Produced Water Samples

Property	PWF-WY1 (Low Salinity)	PWF-TX1 (Low Salinity)	PWF-WY2 (Low Salinity)	PWS-LA3 (High Salinity)	PWS-CA1 (High Salinity)
Hardness (mg/L as CaCO3)	1,580	1,440	2,300	3,970	830
Alkalinity (mg/L)	400	456	1,834	980	2,800
pH	7.4	6.4	6.4	8.2	8.2
Salinity (ppt)	NA	NA	NA	42	21
Dissolved Oxygen (mg/L)	3.0	8.3	2.4	4.0	4.0
Oil and Grease (IR-mg/L)	NA	100	8.4	30	15
Oil and Grease (GR-mg/L)	19	66	8.7	23	17
Total Ammonia (mg/Las N)	5.0	41	0.5	59	38
Sodium (mg/L)	NA	NA	NA	20,000	7,400
Potassium (mg/L)	NA	NA	NA	190	46
Calcium (mg/L)	NA	NA	NA	1,100	240
Magnesium (mg/L)	NA	NA	NA	200	46
Chloride (mg/L)	NA	NA	NA	11,000	12,000
Sulfate (mg/L)	NA	NA	NA	<10	24
Bicarbonate (mg/L)	NA	NA	NA	910	2,500
Iron (mg/L)	NA	21	0.081	1.9	1.4
COD (mg/L)	NA	NA	NA	NA	NA
Conductivity (umhos/cm)	2,870	9,930	3,640	NA	28,100

NA - Not Analyzed

Table D-3. Chemical Properties of Phase 2 Produced Water Samples

Property	PWF-LA5 (Low Salinity)	PWF-LA6 (High Salinity)	PWS-CA2 (High Salinity)	PWS-LA7 (High Salinity)
Hardness (mg/L as CaCO3)	647	14,000	2,400	5,000
Alkalinity (mg/L)	167	141	468	1,500
pH	6.5	7.0	8.0	6.9
Salinity (ppt)	NA	>100	3	59
Dissolved Oxygen (mg/L)	3.3	<2.0	7.5	4.0
Oil and Grease (IR-mg/L)	18	12	24	8.2
Oil and Grease (GR-mg/L)	20	7.3	58	<5.3
Total Ammonia (mg/L as N)	24.8	162	10	52
Sodium (mg/L)	NA	89,000	1,400	22,000
Potassium (mg/L)	NA	370	98	140
Calcium (mg/L)	NA	3,200	200	1,100
Magnesium (mg/L)	NA	810	64	210
Chloride (mg/L)	NA	140,000	2,200	38,000
Sulfate (mg/L)	NA	230	10	3.7
Bicarbonate (mg/L)	NA	210	580	810
Iron (mg/L)	50	36	0.65	14
COD (mg/L)	NA	NA	NA	NA
Conductivity (umhos/cm)	2,770	205,000	7,330	71,600

NA - Not Analyzed

Table D-4. Chemical Properties of Phase 3 Produced Water Samples

Property	PWS-LA9	PWS-LA10	PWS-LA11	PWS-LA12	PWS-LA13
Hardness (mg/L as CaCO3)	14,500	16,400	660	15,700	1,200
Alkalinity (mg/L)	94	14	45	55	480
pH	6.7	6.5	5.3	6.7	7
Salinity (ppt)	100	112	5	171	6
Dissolved Oxygen (mg/L)	3.8	3.8	4.4	4.1	5.6
Oil and Grease (IR-mg/L)	11	8.7	29	39	20
Oil and Grease (GR-mg/L)	15	<5.8	26	26	14
Total Ammonia (mg/Las N)	86	110	34	99	12
Sodium (mg/L)	34,000	38,000	1,500	57,000	1,200
Potassium (mg/L)	290	300	19	330	490
Calcium (mg/L)	2,700	3,600	190	3,200	460
Magnesium (mg/L)	340	1,200	17	620	13
Chloride (mg/L)	62,000	39,000	2,500	110,000	4,400
Sulfate (mg/L)	<1	<1	18	6	10
Bicarbonate (mg/L)	450	77	330	370	510
Iron (mg/L)	6.3	33	77	19	28
COD (mg/L)	3,500	2,100	2,300	8,400	1,000

TOXICITY TESTS

The toxicity test results are presented in a manner that highlights apparent differences in the toxicity of a PW fraction relative to the whole PW. Although both initial (Day 1) and baseline (Day 2) LC50 and EC50 values were determined, comparisons are made primarily against the baseline toxicities to offset any potential artifacts of the additional storage time. In Tables D-5 through D-9, LC50 or EC50 values for the initial (Day 1) and baseline (Day 2) tests are reported along with the 95 percent confidence interval (CI). These EC50 and LC50 values are expressed as the percent concentration of PW (whole or fraction) in dilution water at which 50 percent of the test organisms die (LC50) or exhibit a sublethal effect (EC50). Recognizing that 95 percent CIs around some of these values may constitute a wide concentration range, possible differences (+) or distinct differences (++) in fraction toxicities take into account both the magnitude of the difference in the LC50/EC50 and the 95 percent confidence interval.

Phase 1 Experiments

For the Phase 1 low-salinity PW TIEs, several experimental factors limited calculating LC50 values for some fractions, because of insufficient sample volumes to perform the full range of dilutions (Table D-5). The Phase 1 high-salinity PW TIEs were performed with *Cyprinodon variegatus*, generating LC50s, and *Arbacia punctulata*, generating EC50s for gamete fertilization (Table D-6).

Phase 2 Experiments

In the Phase 2 TIE experiments, PWF-LA4 was originally designated a low-salinity PW, and tested with the freshwater species. The hardness, alkalinity, and conductivity results indicated that this sample contained high levels of total dissolved solids. These levels suggest that it would have been more appropriate to perform toxicity tests with the marine species. The SPE fractionation was not employed for the Phase 2 experiments. Because of the low toxicity of both designated low-salinity

whole PW samples with *Pimephales promelas*, toxicity tests on the fractions were conducted using *Ceriodaphnia dubia* only (Table D-7). For the Phase 2 high-salinity PW TIEs (Table D-8), the *Arbacia* toxicity test was replaced with the *Mysidopsis* test because of the high variability in EC50 values during the Phase 1 tests.

Phase 3 Experiments

For the Phase 3 experiments with high-salinity PW samples, replication was added to the experimental design. Also, LC50 values were determined for each fraction at 24 hr and 48 hr after initiation. The 48-hr LC50 values showed the same general trends as the 24-hr values (Table D-9).

Mysid Salinity Tolerance

The salinity tolerance of *Mysidopsis bahia* was assessed in a separate experiment. The results of tests, ranging from 1 ppt to 55 pt, indicated that salinity levels less than 5 ppt, and greater than 40 ppt began to cause mortality. Sample salinities between 5 and 40 ppt did not appear to be acutely toxic to mysids. Mortality in PW samples within this salinity range can be attributed to toxicants other than salinity. This conclusion is based on the assumption that salinity-stress mortality in samples containing PW was not due to imbalances in ionic composition, but rather to overall ionic strength effects.

Table D-5. Summary of Phase 1 Low-Salinity Fraction Toxicity (24-hr Static Acute Test LC50 Values with *Ceriodaphnia dubia*) Relative to Whole Produced Water (pH*i* denotes *initial* pH of the PW as received)

Produced Water Fraction	PWF-WY1	PWF-TX1	PWF-WY2
Initial (Day 1) LC50	5.8%	8.0%	22%
(95%CI)	(1-10%)	(5-50%)	(1.0-50%)
Baseline (Day 2) LC50	26%	11%	>80%
(95%CI)	(15-50%)	(10-25%)	
pH Adjustment			
pH3	+	0	0
pH11	0	0	0
Nitrogen Aeration			
pH*i*	+	0	0
pH3	+	0	0
pH11	0	+	0
Air Aeration			
pH*i*	+	0	0
pH3	+	+	0
pH3	0	0	0
Filtration			
pH*i*	+	++	0
pH3	+	+	0
pH11	0	+	0
Solid-Phase Extraction			
pH*i*	+	NP	0
pH3	+	NP	0
pH9	0	NP	0
Organic Cmpd. Isolation			
ACE at pH<2	0	+	0
BCE at pH>12	NP	+	0
NCE	NP	+	0
Graduated pH	NP	N	N

"0" - No difference
"+" - Possible difference
"++" - Distinct difference

NP - Test not performed
N - No pattern observed

Table D-6. Summary of Phase 1 High-Salinity Fraction Toxicity (*Arbacia punctulata* Gamete Fertilization Acute Test EC50 Values) Relative to Whole Produced Water (pH*i* denotes *initial* pH of the PW as received)

Produced Water Fraction	PWS-LA3	PWS-CA1
Initial (Day 1) EC50 (95% CI)	17% (10-50%)	NP
Baseline (Day 2) EC50 (95% CI)	100%	42% (40-60%)
<u>pH Adjustment</u>		
pH3	0	0
pH11	0	0
<u>Nitrogen Aeration</u>		
pH*i*	0	0
pH3	0	0
pH11	0	0
<u>Air Aeration</u>		
pH*i*	0	0
pH3	0	0
pH11	0	0
<u>Filtration</u>		
pH*i*	0	0
pH3	0	0
pH11	0	0
<u>Solid-Phase Extraction</u>		
pH*i*	0	0
pH3	0	0
pH9	0	0
<u>Organic Cmpd. Isolation</u>		
ACE at pH<2	0	0
BCE at pH>12	0	+
NCE	0	0
Graduated pH	N	NP

"0" - No difference
"+" - Possible difference
"++" - Distinct difference

NP - Test not performed
N - No pattern observed

Table D-7. Summary of Phase 2 Low-Salinity Fraction Toxicity (24-hr Static Acute Test LC50 Values with *Ceriodaphnia dubia*) Relative to Whole Produced Water (pH*i* denotes *initial* pH of the PW as received)

Produced Water Fraction	PWF-LA5
Initial (Day 1) LC50	8.0%
(95% CI)	(5-50%)
Baseline (Day 2) LC50	>50%
(95% CI)	
pH Adjustment	
pH3	0
pH11	0
Nitrogen Aeration	
pH*i*	0
pH3	0
pH11	0
Air Aeration	
pH*i*	0
pH3	0
pH11	0
Filtration	
pH*i*	0
pH3	0
pH11	+
Organic Cmpd. Isolation	
ACE at pH<2	0
BCE at pH>12	0
NCE	0
Graduated pH	N

"0" - No difference
"+" - Possible difference
"++" - Distinct difference

NP - Test not performed
N - No pattern observed

Table D-8. Summary of Phase 2 High-Salinity Fraction Toxicity (24-hr Static Acute Test LC50 Values with *Mysidopsis bahia*) Relative to Whole Produced Water (pH*i* denotes *initial* pH of the PW as received)

Produced Water Fraction	PWS-LA6	PWS-CA2	PWS-LA7
Initial (Day 1) LC50	6%	7%	5%
(95% CI)	(5-10%)	(5-10%)	(2.0-8.0%)
Baseline (Day 2) LC50	6%	22%	34%
(95% CI)	(1-10%)	(10-50%)	(6.8->50%)
pH Adjustment			
pH3	0	0	0
pH11	0	0	0
Nitrogen Aeration			
pH*i*	0	0	0
pH3	0	0	0
pH11	0	0	0
Air Aeration			
pH*i*	0	0	0
pH3	0	0	0
pH11	0	0	0
Filtration			
pH*i*	0	0	0
pH3	0	0	0
pH11	0	0	0
Organic Cmpd. Isolation			
ACE at pH<2	0	++	0
BCE at pH>12	0	0	0
NCE	+	0	0
Graduated pH	Y	N	N

"0" - No difference
"+" - Possible difference
"++" - Distinct difference

NP - Test not performed
N - No pattern observed
Y - Pattern observed indicating presence of ammonia

Table D-9. Summary of Phase 3 High-Salinity Fraction Toxicity (24-hr Static Acute Test LC50 Values with *Mysidopsis bahia*) Relative to Whole Produced Water. (pH*i* denotes *initial* pH of the PW as received)

Produced Water Fraction	PWS-LA9	PWS-LA10	PWS-LA11	PWS-LA12	PWS-LA13
Initial (Day 1) LC50	15%	8%	16%	6%	1.9%
(95% CI)	(5-50%)	(5-50%)	(1-50%)	(1-10%)	(1-5%)
Baseline (Day 2)	11%	<3%	15%	2%	1.7%
(95% CI)	(6-12%)		(5-25%)	(<1-5%)	(<0.5-8%)
pH Adjustment					
pH3	0	0	0	0	0
pH11	0	0	0	0	0
Nitrogen Aeration					
pH*i*	0	0	0	0	0
pH3	0	0	0	0	0
pH11	0	0	0	0	0
Air Aeration					
pH*i*	0	0	0	0	0
pH3	0	0	0	0	0
pH11	0	0	0	0	0
Filtration					
pH*i*	0	0	0	+	0
pH3	0	0	0	0	0
pH11	0	0	0	0	+
Organic Cmpd. Isolation					
ACE at pH<2	+	0	0	0	0
BCE at pH>12	0	0	++	0	0
NCE	0	+	0	0	0
Graduated pH	N	NP	N	NP	N

"0" - No difference
"+" - Possible difference
"++" - Distinct difference

NP - Test not performed
N - No pattern observed

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