

Summary of Produced Water Toxicity Identification Evaluation Research

Health and Environmental Sciences Department
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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; <i>Mysidopsis bahia</i> substituted for <i>Arbacia punctulata</i>
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 <i>Mysidopsis bahia</i> tested

^aBecause one "Low-TDS" PW sample had high salinity (90 ppt), results evaluated from single sample.

^bBecause 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
<u>Phase 1 - Low TDS</u>				
PWF-WY1	Wyoming	<3	oil	EB,CI,REB,SI,GT
PWF-TX1	Coastal Texas	<3	gas	C
PWF-WY2	Wyoming	<3	oil	CI CB
<u>Phase 1 - High TDS</u>				
PWS-LA3	Offshore/Louisiana	43	oil	EB,REB,SI,GTC
PWS-CA1	Coastal California	41	oil	EB,CI,REB,SI,C, F,GTC
<u>Phase 2 - Low TDS</u>				
PWF-LA5	Coastal Louisiana	<3	oil	CI
PWF-LA4 ^a	Offshore Louisiana	90	oil	None
<u>Phase 2 - High TDS</u>				
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
<u>Phase 3 - High TDS</u>				
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

^aResults not evaluated because PW salinity was too high for tests with freshwater species.

^bResults 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*

Condition	Species	
	<i>Pimephales promelas</i> (Fathead Minnow)	<i>Ceriodaphnia dubia</i> (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:	<i>Cyprinodon variegatus</i>	<i>Mysidopsis bahia</i>
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>i</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>i</i> , 3, and 11	Removal of volatile and oxidizable compounds under effluent pH, acidic, and basic conditions.
Filtration at pHs <i>i</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>i</i>	Isolation of neutral organic compounds.
Solid-phase Extraction (SPE) at pHs <i>i</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.

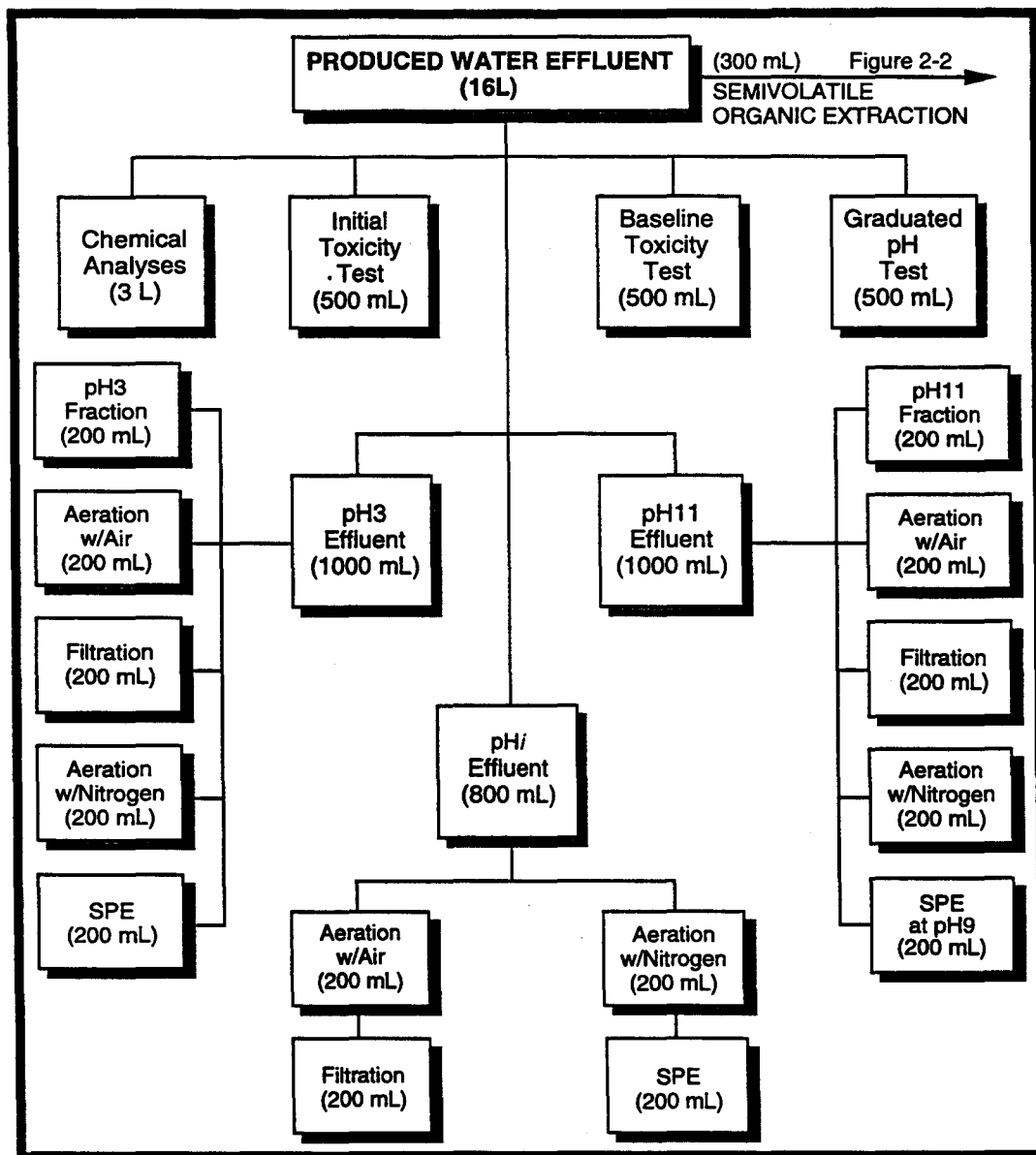


Figure 2-1. Flow diagram of the physical adjustments performed on the produced water samples. (pHi denotes the *initial* pH of the PW as received)

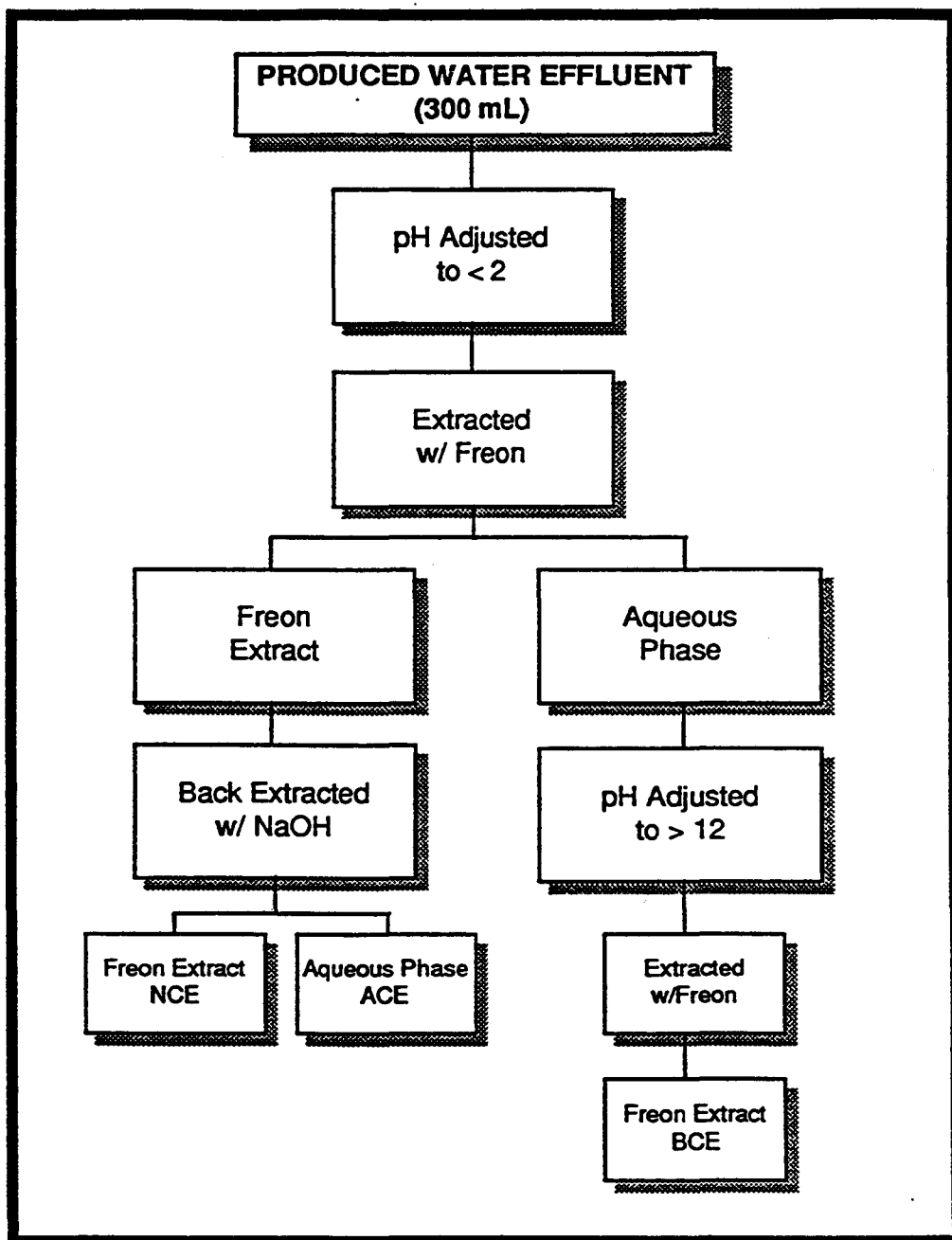


Figure 2-2. The semivolatile organic compound fractionation procedure (ACE/BCE/NCE) performed on the produced water samples.

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).