Work/QA Project Plan

TOXICITY STUDY

OF THE SANTA CLARA RIVER, SAN GABRIEL RIVER AND CALLEGUAS CREEK

Prepared for

Los Angeles Region Water Quality Control Board
101 Centre Plaza Drive
Monterey Park, California
(213) 266-7557

Principal Investigator: David E. Hinton Date 8-20-92
Dr. David Hinton , Univ. of California, Davis

Project Director: David Hanes Date 8-20-92
Dr. David Hanes, Univ. of California, Davis

Contract Manager: Date 8-11-92
Deborah J. Smith, L.A. Regional Board

Regional Board
Quality Assurance Officer: Hiam Tan Date 8-11-92
Hiam Tan, L.A. Regional Board

1. PROJECT NAME:	Site Specific Study of Toxicity in the Santa Clara River, San Gabriel River and Calleguas Creek
2. PROJECT REQUESTED BY:	California Regional Water Quality Control Board, Los Angeles Region
3. DATE OF REQUEST:	May 1, 1991
4. DATE OF PROJECT INITIATION:	March 5, 1992
5. CONTRACT MANAGER:	Deborah J. Smith, Environmental Specialist with California Regional Water Quality Control Board, Los Angeles Region, 101 Centre Plaza Drive, Monterey Park, CA 91754. (213) 266-7557, Fax (213) 266-7600.
6. REGIONAL BOARD QUALITY ASSURANCE OFFICER	Hiam Tan, Quality Assurance Officer with the California Regional Water Quality Control Board, Los Angeles Region, 101 Centre Plaza Drive, Monterey Park, CA 91754. (213) 266-7567, Fax (213) 266-7600

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7. PROJECT DESCRIPTION:

The Santa Clara and San Gabriel Rivers drain large areas in the Los Angeles Region and are representative of effluent-dominated rivers found in Southern California. They serve a multitude of beneficial uses that include propagation and protection of aquatic life. Most dry weather flow in these rivers is due to discharges of reclaimed water from municipal waste water treatment facilities. Industrial point sources and urban runoff also contribute to the flow of these rivers. Minor flow is also contributed by the northern tributaries of the San Gabriel River and areas of rising ground water along the Santa Clara River. Both waterways have some alterations (dams, diversions, concrete linings) and some natural stream channels. The mainstream of the Santa Clara has no lined portions.

Seasonally their flow is dominated by significant amounts of urban and industrial effluent. The 1990 Water Quality Assessment classified most of the Santa Clara and San Gabriel Rivers as "intermediate". The intermediate classifications were based primarily on impairments or threats to beneficial uses of these waters. The tidal prism of the San Gabriel River was classified as impaired due to bioaccumulation of metals in fish. The 1992 Water Quality Assessment revised the classification of the lower San Gabriel River to impaired due to high ammonia and acute toxicity found in an EPA study. Calleguas Creek is also listed as impaired due to high levels of pesticides in sediment and biota.

This project will test for ambient toxicity in waters from the Santa Clara River, San Gabriel River, and Calleguas Creek water sheds. Point and non-point source inputs will be evaluated, in terms of toxicity, to determine contributions, and to assist in the development of appropriate management practices or other controls. Tasks include literature and data review, site selection, chronic bioassays, toxicity identification evaluation (TIE), analyses of water samples, source identification, and recommendations for implementation of control strategies.

A. Objectives

The specific objectives of this study are to:

1. Determine whether aquatic life beneficial uses are impaired in these two rivers.

2. Test the usefulness of chronic toxicity studies, coupled with traditional water quality data, for assessment of stream water quality.

3. Identify specific pollutants and sources responsible for ambient toxicity in these waters.

4. Provide recommendations for specific practices or regulatory controls which can be used for toxicity reduction.

5. Provide background data for the development of site-specific objectives for effluent-dominated inland surface waters.

B. Data Usage

The data produced by this project will be used to determine the extent and sources of impairment to aquatic life beneficial uses in the Santa Clara, San Gabriel, and Calleguas water sheds. This study will be used as a basis for recommendations of changes in specific best management practices and regulatory controls to reduce the toxicity of these rivers. The data will also add to background information for the development of site-specific objectives for effluent-dominated inland surface waters.

C. Monitoring Network Design and Rationale

Sampling Site Selection. Sites selected by the contract manager will reflect known or suspected point and non-point sources in areas where beneficial uses may be threatened or impaired. The Contract Manager and Project Director will coordinate the timing and number of samples to be collected at each site, to inhance, as much as cost will allow, identification of the causes and sources of stream toxicity.

8. TEST PROCEDURES

TEST ORGANISMS

Pimephales promelas

Larvae which are newly hatched will be obtained from Aqua Tox, Hot Springs, Arkansas and the exposures will be initiated before the larvae are more than 24 h old. The animals will be disease free, identified to species, and healthy thus

producing low mortality in controls. They will be exposed to the sample waters at 25 ± 1 °C with a 16 h light, 8 h dark cycle. The animals and their feeding and treatment will meet the guidelines of health, identification, and culture conditions as specified in EPA 600 4-89/001 method 1000.0.

Ceriodaphnia dubia

Cultures originally obtained from Aquatic Science, Davis, California will be cultured in this laboratory using the procedures in EPA 600 4-89/001 method 10002.0 with the exceptions of feeding and age. The animals will be fed 0.2 ml of Silver Cup Trout Chow supernate from 2 h of mixing and 43.3 X 10^6 *Selenastrum* cells (in as little water as possible) per 200 ml test concentration daily. The test organisms will all be released within a 16 h period and used before they are 16 h old. We have found that these younger animals provide a more sensitive test than animals 24 h old and our feeding regimen provides healthy animals. Cultures used for testing are all derived asexually from one animal and that animal is preserved for possible identification. These orgainisms will be raised and exposed at 25 ± 1 °C with a 16 h light, 8 h dark cycle. The *Ceriodaphnia* cultures will be raised in the same room and under the same conditions as those exposed to test water samples, but will be in a separate area.

Selenastrum capricornutm

The alga will be cultured in the UCDATL for the tests. It was originally obtained from University of Texas, Austin Texas and will be maintained during the tests as specified in EPA/600/4-89/001 method 10003.0. The culture and the tests will be housed in separate environmental chambers at 25 ± 1 °C under continuous illumination of 400 ± 40 ft-c. The exposure chamber will rotate the cultures at 100 rpm. Cell densities will be determined with a Coulter Counter. Growth medium will be prepared as specified in EPA/600/4-89/001.

TEST MEASUREMENTS

All water chemistry tests specified in EPA/600/4-89/001will be done. The equipment and chemicals used are the following:

Equipment	Model	Minimum Detectable
YSI Electrical Conductivity	33	10 µMho
VWR pH Meter	34100-642	0.01 units
YSI Dissolved Oxygen	57	0.1 mg/L O_2
Hach Ammonia Kit	NI-8	0.1 mg/L NH_3
Hach Alkalinity Kit	AL-AP	5 mg/L $CaCO_3$
Hach Hardness Kit	5-EP	20 mg/L $CaCO_3$
Hach Chlorine Colorimeter	DR 100	0.02 mg/L
Thermometers	Calibrated with Certified Thermometer	0.5 °C
Rainin HPLC	HP	Controllable Methanol/Water gradient
Coulter Counter	ZM	NA

A summary of organism test conditions follow:

SUMMARY OF RECOMMENDED TOXICITY TEST CONDITIONS FOR THE FATHEAD MINNOW (Pimephales promelas) LARVAL SURVIVAL AND GROWTH TEST (Method 1000.0, EPA 600/4-89/001)

1.	Test type:	Static renewal
2.	Temperature:	25 ±1 °C Continuously recorded
3.	Light quality:	Ambient laboratory illumination
4.	Light intensity:	10-20 µE/m²/s (50-100 ft-c) (ambient laboratory levels)
5.	Photoperiod:	16 h light, 8 hr dark
6.	Test chamber size:	600 ml Glass Beaker
7.	Test solution volume:	250 ml/replicate
8.	Renewal of test concentrations:	Daily, 80-85% renewal using original sample
9.	Age of test organisms:	Newly hatched larvae, less than 24 h old from at least three separate spawnings.
10.	No. larvae per test chamber	15
11.	No. replicate chambers/sample:	4
12.	No. larvae /sample	60

13.	Feeding:	Feed 0.1 ml (700-1000) newly hatched (less than 24 h old) rinsed brine shrimp nauplii three times daily at 4 h intervals or, as a minimum, 0.15 ml twice daily, 6 h between feedings (at the beginning of the work day prior to renewal, and at the end of the work day following renewal). Sufficient larvae are added to provide an excess. Larvae are not fed during the final 12 h.
14.	Cleaning:	Siphon daily with bulbed pipette immediately before test solution renewal
15.	Aeration:	No aeration unless DO concentration falls below 40% saturation (3.3 mg/L). Then the rate will be less than 100 bubbles/min.
16.	Dilution water:	No dilution water will normally be used. Control water, water for QC tests, and, if necessary, water for dilution of highly toxic samples will be moderately hard synthetic water as defined in EPA/600/4-89/001 using reagent grade chemicals in Sierra Spring water. Each batch of SSEPAMH water is routinely tested for hardness, alkalinity, pH, and Electical Conductivity.
17.	Test concentrations:	Samples will be used full strength (undiluted) or one dilution (using Sierra Spring EPAMH) agreed upon by the project director and contract manager

18.	Test duration:	7 days unless an acute endpoint is discovered and a TIE is initiated
19.	Endpoints:	Survival and growth (dry weight).
20.	Test acceptability:	80% or greater survival in controls; Average dry weight of surviving controls equal to or greater than 0.25 mg
21.	Sampling requirement:	Grab samples will be delivered iced, refrigerated at 4 °C upon arrival and the experiment initiated within 36 h as a maximum. Experiments will be initiated upon sample arrival if possible. All testing will be accomplished with this one grab sample.
22.	Sample volume required:	7 L/site
23.	Water chemistry	Initial water samples will be tested for DO, temperature, pH, conductivity, alkalinity, chlorine, and calcium hardness. Selected samples will be tested for ammonia. Each day fresh water samples and water samples after 24 h exposure will be tested for temperature, DO, and pH.

SUMMARY OF RECOMMENDED TOXICITY TEST CONDITIONS FOR THE CERIODAPHNIA SURVIVAL AND REPRODUCTION TEST (Method 1002.0, EPA 600 4-89/001)

1.	Test type:	Static renewal
2.	Temperature:	25 ± 1 °C Continuously recorded
3.	Light quality:	Ambient laboratory illumination
4.	Light intensity:	10-20 μE/m²/s (50-100 ft-c) (ambient laboratory levels)
5.	Photoperiod:	16 h light, 8 hr dark
6.	Test chamber size:	20 ml glass scintillation vial
7.	Test solution volume:	15 ml/replicate
8.	Renewal of test concentrations:	Daily
9.	Age of test organisms:	Less than 16 h; and all released within a 16-h period.[1]
10.	No. neonates per test chamber	1
11.	No. replicate chambers/sample:	10
12.	No. neonates /sample	10

[1]We have found that animals less than 16 h old are more sensitive and give good reproducible results rather than 24 h old animals. The larger range in age does not produce more variability in test results. Young are released overnight and used in the test the next day.

13.	Feeding:	Feed 0.2 ml Trout Chow supernate and 43.3 X 10^6 Selenastrum cells (in as little water as possible) per 200 ml test concentration daily.[2]
14.	Aeration:	None
15.	Dilution water:	No dilution water will be used unless a single dilution is agreed upon by the contract manager and project director. Control water, water for QC tests, and, if necessary, water for dilution of highly toxic samples will be moderately hard synthetic water as defined in EPA/600/4-89/001 using reagent grade chemicals in Sierra Spring water. Each batch of SSEPAMH is routinely tested for hardness, alkalinity, E.C. & pH.
16.	Test concentrations:	Samples will be used full strength (undiluted) unless a single dilution is agreed upon by the project director and contract manager.
17.	Test duration:	Until 60% of control females have three broods (may be more than 7 days or fewer if 100% mortality occurs).
18.	Endpoints:	Survival and reproduction

[2]This feeding regimen is a modification of EPA suggestions. It has produced excellent results in this laboratory.

19.	Test acceptability:	80% or greater survival and an average of 15 or more young per surviving female in the control solutions. At least 60% of surviving control females should have produced their third brood.
20.	Sampling requirement:	Grab samples will be delivered iced, refrigerated at 4 °C upon arrival and the experiment initiated within 36 h as a maximum. Experiments will be initiated upon sample arrival if possible.
21.	Sample volume required:	1400 ml/site

SUMMARY OF RECOMMENDED TOXICITY TEST CONDITIONS FOR THE ALGAL (Selenastrum capricornutum) GROWTH TEST (Method 1003.0, EPA/600/4-89/001)

1.	Test type:	Static
2.	Temperature:	25 ± 1 °C
3.	Light quality:	"Cool White" fluorescent lighting
4.	Light intensity:	86 ± 8.6 μE/m²/s (400 ± 40 ft-c) 3875 > 4306 < 4736 lux
5.	Photoperiod:	Continuous illumination
6.	Test chamber size:	250 ml Erlenmeyer flasks
7.	Test solution volume:	100 ml/replicate
8.	Renewal of test concentrations:	None
9.	Age of test organisms:	4 to 7 days
10.	Initial cell density in test chambers:	10,000 cells/ml
11.	No. replicate chambers/sample:	4
12.	Shaking rate:	100 rpm continuous or twice daily by hand

13.	Dilution water:	No dilution water will normally be used. Control water, water for QC tests, and, if necessary, water for dilution of highly toxic samples will be chemically defined media as specified in EPA/600/4-89/001 using reagent grade chemicals in glass distilled water.
14.	Test concentrations:	Samples will be used full strength (undiluted) unless a single dilution is agreed upon by the project director and contract manager.
15.	Test duration:	96 h (4 days)
16.	Endpoint:	Growth (cell counts via Coulter Counter)
17.	Test acceptability:	More than 2 X 10^5 cells/ml in the controls. Variance of controls less than 20%.
18.	Sample volume required:	400 ml/ site

REFERENCE TOXICANT CONTROLS

Sodium chloride will be used as a reference toxicant to be run simultaneously with the surface water samples. Ceriodaphnia will be exposed to a salt concentration that produces a conductance of 10,000 µmho and standard 0.5 dilutions down to 625 µmho. The Pimephales will be exposed to 10 g NaCl/l and standard 0.5 dilutions to 0.625 g NaCl/l. The dilution water for these two species will be SSEPAMH. Selenastrum will be exposed to 10 g NaCl/l and standard 0.5 dilutions to 0.625 g NaCl/l with EPA Selenastrum chemically defined growth medium (EPA 600 4-89/001) as the diluent. Statistical analyses of the growth and reproduction of these tests will be performed as specified in EPA 600 4-89/001.

HISTOPATHOLOGY

A maximum of 150 samples of fish collected by California Fish and Game from sites selected by the project manager will be preserved and processed for microscopic examination. Small fish will be cut bilaterally to reveal most of the internal organs. Larger fish will be dissected and samples of major internal organs will be taken such as gill, heart, spleen, liver, kidney, and gut. They will be examinied by experienced veterinary fish pathologists for disease, abnormal development, lesions, and other abnormal structures. Fish will be fixed and preserved in 10% formalin, embedded in parafin, sectioned at a thickness of less than 10 microns, and stained with hematoxylin and eosin and some with special stains to determine the nature of lesions found. A pathologist's report will be included as part of the data of this project.

TOXIC IDENTIFICATION EVALUATIONS

Phase I toxic identification evaluations (TIE's) and chemical analysis of the toxic fraction/s will be determined for up to four sites per month when significant mortality is found (mortality significantly greater than controls). TIE procedures are a series of sample manipulations designed to remove or reduce the toxicity of a variety of chemicals. After each manipulation, the sample is again tested for toxicity to determine if toxicity has been removed by the procedure. If toxicity has been reduced, then something is known about the characteritics of the chemical/s causing the toxicity. The manipulations include pH adjusted samples with filtration, solid phase extraction, and aeration. Tests without pH

adjustment include an initial test, baseline test, filtration, solid phase extraction, aeration, EDTA chelation, and oxidant reduction. Tests are also done with gradual pH changes. Each of the 21 or more toxicity tests consists typically of 4 sample dilutions, each with 5 organisms in 10 ml of sample. The initial and baseline tests will be run in duplicate. Tests will be observed for 48 h if necessary. *Ceriodaphnia* will be fed at the beginning of the test only. Methods used will be in accordance with the EPA procedures found in EPA 600 3-88-034 of September 1988.

TIE PRODUCTS

Test	If toxicity Increases	If toxicity decreases
Initial Effluent	NA	NA
Baseline Effluent	Toxicant degradation	Toxicant degradation
pH Adjustment	Increased ionic strength Irreversible reactions	Dilution effect Irreversible reactions pH driven degradation
pH Adj/Filtration	Toxic filter	Precipitated toxin, try to recover from filter
pH Adj/Aeration	Oxidation to toxin	Oxidation, Sparging or surface film - try N_2
pH Adj/C18 Extr	Manipulation problems	Column sequestered toxicant test methanol extract
Oxidant Reduction	Reduced form more toxic	Toxin an oxidizer -test for chlorine
EDTA Chelation	EDTA toxicity	Toxicant a heavy metal
Graduated pH	NA	Toxicity pH dependent If pH6<pH7<pH8 suspect ammonia

TIE's allow one to characterize a toxicant. It is then easier to determine

the type of specific chemical analysis which should be used. In the event that the SPE C18 column removes toxicity, the toxicant will be extracted with a methanol/water gradient and separated into 30 fractions each of which will be tested for toxicity. The toxic fraction can then be analyzed for the specific chemical.

If during the three species test, the only organism affected is Selenastrum, one may want to have the untreated sample analyzed. If the only species affected is Ceriodaphnia or Pimephales, then the affected species may be used for a TIE. If both Pimephales and Ceriodaphnia are affected by a sample, one may choose to run TIE's on both species. Choices will be made by the contract manager and project director in consultation. Choices must be based on usefulness of the test results, capabilities of the laboratory (no more than 4 TIE's/month), and budget for chemical analysis . Controls for the TIE tests will be performed as specified in EPA /600/3-88/034.

CHEMICAL ANALYSIS

When the toxic chemical/s has been isolated and characterized by the TIE process, it is then possible to better determine the proper chemical analytical procedure to identify the toxic chemical/s. The contract manager and project director, in consultation, will determine the number and types of chemical analyses to be done.

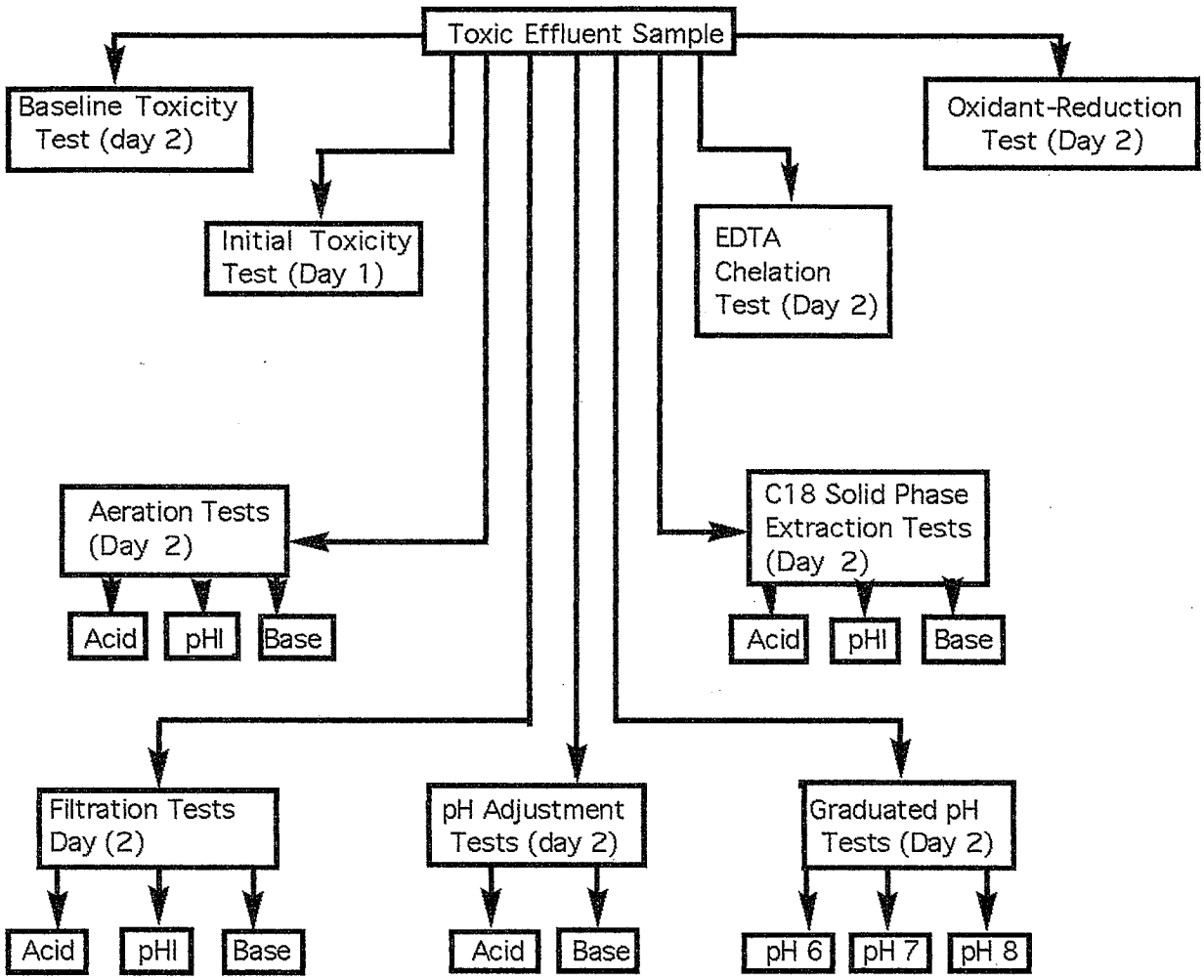
Toxic TIE fractions will be sent to Eureka Laboratories, Inc., 6790 Florin Perkins Road, Sacramento, CA 95828, Tel (916) 381-7953, Fax (916) 381-4013. This laboratory is certified (certification E765) for hazardous waste testing. All testing will follow EPA Methods and the reports from this laboratory will contain the EPA method number used, compounds searched for, concentrations detected, concentration detection limits, instrument identification, date and volume of sample, and identity of the chemist. The laboratory director is Dr. Shao-Pin Yo, Ph.D.

The following documents are available for review at Eureka Laboratories:

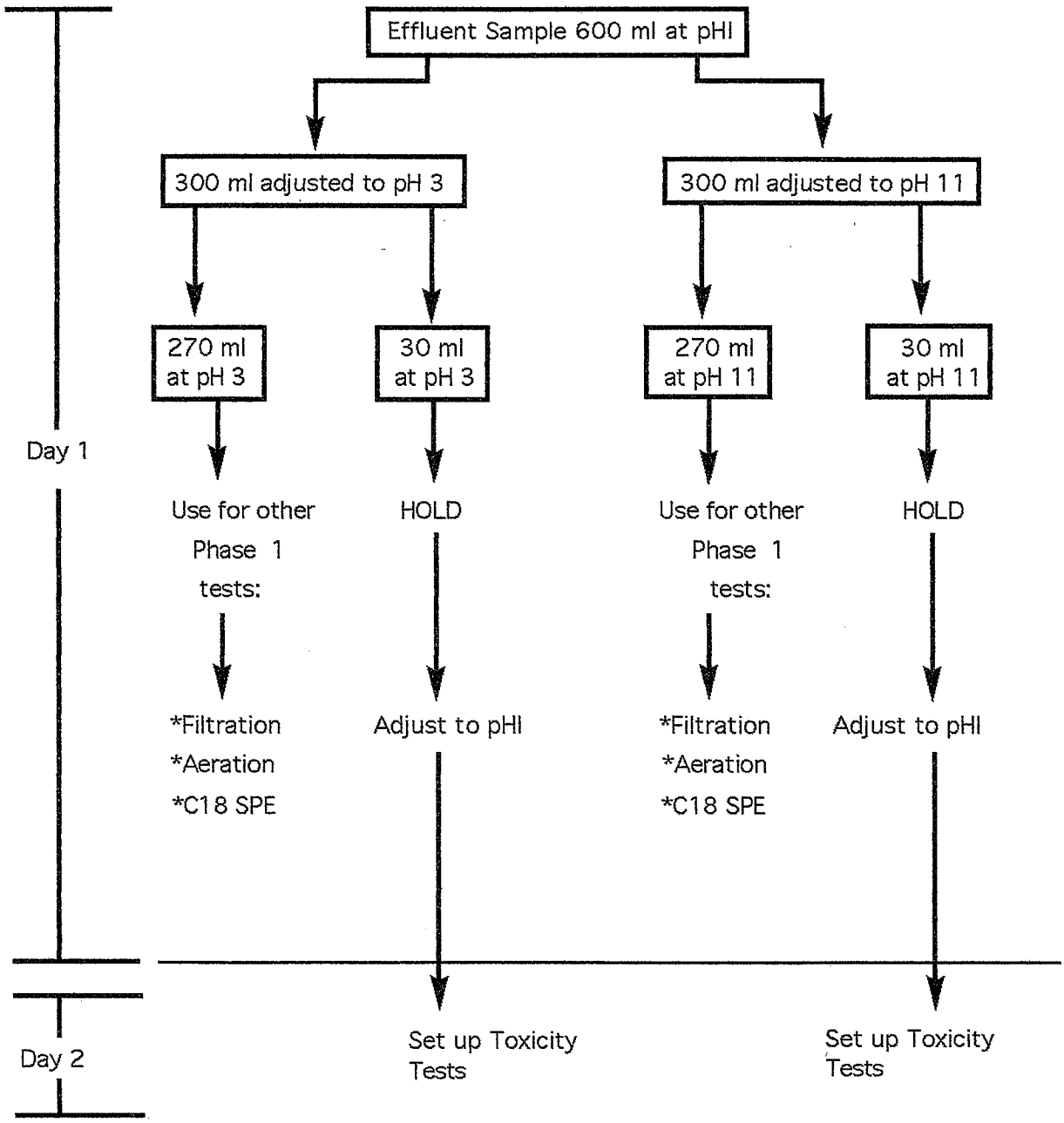
Quality Assurance and Quality Control Manual
Volume 1: Environmental Laboratory testing of Soil and Water Samples
March 1991

Statement of Qualifications
Analytical Laboratory Services
May 1992

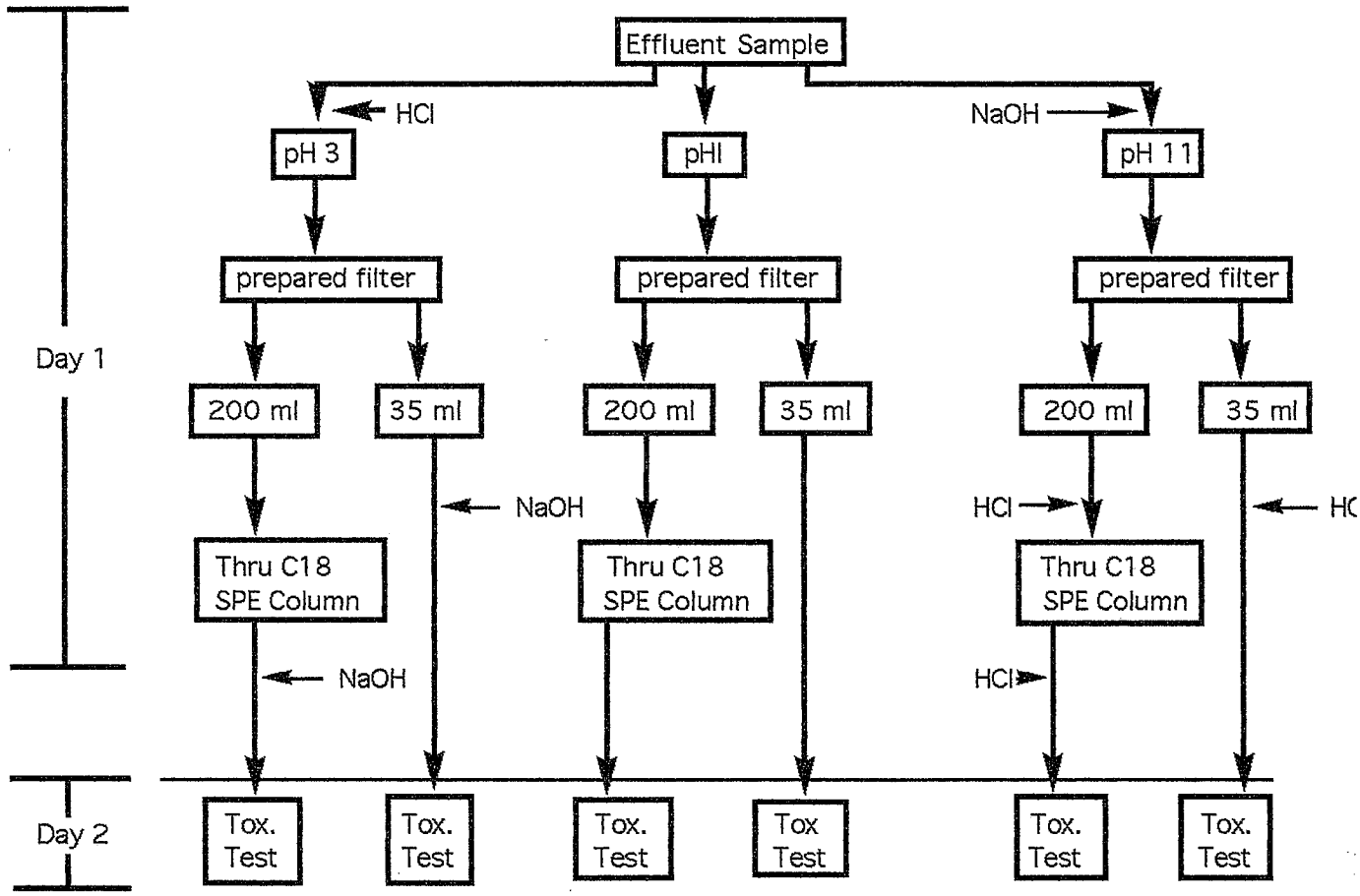
A summary chart of the TIE procedure follows:



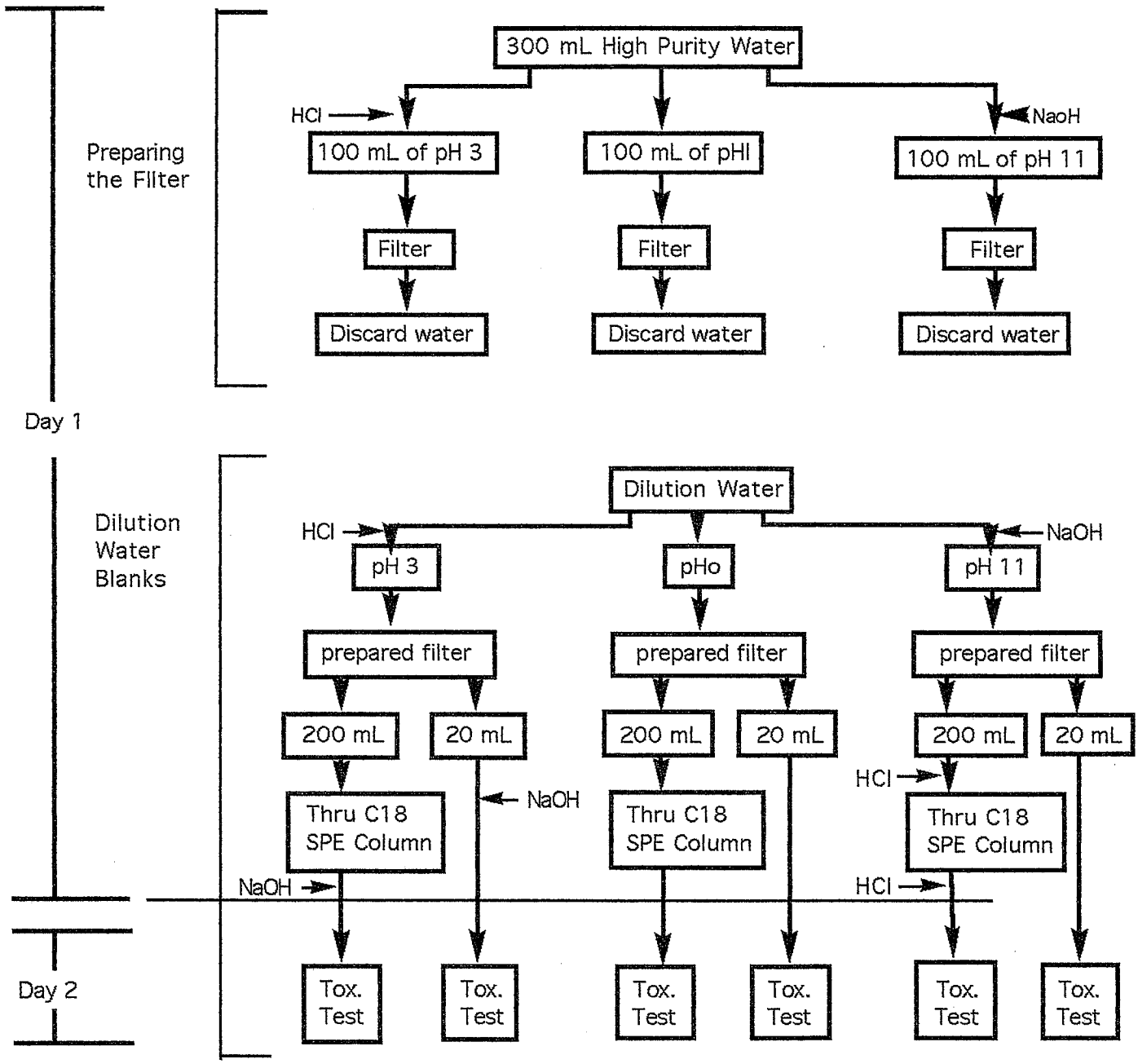
Overview of Phase I effluent characterization tests.



Flow chart for pH adjustment tests.



Overview of filter preparation and dilution water blanks for filtration test and C18 solid phase extraction of test samples.



Overview of filter preparation and dilution water blanks for filtration test and C18 solid phase extraction column test samples.

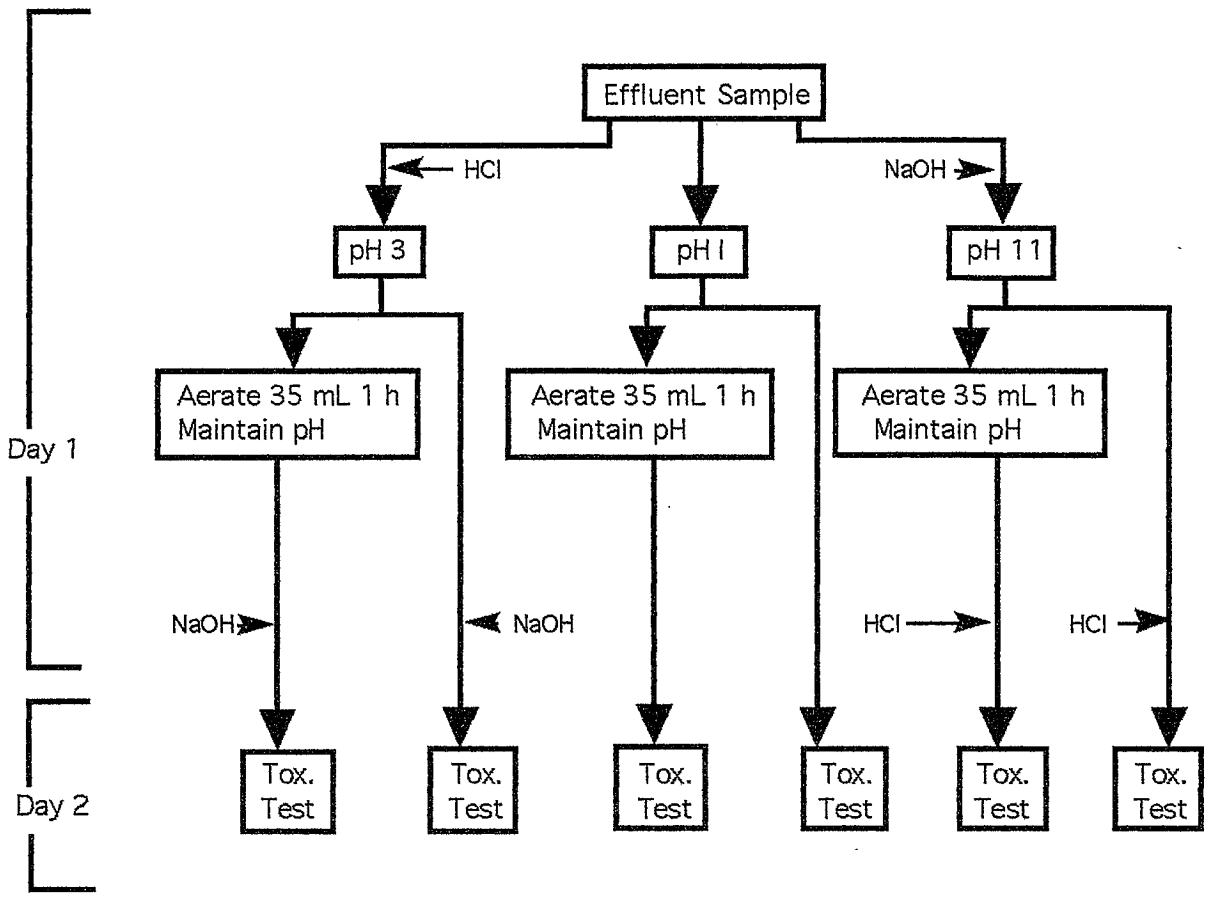
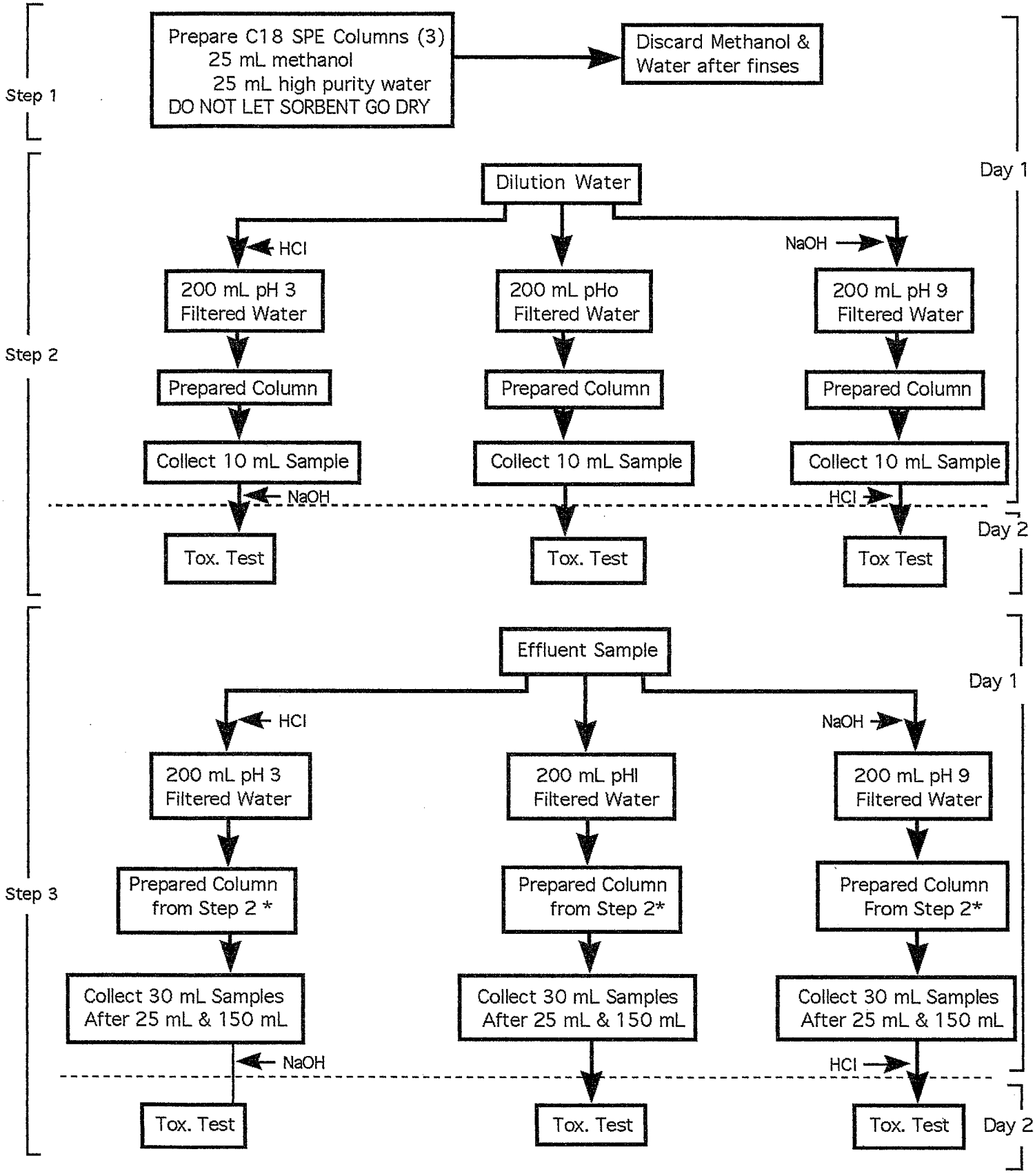


Diagram for preparing aeration test samples,



*Use same column used with the dilution water

Stepwise diagram for C18 solid phase extraction column test samples.

9. Schedule of Tasks and Products[3]

	5 Mar 92	Date of Award
	1 May 92	Quality Assurance Plan Due
	7 May 92	Field Reconnaissance
	2 Jun 92	Collect Fish from Calleguas Cr., Conejos Cr. & Revlon SLough
Wed	3 Jun 92	Collect San Gabriel Area Samples
Thu	4 Jun 92	Test Toxicity of San Gabriel Area Samples
	27 Jun 92	Collect fish from Coyote Cr. and San Gabriel River
	1 Jul 92	Quarterly Report Due
Tue	21 Jul 92	Collect Santa Clara Area Samples
Wed	22 Jul 92	Test Toxicity of Santa Clara Area Samples
Wed	22 Jul 92	Collect Calleguas Creek Samples
Thu	23 Jul 92	Test Toxicity of Calleguas Creek Samples
Wed	9 Sep 92	Collect San Gabriel Area Samples
Thu	10 Sep 92	Test Toxicity of San Gabriel Area Samples
	1 Oct 92	Quarterly Report Due

[3]Dates may be changed upon agreement between the project manager and the project officer. Test dates will require two weeks warning. Coolers, sample bottles/"cubitainers", packing, funnels, filter cloth, and "blue ice" will be sent to the Regional Board 1 week before the sampling date.

Tue	20 Oct 92	Collect Santa Clara Area Samples
Wed	21 Oct 92	Test Toxicity of Santa Clara Area Samples
Wed	21 Oct 92	Collect Calleguas Creek Samples
Thu	22 Oct 92	Test Toxicity of Calleguas Creek Samples
Wed	2 Dec 92	Collect San Gabriel Area Samples
Thu	3 Dec 92	Test Toxicity of San Gabriel Area Samples
	31 Dec 92	Interim Financial Status Report (FSR) Due Standard Form 259 (Rev. 4/88)
	1 Jan 93	Quarterly Report Due Histopathology Due[4]
Tue	19 Jan 92[5]	Collect Santa Clara Area Samples
Wed	20 Jan 92	Test Toxicity of Santa Clara Area Samples
Wed	20 Jan 93	Collect Calleguas Creek Samples
Thu	21 Jan 93	Test Toxicity of Calleguas Creek Samples
Wed	3 Mar 93	Collect San Gabriel Area Samples
Thu	4 Mar 93	Test Toxicity of San Gabriel Area Samples
	1 Apr 93	Quarterly Report Due
Tue	1 Apr 93	Collect Santa Clara Area Samples
Wed	2 Apr 93	Test Toxicity of Santa Clara Area Samples

[4]Assumes Regional Board submittal of fish to the Contractor (summer 1992)

[5]May be compressed to catch one wet weather event or samples may be from more than one river system.

Wed	2 Apr 93	Collect Calleguas Creek Samples
Thu	3 Apr 93	Test Toxicity of Calleguas Creek Samples
	1 May 93	Geographic Source Identification Due
	15 Jun 93	Draft Report Due TIE Completion Due Recommendations Due
	1 Jul 93	Chemical Characterizations Due
	31 Aug 93	Final Financial Status Report Due Final Report Due Public Participation Completed

The San Gabriel and Santa Clara Rivers, their tributaries and Calleguas Creek and its tributaries will have the following sampling sites:

SANTA CLARA RIVER AND TRIBUTARIES

SC-1	Santa Clara River at Saticoy Diversion
SC-2	Santa Clara River at Newhall Ranch Road
SC-3	Santa Paula Creek at Highway 150
SC-4	Sespe Creek at Old Telegraph Road
SC-5	Piru Creek at Diversion below Center Street
SC-6	Santa Clara River at Old Hwy 99 (near Valencia)
SC-7	San Francisquito Canyon Creek (N of Powerhouse)
SC-8	Bouquet Canyon Creek at Falls Campground

CALLEGUAS CREEK AND TRIBUTARIES

C-1	Calleguas Creek at Pacific Coast Highway
C-2	Calleguas Creek at Hueneme-Lewis Road
C-3	Conejo Creek at Cemetery Road Bridge
C-4	Revelon Slough at Wood Road
C-5	Beardsley Wash at Central Avenue
C-6	Duck Pond Agricultural Drain

The San Gabriel River sites will be the following:

The San Gabriel River sites will be the following:

SAN GABRIEL RIVER AND TRIBUTARIES

SG-1	San Gabriel River Tidal Prism (just below end of concrete channel)
SG-2	San Gabriel River - low flow channel (near end of concrete channel - San Gabriel River side)
SG-3	San Gabriel River - low flow channel (near end of concrete channel - Coyote Creek side)
SG-4	Coyote Creek at Willow Street
SG-5	San Gabriel River at Willow Street
SG-6	San Gabriel River at Beverly Blvd.
SG-7	San Jose Creek at Workman Mill Road
SG-8	Walnut Creek at Baldwin Park Blvd.
SG-9	San Gabriel River at Foothill Blvd.
SG-10	San Gabriel River (E. Fork) - above Cattle Canyon Creek
SG-11	North Fork of San Gabriel River
SG-12	West Fork of San Gabriel River
SG-13	variable station
SG-14	variable station

Station locations may be subject to change due to variable flow conditions or other reasons. A maximum of 14 samples/area will be submitted. The Santa Clara River and Calleguas Creek will be considered one area for the purposes of numbers of samples collected.

10. Project Organization and Responsibility

Principle Investigator: Dr. David Hinton

Dr. Hinton has been Professor of Fish Pathology in the Department of Medicine, School of Veterinary Medicine, and Aquatic Toxicologist in the Aquaculture and Fisheries Program at U. C. Davis since 1985. He has numerous publications in fish toxicology, nutrition and pathology. The principle investigator is a full-time employee of the University. He is ultimately responsible for the product, efficiency and safety of the laboratory; that it is properly equipped with instrumentation and personnel. He is also ultimately responsible for approving contracts and the use of contract funds. (916) 752-6413; FAX (916) 752-0414

Vet. Med. Medicine
Univ. of California, Davis
Davis, CA 95616

Laboratory Manager: David Ostrach

David Ostrach has been manager of the Aquatic Toxicology Facility at Science and Application Inc. /JRB Associates (2 yrs) and has owned his own aquatic toxicological consulting firm (5 yrs) and aquaculture facility (3 yrs). He is presently a staff researcher and graduate student at U.C. Davis, Department of Medicine, School of Veterinary Medicine.
The laboratory manager is a full-time employee of the University. The laboratory manager negotiates contracts for the laboratory and oversees the use of contract funds. He also acts as a resource for trouble-shooting and problem solving. When necessary, the laboratory manager may also help out in the capacity of support staff. (916) 752-9315 FAX (916) 752-0414
Vet. Med. Medicine
University of California, Davis
Davis, CA 95616

Project Director: Dr. David Hanes

Dr. Hanes is a professor in the Department of Biology, Sonoma State University and a visiting professor in the Department of Medicine, School of Veterinary Medicine, U.C. Davis. He received a Ph.D in Water Pollution Biology from Oregon State University. The project director has more than 20 years experience teaching toxicology, physiology and neurophysiology. He is very familiar with the equipment and theory of the tests used in these procedures.

The project director is a full-time employee of U.C. Davis. The project director performs the most difficult and non-routine analyses. He provides direct supervision and training of laboratory personnel. Working with the Regional Board contract manager, the project director reviews, selects and approves methods of sampling and analyses as well as develops and implements quality control programs. He may suggest and will consult with the contract manager about changes that may become advisable. Other responsibilities include: writing and revising standard operating procedures, over-seeing disposal of samples at the conclusion of analyses, scheduling sampling, logging incoming samples, tracking sample

custody and laboratory record maintenance. The project director also writes all reports and is in general in charge of carrying out the terms of the contract. (916) 752-0772 FAX (916) 752-0414
Institute of Ecology, Laboratory Facility
Bldg 5
University of California, Davis
Davis, CA 95616

Contract Manager: Deborah Smith

Deborah Smith is an Environmental Specialist with the California Regional Water Quality Control Board, Los Angeles Region. She will work with the project director to assure that the project is meeting its objectives. She is responsible for approving work accomplished and signing off for payments of invoices. She is responsible for the day-to-day management of the contract activities and sample collection and shipment to UCDATL. The contract manager will assure that proper QA/QC guidelines are met. She will suggest and consult with the project director when changes in the project become beneficial such as changes in collecting sites and times. (213) 266-7557 FAX (213) 266-7600
California Regional Water Quality Control Board - Los Angeles Region
101 Centre Plaza Drive
Monterey Park, CA 91754

Support Staff: Erika Gottl

Ms Gottl is graduated from the University of California at Irvine and holds a B.S. in Biology. She has six years of experience in various fields of research including animal surgery and physiology, biochemistry, aquaculture and aquatic toxicology. She is familiar with aquatic toxicological bioassay tests and HPLC. (916) 752-0772 FAX (916) 752-0414
Institute of Ecology, Laboratory Facility
Bldg 5
University of California, Davis
Davis, CA 95616

The support staff performs routine services including cleaning glassware and routine analyses. The current support staff is well qualified to carry out these tests. If new personnel are required, they will work with the project manager until he deems them capable of carrying out analyses on

their own.

Howard Bailey is also available for consultation or emergency support. He is a project manager for a similar test being conducted for the State Water Quality Board. He has been a research biologist (8 yrs) and director of the aquatic toxicology program (4 yrs) at SRI International. He has also been a research associate at Anatec Laboratories, Santa Rosa. Currently Mr. Bailey is a study director at AQUA-Science in Davis, CA and a post graduate researcher and graduate student at U.C. Davis, Department of Medicine, School of Veterinary Medicine. Carol DiGiorgio, the major support staff person for Howard Bailey's project is also available on a reciprocal arrangement for emergency help if necessary.

Responsibilities

Sampling Operation	Deborah Smith
Sampling QC	Deborah Smith
Laboratory Analysis	David Hanes
Laboratory QC	David Hanes
Data Processing	David Hanes
Data Processing QC	David Hanes
Data Quality Review	David Hanes
Performance Auditing	Howard Bailey
System Auditing	Deborah Smith
Regional Board QA	Hiam Tan
Overall Project Coordinator	David Hanes
Contract Manager	Deborah Smith

11. Data Quality Requirements and Assessments

The data produced by this study are toxicity data and as such detection/quantitation limits are unknown until the toxic substances are known. Accuracy and quantitation of toxic substance detection by chemical analysis will conform to a standard of equal to or better than the critria of the State Inland Waters Plan or EPA Gold Book. Precision of toxic testing is possible through replicate samples in the three species tests. Tests using *Ceriodaphnia dubia* will have 10 replicates, those using *Pimephales promelus* will have 4 replicates and those testing with *Selenastrum capricornutum* will have 4 replicates per sample. Precision of toxicity testing will also be monitored by the acceptability of organisms exposed to control water (EPA medium hard in Sierra Spring).

Acceptability in the *Pimephales* tests requires that controls will produce 80% or greater survival and an average dry weight of 0.25 mg. In the *Ceriodaphnia* tests, controls must have 80% or greater survival, an average of 15 or more young per surviving female and at least 60% of surviving control females producing at least three broods. Acceptable *Selenastrum* tests require that control growth produce $2X10^5$ cells/ml with a coeficient of variance less than 20% among replicates.

Sample representativeness will be difficult with a maximum of 4 grab samples throughout a year. The samples will reflect a state of the waterways at the time of sampling. Since the rivers being studied are seasonal in their lower reaches and in some seasons dominated by treated effluent, alternative sites may be sampled where stream flow is inadequate. This study should be representative of streams affected by urban run-off and effluent dominance. Samples on the same day, above and below point and non-point sources should allow characterization of such sources.

The three species tests use either 4 or 10 replicates per sample. Three replicates per sample would allow detection of a 25 percent difference between sample survival/reproduction and control survival/reproduction. This holds true when the certainty of detecting a true significant difference is 80 percent, and the probability of identifying a "non-toxic" sample as "toxic" is 5 percent. This determination is based on the following equation in *Biometry* by Sokal and Rohlf, 1981:

$$n \geq 2(\sigma/\delta)^2 \ (t_{\alpha\,(v)} + t_{2(1-P)(v)})^2$$

where:

n = number of replicates,

σ = assumed standard deviation (8%)

δ = desired detectable difference (25%)

α = significance level (0.05)

v = degrees of freedom of the sample standard deviation. With "a" treatment groups and "n" replicates per treatment, v = a(n-1).

P = desired probability that a difference will be significant. This is the power of the test, which we set at 80 percent.

t = values from a two tailed t-table.

Statistical analysis of mortality, growth and reproduction tests will include those analyses specified in EPA/600/4-89/001. If the variance conforms to a normal distribution using arcsin transformation and Shapiro-Wilks Test, either the T-test with Bonferroni Adjustment or Dunnett's Test will be used for reference toxicant tests. If a heterogeneous variance is found, Steel's Many-One Rank Test or Wilcoxon Rank Sum Test with Bonferroni Adjustment will be used for the reference toxicant tests. Reference NOEC and LOEC estimates will be reported and LC50 by probit analysis. Tests run without multiple dilutions will be compared via a two tailed T-Test to control samples.

12. Sampling Procedures

Project Organization and Responsibilities

All sampling will be performed by personnel from the Planning Unit of the Regional Water Quality COntrol Board, 101 Centre Plaza Drive, Monterey Park, CA. Personnel will be selected and trained by the Contract Manager. The organization of the Regional Board is shown in Figure 1.

Sample Collection

Sample containers will comply with EPA requirements (amber glass bottles or cubitainers). These containers will be either new factory cleaned containers, or previously used containers, cleaned and prepared in accordance with EPA protocols (non-phosphate detergent, acetone, and 10% nitric acid). Sample bottles will be shipped 1 week before sampling to the Regional Board (capped and labeled). Blue ice, filters, funnels, beakers, igloo-type coolers, and chain of custody forms will also be included in the shipment.

Sample volume will be specified by the Project Director at UCDATL. The minimum sample collected should be sufficiently large to supply the needs for all anticipated replicates.

Grab samples will be collected monthly for the purposes of this study.

Three watersheds will be sampled under this study (San Gabriel River, Santa Clara RIver, and Calleguas Creek). See Table above for the list of proposed sampling locations. Sample locations may be modified during the scope of the project. The Contract Manager will notify the Project Manager of any such changes before they occur. A maximum of 28 stations will be samples 4 times per year for one year. Sampling will be staggered so that one river will be sampled each month. Samples will be collected and shipped on Wednesdays. One week notice will be given to UCDATL so that the fathead minnows can be delivered to the laboratory on the date samples arrive.

A representative sample of the flowing stream will be collected near the center of the stream at 1/2 depth, if physically possible. Samples will be collected in such a manner that the sample comes from the water column, and not the surface or benthic areas. If flow is not consistent across the with of the stream, a composite sample will be collected. If several containers are being filled, each container will be filled from a common container to insure homogeneity. Sample containers will be rinsed with river water (3X), and filled without air space. Samples will be filtered with 60 mesh nylon to remove possible invertebrate predators. Each sample will be properly labelled and immediately iced to 4 °C.

A field data sheet and chain of custody form will be filled out at each sample collection site. Copies of these forms are included in Appendix A. These forms will be shipped along with the samples to the UCDATL. A photograph of each site will be taken during the first sampling event, and submitted to the Project Director at a later date.

Upon receipt of the samples at the UCDATL, the samples will be inspected. Any unusual condition of the samples will be noted and sample temperature will be recorded. Each sample container will be assigned a unique code, based upon the collection date and sampling site. This code will be recorded in a notebook, along with the date and time received, the recipient, and the analysis requested. The samples will be immediately placed in a refrigerator at 4 °C. Every effort will be made to initiate the tests on the day of arrival at the lab.

Sample preparation

Unless unforseen circumstances arise, all sample tests will begin on the day of arrival at UCDATL. In no instance, will testing be delayed more

than 48 hrs. after collection. Samples will be stored in a 4 °C refrigerator until used. Samples will be warmed to 25 °C immediately before use. In tests using animals, if samples are supersaturated with oxygen or below 40% saturation, they will be heated and vigorously stirred, but without cavitation or splashing, to not more than 30°C until oxygen concentrations are acceptable. The samples will then be immediately returned to 25 ± 1°C before food or organisms are added. This must be done to assure that oxygen levels are compatable with the life of the test organisms. Samples used in the alga test will be filtered through a 0.45 µm pore diameter filter to remove native algae. At the beginning of a test, samples will be monitored for dissolved oxygen, electrical conductivity, temperature, pH, chlorine, and ammonia.

pH will not be controlled in the test waters. While on test, the water will be exposed to the atmosphere of the laboratory and will reach a pH dependent upon their chemical composition, carbon dioxide concentrations of the laboratory atmosphere and the metabolism of the test organisms.

13. Sample Custody Procedures

Chain of custody forms will be completed at the time of sample collection in the field. The form will contain the date and time samples, date and time received by the laboratory, and any other changes of custody in-between. If the samples are passed to another party, change of possession will be recorded in writing, and attested to by both parties, showing the date and time of the transfer. The form will also contain the sampler and recipients name, address, and phone number, sample type, and location. A sample of the sample/chain of custody form is attached in Appendix A. These forms will be sent to the Regional Board with the sample containers prior to each sampling event.

14. Calibration Procedures and Preventive Maintenance

Facilities, Equipment, and Test Chambers

This laboratory is equipped for a variety of aquatic testing. We perform static acute toxicity tests on aquatic organisms, EPA three species bioassay, toxicity identification evaluations and simple water quality determinations (limited to pH, EC, DO, T, ammonia, chlorine, nitrite, calcium hardness and alkalinity). The building has a chemical laboratory, a positive pressure exposure room with controlled temperature and light,

a negative pressure exposure room with controlled light for static tests in a hooded water bath, and a large controlled light room for running water tests or testing large numbers of fish.

There are also available in the laboratory environmental chambers, some equipped with shakers. These chambers are equipped with maximum-minimum thermometers. A glass still, deionized water, and autoclave are available for water treatment and sterilization. There is also a Coulter Counter and High Performance Liquid Chromatography apparatus available for tests. The HPLC is used to further separate organic, non-polar compounds. Chemicals and kits for the determination of Calcium alkalinity, total hardness, ammonia, nitrite, chlorine, and instruments for the determination of D.O., pH and electrical conductivity. Test chambers for this study consist of borosilicate glass containers and food grade plastic cups.

Chemicals, when appropriate, are reagent grade, color coded if there is an expiration date. They are logged in when received.

Laboratory facilities are clean, climate-controlled and safe. Reagents and solvents are stored according to manufacturers directions.

Instrument	**Calibration/Monitoring**	**Frequency**
Refrigerator	Max-Min Thermometer	Daily
Exposure Chambers	Max-Min Thermometer	Daily
Exposure Room	Recording Thermometer	Contin.
Coulter Counter	5 µm pellets	Weekly
pH Meter	7 & 10 pH buffers	Each use
EC Meter	Standard Solutions	Monthly
D.O. Meter	Air	Each use
Balance	Standard weights	Monthly

15. Documentation, Data Reduction and Reporting

Records will be kept on data sheets with ball-point pen. The data from each sample will be recorded on summary sheets. A copy of the original data sheets and the summary sheet will be sent to the contract manager as soon as practical. The originals will be filed for review.

16. Data Validation

Data Validation and report writing are the responsibility of David Hanes. Data will be transferred from hand-writen data sheets to computerized data sheets by laboratory personnel. The computerized data sheets will then be printed and proof-read against the hand-written sheets. Statistical analysis requires re-entry of data into the computer for the statistical program. Advantage will be taken of this fact and means from the statistical analysis will be compared to means calculated by the computerized data sheets. The final report will be reviewed and approved by Dr. David Hinton, principle investigator, and Deborah Smith, contract manager with the Regional Water Quality Control Board. Statistical outlyers (more than two standard deviations away from the mean) will not be incorporated in the statistics, however they will be reported to the contract manager in the data sheets. Criteria for acceptable tests are those described in EPA/600/4-89/001 and reiterated in section 11 of this document and the test summaries.

17. Performance and System Audits

Performance audits will be conducted by Howard Bailey or Carol DiGorgio. Acceptable performance will be reflected in data generated. Criteria used for proper performance of the tests are found in the section 11 of this document and the test summaries.

The Regional Water Quality Control Board Contract Manager will perform on-site reviews of physical facilities, operational systems and operating procedures as she deems necessary.

18. Corrective Action

If tests do not meet acceptability standards as innumerated in the test summaries and in section 11 of this document, corrective action will be taken. Corrective action is the responsibility of David Hanes, project director. If a chronic test fails, samples will be rerun with the agreement of the contract manager and project director. They will be rerun at no additional charge to the contract. A new grab sample will be required, and it is noted that it will not match samples from other sites on the river. In addition to the criteria for acceptability of tests mentioned above,

corrective action will be taken if the coefficient of variation in the reference toxicity tests is greater than 25 percent or any one test will be declared unacceptable if its coefficient of variation is 50 percent not including outlyers (more than two standard deviations from the mean) or there are more than one outlyer per *Pimephales* or *Selenastrum* test.

19. Reports

Quarterly progress reports will be produced by the project director. These will include work performed during the quarter, the results of completed tests, QA/QC failures, reference toxicant results, and problems arising during testing and their possible significance on data produced. If problems occur, a discussion of corrective actions taken will be included in the report. The contractor will submit quarterly reports by 1 Jul 92, 1 Oct 92, 1 Jan 93, and 1 Apr 93.

An interim Financial Status Report will be produced by the project director and submitted by 31 Dec 92 and a final Financial Status Report will be submitted 1 Aug 93.

A final report on the toxicity of effluent dominated streams will be produced and due 1 Aug 93. It will contain the following information:

1. A review of existing Regional Board data on the rivers tested.

2. A summary of all work performed and data analyzed under this project. The data and statistical analyses will be provided as raw data and in summary tables or figures.

3. The results of toxicity identification evaluations and specific chemical analyses characterizing specific chemicals or groups of chemicals that represent the greatest toxic load of sampled water and an assessment of the impact these toxicants have on the aquatic community.

4. A summary of histopathology results from samples of fish supplied by the contract manager.

5. Maps of the watersheds showing general land use, storm drain inputs, tributaries, sampling sites, suspected point and non-point sources of pollutants and possible routes of conveyance of toxic pollutants. A list of probable sources of toxics will be included in the

report.

6. A series of recommendations that will most effectively abate toxicity from point and non-point sources. Where possible specific agencies will be targeted for implementation of these programs/activities. Recommendations will be prioritized with cost and completion time estimates.

7. A summary of public and agency comment on recommendations for implem

APPENDIX A

CALIFORNIA REGIONAL WATER QUALITY CONTROL BOARD
Los Angeles Region
101 Centre Plaza Drive
Monterey Park, CA 91754

TOXICITY BIOASSAY FIELD SHEET

Sampler _____

Waterbody Name _____

Location _____

Date of Collection _____

Time of Collection _____

Field Conditions

Weather _____

Stream Depth (approx.) _____ Width (approx.) _____

Bottom Substrate _____

Field Chemistry (Hydrolab)

Color	_____	Temp.	_____ C
pH	_____	DO	_____ mg/L
Conduct.	_____ mS/cm		_____ % sat.

Were water quality samples taken during this site visit? Y N

Circle:

Gen. Mineral	Coliform	VOCs
TDS, Cl, SO4, B	BOD	TPH
NO3, NO2, NH3	MBAS	Pesticides
P	Metals	Other _____

Predominant Land Use in the area _____

Likely source(s) of water in stream:

____ Rising GW
____ Reclaimed water (municipal effluent)
____ Urban runoff
____ Agricultural runoff
____ Natural runoff (i.e. snowmelt, rainfall)
____ Releases from upstream dam
____ Unknown

DJS 5/6/92

CALIFORNIA REGIONAL WATER QUALITY
CONTROL BOARD
LOS ANGELES REGION
101 CENTRE PLAZA DRIVE
MONTEREY PARK, CA 91754 (213) 266-7557

Chain of Custody/ Sign in Record

Bioassay samples:
Dr. David Hanes / Erika Gottl
Aquatic Toxicology Laboratory, Bldg. 5
Institute of Ecology, Laboratory Facility
University of California, Davis
Davis, CA 95616 (916) 752-0772

Weather Conditions: _____

Sampling Information

Sample ID#	Source	Sample Type	Date	Hour	Collect by
		GRAB			
		GRAB			
		GRAB			
		GRAB			
		GRAB			
		GRAB			
		GRAB			
		GRAB			
		GRAB			
		GRAB			
		GRAB			
		GRAB			
		GRAB			
		GRAB			
		GRAB			

Date Rec'd:_____ Time Rec'd:_____ Rec'd by:_____
Date Rels'd:_____ Time Rels'd:_____ Rels'd by:_____
Date Rec'd:_____ Time Rec'd:_____ Rec'd by:_____

FAT HEAD SURVIVAL Data Sheet

Sample Date:	
Test date:	

Treatment	Rep	Day 1	2	3	4	5	6	7
	1							
	2							
	3							
	4							
Initial pH								
Final pH								
Initial DO								
Final DO								
Temp								
Date								
ID								

	Intial	Final
EC		
Alkalinity		
Hardness		
Chlorine		
Date		
ID		

Comments:

Treatment	Rep	Day 1	2	3	4	5	6	7
	1							
	2							
	3							
	4							
Initial pH								
Final pH								
Initial DO								
Final DO								
Temp								
Date								
ID								

	Intial	Final
EC		
Alkalinity		
Hardness		
Chlorine		
Date		
ID		

Comments:

Treatment	Rep	Day 1	2	3	4	5	6	7
	1							
	2							
	3							
	4							
Initial pH								
Final pH								
Initial DO								
Final DO								
Temp								
Date								
ID								

	Intial	Final
EC		
Alkalinity		
Hardness		
Chlorine		
Date		
ID		

Comments:

Cerio Data Sheet

Sample Date:
Test date:

		Replicate										Initial	Final
Treatment	Day	1	2	3	4	5	6	7	8	9	10	EC		
	1													
	2											pH		
	3													
	4											DO		
	5													
	6											Temp		
	7													
	8											NH3		
	9											Date		
Temp												ID		
DO												Comments:		
Date														
ID														

Treatment	Day	1	2	3	4	5	6	7	8	9	10	EC		
	1													
	2											pH		
	3													
	4											DO		
	5													
	6											Temp		
	7													
	8											NH3		
	9											Date		
Temp												ID		
DO												Comments:		
Date														
ID														

Treatment	Day	1	2	3	4	5	6	7	8	9	10	EC		
	1													
	2											pH		
	3													
	4											DO		
	5													
	6											Temp		
	7													
	8											NH3		
	9											Date		
Temp												ID		
DO												Comments:		
Date														
ID														

Sample Date:
Test date:

ALGAL GROWTH TEST Data Sheet

Treatment	Replicate Cell Densities			Treatment Mean	Comments:
	1	2	3		
	Temp		Conductiv.		
	pH		Chlorine		
	Alkalinity		Date		
	Hardness		I.D.		

Treatment	Replicate Cell Densities			Treatment Mean	Comments:
	1	2	3		
	Temp		Conductiv.		
	pH		Chlorine		
	Alkalinity		Date		
	Hardness		I.D.		

Treatment	Replicate Cell Densities			Treatment Mean	Comments:
	1	2	3		
	Temp		Conductiv.		
	pH		Chlorine		
	Alkalinity		Date		
	Hardness		I.D.		

Treatment	Replicate Cell Densities			Treatment Mean	Comments:
	1	2	3		
	Temp		Conductiv.		
	pH		Chlorine		
	Alkalinity		Date		
	Hardness		I.D.		

Treatment	Replicate Cell Densities			Treatment Mean	Comments:
	1	2	3		
	Temp		Conductiv.		
	pH		Chlorine		
	Alkalinity		Date		
	Hardness		I.D.		

Treatment	Replicate Cell Densities			Treatment Mean	Comments:
	1	2	3		
	Temp		Conductiv.		
	pH		Chlorine		
	Alkalinity		Date		
	Hardness		I.D.		