

**TREATABILITY STUDY WORK PLAN  
INTRINSIC BIOREMEDIATION  
SITES 3 AND 13  
NAVAL AIR STATION ALAMEDA  
ALAMEDA, CALIFORNIA**

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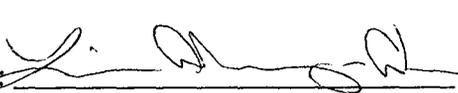
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ALAMEDA, CALIFORNIA**

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# 1. INTRODUCTION

This work plan describes the Treatability Study that will be conducted by the University of California at Berkeley (UCB) to evaluate whether intrinsic bioremediation is degrading the hydrocarbons found in the soil and groundwater at Sites 3 and 13 of Naval Air Station Alameda (NAS Alameda). A draft work plan for the Treatability Study dated February 7, 1996 was submitted to the US Navy for review. The work plan was reviewed by the Navy, Clean Contractor (PRC Environmental Management, Inc.), and the US EPA. Their comments and responses by UCB are included in Appendix D.

Conventional methods are severely limited in providing conclusive evidence to document intrinsic bioremediation because the complex, heterogeneous nature of subsurface environments does not permit complete characterization of all transport and reaction mechanisms which result in contaminant loss. However, researchers at UCB, Lawrence Berkeley National Laboratory (LBNL), and Lawrence Livermore National Laboratories (LLNL) have developed a range of field monitoring techniques to provide overlapping lines of evidence to measure the occurrence of intrinsic bioremediation. These field monitoring techniques, together with modeling analyses, will be utilized as part of the Treatability Study to estimate the rate of bioremediation at Sites 3 and 13. The field monitoring and modeling activities are described in this work plan. Other mechanisms that may be responsible for contaminant loss include volatilization, adsorption, and dilution. Measurements of hydrocarbon concentrations in gas, solid, and liquid phases will allow estimation of hydrocarbon partitioning while measurements of groundwater movement will allow for dilution estimations.

Site 3 was selected for investigation because soil and groundwater at this site contains petroleum hydrocarbons that can potentially be biodegraded by indigenous microorganisms. Site 3 is an abandoned fuel storage area where a leak of aviation fuel from underground storage tanks took place between 1975 and 1978. Site 13 was selected for investigation because it contains a mixture of oil refinery residuals and lighter hydrocarbons, which may be biodegradable in combination. This site is an abandoned oil refinery where both oily materials and gasoline hydrocarbons have been identified in the subsurface. As part of the Steam Enhanced Extraction (SEE) Pilot-Scale Treatability Study to be conducted by UCB, steam will be injected to and extracted from the subsurface at Site 13 to reduce hydrocarbon levels in the oil materials and subsurface soil (BERC, 1996a). The Treatability Study described in this Work Plan includes activities to perform a biological characterization of the site prior to the implementation of the SEE Treatability Study.

## **1.1 DESCRIPTION OF INTRINSIC BIOREMEDIATION**

Bioremediation is a naturally occurring process that is capable of breaking down chemicals in the subsurface. Successful bioremediation involves indigenous, or naturally occurring, organisms break down hazardous substances into less toxic or nontoxic compounds. In order for microorganisms to grow and reproduce they require a source of energy and a source of carbon as well as nutrients such as nitrogen and phosphorous. In environments where indigenous microorganisms are capable of using contaminants for energy or carbon sources, in situ bioremediation may be possible. In situ bioremediation may also be facilitated by microorganisms that are capable of breaking down contaminants without deriving energy from them, provided that an alternate substrate is available to support cellular growth. In this case the microorganisms cometabolize the contaminants. In situ bioremediation, therefore defines the process by which indigenous microorganisms metabolize contaminants, transforming them to less toxic or non-toxic compounds under environmental conditions.

## **1.2 OBJECTIVE OF TREATABILITY STUDY**

The Treatability Study described in this work plan evaluates the process of intrinsic bioremediation as a remedial alternative for reducing the hydrocarbon levels in the soil and groundwater at Sites 3 and 13. The results of this Treatability Study will be used by the U.S. Navy in a future engineering evaluation/cost analysis or feasibility study to develop general response actions for Sites 3 and 13. Development of the response actions will consider the following criteria when comparing intrinsic bioremediation other remedial alternatives:

- Overall protection of human health and the environment
- Compliance with applicable or relevant and appropriate requirements
- Long-term effectiveness and permanence
- Reduction of toxicity, mobility, and volume through treatment
- Short-term effectiveness
- Implementability
- Cost
- State acceptance
- Community acceptance

The Treatability Study will provide data to evaluate the occurrence and rate of degradation of hydrocarbons present at each site and follow the progress of intrinsic bioremediation by examining three indirect lines of evidence (NRC, 1993):

- Loss of hydrocarbons in the bioactive area
- Laboratory confirmation of microbial potential
- Field confirmation of microbial activity.

The loss of hydrocarbons in the bioactive area is most often documented by following the disappearance of hydrocarbons with time or distance by collection of a series of soil samples. As part of this Treatability Study, partial evidence for contaminant loss will be collected from previous site characterization studies conducted at both sites. However, this Treatability Study will primarily use innovative analytical techniques, including physical/chemical and isotopic assays, to demonstrate the loss of hydrocarbons in the bioactive area by sampling at discrete intervals along the contaminant plane over several periods of time. This innovation is promising for following the biodegradation of both light hydrocarbons and high molecular weight hydrocarbons found in tars, pitches, and refinery tank bottoms such as those at Site 13.

Traditional enrichment culture techniques and microcosm assays will provide laboratory confirmation of microbial potential. Whereas most treatability studies only quantify bacterial hydrocarbon degraders, this study will also quantify fungi and protozoa. Like bacteria, fungi can degrade hydrocarbons, including the more carcinogenic polycyclic aromatic hydrocarbons (PAH) that are difficult for bacteria to degrade. The presence of large numbers of protozoa will confirm that large numbers of bacteria, the food source for protozoa, are actively being produced (Madsen et al, 1991).

The last of the three lines of evidence, field confirmation of microbial activity, is generally the most difficult to obtain. This Treatability Study will utilize direct epifluorescent microscopy, a method that utilizes fluorescent stains that bind directly to microbial cells within subsurface soil samples, to confirm that organisms capable of in situ bioremediation are present in the field. Isotopic assays will also provide confirmation of in situ bioremediation in the field.

Modeling of natural intrinsic fate and transport processes, including bioremediation, will be used to predict the future concentrations of residual hydrocarbons in the soil and groundwater. The results may support a decision to allow natural processes to remediate, in place, the hydrocarbons at Sites 3 and 13 over a period of several decades if it can be demonstrated that leaving the contaminants in place for this time period would pose no immediate threat to public health or the environment.

Regulatory guidance has recently changed to acknowledge that no matter how long they are operated or how much they cost, traditional remediation technologies are ineffective at some sites.

For instance a study by the National Research Council (NRC, 1994) found that pump and treat systems operated for decades have been unable to restore underground aquifers to their original condition. The San Francisco Bay Regional Water Quality Control Board (RWQCB) recognizes that natural bioremediation is an acceptable remedial alternative if the source has been removed and the State Water Resources Control Board is currently developing state-wide policy addressing the containment zone policy.

### **1.3 PARTNERSHIP AGREEMENT**

On August 24, 1994, the U.S. Navy and UCB entered into a partnership that provides the framework for exploring the application of innovative environmental restoration technologies developed by UCB, LLNL, and LBNL to Installation Restoration (IR) sites at NAS Alameda. As part of the partnership agreement, UCB established the Berkeley Environmental Restoration Center (BERC) as a coordination office to administer the contract for UCB. The partnership is governed by Contract No. N62474-94-7430.

Delivery Order Number 5 (DO 5) to the partnership agreement authorizes the examination of intrinsic in situ bioremediation at Sites 3 and 13. Specific tasks that are authorized include:

1. Preparation of a Treatability Study Work Plan for Sites 3 and 13;
2. Implementation of the Treatability Study; and  
Preparation of a Treatability Study Report.

This work plan is written by UCB to fulfill the requirements of Task 1 and describes the investigation that will be implemented to fulfill the requirements of Task 2. At the completion of the Treatability Study, UCB will prepare a report to fulfill the requirements of Task 3.

During implementation of the Treatability Study, LBNL will be responsible for conducting the following assays:

- Microcosm assays
- Stable isotope ratio monitoring

LLNL will be responsible for conducting the following assays:

- Microbial enrichment
- <sup>14</sup>C radio-isotope tracking
- Field physical/chemical assays

UCB will be responsible for project oversight and coordination, conducting modeling to predict the rate of intrinsic bioremediation, and the following assay:

- Direct epifluorescent microscopy

#### **1.4 TREATABILITY STUDY DECISION PROCESS**

A review of the initial site characterization data for Sites 3 and 13 (summarized in Section 2) indicated that intrinsic bioremediation would be an appropriate alternative to consider for these sites. The petroleum hydrocarbons that have been found at Site 3 are of the type that are typically biodegradable and historic site sampling suggests that the hydrocarbon concentrations at Site 3 are decreasing naturally.

The hydrocarbons at Site 13, a combination of gasoline and JP5 from recent spills and heavy residuals from an old refinery, are expected to be more difficult to degrade biologically. Yet intrinsic bioremediation of these hydrocarbons may be sufficient to prevent the spread of mobile fractions and to eventually transform most, or possibly all, of the hydrocarbons to biomass, carbon dioxide, and water.

Thus, based on the initial site characterization, this draft work plan was prepared to describe the Treatability Study that will be performed at Sites 3 and 13 . This draft work plan will be reviewed by the U.S. Navy, regulatory agencies, the Restoration Advisory Board (RAB), and the BRAC Closure Team (BCT). After these reviews, the U.S. Navy will authorize preparation of the final work plan. After considering reviews of the Final Work Plan, the U.S. Navy will authorize implementation of the Treatability Study. The decision process is diagrammed on Figure 1-1.

The final report for the Treatability Study will also be reviewed by the U.S. Navy, regulatory agencies, the RAB, and the BCT. The final report may be considered by the U.S. Navy and their feasibility study contractor in either an engineering evaluation and cost analysis for an interim removal or remedial action or a feasibility study for a final remedial action.

## 1.5 RELATED DOCUMENTS

BERC has developed program level documents to describe procedures to be followed on projects implemented under the partnership agreement. These include:

- The Contractor Quality Control Program Plan;
- The Program Health and Safety Plan, Volumes I and II;
- Standard Operating Procedures; and
- Standard Quality Procedures.

Work conducted at Sites 3 and 13 will be conducted in accordance with the general procedures described in these program level documents and as more specifically described in this Work Plan. Applicable Standard Operating Procedures (SOPs) and Standard Quality Procedures (SQPs) for this project are identified in Table 1-1. The program level documents are updated periodically and the most recent version of each document is maintained in the BERC office. Project personnel will be trained in the use of these documents and this Work Plan and the training will be documented on the Training Attendance Record, Figure 1-2.

## 1.6 ORGANIZATION OF WORK PLAN

Section 1 of this Work Plan presents the objective of the Treatability Study to be performed for Sites 3 and 13, as well as the contracting mechanisms that authorize the work and related documents that need to be referred to during implementation of the study. A brief background of NAS Alameda as well as Sites 3 and 13 is presented in Section 2 with a discussion of previous investigations, site hydrogeology, soil and groundwater quality, and probable exposure pathways. Section 3 describes the conceptual approach of the Treatability Study and provides a brief description of the innovative and newly developed assays that have been prepared by UCB, LLNL, and LBNL and will be used during the Treatability Study. The Field Sampling Plan, detailing the field activities to be conducted, is presented in Section 4 and the Quality Assurance Project Plan is included in Section 5. Modeling that will be conducted to evaluate and predict the long term effectiveness of bioremediation at Sites 3 and 13 is described in Section 6. Reporting is described in Section 7 and project management, including a project schedule and project organization chart is described in Section 8. References are listed in Section 9.

Appendix A contains the project specific SOPs that have been developed for the Treatability Study. These SOPs supplement those that are included in the program level SOPs (BERC, 1996b); they have not previously been submitted to the U.S. Navy and reviewers. The site specific health and

safety plan for field work related to the Treatability Study is included in Appendix B. Resumes for project personnel are included in Appendix C.

## **2. BACKGROUND**

### **2.1 NAS ALAMEDA DESCRIPTION**

NAS Alameda is located on the western end of Alameda Island (Figure 2-1). The base, rectangular in shape, is approximately 2 miles long and 1 mile wide, and occupies 2,634 acres. Approximately 1,526 acres of NAS Alameda are land and 1,108 acres are bay.

Much of the land now occupied by NAS Alameda was once covered by the waters of San Francisco Bay or was tidal flats. Much of the base was gradually filled using hydraulically placed dredge spoils from the surrounding San Francisco Bay, the Seaplane Lagoon at NAS Alameda, and the Oakland Channel.

The Army acquired the NAS Alameda site from the city of Alameda in 1930 and began construction activities in 1931. The U.S. Navy acquired title to the land from the Army in 1936 and began building the air station in response to the military buildup in Europe prior to World War II. After the 1941 entry of the United States into the war, more land was acquired adjacent to the air station. Following the end of the war, NAS Alameda returned to its original primary mission of providing facilities and support for fleet aviation activities.

### **2.2 SITE 3**

Site 3 consists of an abandoned fuel storage area located in an inner island of Atlantic Avenue, approximately 200 feet west of the East Gate (Figure 2-1). Four partially buried concrete tanks lined with carbolite and one partially buried steel tank were previously present at the two acre site. Each tank previously contained aviation gasoline (AVGAS) and had a nominal capacity of 100,000 gallons. In 1975 it was discovered that three of the concrete tanks had leaked and in 1978 the fourth concrete tank was found to be leaking (PRC and MW, 1993b). The four concrete tanks were reportedly destroyed and buried in place. No information is available on the timing of the tank destruction or the fate of the steel tank.

Based on tank inventories, NAS Supply Fuels Branch personnel estimated that approximately 365,000 gallons of AVGAS may have escaped from the fuel storage area in the 1960s and early 1970s. The escaped fuel caused serious vapor problems in adjacent underground utilities resulting in an explosion and fire in 1977 that injured a contractor. Between 1978 and 1983, high AVGAS

vapors also caused several evacuations of the building located immediately north of Site 3 (PRC and MW, 1995a).

Currently, Site 3 is landscaped and used for an aircraft exhibit consisting of a naval aircraft that is centrally mounted on a pedestal in a grass covered area.

### **2.2.1 Previous Investigations, Site 3**

Three site investigations were conducted at Site 3 in 1979, 1985, and 1990. The location of each groundwater monitoring well installed during these investigations is shown on Figure 2-2. In 1979, Kennedy Engineers investigated the extent of AVGAS in the subsurface through the installation and sampling of 18 groundwater monitoring wells; the monitoring wells are designated with the prefix "OW" on Figure 2-2 (Monitoring Wells OW-8 and OW-32 are located outside of the map area). In 1985, Wahler Associates installed and sampled Monitoring Wells WA-7, WA-8, and WA-9 (Wahler, 1985). During this investigation, Wahler also sampled 12 of the monitoring wells installed by Kennedy. The current status of the monitoring wells installed in 1980 and 1985 is not known; several monitoring wells installed in 1979 could not be found during the 1985 investigation. Canonie installed and sampled Monitoring Wells MW97-1, MW-97-2, and MW97-3 and conducted a soil vapor survey in 1990 (Canonie, 1990a).

### **2.2.2 Site Hydrogeology, Site 3**

The geologic units encountered at Site 3 consist of artificial fill, Bay Mud, and the Merritt Sand. The artificial fill and Merritt Sand comprise the first and second water bearing zones of the shallow aquifer at NAS Alameda (PRC and MW, 1995b). Where present, the Bay Mud separates the two water bearing zones. However the Bay Mud is not continuous beneath Site 3. The relationship of the geologic units discussed is illustrated on the geologic cross section presented on Figure 2-3.

Based on the results of the investigation conducted in 1990 (Canonie, 1990), Site 3 is immediately underlain by 7 to 12 feet of artificial fill consisting of an upper layer of silty sand. Portions of the fill are also comprised of dark gray sandy clay, silty clay, and a deeper silty sand. Where present, approximately 3 to 4 feet of Bay Mud consisting of dark gray to gray silty clay underlies the fill. The artificial fill is deepest where the Bay Mud is absent.

The Merritt Sand underlies the Bay Mud at depths of approximately 10 to 12 feet below ground surface (bgs). Where the Bay Mud is absent (near Boring MW97-2), the Merritt Sand underlies the artificial fill at a depth of approximately 12 feet bgs. At this location, the fill extends below the original top of the Merritt Sand. The Merritt Sand is composed of orange brown mottled fine clayey sand with abundant iron oxide stains.

Groundwater flow directions observed during the 1990 investigation were towards the east under a gradient of 0.006 feet per foot. This flow direction is inconsistent with the flow directions in the eastern portion of NAS Alameda and may not be representative of actual flow directions because the wells are screened in different types of materials. Depths to groundwater in 1990 ranged from 5.94 to 6.04 feet (Canonie, 1990a).

### **2.2.3 Soil and Groundwater Quality, Site 3**

During the 1979 investigation, AVGAS was identified in soil samples from Borings OW-1, OW-16, and OW-23 at concentrations of 1,100 milligram per kilogram (mg/kg), 9,200 mg/kg, and 7,600 mg/kg, respectively. AVGAS was not identified in soil samples from the remaining borings; the detection limit was 720 mg/kg. The identification of AVGAS was made on the basis of matching the sample gas chromatographs to standard curves produced by analysis of known fuel types. A pentane extraction was used to prepare the samples. No soil analytical results were presented for the 1985 investigation.

During the 1990 investigation, soil samples were analyzed for volatile organic compounds (VOCs), semivolatile organic compounds (SVOCs), total recoverable petroleum hydrocarbons (TRPH), ethylene dibromide (EDB), metals, and the general chemical characteristics. Methylene chloride, acetone, toluene, and carbon disulfide were identified, however none of their concentrations exceeded the preliminary comparison level of 1 mg/kg (PRC and MW, 1995a). Pyrene and benzo (g,h,i) perylene were the only SVOCs identified. Both of these chemicals are polynuclear aromatic hydrocarbons (PAHs) and their total concentrations did not exceed the preliminary comparison level of 10 mg/kg. TRPH was identified in nine of 16 soil samples; the concentration exceeded the preliminary comparison level of 100 mg/kg only in soil samples from Borings MW97-1 and MW97-3; the concentrations were 129 and 133 mg/kg, respectively. EDB, an antiknock additive for gasoline, was not identified in any of the soil samples. According to the reference reviewed, the concentrations of 13 metals exceeded the background levels identified for NAS Alameda. However, only the concentrations of copper and magnesium exceeded the typical levels found in naturally occurring soil samples. Only those metals occurring at levels greater than naturally occurring levels would be considered for remediation. The pH of the soil samples analyzed ranged from 7.2 to 9.0. Analytical results for the other general chemical characteristics are presented in Table 2-1.

Groundwater samples collected during the 1979 investigation were analyzed for AVGAS using the procedures described for the soil samples. AVGAS was identified in six of the groundwater samples analyzed (from Monitoring Wells OW-1, OW-2, OW-14, OW-21, OW-23, and OW-28)

at concentrations ranging from 4 to 41 milligrams per liter (mg/l). The groundwater sample from Monitoring Well OW-6 contained 1,410 mg/l of oil and grease range hydrocarbons. They were reported to be a "heavy, dark, highly viscous oil". AVGAS was not identified in the groundwater sample from this well.

During the 1985 investigation, the hydrocarbon content of the groundwater samples was quantitated by comparison of the gas chromatograms to a standard produced from a commercial unleaded gasoline. The groundwater sample from Monitoring Well OW-14 contained 7.5 mg/l of gasoline range hydrocarbons. This was the only groundwater sample with a hydrocarbon concentration greater than the preliminary comparison level of 1 mg/l (PRC and MW, 1995a). Hydrocarbons were also identified in a groundwater sample from a utility trench at 3,900 mg/l. The location of this sample is shown on Figure 2-2. Free product was also identified on the groundwater surface in the utility trench at the location indicated on Figure 2-2. The presence of a heavy black fluid in Monitoring Well OW-6 was confirmed during this investigation.

During the 1990 investigation, groundwater samples from Monitoring Wells MW97-1, MW97-2, and MW-97-3 were analyzed for VOCs, SVOCs, oil and grease, metals, and general chemical characteristics. VOCs, SVOCs, and oil and grease were not identified in the groundwater samples. Of the metals analyzed, only sodium exceeded the background level in one groundwater monitoring well. No values for lead concentrations in groundwater were presented. The pH of the groundwater ranged from 6.8 to 7.4 and the TDS concentrations ranged from 1,280 to 22,300 mg/l. Analytical results for other general chemical characteristics are summarized in Table 2-2.

In 1979 Kennedy also conducted a fuel vapor survey in the electrical duct manholes, storm drain manholes, and sanitary sewer manholes surrounding Site 3. During this survey, organic vapor concentrations greater than 10,000 ppm were identified. There was no clear pattern to the fuel vapors identified in the survey. Kennedy concluded that the bulk of the AVGAS previously released was transported away from the site through infiltration to the storm and sanitary sewers (PRC and MW, 1995a).

To characterize the extent of petroleum hydrocarbons remaining in the soil, Canonie conducted a soil vapor survey in 1990 using a grid with a 100 foot spacing; a total of 121 soil vapor samples were collected and analyzed for benzene, toluene, ethylbenzene, and xylenes (BTEX) and total hydrocarbons (THC). Detected benzene concentrations ranged from 0.04 to 73,000 ug/l and THC concentrations ranged from 0.2 to 960,000 ug/l. Figures 2-4 and 2-5 show the distribution of these compounds identified in the soil vapor samples. The distribution of benzene and THC

concentrations are similar with the highest concentrations identified slightly to the northwest of Site 3. Detectable levels of benzene and THC extend to the north and west.

In summary, elevated hydrocarbon levels appear to be present beneath the landscaped portion of Site 3 and to the north and east. Previous investigations have identified AVGAS in soil samples at concentrations up to 9,200 mg/kg (from Borings OW-1, OW-16, and OW-23). TRPH was also identified in soil samples from this area (from Borings MW97-1 and MW97-3). AVGAS was also identified in groundwater samples from monitoring wells in this area (Monitoring Wells OW-1, OW-2, OW-14, OW-21, and OW-23) at concentrations ranging from 4 to 41 mg/l. Results of the soil vapor study also show elevated concentrations of THC and benzene beneath the landscaped portion of Site 3 and to the north and east.

#### **2.2.4 Probable Transport Pathways, Site 3**

The primary contaminants identified at Site 3 include petroleum related compounds present in the soil and groundwater. Site 3 is generally landscaped or covered with pavement. Under existing conditions, exposure to petroleum vapors could occur through volatilization. Direct contact with soil or groundwater would not be expected unless the ground were disturbed. Petroleum compounds have been identified in the groundwater and compounds present in the soil could be leached to the groundwater with infiltration of water from the ground surface.

Under reuse of this site, future site occupants could potentially be exposed to petroleum compounds through volatile compounds that could be released to indoor air, outdoor air, and through volatilization from groundwater. If the soil were disturbed, exposure to petroleum compounds could also occur through direct contact with soil or groundwater. Ground disturbing activities could also produce airborne soil particulates; individuals could be exposed to petroleum compounds present in the particulates through direct contact, inhalation, and ingestion. Additional exposure could occur if the particulates are deposited at nearby sites. Uptake to fruits and vegetables is also possible under a future use scenario where food crops may be grown.

Biodegradation of the compounds would be expected to diminish each of these transport and exposure pathways by transformation of the hydrocarbons present in the soil and groundwater to nontoxic products. If complete transformation is not demonstrated with the Treatability Study described in this Work Plan, the results of the assays and modeling can be used to evaluate which transport and exposure pathways are still present based on the characteristics of the petroleum compounds remaining in the soil and groundwater following transformation.

## 2.3 SITE 13

Site 13 consists of approximately 30 acres located in the southeast corner of NAS Alameda (Figure 2-1). This site is the former location of the Pacific Coast Oil Works refinery, which operated between 1879 and 1903. Refinery wastes and asphaltene residues were dumped at the site during the 24-year history of the refinery. The refinery consisted of pump and lubricating houses, stills, two laboratories and agitators, as well as approximately 19 above-ground iron oil storage tanks, six underground iron storage tanks, and a storage area containing drums of oil.

The section of Site 13 that is bordered by Avenues K and L and 9th and 11th Streets (Figure 2-6) is the location where the Treatability Study will be implemented because this is the area where the highest concentrations of refinery wastes have been identified. The sections of the Pacific Coast Refinery that were located on that section of Site 13 include an oil storage area, a lubricating building, bleaching tanks and several large iron oil tanks (PRC and MW, 1993b).

The location of the Pacific Coast Refinery was originally bound on the north by what is now K Avenue, on the east by Central Avenue, and to the south and west by the historic bay boundary. The historic bay boundary is shown on Figure 2-7 (PRC and MW 1993b). At the time of the refinery operation, the edge of the bay extended from the intersection of 9th Street and Avenue K, south along 9th Street 250 feet, and to the southeast where it crossed Avenue L approximately 300 feet east of 9th Street. The south west part of Site 13 was originally bay that was filled between 1942 and 1946.

The area once occupied by the refinery was later surfaced by the U.S. Navy. Sometime in the 1940s, a surface rupture occurred as a result of vapor pressure buildup from underground hydrocarbons and refinery wastes. To remove contaminated soil and reduce the risk of future rupture, the U.S. Navy excavated an area of approximately 30 by 30 feet (depth not recorded), and a concrete slab was placed in the bottom of the excavation which was then backfilled and resurfaced (Canonie, 1990b). The location of the removal was not available in the information reviewed.

Several naval facilities now exist on the site of the former oil refinery (Figure 2-6). A former on-base annex service station, Building 547 (Site 7C), is located in the northeast corner of the former oil refinery area. In the northwest corner is a hazardous waste storage yard (Site 19), which is currently in operation. A missile rework facility is housed in Building 530 (Site 10B), which is located in the southern portion of the former oil refinery area. The CANS C-2 Area (Site 16), a

storage area for large shipping containers containing paints, solvents, acids, bases, and transformers containing PCBs is located immediately to the south of Site 13.

During a previous removal action, approximately 104 cubic yards of soil exhibiting a low pH and containing high lead concentrations was removed from the area around Boring B-7 (Figure 2-7). Approximately another 50 cubic yards was expected to be removed after September 1994. For this removal action, soil containing lead at concentrations greater than 100 mg/kg were removed from the southern portion of Site 13 (PRC and MW, 1993a). The excavation area is shown on Figure 2-7.

In February 1991, a JP-5 release occurred on the east side of Building 397 (Figure 2-6), a jet engine test cell. Following a period of heavy rains, several storm drain manholes overflowed, resulting in an accumulation of free product; twelve manholes in the area were found to contain floating product. The storm drain lines south of Building 397 were reportedly extensively damaged during the 1989 Loma Prieta earthquake and groundwater in the area may have been impacted by JP-5 leaking from the damaged storm sewer lines. (PRC and MW, 1994b)

### **2.3.1 Previous Investigations, Site 13**

Five site investigations were conducted at Site 13 in 1989, 1990, 1991, and 1994. Soil borings and groundwater monitoring wells were installed throughout all of Site 13 as part of these investigations. The location of each boring and groundwater monitoring well installed within or adjacent to the block bound by Avenues K and L and 9th and 11th streets is shown on Figure 2-7 along with the location of the four borings drilled by UCB for collection of soil samples for the laboratory treatability tests. In addition, the location of two monitoring wells observed in the field are shown; a reference documenting the installation of these wells has not been identified. The previous investigations are described as follows:

- In 1989, Harding Lawson drilled and took soil samples from three borings (B-1 to B-3) to investigate the nature of petroleum hydrocarbons that were detected during the planned construction of the Intermediate Maintenance Facility. Upon detection of total petroleum hydrocarbons as JP-5 (TPH<sub>jp5</sub>) concentrations as high as 8600 mg/kg in some soil samples, an additional 15 soil borings (B-4 to B-18) were drilled. Soil Boring B-14 was converted to Monitoring Well MW-1 (HLA, 1989).
- In 1990, Canonie performed an investigation at Site-13 to determine if chemicals from the former refinery were leaching into the groundwater. Three groundwater monitoring wells

(MWOR-1 through MWOR-3) and seven soil borings (Bor-8, Bor-9, Bor-10, Bor-11, Bor-13, Bor-14, and Bor-19) were installed (Canonie, 1990b).

- In 1991 and 1992, PRC Environmental Management and J.M. Montgomery Consulting Engineers investigated the area surrounding Boring B-7 (installed in 1989 by Harding Lawson) to evaluate the extent of the low pH and elevated concentrations of lead identified in soil samples from this boring. Two groundwater monitoring wells (M-IMF-01 and M-IMF-02) and eleven soil borings (B-IMF-01 to B-IMF-11) were all drilled within 50 feet of Boring B-7 (PRC and JMCC, 1992; PRC and MW, 1993a).
- In March and April 1994, PRC conducted a Site Characterization and Analysis Penetrometer System (SCAPS) project to evaluate the extent of refinery wastes at Site 13. The SCAPS project included the advancement of 26 SCAPS push holes (ALA13P01 through ALA13P23, ALA13P25, ALA13P26, and ALA13P37) and seven hollow stem auger borings (ALA13PB38 through ALA13PB43 and ALA13PB45) (PRC, 1994).
- In 1994, PRC conducted additional investigation to further characterize the extent of soil and groundwater contamination. As part of this investigation, Monitoring Wells M13-06 and M13-07 were installed and three GeoProbe investigations (13GB004 through 13GB006) were carried out just to the east of 9th Street (PRC and MW, 1995a).

### **2.3.2 Site Hydrogeology, Site 13**

The geologic units encountered at Site 13 consist of artificial fill, Bay Mud, and the Merritt Sand. The artificial fill and Merritt Sand comprise the first and second water bearing zones of the shallow aquifer at NAS Alameda (PRC and MW, 1995b). Where present, the Bay Mud separates the two water bearing zones. However the Bay Mud is not continuous beneath Site 13.

Site 13 is immediately underlain by artificial fill to depths of 5 to 12 feet bgs; this unit generally consists of sand and silty sand. Where present, Bay Mud consisting of dark gray silty clay, is typically encountered at 9 to 12 feet bgs. The Merritt Sand underlies the Bay Mud and directly underlies the artificial fill where the Bay Mud is absent. The depth to the top of the Merritt Sand ranges from five to 12 feet bgs. The cross section provided in Figure 2-8, constructed from borings installed as part of the SCAPS project, illustrates the relationship of these geologic units immediately to the south of the planned treatment area for the SEE Pilot Scale Treatability Study.

The depth to groundwater at Site 13 ranges from 4 to 7.5 feet bgs (PRC and MW, 1995b). Local groundwater directions and gradients vary. However, groundwater at Site 13 generally flows to the southwest with an average hydraulic gradient of 0.001 to 0.003 feet per foot. Hydraulic conductivities measured on soil samples from Site 7C, located adjacent to Site 13, were 3.0E-07 cm/sec for a sample of Merritt Sand from a depth of 10.5 feet and 1.0E-03 cm/sec for a sample of hydraulic fill from a depth of 2 feet. The hydraulic conductivity for the Merritt Sand is questionable because the value reported is typical of the hydraulic conductivity for a clay which would typically have a lower hydraulic conductivity than a sandy material.

### **2.3.3 Soil and Groundwater Quality, Site 13**

#### **2.3.3.1 Soil Quality, Site 13**

During the 1989 investigation described in Section 2.3.1, soil and water samples were analyzed for total petroleum hydrocarbons (TPH), SVOC, oil and grease, BTEX, and pH. Petroleum hydrocarbons were identified in soil samples from 15 of the 18 borings. Borings B-9 and B-10, located on the far west side of Site 13, did not contain detectable hydrocarbons. The other boring where hydrocarbons were not found was Boring B-2 located approximately 80 feet south west of Building 397. In the soil sample from 4.5 to 5 feet bgs in Boring B-7 lead was identified at 13,000 mg/kg, total petroleum hydrocarbons as gasoline (TPHg) was identified at 16,000 mg/kg, total petroleum hydrocarbons as diesels (TPHd) was identified at 76,000 mg/kg, and oil and grease was identified at 120,000 mg/kg; this soil sample also had a pH of 1.6. None of the other soil samples taken from the other borings had a pH that was less than 5.5. The next highest lead level found in the soil samples was 140 mg/kg for a soil sample taken at Boring B-8 (HLA, 1989).

During the 1991 and 1992 investigation to evaluate the extent of lead and low pH soil identified in the soil sample from Boring B-7, soil samples from 11 soil borings and Monitoring Well M-IMF-02 were analyzed for pH and lead. Of the 20 soil samples from within ten feet of B-7 (from B-IMF-09 through B-IMF-11 and M-IMF-02), seven had a pH of less than 5.5 and only thirteen had a pH of greater than 5.5. Of the 47 soil samples taken between 10 and 50 feet from B-7, none had a pH of less than 4 and 38 had a pH higher than 7. Six of the thirty-five soil samples analyzed had lead concentrations greater than 100 mg/kg. The highest lead level detected was 1980 mg/kg for a soil sample taken at 3 feet at Boring B-IMF-10. Soil samples were also taken using a hand auger to determine if the pH readings seen at Boring B-7 in the 1989 Harding Lawson Investigation were accurate. A soil pH of 0.7 was found in soil samples taken 4 feet bgs next to the location of Boring B-7. During this investigation, two soil samples, one from eight feet in Boring B-IMF-01 and another from four feet in Boring B-IMF-06, were analyzed for base/neutral/acid semivolatiles organic compounds and none were identified (PRC and JMMC, 1992).

In 1994 a removal action was conducted to excavate soils with lead levels greater than 100 mg/kg. During the removal action, field screening for lead concentrations and the pH of soil samples were used to determine the extent of the excavation (PRC and MW, 1993a). The limits of excavation were not available from the literature reviewed. However, the planned area for the removal action was located approximately 200 feet from the proposed area for the Preliminary Screening phase of this Treatability Study.

During the 1990 Canonie investigation, soil samples were analyzed for TRPH, VOCs, SVOCs, metals, pesticides, and pH. With the exception of soil samples from Borings Bor-9 and Bor-19, the TRPH concentrations identified were all less than 100 mg/kg. Boring Bor-9 is located 60 feet southwest of Monitoring Well MW-1. Soil Boring B-19 is located at the intersection of Avenue L and Eleventh Street at the southeast corner of Site 13. TRPH was identified at 4,360 mg/kg in the soil sample from 6.5 feet in Boring Bor-9 and at 3,600 mg/kg in the soil sample from 11 feet in Boring Bor-19. VOCs identified in the soil at concentrations greater than 1 mg/kg were methylene chloride, benzene, ethylbenzene, toluene, and xylenes. SVOCs detected at concentrations greater than 10 mg/kg included Di-n-butyl-naphthalate and 2-methylnaphthalene. Pesticides were detected in concentrations less than 0.035 mg/kg and were identified in soil samples from Borings Bor-8 and Bor-9. The pH was greater than 5.5 in all of the 14 soil samples analyzed for pH (Canonie, 1990b).

During the 1994 PRC investigation, three soil samples from Monitoring Well M13-06 were analyzed. VOCs and TPH were not identified in soil samples from this boring (PRC and MW, 1995a).

During the 1994 SCAPS project, petroleum hydrocarbon concentrations in the soil were measured using an in-situ fluorometer. The results of these measurements were validated by traditional laboratory analysis of a total of 45 soil samples from locations ALA13-PB38 through ALA13-PB43 and ALA13-PB45 for TRPH, TPHd, TPHg, TPHjp5, and total petroleum hydrocarbons as motor oil (TPHmo) and pH. The pH of the soil samples ranged from 1.1 to 9.3. The locations of soil samples with a pH lower than 5.5 are indicated in Figure 2-9. The soil sample from 7 to 7.5 feet bgs in Boring ALA13-PB41 had a pH of 1.1. The highest concentrations of TPHd and lead were also identified in this soil sample. The concentrations were 170,000 mg/kg and 413 mg/kg, respectively.

In the zero to five foot depth, soil pH values that were less than 5.5 were identified only in soil samples from Boring B-IMF-06 to the north of Boring B-7. In the 5 to 10 foot depth, low pH soil

was only identified in soil samples from the vicinity of Boring B-7 and at SCAPS Boring ALA13-PB41 (Figure 2-9). The soil around Boring B-7 has been removed.

Hydrocarbons in the form of TPH, TRPH, or oil and grease are mostly concentrated in the central and eastern sections of the site. Figure 2-10 illustrates the distribution of hydrocarbons in soil samples from the zero to five foot depth. Hydrocarbon concentrations greater than 10,000 mg/kg were identified in soil samples from within five feet of the ground surface at Monitoring Well MW-1 (Boring B-14), near the southeastern corner of Building 39, and in the vicinity of Boring B-7. Figure 2-11 shows the distribution of hydrocarbons in soil samples from the five to ten foot depth; the levels above 10,000 mg/kg were also clustered around B-7 and B-14. Areas of hydrocarbon levels between 1000 and 10,000 mg/kg extend several hundred feet between B-7 and B-14. Figure 2-12 shows the distribution of hydrocarbons in soil samples from the 10 to 15 foot depth. At this depth, there were no soil samples with concentrations greater than 10,000 mg/kg. The region with hydrocarbon concentrations between 1,000 and 10,000 mg/kg extends from north centrally located SCAPS Boring ALA13-PB45 to Boring Bor-19, located in the south east part of Site 13. The distribution of hydrocarbons in soil samples from the 15 to 20 foot depth is illustrated on Figure 2-13. The only soil sample from this depth that had hydrocarbon concentrations greater than 1000 mg/kg was from Boring B14.

The distribution of lead identified in soil samples from the zero to five foot depth is illustrated on Figure 2-14. Lead concentrations greater than 100 mg/kg were identified in Borings B-7 and B-8. Lead concentrations were greatly reduced in the five to ten foot depth and lead was not identified at concentrations greater than 100 mg/kg in any soil samples from depths greater than 10 feet.

In summary, TPHg, TPHd, oil and grease, VOCs, SVOCs, lead, and some pesticides have been identified in soil samples from Site 13. The highest hydrocarbon concentrations identified were 120,000 mg/kg of oil and grease and 76,000 mg/kg of TPHd. Hydrocarbons were identified at the greatest depth in soil samples from Boring ALA13-P13; this location was selected for implementation of the SEE Pilot Scale Study.

Soil from the vicinity of Boring B-7 exhibited low pH values and high lead concentrations. This soil was removed during a removal action. Soil from the vicinity of Boring ALA13-PB41 exhibited a low pH and this soil remains in place.

### ***2.3.3.2 Groundwater Quality, Site 13***

In the 1989 Harding Lawson investigation, groundwater from Monitoring Well MW-1 (Boring B-14) was analyzed for VOCs, TPHd, oil and grease, base/neutral/acid compounds (BNAs) and

dissolved metals. Benzene was identified at a concentration of 0.44 mg/l and no other VOCs or SVOCs were identified at a concentration greater than 0.005 mg/l. Lead was identified at 0.05 mg/l; TPHg was identified at 11 mg/l; and oil and grease was identified at 60 mg/l.

During the 1991 and 1992 PRC investigations, groundwater samples taken from the borings closest to Boring B-7 had pHs of 0.9, 2.8, 6.7, and 3.0. Lead was identified in the groundwater at concentrations ranging from 0.0015 to 1.77 mg/l in water samples taken from these borings. The lowest pH and the highest dissolved lead levels were found in groundwater from Boring B-IMF-09. This soil boring was located approximately 8 feet north of Boring B-7.

The pH of groundwater samples from Monitoring Wells MWOR-1 through MWOR-3 (sampled in 1990) ranged from 6.8 to 7.8. Methylene chloride was identified in the groundwater samples from MWOR-1 and MWOR-3 and DDT was identified in the groundwater sample from Monitoring Well MWOR-1.

During the 1994 SCAPS investigation, water samples from Borings ALA13-PB40, ALA13-PB43, and ALA13-PB45 were analyzed for TPHd, TPHg, TPHjp5, TPHmo, pH, and metals. The TPH levels were all less than 0.1 mg/l and the pH levels were all between 5.6 and 6. Lead levels were equal or less than 0.001 mg/l.

During the 1994 site investigation, the groundwater from the GeoProbe investigations was analyzed for VOCs, TPHd, and TPHg. These compounds were not identified in groundwater samples from GeoProbe Well 13GB005. Water samples collected from Monitoring Wells M13-06 and M13-07 as well as four of the previously installed Monitoring Wells (MW-1 and MWOR-1 through MWOR-3) were analyzed for VOCs, SVOCs, TPHd, TPHg, metals, general chemicals, total organic carbon, and pesticides/PCBs. No VOCs, SVOCs, or pesticides were identified in any of the groundwater samples from the area of interest. The highest TPHd concentrations identified in any of the groundwater samples was 10 mg/l identified in the groundwater sample from Monitoring Well MW-1. TPHd was identified at 1.75 mg/l and 2.0 mg/l in the groundwater samples from Monitoring Well M13-06 and M13-07 but was not identified in groundwater samples from the other three wells. The groundwater sample from Monitoring Well M13-07 (located adjacent to the area of interest) also contained detectable levels of VOCs and phenols.

In summary, TPHg, TPHd, benzene, oil and grease, and lead have been identified in the groundwater at Site 13, primarily in the vicinity of Monitoring Well MW-1. Methylene chloride and DDT have also been identified in the groundwater. With the exception of groundwater samples

obtained from the vicinity of Boring B-7 where a soil removal action was conducted, the pH of the groundwater at Site 13 generally varies from 5.6 to 7.8.

### **2.3.4 Probable Transport Pathways, Site 13**

Potential sources of soil and groundwater contamination at Site 13 include historic oil refinery operations and wastes as well as the JP-5 release on the east side of Building 397. The site is currently unpaved. Under existing conditions, exposure to contaminants present in the soil would not be expected unless the ground were disturbed. Petroleum hydrocarbons have been identified in the groundwater and compounds identified in the soil could be transported to the groundwater by infiltration of water from the ground surface.

Under reuse of this site, future site occupants could potentially be exposed to the contaminants present through inhalation of compounds that could be volatilized to indoor or outdoor air. If soil were disturbed, exposure to the contaminants could also occur through direct contact with or ingestion of soil or groundwater. Ground disturbing activities could also produce particulates; individuals could be exposed to these particulates through direct contact, inhalation, and ingestion (indirectly). Additional exposures could occur if the particulates are deposited at nearby sites.

Biodegradation of the compounds would be expected to diminish each of these transport and exposure pathways by transformation of the hydrocarbons present in the soil and groundwater to nontoxic products. If complete transformation is not demonstrated with the Treatability Study described in this Work Plan, the results of the assays and modeling can be used to evaluate which transport and exposure pathways are still present based on the characteristics of the petroleum compounds remaining in the soil and groundwater following transformation.

## **3. TESTS AND PROCEDURES**

This section presents the conceptual approach to the Treatability Study and a brief description of the assays to be performed by UCB, LLNL, and LBNL; full descriptions of these assays with the test methodology and Quality Assurance/Quality Control (QA/QC) requirements are presented in SOPs prepared for this project and included in Appendix A. The title and number of each SOP prepared is included in Table 1-1.

### **3.1 CONCEPTUAL APPROACH**

The Treatability Study will follow the progress of intrinsic biodegradation at Sites 3 and 13 using monitoring techniques to provide the following three overlapping lines of evidence (NRC, 1993) for the microbially mediated destruction of hydrocarbons:

- Contaminant loss in the bioactive area - This type of evidence is usually obtained by collecting and analyzing samples from a contaminated area over time to demonstrate that the levels of contaminants are decreasing. This Treatability Study will utilize discrete sampling as well as new isotope monitoring techniques to measure the byproducts of biodegradation in order to document whether biodegradation is occurring.
- Laboratory confirmation of microbial potential - Microorganisms that degrade hydrocarbons must be present at a site for bioremediation to occur. Well tested field sampling and laboratory techniques will be used to demonstrate that microorganisms that degrade hydrocarbons are present in field samples from Sites 3 and 13.
- Field confirmation of microbial activity - Although microorganisms found at a site may degrade hydrocarbons in the laboratory, they do not necessarily degrade them under the conditions that exist in the field. Several new techniques will be employed to demonstrate that microbial processes, rather than physical or chemical processes, are actually responsible for observed decreases in hydrocarbon concentrations. This type of evidence will be obtained by selectively staining and counting the actual microorganisms present in field samples to identify the active microbial population. Measurement of the isotope ratios of hydrocarbon breakdown products will also provide field confirmation of microbial activity.

The Treatability Study will involve three phases of field work and subsequent assays to demonstrate the occurrence and rate of biodegradation and to confirm contaminant stimulated bacterial growth at Sites 3 and 13. The phases are described as follows:

- Preliminary Screening - During this phase soil gas and groundwater samples will be collected from hand augered borings and assayed using isotope monitoring techniques to measure the byproducts of biodegradation. This phase is necessary to better define the boundary of the plumes at Sites 3 and 13 and to provide preliminary evidence that biodegradation is occurring at each site before proceeding to subsequent phases of the Treatability Study.
- Background Level and Contaminant Area Characterization - During this phase, soil gas, groundwater, and soil samples will be collected and assayed. The purpose of this sampling is to establish levels of contaminant concentration, to characterize the microbial communities, and to identify the environmental conditions (i.e. aerobic-vs-anaerobic) in both contaminated and

uncontaminated areas. The results will be compared and used in modeling to estimate the occurrence and rate of biodegradation at Sites 3 and 13.

- Periodic Monitoring - During this phase, soil gas and groundwater samples will be collected from locations adjacent to the locations sampled for the Background Level and Contaminant Area Characterization phase on two separate occasions. The sampling will be used in modeling to assess the effects of seasonal changes in temperature, soil moisture, and groundwater levels on subsurface biological activity.

Six types of field and laboratory assays will be utilized during the Treatability Study to provide data to support each line of evidence identified above. The assays are summarized in Table 3-1 with a brief description of the information obtained and the line of evidence supported by each assay. As shown in Table 3-1, at least two assays are conducted to support each line of evidence; this will reduce the uncertainty due to heterogeneous field conditions. The assays to be conducted include:

- Field physical/chemical assays
- Microbial enrichment
- Microcosm assays
- Direct epifluorescent microscopy
- Stable isotope ratio monitoring
- <sup>14</sup>C Radio-isotope tracking

Field physical/chemical assays will be performed on soil and groundwater samples collected during the Background Level and Contaminant Area Characterization. Measured characteristics will include contaminant concentrations, potential electron acceptors, available nutrients, trace metals, pH, conductivity, and soil moisture content. Physical and chemical conditions conducive to high rates of microbial hydrocarbon degradation include permeable soils, aerobic environments, adequate supplies of essential nutrients like nitrogen and phosphorous, and neutral pHs.

Microbial enrichment and microcosm assays will be used to provide laboratory confirmation of microbial activity. Microbial enrichment will be used to identify the types of microorganisms present in the field samples, including those that have been classified as hydrocarbon degraders. A diverse microbial population, including large numbers of protozoa that feed on hydrocarbon degrading bacteria, can be a strong indicator that intrinsic bioremediation is actively occurring.

Microcosm assays will be used to confirm that the indigenous microbial communities at Sites 3 and 13 have the potential to transform the contaminants present at each site and to evaluate factors that may limit effective bioremediation. With this assay, radiolabeled petroleum compounds that are representative of the contaminants present at each site will be added to soil samples in laboratory microcosms; these compounds will provide a food source for the microorganisms. The loss of these substrates and the concurrent production of degradation products will be measured in the laboratory along with the isotopic ratios of the transformed compounds.

Direct Epifluorescent Microscopy assays will be conducted on soil samples to provide field confirmation of microbial activity. Selected biological stains will be used on soil samples to differentiate total microbial numbers from inert soil solids and to identify the total active and inactive fractions of the microbial cells. Significant differences in overall biomass estimates, especially in active biomass estimates, between samples from contaminated zones and those from noncontaminated zones, would provide strong evidence that bioremediation of the contaminants is occurring and that the microorganisms are responsible.

Isotopic assays will also be conducted on soil gas and groundwater samples collected during each phase of the investigation. These analyses provide a determination of isotopic ratios in the soil gas and groundwater. Isotopic ratios, taken together with results provided from the other assays can demonstrate both that hydrocarbons have degraded, and that microorganisms were responsible for the observed degradation.

Throughout the Treatability Study, data obtained from the assays will be used to develop model inputs for extrapolation of broad soil gas and groundwater supplies of substrates, nutrients, and/or oxygen to the microbial communities. Ultimately, the modeling will be used to evaluate the rate and occurrence of biodegradation at Sites 3 and 13 and to predict the time course of hydrocarbon disappearance from each site.

## **3.2 LABORATORY MICROBIAL CHARACTERIZATION**

### **3.2.1 Microbial Enrichment**

Enrichment and isolation techniques (SOP 24.1) will be used on soil samples to provide laboratory confirmation of in situ bioremediation. These techniques rely on the nutritional requirements of microorganisms to differentiate and quantify specific members of the soil microbial biomass. The number and distribution of the microbial population in samples from contaminated zones will be

correlated with the population in soil samples from uncontaminated zones in order to evaluate the occurrence of stimulated microbial growth due to contaminant degradation.

Enrichment techniques will also be used to quantify higher microorganisms (i.e. the protozoa and fungi) and to distinguish the taxonomic and metabolic diversity among the bacterial population. The abundance and distribution of heterotrophic bacteria, actinomycetes and fungi will be determined using colony counts on selective agars in accordance with standard methods. Most probable number (MPN) dilutions will be employed to provide information on microbial distribution. The occurrence of enhanced populations of protozoans and fungi within the contaminated zones relative to the noncontaminated zones is a strong indication of bioremediation potential (Madsen et al, 1991).

### 3.2.2 Laboratory Microcosm Assays

Environmental analogs (microcosms) that mimic natural settings are extremely useful as experimental laboratory models to monitor ongoing microbial processes under pseudo-environmental conditions (Atlas and Bartha, 1992). They will be used with soil from Sites 3 and 13 to determine if indigenous microbes have the potential to transform contaminants present at Alameda and to evaluate factors that may limit effective bioremediation. The method is described in SOP 24.2. Radiotracer assays and mass balances will be used to ascertain whether microorganisms from site samples express contaminant degradation behavior in small laboratory vessels maintained under carefully controlled conditions.  $^{14}\text{C}$  labeled substrates representative of the contaminants of interest (e.g. toluene for gasoline contaminants, phenanthrene for PAH contamination) will be added to NAS Alameda soil microcosms to indicate biologically mediated transformations of specific contaminants. Radiolabeled petroleum hydrocarbons that span a broad range of solubilities and are representative of in situ hydrocarbon contamination at Sites 3 and 13 will be used. The final selection of representative hydrocarbons will be made after the screening phase is completed at each site.

During the assay, the evolution of radiolabeled  $^{14}\text{CO}_2$  will be monitored using isotope monitoring techniques described in SOP 24.4; intermediate metabolites will be analyzed by gas and liquid chromatography. In a portion of the microcosms, the actual depletion of contaminants from the soil matrix will be measured using EPA approved solvent extraction techniques combined with chromatography. In addition, the stable carbon isotope ratios ( $^{13}\text{C}/^{12}\text{C}$ ) of the  $\text{CO}_2$  evolved and of the organic carbon in the soil at the beginning and end of each microcosm experiment will be measured using isotope monitoring techniques described in SOP 24.4. These results will provide additional information with respect to overall degradation rates during the microcosm assays. They

will also be helpful for evaluating field isotopic data. These studies will provide laboratory confirmation of indigenous microbial ability to degrade mixtures of compounds found at Sites 3 and 13.

### **3.3 SUBSURFACE MICROBIAL CHARACTERIZATION**

#### **3.3.1 Direct Epifluorescent Microscopy**

While enrichment methods and microcosm assays are effective for laboratory confirmation of in situ microbial activity, the use of stains that directly bind to microbial cells within subsurface soil samples have proven to be more effective and appropriate for establishing field confirmation (evidence category 3) of in situ bioremediation (NRC, 1994). Stains considerably reduce the potential for inaccuracies in estimating microbial numbers in subsurface soils because they are a direct visualization technique. Both established and innovative methods of direct epifluorescent microscopy will be used to provide quantification and characterization of subsurface microbial activity (SOP 24.3). Fluorescent stains specific for different microbial proteins and DNA will be used to determine total biomass in subsurface samples.

Determination of the different microbial factions that comprise the soil biomass is particularly important because community structure can serve as a meaningful indicator of adapted biodegradation activities. Fluorochrome stains are now available that are specific for different microbial families and thus allow for representative estimates of the range of populations in environmental samples. Well tested fluorochrome stains will be used as biomarkers of three major factions that comprise the soil microbial biomass: Fluorescein Isothiocyanate (FITC) - to determine total bacterial biomass; Calcofluor M2R - to determine fungal biomass; and Schaffer-Fulton spore stains - to estimate the quantities of spores present in environmental samples.

In addition to the total microbial population, an important parameter for assessing in situ bioremediation is determining the viable or active fraction of that population. To visually determine microbial activity in situ, redox dyes (viability stains) that serve as non-specific substrates for microbial respiration will be employed to stain active biomass within soil samples. 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) will be used to quantify active bacterial cells while fluorescein diacetate will be used to quantify active fungi. These redox dyes will be used in conjunction with specific fluorescent stains to provide for direct microscopic confirmation of active and inactive fractions of the soil microbial biomass

### 3.3.2 Stable Isotope Ratio Monitoring

Microbial degradation of petroleum hydrocarbons can result in the production of carbon dioxide ( $\text{CO}_2$ ), methane ( $\text{CH}_4$ ) and nitrous oxide ( $\text{N}_2\text{O}$ ) in soil gases and  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and  $\text{HCO}_3^-$  in groundwater. Measurements of changes in the levels of these microbial byproducts in soil gas and groundwater samples represent a method for monitoring in situ microbial activity (Aggarwal and Hinchee, 1991, Baedecker, et al, 1993, Ostendorf and Kampbell, 1991) There are, however, other sources of these compounds in the subsurface besides microbial contaminant degradation (e.g., root respiration, chemical reactions, and atmospheric contamination). In addition, some microbial degradation products may result from the breakdown of natural substrates rather than subsurface contaminants.

In order to identify the sources of these metabolic byproducts, the isotopic composition of targeted degradation products will be measured. The primary constituents of these products: hydrogen, carbon, nitrogen and oxygen, have at least two naturally-occurring stable isotopes. By use of isotope ratio mass spectrometry, variations in the ratios of the stable isotopes of these elements can be measured to better than 1 part in 10,000 or 0.1‰. Natural ratios for the  $^2\text{H}/^1\text{H}$  (or  $\delta\text{D}$ ),  $^{13}\text{C}/^{12}\text{C}$  ( $\delta^{13}\text{C}$ ),  $^{15}\text{N}/^{14}\text{N}$  ( $\delta^{15}\text{N}$ ) and  $^{18}\text{O}/^{16}\text{O}$  ( $\delta^{18}\text{O}$ ) are approximately 700‰, 100‰, 50‰, 100‰, respectively where ‰ signifies parts per thousand. For many of the microbial byproducts discussed above, there are significant differences between the stable isotope compositions of compounds produced from microbial degradation of hydrocarbons, the compounds produced from chemical reaction, and the compounds produced from atmospheric contact. This is based upon the fact that biological reactions favor molecules containing light isotopes (e.g.  $^{12}\text{C}$ ,  $^{14}\text{N}$ ) over heavy isotopes ( $^{13}\text{C}$ ,  $^{15}\text{N}$ ), whereas abiotic reactions do not exhibit as strong a tendency. Therefore, the products of biologically mediated contaminant degradation can be effectively differentiated from contaminant migration or abiotic reactions by the evaluation of the stable isotope ratios in soil gas and groundwater (Aggarwal and Hinchee, 1991). For this Treatability Study, the stable isotope ratios of contaminant degradation products will be measured in order to provide field confirmation of in situ biological activity.

### 3.3.3 $^{14}\text{C}$ Radio-isotope Tracking

A second isotopic technique that can be used to assess biological activity at NAS Alameda sites is the tracking of radio-isotopes such as  $^{14}\text{C}$  (SOP 24.4).  $^{14}\text{C}$  is a radioactive isotope of carbon produced by interaction of cosmic rays with the upper atmosphere. Because of the relatively short half-life of  $^{14}\text{C}$  (5730 years), it is essentially undetectable in natural samples formed more than

40,000 years ago. Organic contaminants are exclusively manufactured from those fossil carbon sources and because of this, the carbon involved with biological transformations of these compounds will be largely depleted in  $^{14}\text{C}$ . Soil gas  $\text{CO}_2$  and  $\text{HCO}_3^-$  in groundwater sampled in areas where fossil fuel derived contaminants are being actively broken down by microbial transformation will be correspondingly depleted of  $^{14}\text{C}$ , as compared to  $\text{CO}_2$  and  $\text{HCO}_3^-$  produced by the metabolic breakdown of relatively young soil organic carbon (Conrad et al, 1994 and Conrad et al, 1996). Trace level radio-isotope measurements will be used in this Treatability Study to differentiate between biodegradation of natural organic matter and that of anthropogenic contaminants in order to track the products of fossil fuel degradation in water and soil samples from the field. This will provide direct evaluation of in situ bioremediation.

### **3.3.4 Physical / Chemical Assays**

Together with the microbial assays, a comprehensive set of analyses will be performed to assess the subsurface physical/chemical conditions that are critical to microbial growth. These analyses will determine the presence and in situ concentrations of organic contaminants, primary respiration substrates (oxygen, nitrate, and iron), and selected nutrients (nitrogen and phosphorus). In addition to substrates, the subsurface temperature, pH, salinity (conductivity), soil moisture content, and the oxidation/reduction conditions will be assessed. Collectively these assays provide basic information on growth conditions and the possibility of substrate, nutrient or other chemical limitations to intrinsic bioremediation. These assays are particularly applicable to NAS Alameda sites 3 and 13 where higher concentrations of readily degradable contaminants are present.

## **4. FIELD SAMPLING PLAN**

Soil, soil gas, and/or groundwater samples will be collected and analyzed during the Preliminary Screening phase, the Background Level and Contaminant Area Characterization phase, and the Periodic Monitoring phase of the Treatability Study. Different sampling methods will be used during each of the three phases and these methods are described below. The Site Health and Safety Plan that has been prepared for these field activities is included in Appendix B.

### **4.1 PRELIMINARY SCREENING**

During this phase of the Treatability Study, a minimum of three soil borings will be installed at each site in the locations shown on Figures 2-4 and 2-10 for the collection of soil samples. Soil gas and groundwater samples will also be collected from stainless steel tubing inserted into the soil adjacent to each soil boring location. One soil boring at each site will be located outside of the area

expected to be contaminated and two soil borings at each site will be located within the area expected to be contaminated. Soil gas and groundwater samples will also be collected at three intermediate locations.

Boring locations for this phase were selected on the basis of the results of previous investigations at Sites 3 and 13. As indicated on Figure 2-5, Boring 3PS1 at Site 3 is located outside of the area where elevated THC and benzene levels were indicated during the previous soil vapor survey at the site. Borings 3PS2 and 3PS3 are located along a line with this boring in areas where the highest THC and benzene levels were identified during the soil vapor survey. As indicated on Figure 2-10, Boring 13PS1 at Site 13 is located outside of the area where hydrocarbons have previously been identified in the soil. Borings 13PS2 and 13PS3 are located along a line with this boring in areas where the highest hydrocarbon concentrations were previously identified in the soil.

As stated above, the existing estimate of contaminant boundaries is based on the results of previous investigations. However, conditions could have changed over time due to migration or breakdown of contaminants since the time that the sampling was conducted. Prior to proceeding to the Background Level and Contaminant Area Characterization phase it will be necessary to establish the existing contaminant boundaries so that the locations for that phase of the Treatability Study can be accurately selected.

The data obtained from each location sampled during the Preliminary Screening phase will be reviewed and compared to identify the existing boundary of contamination at each site prior to initiation of the Background Level and Contaminant Area Characterization phase. If additional data are required to identify this boundary, additional locations may be sampled to provide better delineation. The specific locations will be determined on the basis of field analyses done at the time of sampling.

#### **4.1.1 Soil Gas Sampling Methods**

Prior to installation of soil borings, soil gas samples will be collected adjacent to each of the boring locations and at three intermediate locations. At each location, 1/4" stainless steel tubing will be driven directly into the soil to two depths to allow collection of soil gas samples from sampling intervals within the unsaturated zone and the capillary fringe. Soil gas sampling methods are described in Section 4.4. If necessary to characterize the flux of gasses within the unsaturated zone, soil gas samples may also be collected from intermediate depths by driving additional steel tubing and collecting soil gas samples as described in Section 4.4.

### **4.1.2 Groundwater Sampling Methods**

Prior to installation of soil borings, groundwater samples will also be collected from each soil gas sampling site by driving 1/4" steel tubing directly into the soil to the desired sampling depth. Groundwater samples will be collected from the top of the water table and an intermediate depth using the methods described in Section 4.5.

### **4.1.3 Soil Boring Methods**

A hand held coring tool will be used to install each boring to a total depth of 6 feet. This depth is approximately one foot below the anticipated depth of the water table and will allow the collection of soil samples from the vadose zone, capillary fringe, and from below the water table. Upon completion of sampling, the boring will be abandoned as described in Section 4.8. Drill cuttings will be contained in 55-gallon drums and appropriately labeled for subsequent disposal.

### **4.1.4 Soil Sampling Methods**

During advancement of each boring, soil samples will be collected with a 2.5 cm diameter hand coring tool. Upon retrieval from the boring, each core will be photographed and logged using a standard core log form (included in SOP 24.4, Appendix A) noting changes in color, soil composition and contaminant content.

### **4.1.5 Analytical Plan**

Soil gas, groundwater, and soil samples from each location will be characterized using the Stable Isotope Ratio Monitoring and <sup>14</sup>C Radio Isotope Tracking assays (SOP 24.4). Table 4-1 summarizes the number of samples to be characterized by this assay during the Preliminary Screening phase.

### **4.1.6 Decontamination Procedures**

All sampling and augering equipment used during the Preliminary Screening phase will be decontaminated prior to and between uses using the decontamination procedures for non-sterile sampling described in Section 4.6.

## **4.2 BACKGROUND LEVEL AND CONTAMINANT AREA CHARACTERIZATION**

During this phase of the Treatability Study, four soil borings will be installed within the contaminated area and two soil borings will be installed within the background area at each site; the locations for the borings will be selected on the basis of the results of the Preliminary Screening

phase described in Section 4.1. Soil samples and groundwater samples for physical/chemical characterization will be collected from these borings. Soil gas and groundwater samples for isotope assays will be collected from adjacent probes.

Soil gas and groundwater samples will be collected from additional locations and analyzed to provide additional information on contaminant fate and the extent of subsurface biological activity; the locations for these additional samples will be identified in the field on the basis of the results of the initial samples. If large differences of biological activity are indicated between two existing sampling locations, an additional location will be placed between them to provide better delineation of the biologically active zone. If no large difference in biological activity is indicated, the additional sampling locations will be randomly selected.

#### **4.2.1 Soil Gas Sampling Methods**

Soil gas samples will be collected from adjacent to each soil boring location prior to installation of the boring. To collect the samples, 1/4" stainless steel tubing will be driven directly into the soil to three depths to allow collection of soil gas samples from the planned soil sampling intervals within the shallow soil zone, vadose zone, and the capillary fringe. Soil gas sampling methods are described in Section 4.4. If necessary to characterize the flux of soil gas within the unsaturated zone, soil gas samples may also be collected from intermediate depths by driving additional steel tubing and collecting soil gas samples as described in Section 4.4.

In addition, at three locations per site, a series of more closely spaced soil gas and groundwater samples will be collected in order to constrain diffusion calculations that will be performed as part of the modeling effort described in Section 6. The exact number and spacing of these samples will be determined after data from the Preliminary Screening phase has been collected and evaluated.

#### **4.2.2 Soil Boring Methods**

Borings for soil and groundwater sample collection will be drilled using a truck mounted drilling rig equipped with six to eight-inch outside diameter hollow-stem augers or a portable, hydraulically driven soil coring system. The portable soil coring system utilizes two nested sampling rods that are driven simultaneously. The small-diameter inner rod is used to obtain and retrieve sample cores. The larger rod serves as a temporary drive casing to prevent soil caving into the boring.

At each planned location, a test boring will be drilled and continuous soil samples will be collected in an acrylic sampling tube to visually identify the subsurface lithology, zone(s) of contamination, and depth of the water table. The information obtained will be used to identify the best sampling intervals for collection of soil samples to be assayed.

Upon completion of sampling, the drilling equipment will be removed from the boring and the boring will be abandoned as described in Section 4.8. Any drill cuttings and discarded samples will be contained in 55-gallon drums and appropriately labeled for subsequent disposal. Drill cuttings are not produced using the portable, hydraulically driven soil coring system.

#### **4.2.3 Soil Sampling Methods**

Soil samples to be assayed will be collected from depths that represent the shallow soil, vadose zone, capillary fringe zone, a shallow water table depth, and median water table depth. Soil samples from borings drilled with hollow stem augers will be collected using a two-inch diameter split spoon sampler lined with three-inch long precleaned stainless steel or brass tubes. This soil sampling procedure is described in SOP 3.2. Soil samples from borings drilled with the portable, hydraulically driven coring system will be collected in 1-1/2 inch diameter by three-inch long precleaned stainless steel or brass tubes using a sample barrel attached to the inner rod which is advanced during drilling. All recovered soils will be logged in the field by the project geologist, under the supervision of a registered geologist, using the Unified Soil Classification System (USCS).

#### **4.2.4 Groundwater Sampling Methods**

Groundwater samples for characterization by field physical/chemical assays will be collected from within the shallow water table depth and the intermediate water table depth in each boring by placing a prefilter packed 2-inch diameter slotted PVC within the boring and pumping a minimum of three casing volumes of liquid with a submersible pump. The groundwater sample will then be collected directly into the appropriate sampling containers. Purged groundwater will be contained in 55 gallon drums and appropriately labeled for subsequent disposal.

Groundwater samples for isotopic assays from adjacent to and between the soil boring locations will be collected by driving 1/4" steel tubing to the shallow water table and an intermediate depth within the saturated zone then using the groundwater sampling methods described in Section 4.5.

#### **4.2.5 Analytical Plan**

Soil samples collected during the Background Level and Contaminant Area Characterization phase will be characterized by microbial enrichment, field physical/chemical, direct epifluorescent microscopy, and microcosm assays (SOPs 24.1, 24.5, 24.3, and 24.2). Groundwater and soil gas samples will be characterized by field physical/chemical and isotopic assays (SOPs 24.5 and

24.4). Table 4-1 includes the number of samples that will be characterized by each assay during this phase.

#### **4.2.6 Decontamination Procedures**

Soil sampling equipment used for the collection of soil samples for microbial analyses (microbial enrichment, microcosm, and direct epifluorescent assays) will require sterilization prior to use to prevent the introduction of microorganisms from other sources. Sterilization procedures are described in Section 4.7.

Drilling equipment and sampling equipment for the collection of samples for nonmicrobial analyses (isotope and field physical/chemical assays) do not require sterilization. They will be decontaminated prior to and between uses according to the procedures described in Section 4.6.

### **4.3 PERIODIC MONITORING**

Soil gas and groundwater samples will be collected at two additional times near the sampling sites chosen for the Background Level and Contaminant Area Characterization phase (Section 4.2). These two additional sample sets, together with the sample set obtained from the Background Level and Contaminant Area Characterization phase, will allow assessment of the effects of seasonal changes in temperature, soil moisture and groundwater levels on subsurface biological activity. Nominally, the goal will be to have one set of samples collected prior to the rainy season, one set during the height of the rainy season and one set shortly after the rainy season, although time constraints may not allow this exact schedule. Periodic sampling will provide information on changes in material fluxes over time.

During the Periodic Monitoring phase, soil gas samples will be collected from within the unsaturated zone and the capillary fringe. Soil gas sampling methods are described in Section 4.4. One groundwater sample will also be collected from the top of the saturated zone at each soil gas sampling site during this phase. Groundwater sampling methods are described in Section 4.5. The soil and groundwater samples will be characterized using isotope assays (SOP 24.4). Between uses, the sampling equipment will be decontaminated using the non-sterile procedures described in Section 4.6.

### **4.4 SOIL GAS SAMPLING METHODS**

During each phase of the Treatability Study, soil gas samples will be collected using a peristaltic pump to purge at least three tubing volumes of soil gas through Teflon or other nonadsorbing

tubing at a flow rate of  $\leq 100$  cc/minute. Approximately one liter of soil gas will then be collected directly into a Tedlar bag.

#### **4.5 GROUNDWATER SAMPLING METHODS FOR ISOTOPE ASSAYS**

During each phase of the Treatability Study, groundwater samples for isotope assays will be collected using a peristaltic pump to purge approximately three tubing volumes of groundwater through Teflon or other nonadsorbing tubing into a volumetrically scored liquid container. Approximately 100 ml of groundwater will then be collected directly into the sample vials. Where groundwater nitrate will be analyzed, additional water will be pumped and passed through anion columns in the field. Both groundwater samples and anion columns will be tightly capped and stored at  $<4^{\circ}\text{C}$  until analyses can be completed. Purged groundwater will be contained and transferred to a 55 gallon drum for collection of all liquid Treatability Study field wastes and appropriately labeled for subsequent disposal.

#### **4.6 DECONTAMINATION PROCEDURES FOR NON-STERILE SAMPLING**

All sampling and augering equipment used for non-sterile sampling (samples for characterization by isotope and field physical/chemical assays) will be decontaminated prior to and between uses to minimize the potential for introduction of off-site contaminants as well as cross contamination of samples. The equipment will be decontaminated by washing with a solution of tap water and non-phosphate detergent such as Lacunas or equivalent. Next the equipment will be rinsed in succession with tap water, isopropanol, and deionized water. Wastewater generated during decontamination will be containerized in 55 gallon DOT approved drums and appropriately labeled. These decontamination procedures are described in SOP 6.2.

#### **4.7 DECONTAMINATION PROCEDURES FOR STERILE SAMPLING**

Sampling equipment used for the collection of soil samples for microbial assays (microbial enrichment, microcosm, and direct epifluorescent microscopy assays) will be sterilized. To sterilize the soil samplers and brass tubes, the ends will be covered with foil and they will be autoclaved at 18 psi and  $121^{\circ}\text{C}$  for 20 minutes. Plastic caps for the sample tubes will be sterilized by immersion in 80% ethanol and wrapped in sterilized foil. Upon sterilization, the caps and the autoclaved soil samplers will be kept in sterile plastic bags and stored in a container cleaned with bleach. To prevent contamination of the soil samplers and caps, they will be kept in the sealed containers until ready for use in the field.

Between uses, the non-sterilized soil sampling equipment will be steam cleaned, washed with 1% bleach solution or 80% ethanol and steam cleaned again. The sampler will be allowed to cool to ambient temperatures before loading sterile brass sample tubes.

#### **4.8 BORING ABANDONMENT**

At the completion of sampling during each phase of the Treatability Study, borings used for collection of soil and groundwater samples as well as those for the collection of soil gas samples will be appropriately abandoned by filling the boring with a cement slurry containing no more than five percent bentonite.

#### **4.9 WASTE DISPOSAL**

Waste materials to be disposed of during the Treatability Study include drill cuttings, decontamination fluids, and purged groundwater. These materials will be containerized in DOT - approved 55 gallon drums and sampled for the parameters indicated in Table 4-2 for disposal. These analyses will be performed by an outside commercial laboratory. In the absence of existing on-site treatment facilities, off-site disposal is considered the most economical disposal method for the small quantities of waste that will be produced.

#### **4.10 FIELD NUMBERING**

All sample locations used during the Treatability Study will be identified by a unique number consisting of the site number followed by two letters indicating the phase of the Treatability Study during which the sample was collected and then a sample location number (i.e. 3PS1, 3PS2... and 13PS1, 13PS2...). Sample locations at each site will be numbered sequentially as they are chosen. The letters to be used are as follows:

- Preliminary screening phase - PS
- Background level determination - BG
- Contaminant area characterization - CA
- First periodic sampling interval - FP
- Second periodic sampling interval - SP

Samples collected during the Treatability Study will be numbered with a unique alphanumerical identification as specified in SOP 17.2. Soil samples from borings will be identified with the identifier of the sample location followed by a dash, then the letters SO, then a dash, then the depth of the top of the sampling interval in cm (i.e. 3CA1-SO-6.5). Groundwater samples will be identified with the identifier of the sample location followed by a dash, then the letters GW, then a dash, then the depth of the top of the sampling interval in cm (i.e. 3CA1-GW-6.5). Soil gas

samples will be identified with the identifier of the sample location followed by a dash, then the letters SG, then a dash, then the depth of the top of the sampling interval in cm (i.e. 3CA1-SG-6.5).

#### **4.11 SAMPLE HANDLING**

After collection, each sample will be labeled using indelible ink with the following information as specified in SOP 17.1:

- Project name
- Date and time of collection
- Sample location
- Sample identification number
- Collector's name
- Preservatives used, if any

The samples will be handled and packaged in the field following the requirements of SOP 2.1. The sample container will be tightly sealed immediately following collection of the sample and a piece of custody tape will be placed over or around the cap. Sample containers and storage requirements are identified in Table 4-3.

Each sample will then be placed in a seam-sealing polyethylene bag and excess air will be removed. Samples will be placed in a cooler with ice. The samples will be picked up daily by the laboratory or delivered daily to the laboratory by field personnel using proper chain-of-custody procedures described in SOP 1.1. Documentation of final disposition of all samples collected will be provided to the U.S. Navy.

### **5. QUALITY ASSURANCE PROJECT PLAN**

This section presents the quality assurance/quality control procedures to be followed for the Treatability Study at Sites 3 and 13. The project quality objectives are to provide field and laboratory data of sufficient quality to demonstrate the occurrence of intrinsic bioremediation at each site. The specific objectives are to:

- establish quality control criteria to monitor and assess the quality of measurement data.

- meet the project objectives (identified in Section 1.2) so that data can be used for their intended purpose.

## **5.1 DATA QUALITY OBJECTIVES**

Data quality objectives (DQOs) for this project have been established in accordance with the procedures described in Section 8.3.3 of the Contractors Quality Control Program Plan (CQCPP). Identification of DQOs includes seven stages. Each stage of this process is identified on Table 5-1 with a description of how the DQOs are met for this Treatability Study. These DQOs describe how the data will be applied to provide specific information required for the Treatability Study and how it will be used to make decisions regarding the precision, accuracy, representativeness, completeness, and comparability of the data obtained.

The DQO process is generally based on obtaining data for use in risk assessment and remediation decision making, but generally applies to all data collection activities. The data obtained from this Treatability Study are primarily intended to evaluate a remedial alternative, intrinsic bioremediation, rather than the nature and extent of contamination, as in a remedial investigation. The data quality required for evaluation of remedial alternatives is generally less than that required for evaluating the nature and extent of contamination.

## **5.2 QUALITY CONTROL CRITERIA**

Project quality control criteria will include precision, accuracy, representativeness, completeness, and comparability (PARCC) parameters. Project specific limits for the assays are addressed in the SOPs prepared for each assay (Appendix A), as appropriate. Each of the participating laboratories (from UCB, LLNL and LBNL) will be independently responsible for quality control and the quality control data will be independently reviewed by the project chemist.

### **5.2.1 Analytical Procedures**

The analytical program for the Treatability Study includes the innovative and recently developed chemical and microbial assays described in Section 3 as well as standard analytical techniques. These assays include:

- Microbial enrichment
- Microcosm assays
- Direct epifluorescent microscopy
- Stable isotope ratio monitoring

- <sup>14</sup>C Radio-isotope tracking
- Field and laboratory physical/chemical assays

The number of samples that will be characterized using each assay and the responsible laboratory is summarized in Table 4-1. All assays will be performed by qualified and experienced graduate students, scientists or technicians under the direction of a principal investigator. All methods and results will be supervised and reviewed by the senior staff scientists and/or university professors at the respective laboratories.

The isotope and microcosm assays also include analysis of the soil samples using standard EPA methods. These methods are identified in SOPs 24.2 and 24.4. The standard analyses will be performed at the LBNL and UCB laboratories under the supervision of a Co-Principal Investigator.

Wastes produced during field activities for the Treatability Study will also be characterized for disposal purposes using standard analytical techniques at a commercial laboratory. The analyses that will be performed are summarized in Table 4-2.

### 5.3 SAMPLE CUSTODY

Throughout field sampling activities, proper chain of custody procedures will be followed to demonstrate that samples were obtained from the locations stated and that they have reached the laboratory without alteration. Documentation of this will be accomplished using the Chain of Custody Record (COC) provided in Figure 5-1. Chain of custody procedures will be implemented in accordance with SOP 1.1 and as described in Section 9.6 of the CQCPP. Copies of the completed COCs will be provided to the Project Chemist and maintained in the project files by serial number.

### 5.4 CALIBRATION AND MAINTENANCE OF MEASURING AND TEST EQUIPMENT

Anticipated laboratory and field measuring and test equipment (M&TE) that will require calibration is identified in the SOPs included in Appendix A. Calibration and preventive maintenance procedures for this equipment, and any additional equipment that may be required for the project, are addressed in Section 15 of the CQCPP and in SQP 8.2, *Calibration and Maintenance of Measuring and Test Equipment*. Specific calibration and preventative maintenance procedures for each assay are described in the SOPs provided in Appendix A. The Contractor Project Manager

will ensure that all equipment used in activities affecting quality will be calibrated according to these methods and procedures; laboratory directors, laboratory personnel, field superintendents, and field personnel will be responsible for implementing the procedures. Each item in the calibration program will be uniquely identified to assure its calibration status and identify the recalibration due date.

M&TE will be calibrated prior to use at the project site and at prescribed intervals thereafter, including at the completion of field work each day, in accordance with the manufacturers recommendations. Calibrations will be performed by trained and qualified personnel. Records of calibration will be maintained by the Project Chemist for the items used on site. The laboratories will be required to implement an effective and documented document control program for M&TE used to perform the analyses. The calibration program will be audited by the project chemist to verify conformance to laboratory protocols and project requirements. Calibration of each piece of equipment will be recorded on the Test Equipment List and Calibration form provided on Figure 5-2.

## **5.5 QUALITY CONTROL SAMPLES**

QC sampling will be performed for this project to monitor and assess the quality of laboratory and field procedures. QC samples to be collected include appropriate field and laboratory blanks and laboratory replicate samples. Laboratory replicate samples will be characterized at the frequency summarized in Table 5-2.

Laboratory QC samples for the conventional laboratory methods include method blanks, laboratory control samples, laboratory duplicate samples, and surrogate spikes. The frequency and method for analyses of these samples is addressed in Section 10 of the CQCPP.

## **5.6 SAMPLE COLLECTION, PRESERVATION AND HOLDING TIMES**

Samples to be collected during the Treatability Study include soil gas, soil, and groundwater samples. The numbers of samples to be analyzed and the required analyses are specified in Tables 4-1 and 4-2. The required containers, preservation methods, and holding times for the Treatability Study assays are summarized in Table 5-3 and specified in SOPs 24.1 through 24.4 in Appendix A. The required containers, preservation methods, and holding times for the waste samples are summarized in Table 5-4.

## **5.7 SAMPLE COLLECTION LOGBOOK.**

A sample collection logbook will be filled out for all samples collected. The sample collection logbook will include the following information:

- collection date and time
- project name
- unique sample number
- sample location and type
- container type and preservative
- compositing information
- depth of sample
- weather
- field observations
- problems encountered
- name of sample collector

Copies of the sample collection logbooks will be given to the Project Chemist to be filed in the project files.

## **5.8 DATA REDUCTION, VALIDATION, AND REPORTING**

The procedures and data for each assay will be documented in bound laboratory notebooks and Excel spreadsheets. Recorded data will be transferred to the computer within one month of collection and cross checked for accuracy. Data will be reported on the forms specified in each SOP included in Appendix A or other specific data management procedures for each assay as addressed in these SOPs.

In accordance with the requirements of Section 10.9.1 of the CQCPP, each laboratory will reduce the analytical data for standard analyses using procedures described in U.S. EPA document SW-846 (U.S. EPA, 1994c). The data will be verified by the laboratory and the Project Chemist according to the requirements of Section 10.9.2 of the CQCPP.

The laboratory will report all results in laboratory reports which will include the following at a minimum:

- a case narrative
- copies of COC forms
- analytical results for all samples included on the COC including dilutions and reanalyses and the laboratory detection limits used.
- analytical results for all required laboratory quality control samples

Independent of the laboratory review, the Project Chemist will perform data validation for ten percent of all analyses performed by standard analytical methods as specified in Section 10.10 of the CQCPP. The procedures to be used for data validation are contained in USEPA Contract Laboratory Program National Functional Guidelines Organic Data Review (U.S. EPA, 1994a) and in USEPA Contract Laboratory Program National Functional Guidelines for Inorganic Data Review (U.S. EPA, 1994b). For parameters that are not included in these guidelines, the evaluation will be performed following HAZWRAP in DOE-HWP-65/RI.

## **5.9 QUALITY CONTROL MEETINGS**

The Project Chemist will ensure that the following meetings are conducted in accordance with the requirements of Section 3.0 of the CQCPP:

- A coordination and mutual understanding meeting, held prior to the start up of the Treatability Study.
- Quality control meetings, held quarterly following the start up of the Treatability Study. The Project Chemist will submit meeting notes to the U.S. Navy Remedial Project Manager within one week of each meeting.

## **6. APPROACH TO MODELING**

Intrinsic bioremediation may be limited by a number of factors including the availability of microorganisms, substrates, nutrients, and most frequently, electron acceptors that promote oxidation, such as oxygen. Quantitative modeling will be used to assess the transport of hydrocarbon substrates and oxygen to microbial communities. Modeling of in-situ bioremediation process requires either an assessment of microbial transformation rates under ambient conditions or

determination of substrate delivery rates and formation rates of transformation products. The approach here is to use the latter approach.

The conceptual model for intrinsic bioremediation is shown in Figure 6-1. Overall, the hydrocarbons (HC) are reacted with molecular oxygen (O<sub>2</sub>) to form carbon dioxide (CO<sub>2</sub>), water, and provide bacterial growth. Hydrocarbons trapped below the water table will have oxygen supplied by groundwater flowing into the contaminated site. Additionally, some hydrocarbons and carbon dioxide will be carried by the groundwater flow leaving the contaminated site. Many of the hydrocarbons of concern will partition from the groundwater into the soil gas in the vadose zone and diffuse towards locations with lower concentrations because they are volatile. In Figure 6-1, upward diffusion is indicated because the atmosphere has very low concentrations of these compounds. Also within the vadose zone, molecular oxygen will diffuse downward to a region where an active microbial "filter" is expected to form as a passive barrier. Such in-situ biofilters have been reported in the literature for similar sites (Ostendorf and Kampbell, 1991) and have been in evidence at site 7C at NAS Alameda. The microbial biofilter oxidizes the hydrocarbons to carbon dioxide and water, with the carbon dioxide diffusing to the atmosphere where the concentration is much lower.

While quantitative models of the above processes of groundwater flow and gas phase diffusion can be very complex, our initial approach is to use a practical model in order to understand the experimental observations and make future estimates. A one-dimensional model based on Fick's law for molecular diffusion will be used to simulate the transport of hydrocarbons, oxygen, and carbon dioxide within the vadose zone of the soil. One dimensional groundwater flow will be modeled by Darcy's law to quantify the transport of oxygen dissolved in groundwater to the contaminated site, and to predict the resulting amounts of carbon dioxide and hydrocarbons in the groundwater leaving the site.

The simultaneous measurements of hydrocarbons, oxygen, and carbon dioxide (obtained during the treatability study) will provide an assessment of substrates, the dominant electron acceptor and the chief transformation product, respectively. Profiles of these quantities are input to the models to provide a three fold check of in situ degradation rates and verification of reaction stoichiometry. Estimated groundwater flow velocities are also needed as input to the model.

Soil gas transport by molecular diffusion is readily estimated. Only published values of gas phase molecular diffusivities are needed, along with simple measurements of water content in the vadose zone, to assess the effective diffusivity in the soil (Karimi et al, 1987). In the vadose or unsaturated zone, oxygen supply rates will be determined from measurements of oxygen profiles in the soil column following the approach of Ostendorf and Kampbell (1991). This approach is

easily extended to measuring the release of carbon dioxide to the atmosphere by simultaneous measurement of gas phase carbon dioxide levels combined with isotopic assays to determine the contribution of fossil hydrocarbon compared to the degradation of the more recent plant and animal matter (obtained using the methods identified in SOP 24.4).

Assuming that the water table is stationary, measurements of gas phase composition will be done at a spatial resolution to be determined by the results of soil gas sampling performed during the Preliminary Screening phase (Section 4.1) and implemented during Background Level and Contaminant Area Characterization phase (Section 4.2). If the water table is influenced by tidal action, the water table fluctuations may affect groundwater flow patterns and soil gas exchange. However, little tidal influence is expected at Sites 3 and 13 because they are located away from the shoreline and storm drains (PRC, 1995b).

If available, the groundwater modeling effort may utilize results generated by PRC in their fate and transport modeling effort. Either those modeling results or simple mass balance models calibrated with site water level history at monitoring wells will provide groundwater flow velocities through Sites 3 and 13.

Groundwater flow may be an important transport mechanism because groundwater carries oxygen to the site of biodegradation and carries away the decay product carbon dioxide and any organic products. If a complex flow regime is indicated, two dimensional modeling may be required. Factors which would require the use of a more complex two dimensional model include:

- a complex groundwater flow regime which may be influenced by infiltration, precipitation, and tidal pumping action; and
- complex boundary conditions, including the storm water collection system which may be a localized source of groundwater exchange.

The modeling effort will utilize input parameters having considerable uncertainty, such as the hydraulic conductivity of the water bearing materials and the effect of tidal variations in groundwater levels. A sensitivity analysis will be performed to determine the most important parameters and the level of accuracy needed for each parameter to provide an accurate model.

Field data are needed to provide an accurate assessment of many parameters that will be used to characterize the water bearing materials. The following information will be required:

- site specific hydraulic conductivities for the water bearing materials at Sites 3 and 13; and

additional water level data from selected monitoring wells to assess recharge and local groundwater flows induced by activities at NAS Alameda and the City of Alameda.

We have assumed that the U.S. Navy will supply this information or that reasonable estimates will be available from ongoing aquifer testing (PRC, 1995b). Provisions for obtaining this information are not included in the scope of work for the assessment of intrinsic bioremediation. Measurements of water levels will need to be made to the nearest 0.01 foot.

In addition to modeling of the transport of the contaminants of concern at Sites 3 and 13, the selective weathering of the contaminants will be evaluated, if warranted. For example, if polyaromatic hydrocarbons are of interest, then there might be a selective leaching by groundwater flow and biodegradation of naphthalene and phenanthrene compared to benzo-(a)-pyrene.

Times for destruction of contaminants present at each site are then estimated from modeled rates of degradation and the known extent of contamination. The results of the modeling will be interpolated to represent the contaminated site and arrive at an overall rate of contaminant removal. With this removal rate and measured concentrations of contaminants in various regions of the site, we can estimate how long the site will take to remediate.

## **7. REPORTING**

At the completion of the Treatability Study a report will be submitted to the U.S. Navy for review by the U.S. Navy, regulatory agencies, the RAB, and the BCT. The report will include an analysis and interpretation of the assays and modeling results. Prior to submittal, the report will receive a peer review. The review will be documented according to the methods specified in Section 12.3 of the CQCPP.

## **8. PROJECT MANAGEMENT**

### **8.1 SCHEDULE**

The implementation schedule for the Treatability Study is presented in Figure 8-1. Staging for the project will require approximately two months following notice to proceed from the U.S. Navy. The Preliminary Screening phase for Site 13 will begin at the completion of the Staging. Field sampling will be conducted during this phase and it will require approximately two weeks to complete the isotope assays. The data obtained during this phase will be reviewed to select appropriate sampling locations for the Background Level and Contaminant Area Characterization phase which will begin at Site 13 one month following the completion of staging.

The number of samples that can be handled at each laboratory during the Background Level and Contaminant Area Characterization phase will limit the number of samples collected in the field at one time. To avoid overloading the laboratories, two soil borings will be drilled every other week as indicated on Figure 8-1. Laboratory assays will be ongoing during this period and the last assays (microcosm and isotope assays) will be completed six weeks following the completion of field sampling.

The data obtained from the Treatability Study at Site 13 will be reviewed to identify any problems or procedures that may require refinement, and the Treatability Study at Site 3 will begin one month following the completion of all laboratory assays for Site 13. The Treatability Study at this site will follow the same phases as the study at Site 13.

Throughout the entire Treatability Study, modeling will be conducted to predict the rate and occurrence of bioremediation at Sites 3 and 13. All modeling will be completed within two months following the completion of all laboratory assays conducted for Site 3. The final report will be completed within three months following the completion of all laboratory assays conducted for Site 3.

## **8.2 ORGANIZATION AND RESPONSIBILITIES**

The organization chart for this Treatability Study is shown on Figure 8-2. The responsibilities for each of the positions identified on the organization chart are discussed in Section 2 of the CQCPP. Resumes for each person assigned to the project are included in Appendix C.

The Principal Investigator for this project will be Dr. Lisa Alvarez-Cohen of UCB. She will be responsible for technical oversight of the project and will be assisted by Co-Principal investigators from UCB, LLNL and LBNL. Dr. Alvarez-Cohen will also be in charge of all microbial analyses using direct epifluorescent methods. These assays will be carried out at the Civil and Environmental Engineering Laboratory at UCB.

Dr. Mark Conrad of the LBNL Earth Sciences Division will be the Co-Principal Investigator in charge of isotope monitoring. Dr. Conrad is the supervisor of the stable isotope facilities at Berkeley Center for Isotope Geochemistry (BCIG). The BCIG is a joint University of California Berkeley - Lawrence Berkeley National Laboratory facility directed by Professor Donald J. DePaolo of the Department of Geology and Geophysics at UCB and the Earth Sciences Division at LBNL. Dr. Conrad will directly oversee stable isotope monitoring assays. Dr. Paul Daley of

LLNL will be Co-Principal Investigator in charge of  $^{14}\text{C}$  radio isotope tracking assays. These assays will be performed at LLNL.

Paula Krauter of the Environmental Restoration Division of LLNL will be the Co-Principal Investigator in charge of the microbial enrichment assays. Dr. Hoi-Ying Holman of LBNL will be the Co-Principal investigator in charge of microcosm assays.

Dr. Jim Hunt of UCB will be Co-Principal Investigator of modeling.

Mary L. McDonald of E2 Consulting Engineers will act as the Contractor Project Manager. She will be responsible for overseeing implementation of the project and procuring necessary subcontractors. Ms. McDonald is supported by several technical and administrative positions as indicated on the organization chart.

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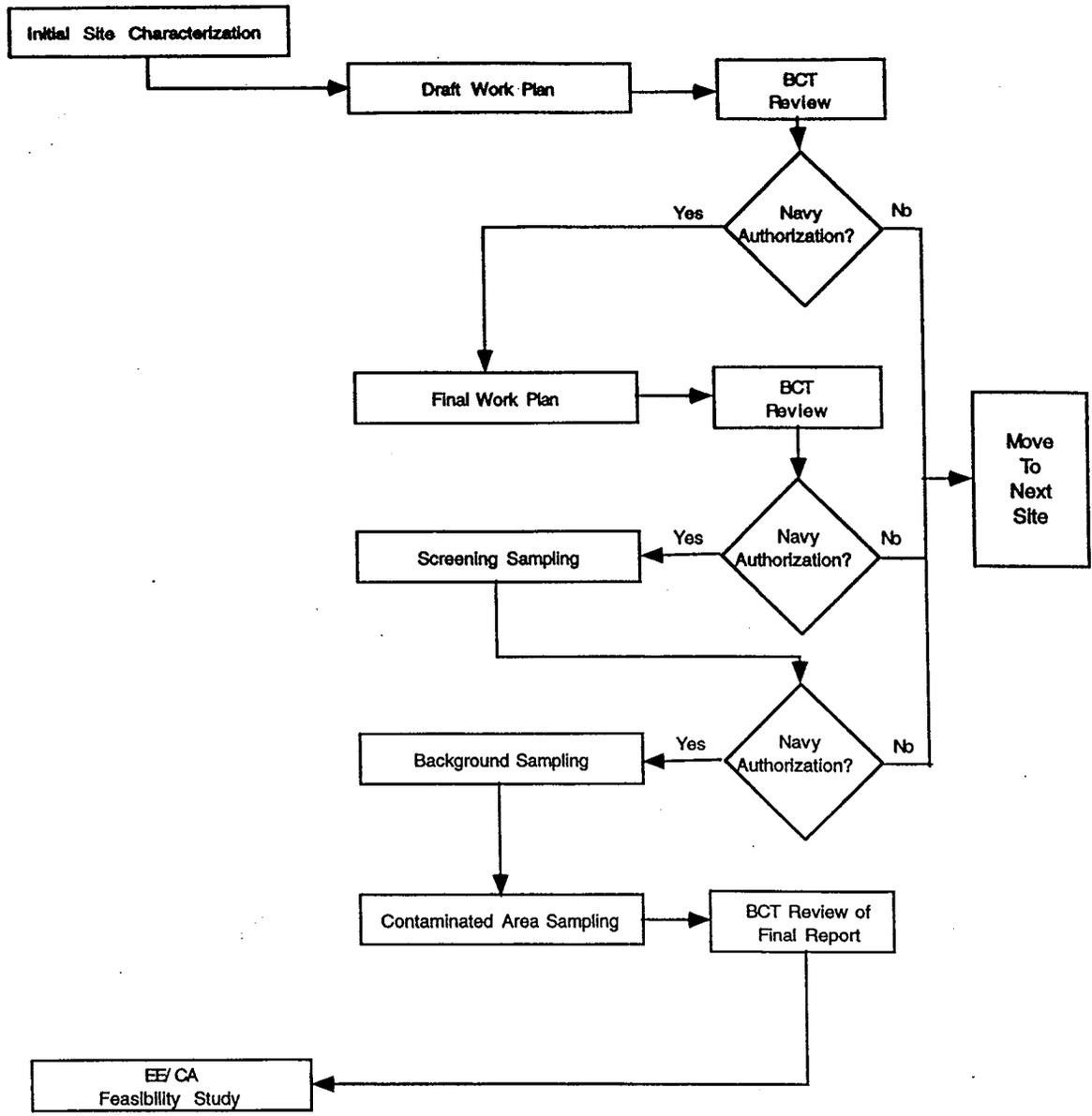
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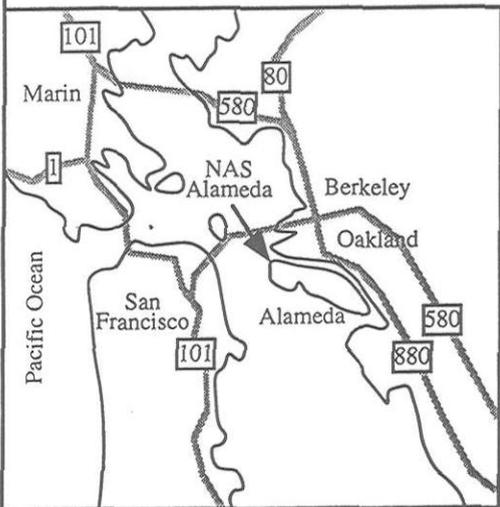
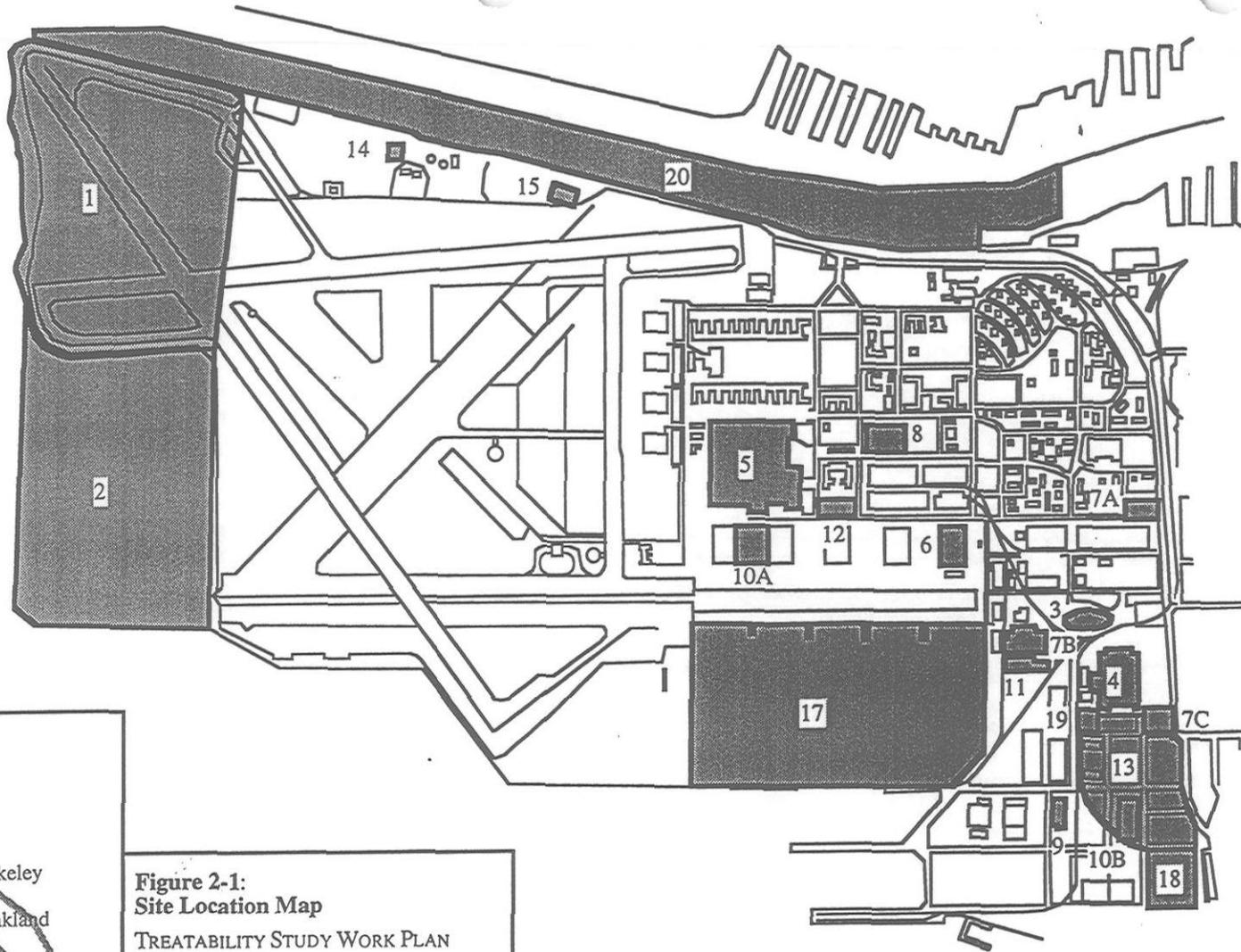
# FIGURES



**Figure 1-1:**  
**Treatability Study Decision Process**  
 TREATABILITY STUDY WORK PLAN  
 NAS ALAMEDA

Drawn By: WJAS	Date: 9 February 1996
Approved By: <i>[Signature]</i>	Project: Intrinsic Bioremediation
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**Figure 2-1:  
Site Location Map**

TREATABILITY STUDY WORK PLAN  
NAS ALAMEDA

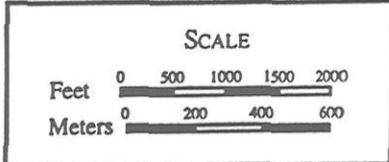
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MTI

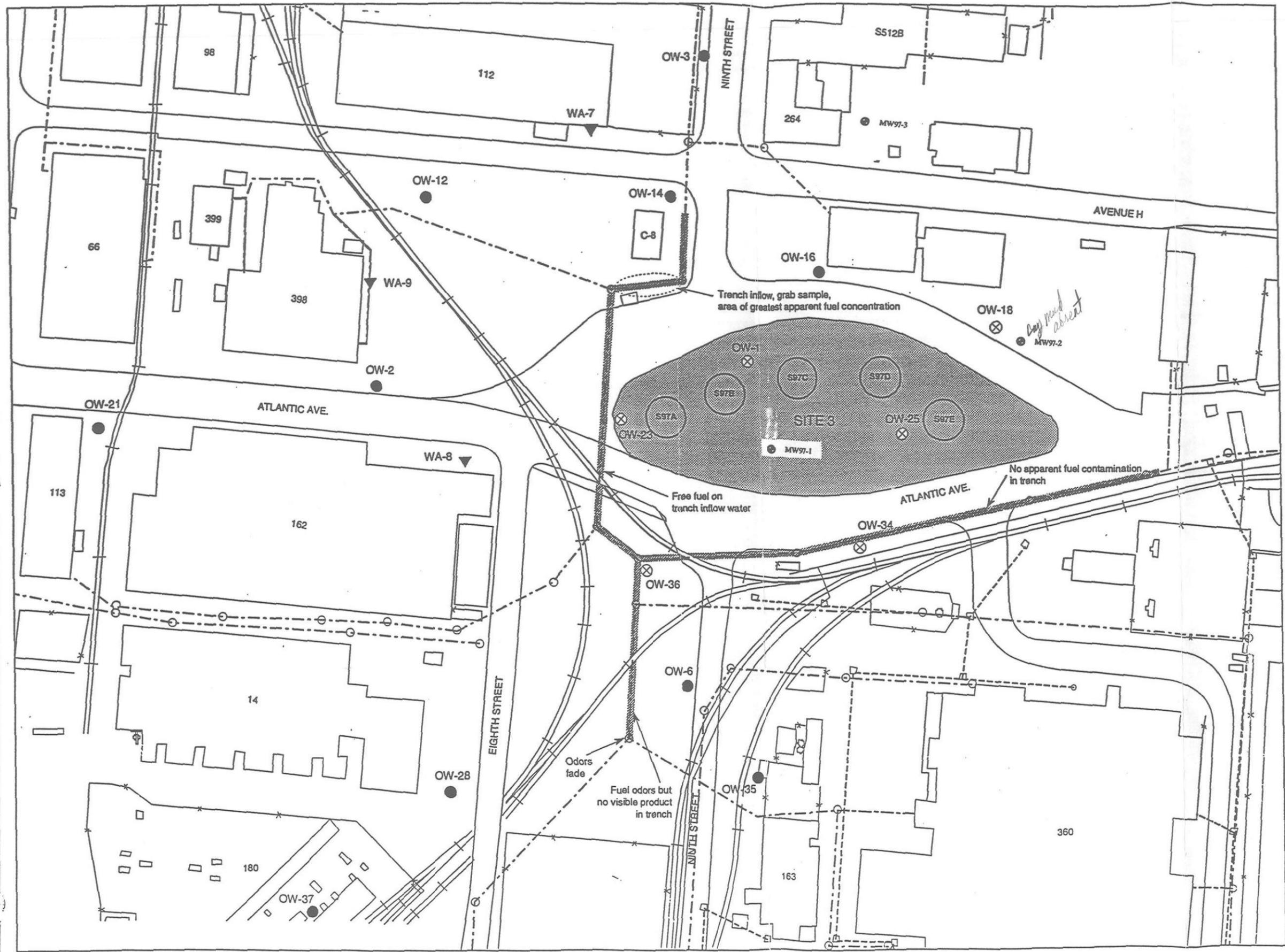
Date:  
9 February 1996

Approved By:  
*MLK*

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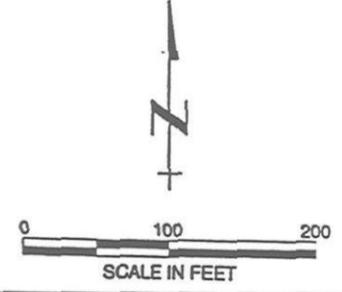




- LEGEND:**
- \*— Fence
  - - - Sanitary Sewer Line
  - Storm Sewer Line
  - +— Railroad
  - Manhole
  - ▬ Trench
  - Catch Basin
  - ▼ Wahler Well
  - Kennedy Well (Found by Wahler)
  - ⊗ Kennedy Well (Not Found by Wahler)
  - (SS7A) Former Fuel Storage Tank
  - Canonic Monitoring Well

**NOTES:**  
 Boring and monitoring well locations were obtained from a base map provided by Canonie Environmental, Inc. The individual locations were digitized onto a base map CAD file provided by NAS Alameda.

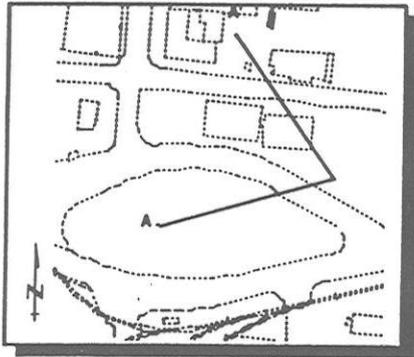
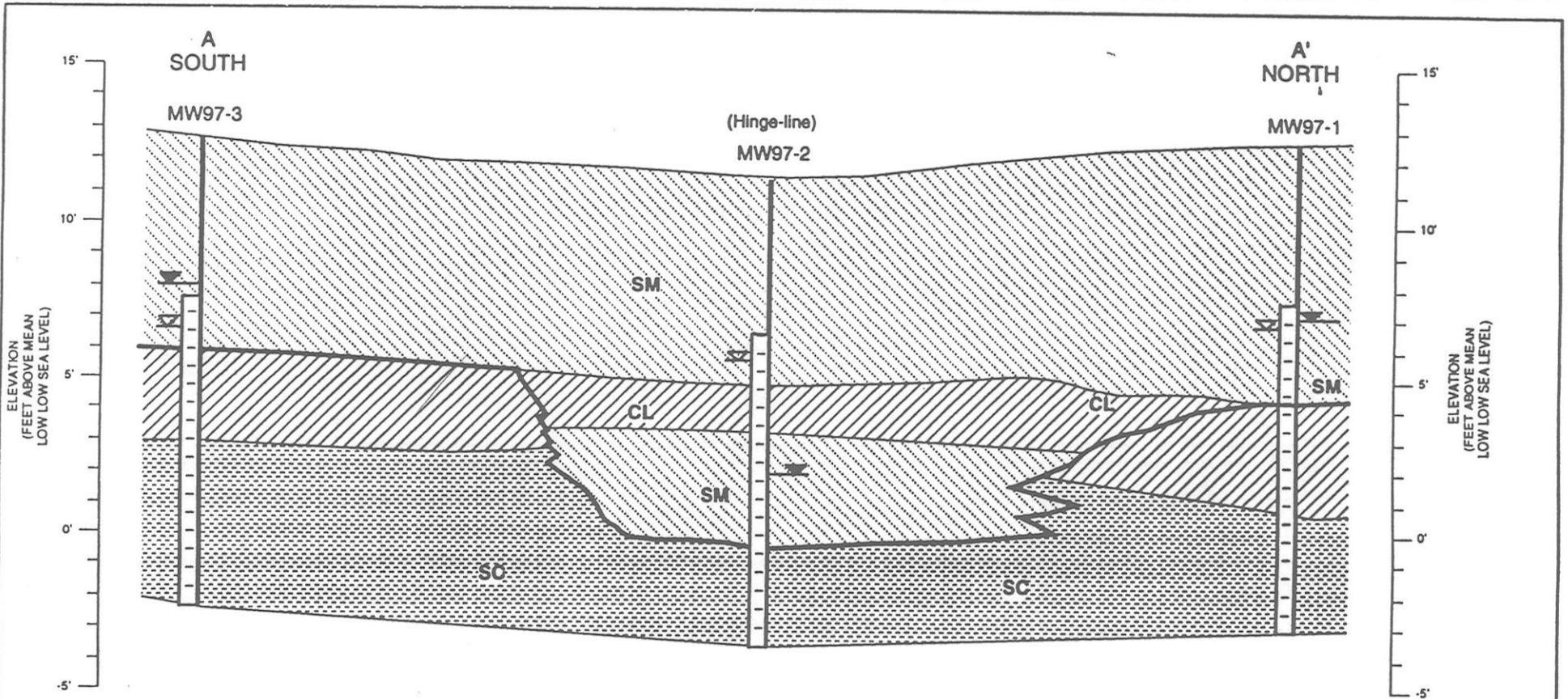
Trench location and comments, Kennedy and Wahler monitoring well locations approximated from Wahler Associates, Figure 6, May, 1985. Storage tank locations approximated from Canonie (1991).



**Figure 2-2:  
 Sampling Location Map, Site 3  
 TREATABILITY STUDY WORK PLAN  
 NAS ALAMEDA**

Drawn By:	Date: 9 February 1996
Approved By: <i>MW</i>	Project: Intrinsic Bioremediation

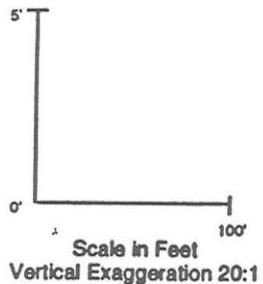
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This cross section is based on logs of wells and borings drilled and logged by Canonic Environmental, Inc. All water levels were measured by Canonic Environmental, Inc.

**LEGEND:**

- GP Gravelly Sand
- SP Sand
- SM Silty Sand
- SC Clayey Sand
- CL Clay
- AS Asphalt
- Approximate Fill/Native Sediment Interface
- Water Level During Water Sampling
- First Water During Drilling
- Monitoring Well
- Screened Interval



**Figure 2-3:**  
**Geologic Cross Section, Site 3**  
 TREATABILITY STUDY WORK PLAN  
 NAS ALAMEDA

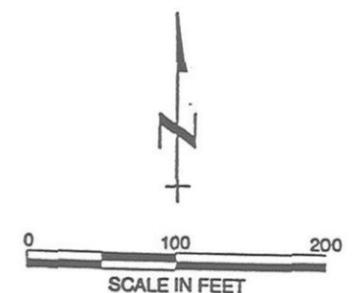
Drawn By: JH	Date: 9 February 1996
Approved By: <i>MCA</i>	Project: Intrinsic Bioremediation

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- LEGEND:**
- ◆ Canonie Monitoring Well Location
  - Canonie Soil Gas Survey Point
  - Benzene Concentration (µg/L)
  - \* 3PS1 Planned Sampling Location, Preliminary Screening Phase

**NOTE:**  
 Boring and monitoring well locations were obtained from a map provided by Canonie Environmental, Inc. The individual locations were digitized onto a base map CAD file provided NAS Alameda.



**Figure 2-4:**  
 Distribution of Benzene concentrations identified in soil vapor samples and planned sampling locations  
 TREATABILITY STUDY WORK PLAN  
 NAS ALAMEDA

Drawn By: JH	Date: 9 February 1996
Approved By: <i>[Signature]</i>	Project: Intrinsic Bioremediation

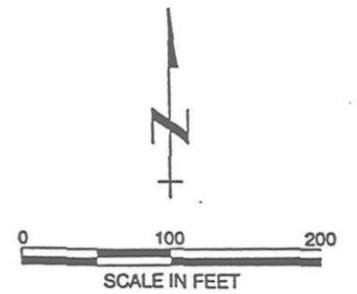
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**LEGEND:**

- ⊕ Canonie Monitoring Well Location
- Canonie Soil Gas Survey Point
- 0.1- THC Concentration (mg/L)

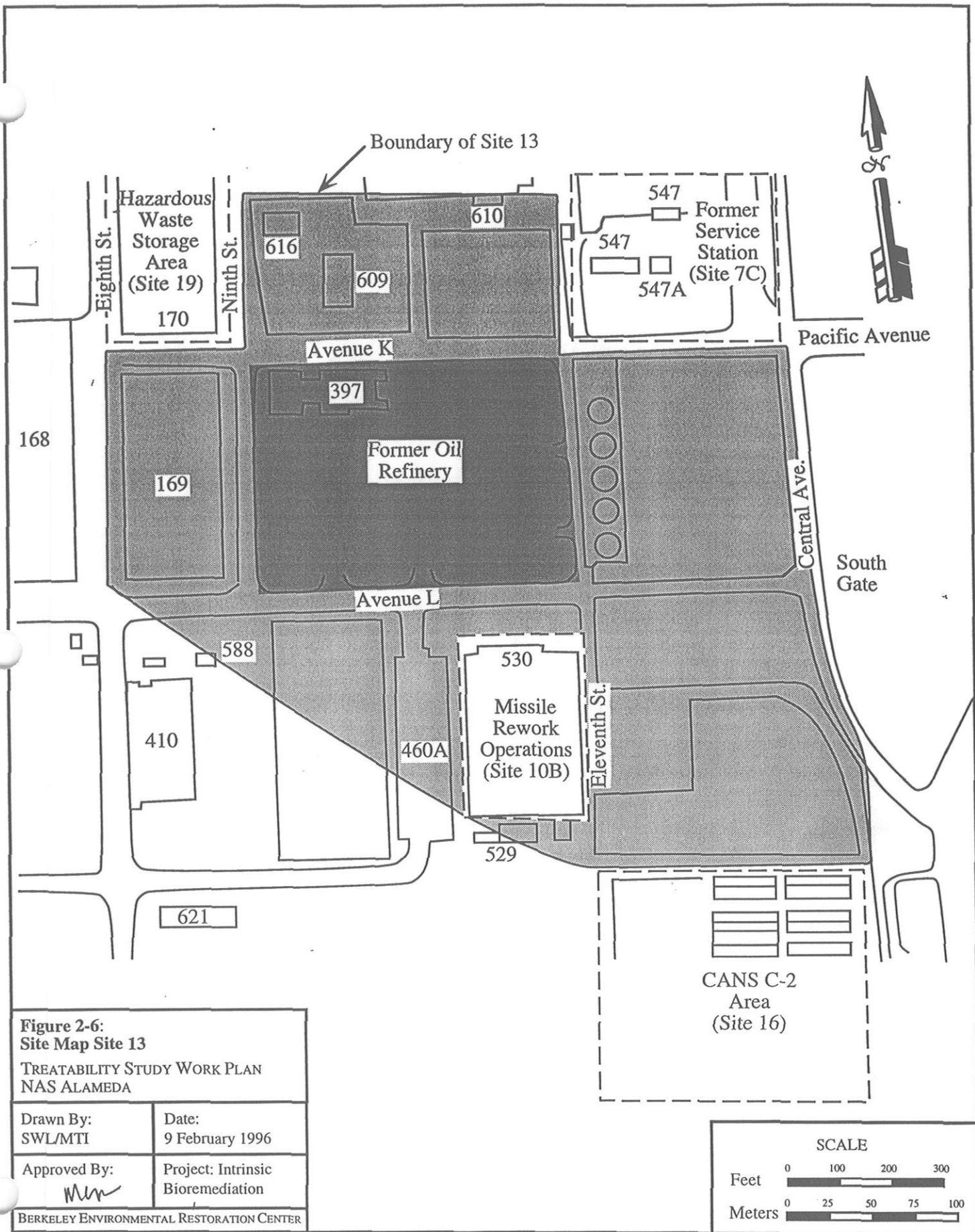
**NOTE:**  
 Boring and monitoring well locations were obtained from a base map provided by Canonie Environmental, Inc. The individual locations were digitized onto a base map CAD file provided by NAS Alameda.



**Figure 2-5:**  
**Distribution of THC Concentrations**  
**Identified in Soil Vapor Samples**  
 TREATABILITY STUDY WORK PLAN  
 NAS ALAMEDA

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Approved By: <i>[Signature]</i>	Project: Intrinsic Bioremediation

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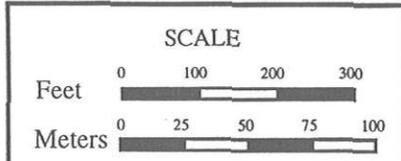


**Figure 2-6:**  
**Site Map Site 13**  
 TREATABILITY STUDY WORK PLAN  
 NAS ALAMEDA

Drawn By: SWL/MTI	Date: 9 February 1996
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Approved By: 	Project: Intrinsic Bioremediation
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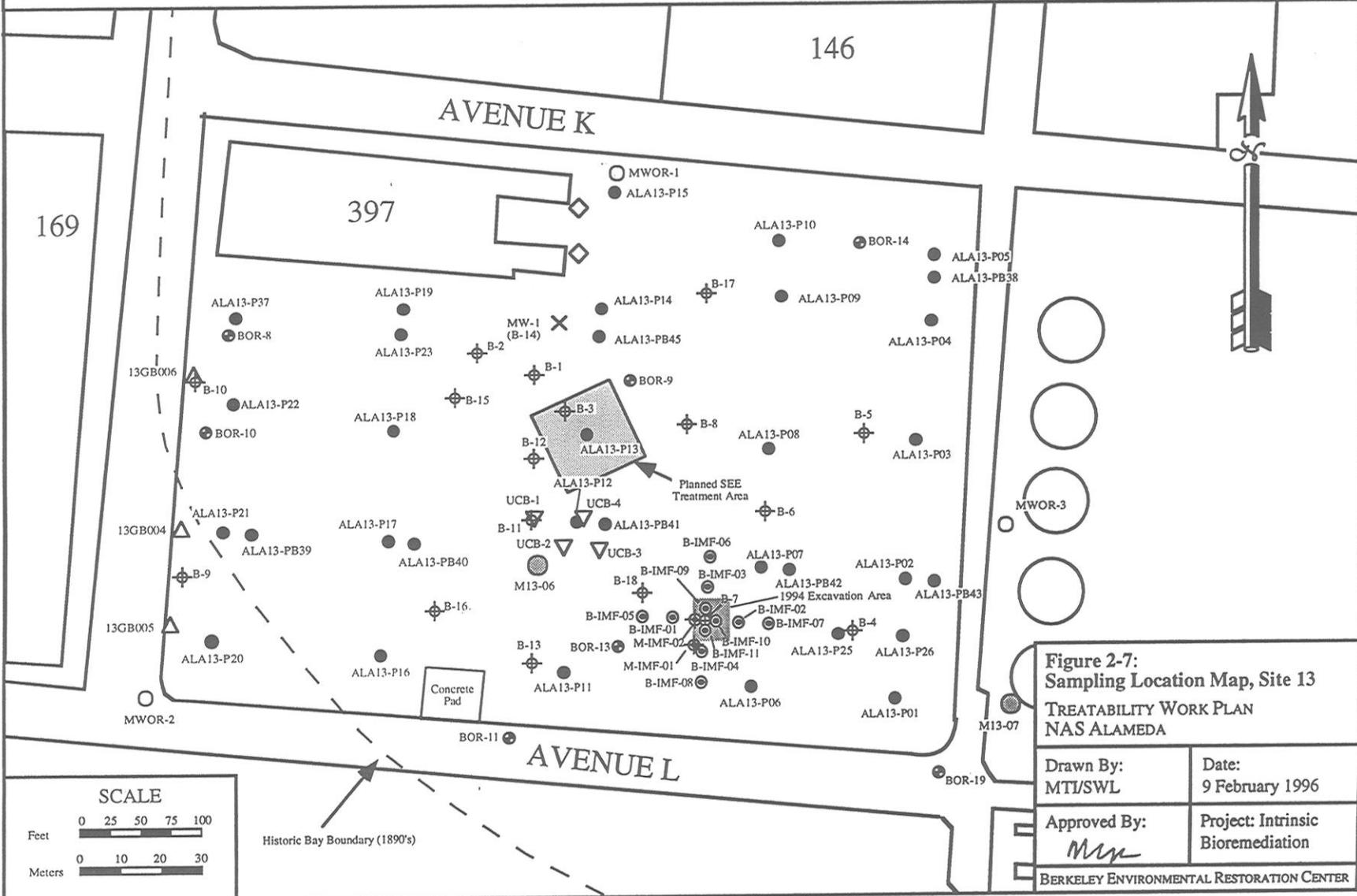
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# NAS Alameda Site 13 map of Water Monitoring and Soil Borings

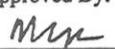
## Legend

-  Harding-Lawson Boring (HLA, 1989)
-  PRC Soil Boring (PRC and JMMC, 1992)
-  Canonic Monitoring Well (Canonic, 1990)
-  SCAPS Push Location and Borehole (PRC, 1994)
-  Harding Lawson Monitoring Well (HLA, 1989)
-  PRC Monitoring Well (PRC and JMMC, 1992)
-  Canonic Soil Boring (Canonic 1990)
-  Geoprobe Location (PRC and MW 1995a)
-  UCB Soil Boring
-  PRC Monitoring Well (PRC and MW, 1995a)
-  Unknown Water Monitoring Well (CA, 1995)

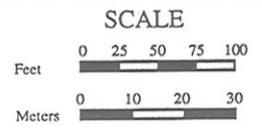


**Figure 2-7:**  
**Sampling Location Map, Site 13**  
**TREATABILITY WORK PLAN**  
**NAS ALAMEDA**

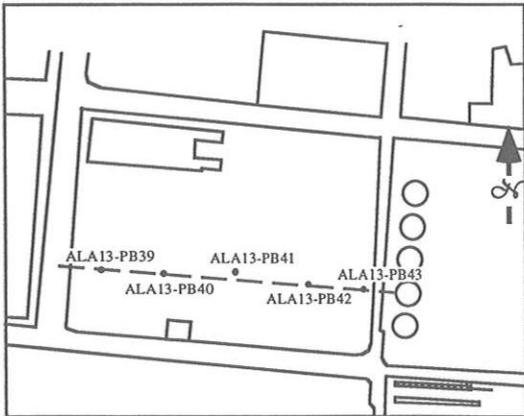
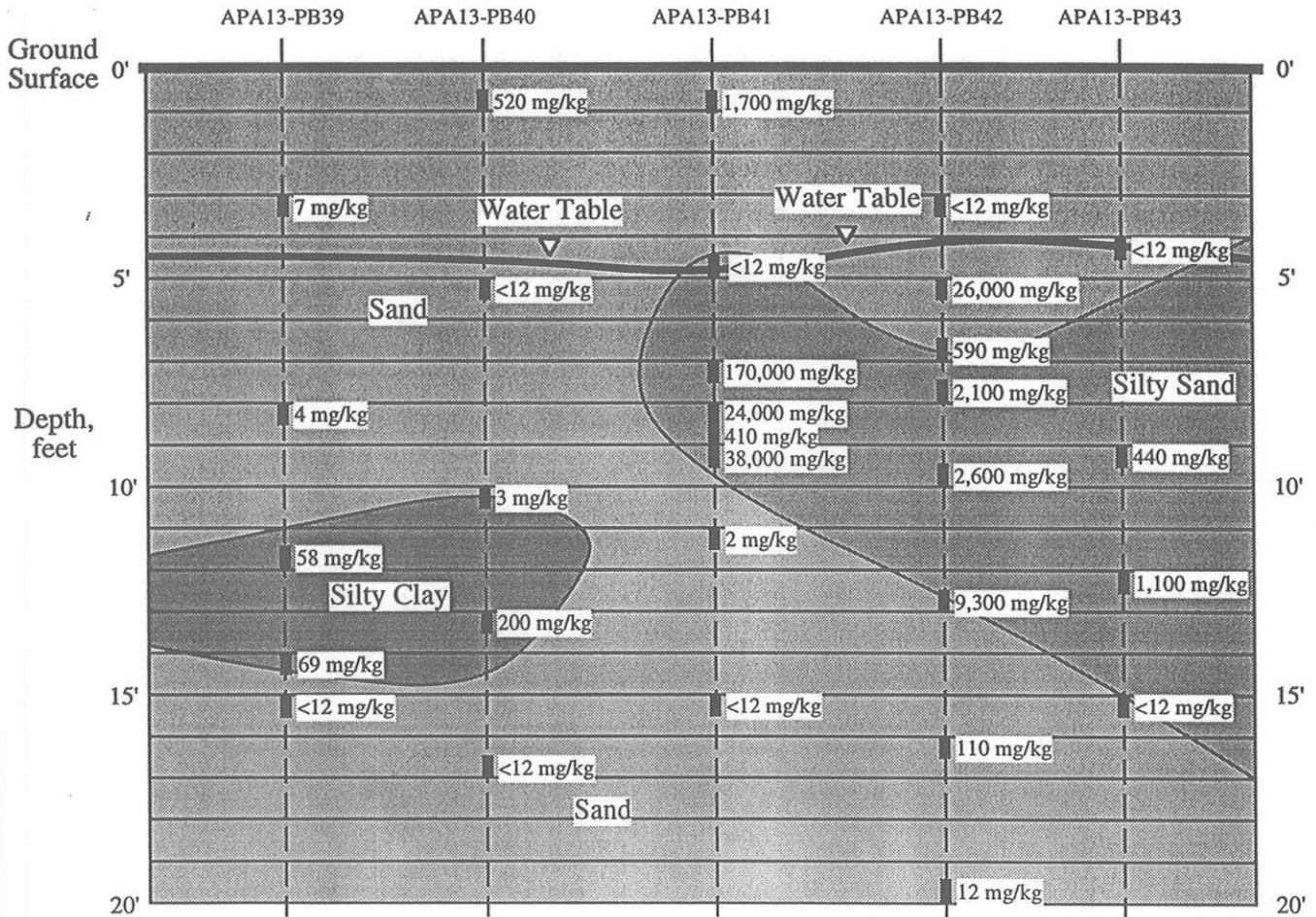
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 MTI/SWL  
 Date:  
 9 February 1996

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# Simplified Geologic Cross-Section of Site 13



**LEGEND**

- Soil sample interval and the highest TPH concentration identified in the soil sample, mg/kg

**HORIZONTAL SCALE**

Feet: 0 25 50 75 100

Meters: 0 10 20 30

**Figure 2-8:  
Geologic Cross Section, Site 13**  
TREATABILITY STUDY WORK PLAN  
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# Legend

- Harding-Lawson Boring (HLA, 1989)
- Harding Lawson Monitoring Well (HLA, 1989)
- UCB Soil Boring
- Unknown Water Monitoring Well (CA, 1995)
- Canonic Monitoring Well (Canonic, 1990)
- Canonic Soil Boring (Canonic 1990)
- SCAPS Push Location and Borehole (PRC, 1994)
- Geoprobe Location (PRC and MW, 1995a)
- PRC Soil Boring (PRC and JMMC, 1992)
- PRC Monitoring Well (PRC and JMMC, 1992)
- PRC Monitoring Well (PRC and MW, 1995a)

**pH Level**

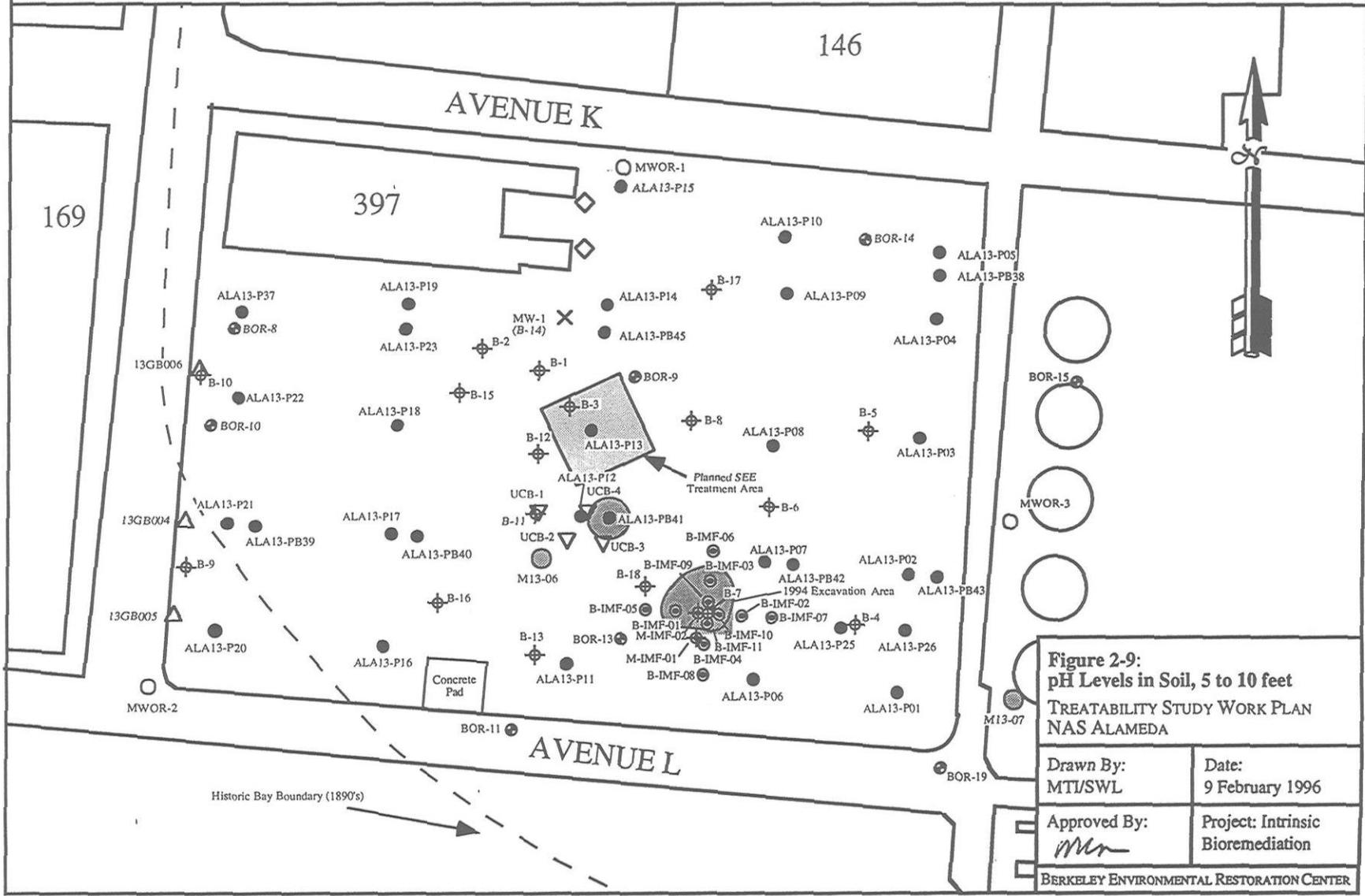
pH < 5.5

pH > 5.5

**Scale**

Fect

Meters



**Figure 2-9:**  
**pH Levels in Soil, 5 to 10 feet**  
 TREATABILITY STUDY WORK PLAN  
 NAS ALAMEDA

Drawn By: MTI/SWL	Date: 9 February 1996
Approved By: <i>MTI</i>	Project: Intrinsic Bioremediation

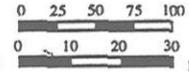
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**Legend**

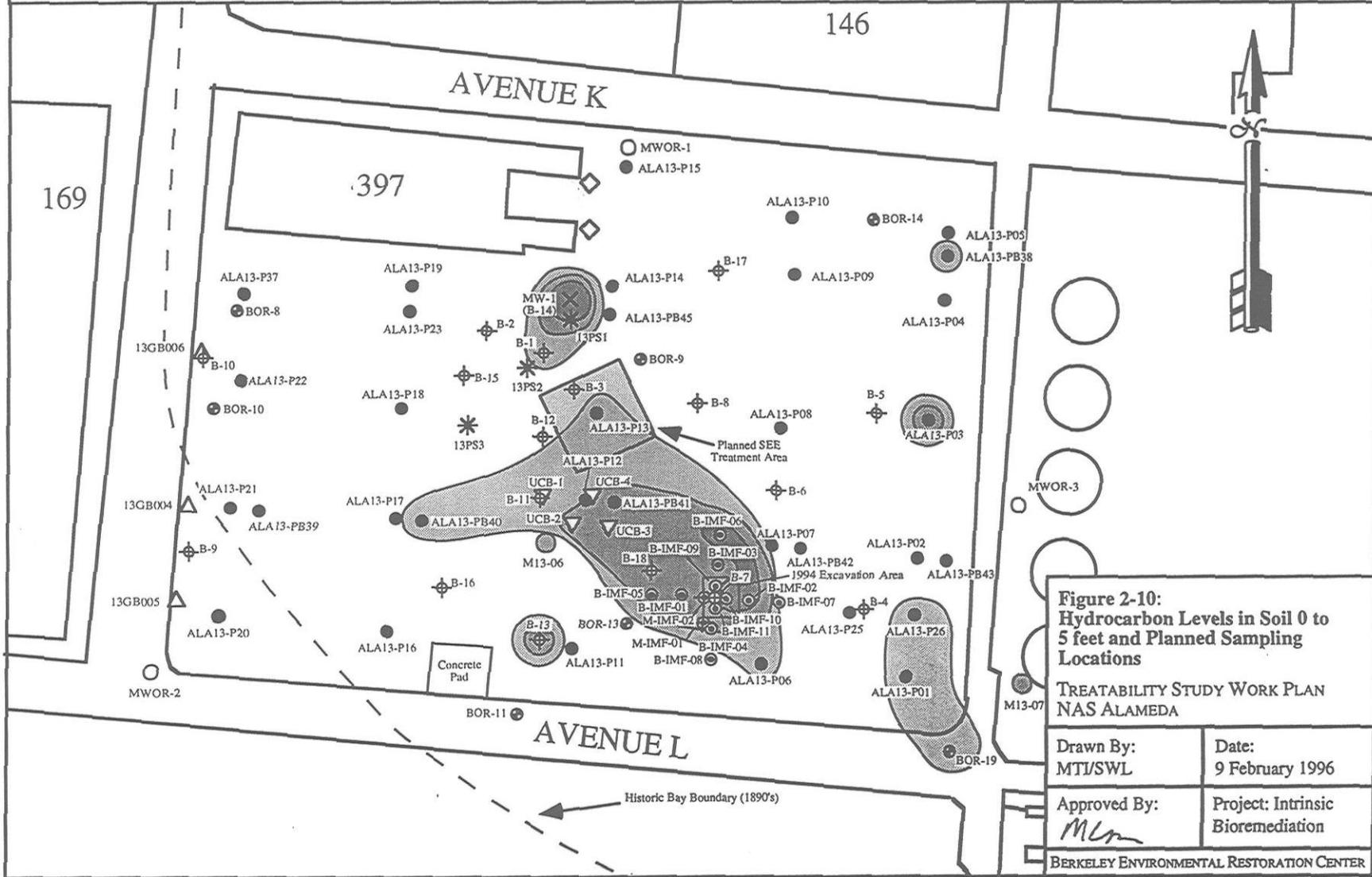
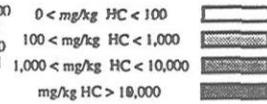
- Harding-Lawson Boring (HLA, 1989)
- Harding Lawson Monitoring Well (HLA, 1989)
- UCB Soil Boring
- Unknown Water Monitoring Well (CA, 1995)
- Canonic Monitoring Well (Canonic, 1990)
- Canonic Soil Boring (Canonic 1990)
- SCAPS Push Location and Borehole (PRC, 1994)
- Geoprobe Location (PRC and MW, 1995a)
- PRC Soil Boring (PRC and JMMC, 1992)
- PRC Monitoring Well (PRC and JMMC, 1992)
- PRC Monitoring Well (PRC and MW, 1995a)
- Planned Sampling Location, Preliminary Screening Phase

**SCALE**

Feet  
Meters



**HYDROCARBON LEVEL**



**Figure 2-10:**  
Hydrocarbon Levels in Soil 0 to 5 feet and Planned Sampling Locations

TREATABILITY STUDY WORK PLAN  
NAS ALAMEDA

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MTI/SWL

Date:  
9 February 1996

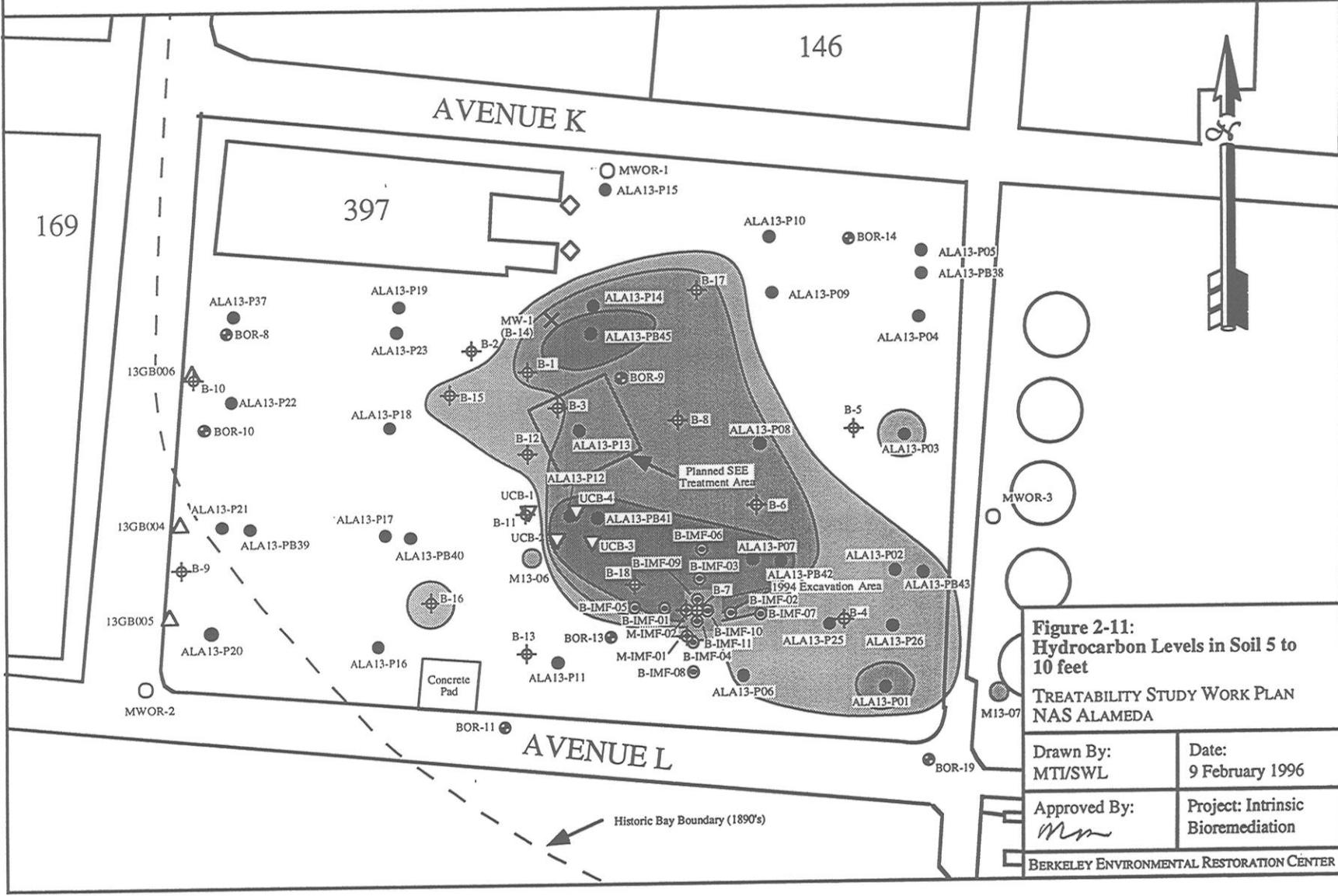
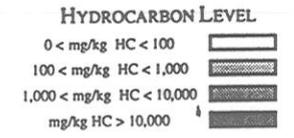
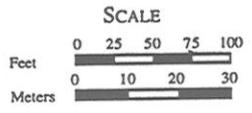
Approved By:  
*MLM*

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**Legend**

- ⊕ Harding-Lawson Boring (HLA, 1989)
- ⊗ Harding Lawson Monitoring Well (HLA, 1989)
- ▽ UCB Soil Boring
- ◇ Unknown Water Monitoring Well (CA, 1995)
- Canonic Monitoring Well (Canonic, 1990)
- ⊙ Canonic Soil Boring (Canonic 1990)
- SCAPS Push Location and Borehole (PRC, 1994)
- △ Geoprobe Location (PRC and MW, 1995a)
- ⊙ PRC Soil Boring (PRC and JMMC, 1992)
- ⊕ PRC Monitoring Well (PRC and JMMC, 1992)
- ⊙ PRC Monitoring Well (PRC and MW, 1995a)



**Figure 2-11:**  
**Hydrocarbon Levels in Soil 5 to 10 feet**  
 TREATABILITY STUDY WORK PLAN  
 NAS ALAMEDA

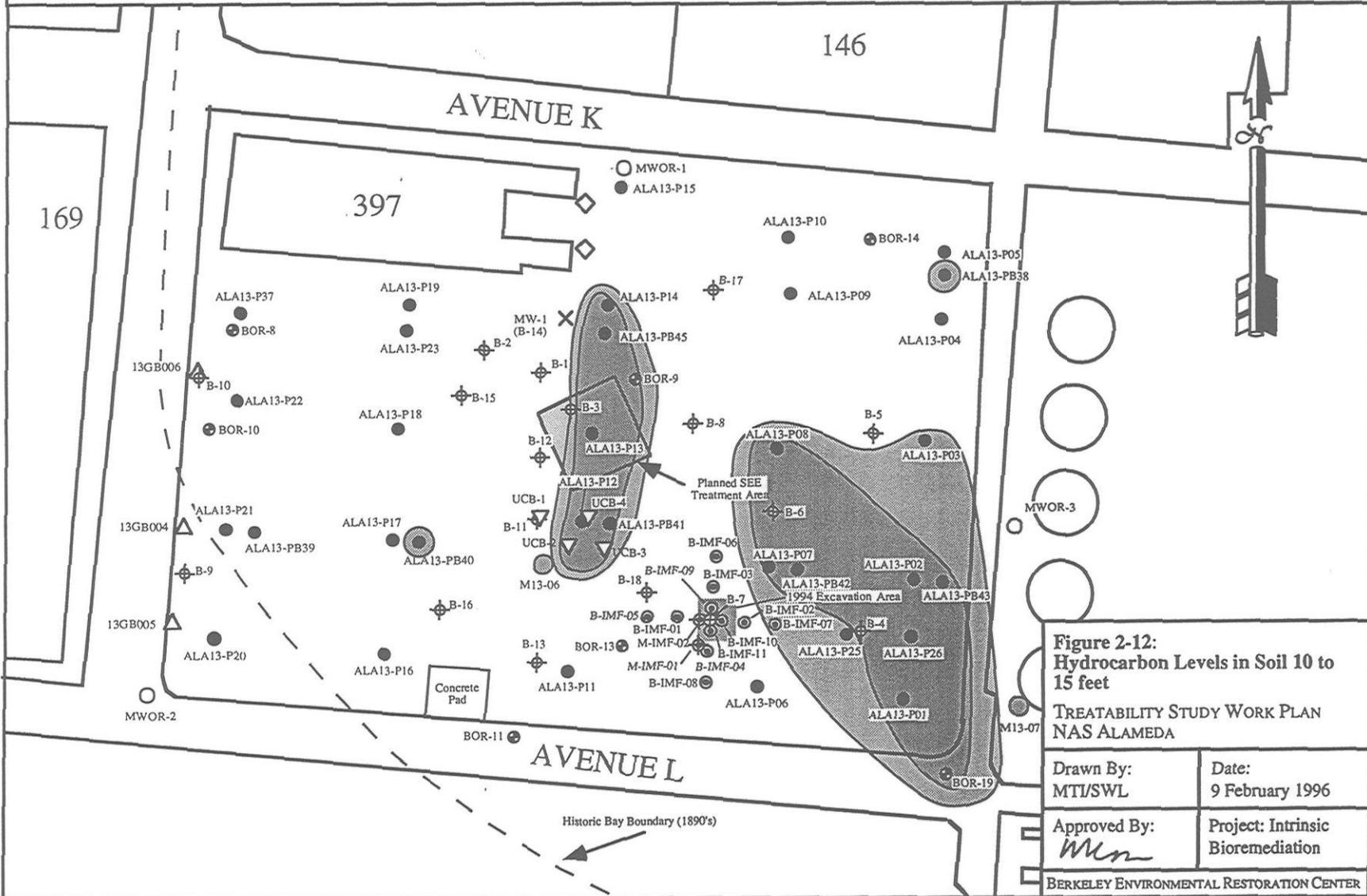
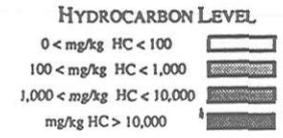
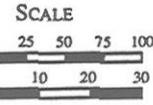
Drawn By: MTI/SWL	Date: 9 February 1996
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Approved By: <i>Mm</i>	Project: Intrinsic Bioremediation
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# Legend

- Harding-Lawson Boring (HLA, 1989)
- Harding Lawson Monitoring Well (HLA, 1989)
- UCB Soil Boring
- Unknown Water Monitoring Well (CA, 1995)
- Canonie Monitoring Well (Canonie, 1990)
- Canonie Soil Boring (Canonie 1990)
- SCAPS Push Location and Borehole (PRC, 1994)
- Geoprobe Location (PRC and MW, 1995a)
- PRC Soil Boring (PRC and JMMC, 1992)
- PRC Monitoring Well (PRC and JMMC, 1992)
- PRC Monitoring Well (PRC and MW, 1995a)



**Figure 2-12:**  
Hydrocarbon Levels in Soil 10 to 15 feet

TREATABILITY STUDY WORK PLAN  
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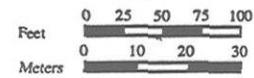
Drawn By: MTI/SWL	Date: 9 February 1996
Approved By: <i>WLn</i>	Project: Intrinsic Bioremediation

BERKELEY ENVIRONMENTAL RESTORATION CENTER

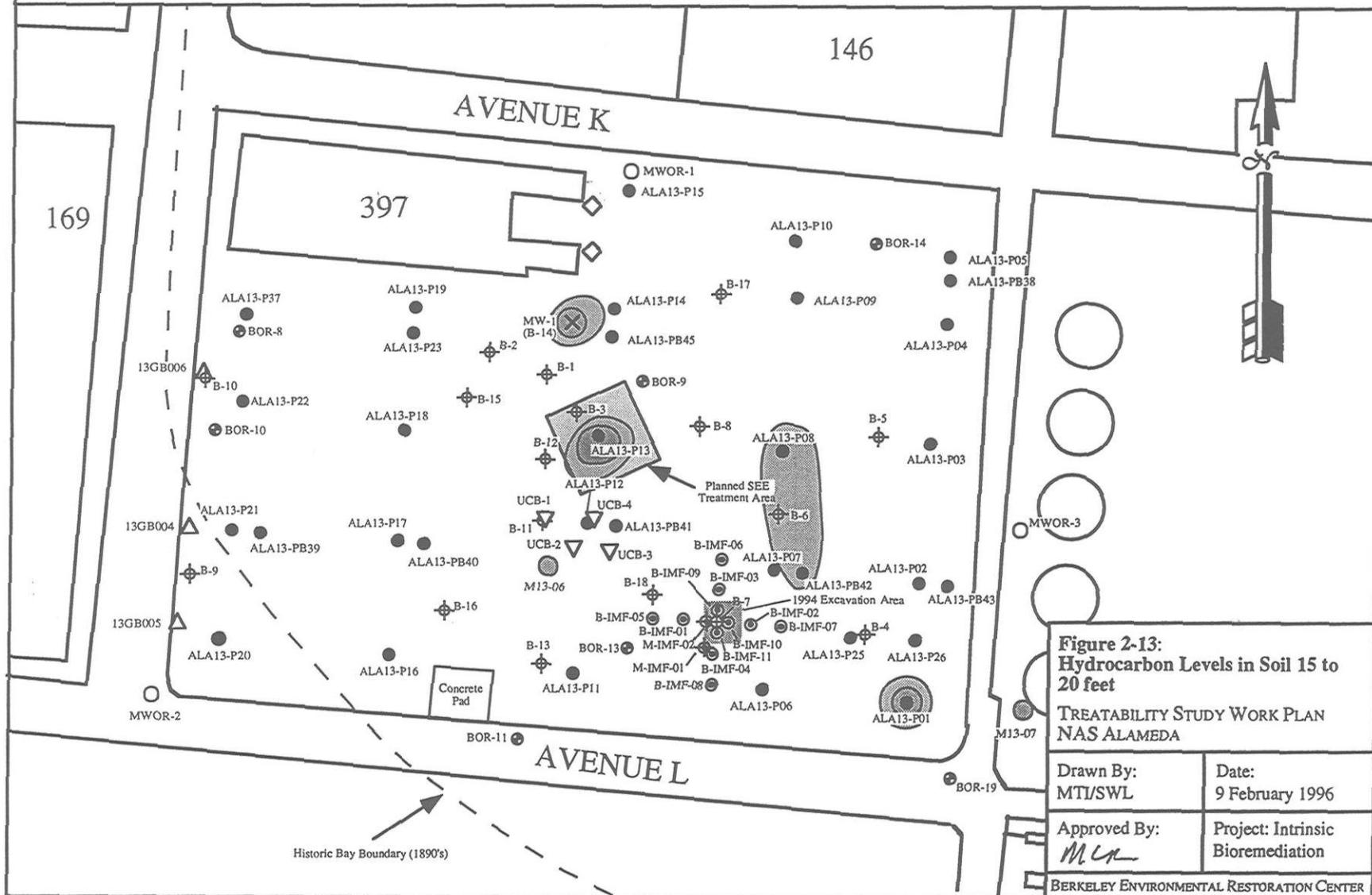
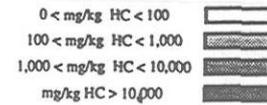
# Legend

- Harding-Lawson Boring (HLA, 1989)
- Harding Lawson Monitoring Well (HLA, 1989)
- UCB Soil Boring
- Unknown Water Monitoring Well (CA, 1995)
- Canonic Monitoring Well (Canonic, 1990)
- Canonic Soil Boring (Canonic 1990)
- SCAPS Push Location and Borehole (PRC, 1994)
- Geoprobe Location (PRC and MW, 1995a)
- PRC Soil Boring (PRC and JMMC, 1992)
- PRC Monitoring Well (PRC and JMMC, 1992)
- PRC Monitoring Well (PRC and MW, 1995a)

## SCALE



## HYDROCARBON LEVEL



**Figure 2-13:**  
**Hydrocarbon Levels in Soil 15 to 20 feet**

TREATABILITY STUDY WORK PLAN  
NAS ALAMEDA

Drawn By:  
MTI/SWL

Date:  
9 February 1996

Approved By:  
*MLA*

Project: Intrinsic  
Bioremediation

BERKELEY ENVIRONMENTAL RESTORATION CENTER

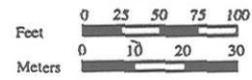
# Legend

- ⊕ Harding-Lawson Boring (HLA, 1989)
- ⊗ Harding Lawson Monitoring Well (HLA, 1989)
- ▽ UCB Soil Boring
- ◇ Unknown Water Monitoring Well (CA, 1995)

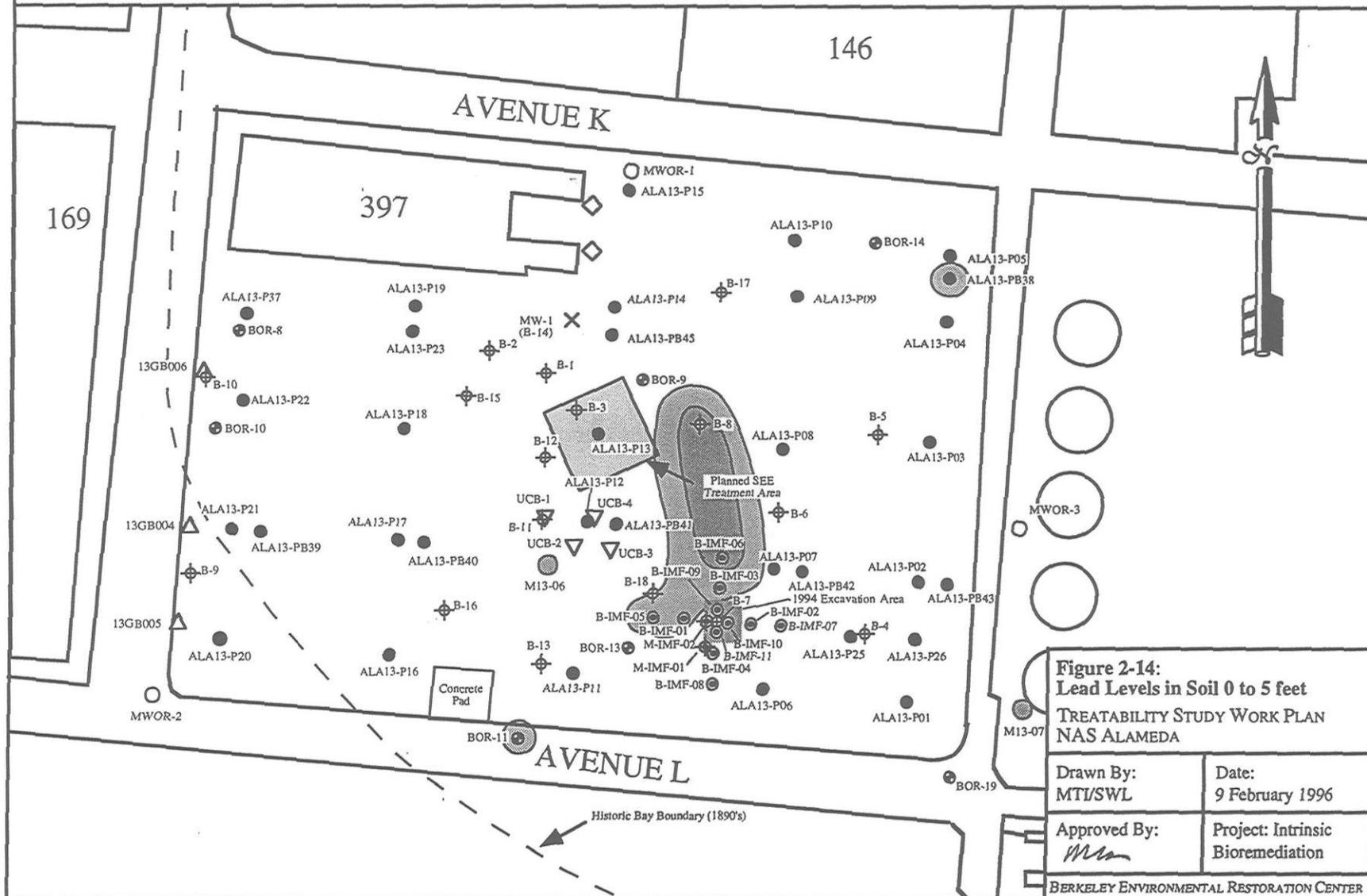
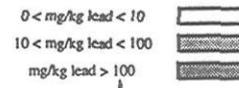
- Canonic Monitoring Well (Canonic, 1990)
- Canonic Soil Boring (Canonic 1990)
- SCAPS Push Location and Borehole (PRC, 1994)
- △ Geoprobe Location (PRC and MW, 1995a)

- ⊕ PRC Soil Boring (PRC and JMMC, 1992)
- ⊕ PRC Monitoring Well (PRC and JMMC, 1992)
- ⊕ PRC Monitoring Well (PRC and MW, 1995a)

## SCALE



## LEAD LEVEL



**Figure 2-14:**  
**Lead Levels in Soil 0 to 5 feet**  
 TREATABILITY STUDY WORK PLAN  
 NAS ALAMEDA

Drawn By: MTI/SWL	Date: 9 February 1996
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Approved By: <i>[Signature]</i>	Project: Intrinsic Bioremediation
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BERKELEY ENVIRONMENTAL RESTORATION CENTER



BERKELEY  
ENVIRONMENTAL  
RESTORATION  
CENTER

# TEST EQUIPMENT LIST AND CALIBRATION

PROJECT NAME: \_\_\_\_\_

PROJECT NO. \_\_\_\_\_

EQUIPMENT NAME: \_\_\_\_\_

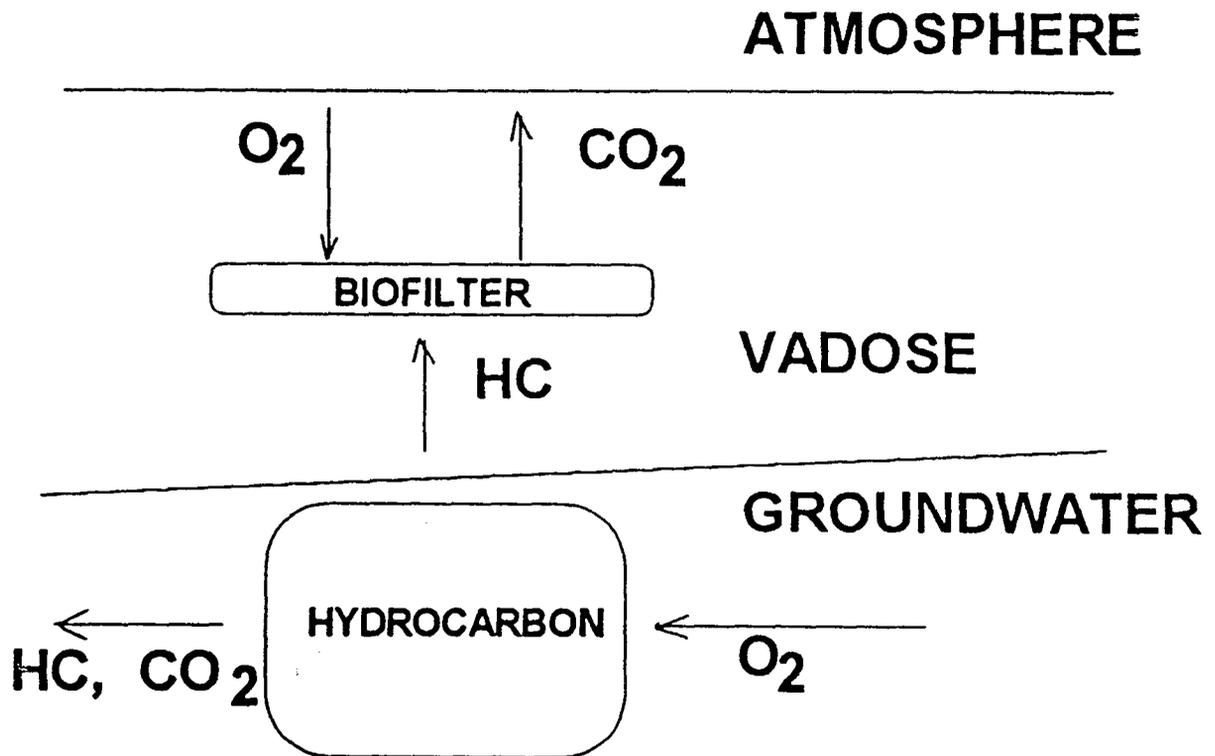
EQUIPMENT TOLERANCE: \_\_\_\_\_

Equipment Number and Use (Screening or Analytical)	Equipment Name (manufacturer and Model ID)	Date and Time (of Calibration)	Calibration Standard Used (manufacturer and Lot Number)	Equipment Reading (Include Units and Tolerances)	Comments (and/or Observations)	Initials (of Person)

**Figure 5-2:  
Test Equipment List and  
Calibration Form**  
TREATABILITY STUDY WORK PLAN  
NAS ALAMEDA

Drawn By: \_\_\_\_\_ Date: 9 February 1996

Approved By: *Mr* Project: Intrinsic Bioremediation



**Figure 6-1:**  
**Conceptual Model**  
 TREATABILITY STUDY WORK PLAN  
 NAS ALAMEDA

Drawn By:  
 JH

Date:  
 9 February 1996

Approved By:  
*mm*

Project: Intrinsic  
 Bioremediation

**Figure 8-1**  
**Treatability Study Implementation Schedule**  
**Intrinsic Bioremediation**

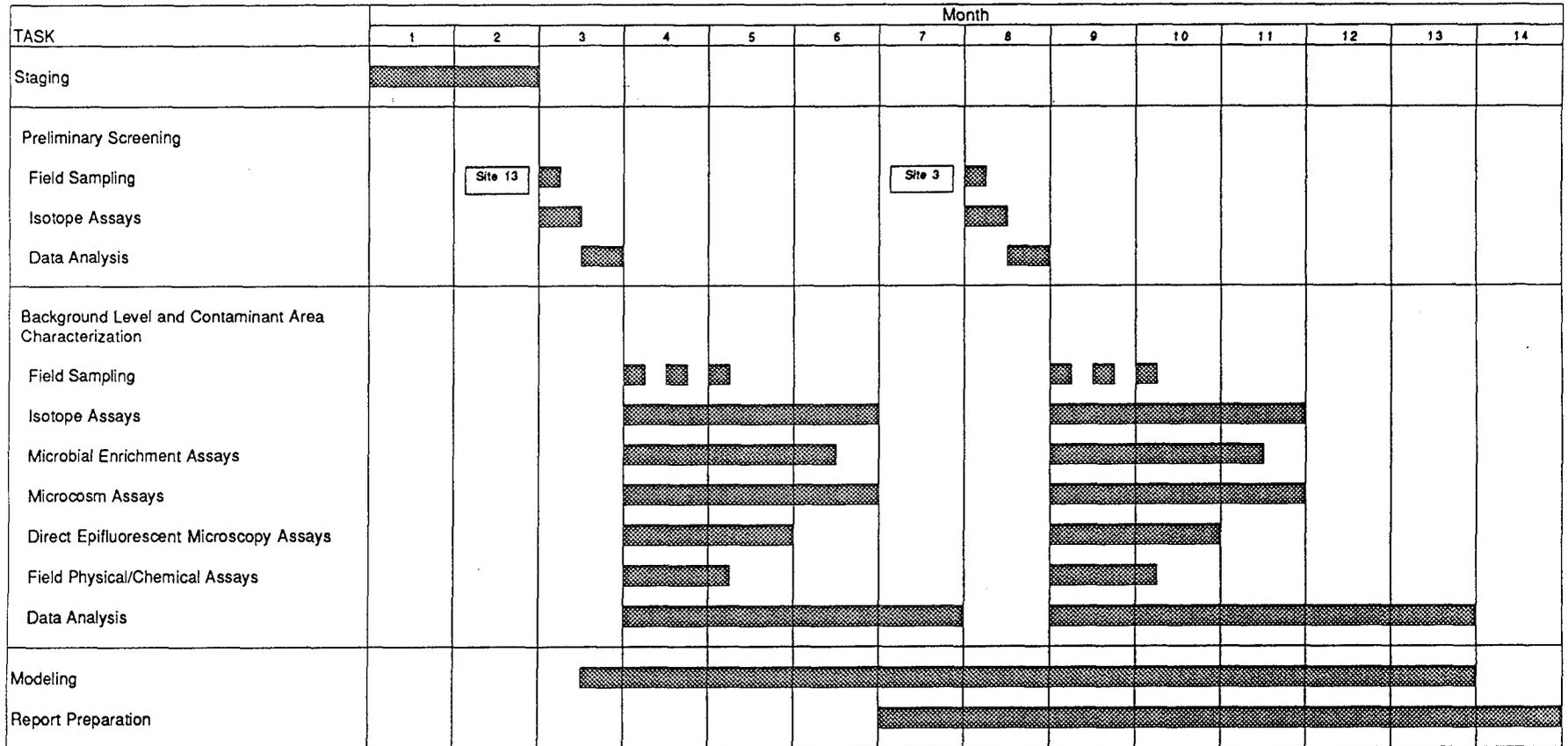
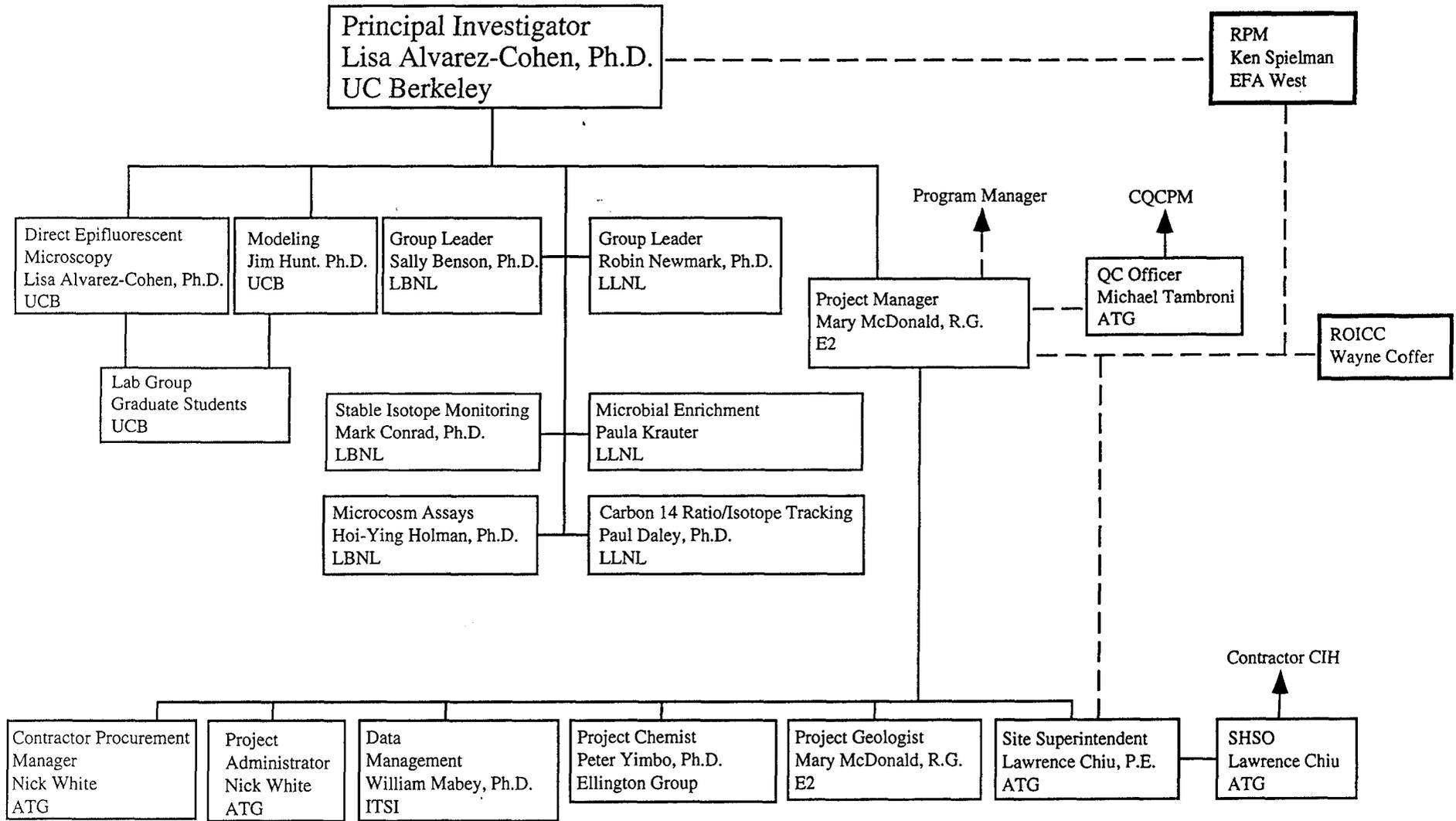


Figure 8-2

# Treatability Study Organization Chart Intrinsic Bioremediation

Berkeley Environmental Restoration Center, UC Berkeley



**LEGEND**

———— : Lines of Authority  
 - - - - - : Lines of Communication

RPM = Remedial Project Manager  
 ROICC = Resident Officer In-Charge of Construction  
 LLNL = Lawrence Livermore National Laboratory  
 LBNL = Lawrence Berkeley National Laboratory

ATG = Allied Technology Group, Inc.  
 E2 = E2 Consulting Engineers  
 SHSO = Site Health & Safety Officer  
 ITSI = Innovative Technical Solutions, Inc.

# TABLES

**Table 1-1  
Applicable Standard Operating Procedures and Standard Quality  
Procedures**

<b>Title</b>	<b>Number</b>
<b>STANDARD OPERATING PROCEDURES</b>	
Chain of Custody	1.1
Sample Handling, Packaging and Shipping	2.1
Subsurface Soil Sampling While Drilling	3.2
Drilling and Heavy Equipment Decontamination	6.2
Sample Labeling	17.1
Sample Numbering	17.2
Microbial Enrichment	24.1
Microcosm Assays	24.2
Direct Epifluorescent Microscopy	24.3
Isotope Monitoring	24.4
Field Physical/Chemical Assays	24.5
<b>STANDARD QUALITY PROCEDURES</b>	
Project Self Assessment	3.1
Indoctrination and Training	3.2
Document Control	4.1
Records Management	4.2
Preparation, Revision and Approval of Plans and Procedures	5.1
Preparation, Review, and Approval of Procurement Documents	6.1
Calibration and Maintenance of Measuring and Test Equipment	8.2
Control of Tests	9.1
Nonconformance Control	10.1
Corrective Action	10.2
Stop Work Order	10.3
Field Work Variance/Request for Information	11.1
Quality Audits	12.1
Quality Surveillances	12.3

TABLE 2-1

SUMMARY OF PREVIOUS ANALYTICAL RESULTS

GENERAL CHEMICAL CHARACTERISTICS IN SOIL SAMPLES, SITE 3

	MW97-1	MW97-2	MW97-3
	07/26/90	08/27/90	07/26/90
Parameter Reported	4-4.5 ft	4-4.5 ft	4-4.5 ft
<b>Miscellaneous Measurements</b>			
Ash (%)	95.6	93.1	98.3
Chloride (mg/kg)	29.2	7550	14.8
Exchangeable Ammonium-N (mg/kg)	<25.0	84	<25.0
Nitrate (as Nitrogen) (mg/kg)	<0.120	0.18	2.24
Sulfate (mg/kg)	42.1	1210	89.8
Total Kjeldahl Nitrogen (mg/kg)	174	1400	196
Total Phosphorus (mg/kg)	675	1230	922
<b>Total Organic Carbon</b>			
Total Organic Carbon (mg/kg)	1090	13500	1260
	MW97-1	MW97-2	MW97-3
	07/26/90	08/27/90	07/26/90
	3-3.5 ft	3.5-4 ft	3.5-4 ft
<b>Characteristic Measurements</b>			
pH (Units)	9	7.2	7.5

Notes: NA = Not Analyzed  
 < = Detection Limit  
 mg/kg = milligrams per kilogram  
 Data not validated by JMM

SOURCE: PRC, 1995a

TABLE 2-2

SUMMARY OF PREVIOUS ANALYTICAL RESULTS

GENERAL CHEMICAL CHARACTERISTICS IN GROUNDWATER SAMPLES, SITE 3

	MW97-1	MW97-2	MW97-3
	08/30/90	08/31/90	10/18/90
Parameter Reported	0-0 ft	0-0 ft	0-0 ft
<b>Miscellaneous Measurements</b>			
Alkalinity, bicarb (as CaCO <sub>3</sub> ) (mg/L)	1550	2430	410
Alkalinity, total (as CaCO <sub>3</sub> ) (mg/L)	1550	2430	410
Total Dissolved Solids (mg/L)	6440	22300	1280
Total Hardness (as CaCO <sub>3</sub> ) (mg/L)	920	4050	614
<b>Total Organic Carbon</b>			
Total Organic Carbon (mg/L)	53.8	79.5	11
<b>Characteristic Measurements</b>			
Dissolved Oxygen (mg/L)	5.4	3.2	7
pH (Units)	7.4	6.8	7.1
<b>Cations/Anions</b>			
Specific Conductance (umhos/cm)	11300	36000	2000

Notes: NA = Not Analyzed  
 < = Detection Limit  
 mg/L = milligrams per liter  
 Data not validated by JMM

SOURCE: PRC, 1995a

**Table 3-1**  
**Applicability of Assays to Lines of Evidence**

<b>Assay</b>	<b>Description</b>	<b>Line Of Evidence</b>
<b>Microbial Enrichment</b>	Differentiates specific members of the soil microbial biomass.	Laboratory confirmation of microbial activity
<b>Microcosm Assay</b>	Determines the potential of indigenous communities to destroy contaminants.	Laboratory confirmation of microbial activity
<b>Direct Epifluorescent Microscopy</b>	Quantifies the numbers of total and active microorganisms within the contaminant plume for comparison with the background area.	Field confirmation of microbial activity
<b>Stable Isotope Ratio Monitoring</b>	Measures presence and differentiates between biotic/abiotic origin of nitrate, nitrite and bicarbonate in groundwater as well as methane, carbon dioxide and other compounds in soil gases. These compounds result from many processes within the subsurface including bioremediation.	Field confirmation of microbial activity Contaminant loss in bioactive area
<b><sup>14</sup>C Radio-isotope Tracking</b>	Monitors <sup>14</sup> C carbon in degradation reactants and products, provides differentiation between biodegradation products of contaminants and those of natural organic matter.	Field confirmation of microbial activity Contaminant loss in bioactive area
<b>Field Physical/Chemical Assays</b>	Provides characterization of the subsurface environment to determine whether it is conducive to microbial degradation.	Contaminant loss in bioactive area

**Table 4-1 Analytical Plan**

Parameter	SOP	Responsible Laboratory	Number of Samples								
			Preliminary Screening			Background Level			Contaminant Area		
			Soil Gas	Soil	Ground-water	Soil Gas	Soil	Ground-water	Soil Gas	Soil	Ground-water
<b>Microbial Enrichment</b>	24.1	LLNL	0	0	0	0	10	0	0	20	0
<b>Microcosm Assays</b>	24.2	LBNL	0	0	0	0	10	0	0	20	0
<b>Direct Epifluorescent Microscopy</b>	24.3	UCB	0	0	0	0	10	0	0	20	0
<b>Stable Isotope Ratio Monitoring</b>	24.4	LBNL	6	3	6	6	0	4	12	0	8
<b><sup>14</sup>C Radio-Isotope Tracking</b>	24.4	LLNL	6	3	6	6	0	4	12	0	8
<b>Field Physical/Chemical Assays</b>	24.5	LLNL	0	0	0	0	10	4	0	20	8

Notes: For the Preliminary Screening, one soil sample from above the water table, two soil gas samples, and two groundwater samples from each boring location will be characterized.  
 For the Background Level and Contaminant Area Characterization, five soil samples, three soil gas samples, and two groundwater samples from each boring location will be characterized.

**Table 4-2**  
**Laboratory Analyses for Soil and Liquid Disposal**

<b>Parameter</b>	<b>Site</b>	<b>Analytical Method</b>
Total petroleum hydrocarbons as gasoline	3/13	8015M
Total petroleum hydrocarbons as diesel	3/13	8015M
Total recoverable petroleum hydrocarbons	13	418.1
Volatile organic compounds	3/13	8260
Polynuclear aromatic hydrocarbons	3/13	8100
Organochlorine pesticides	13	8080
Lead	13	6010
pH	13	9040

Reference for analytical methods: U.S. EPA, 1994c.

**Table 5-1  
Summary of Data Quality Objectives for Intrinsic Bioremediation Treatability Study**

<b>Stage of DQO Process</b>	<b>Description of Activities</b>
<b>Statement of Problem</b>	The Treatability Study will obtain evidence to demonstrate whether intrinsic remediation of hydrocarbons is occurring at Sites 3 and 13. The Treatability Study is funded through Delivery Order 5 to the U.S. Navy and UCB Partnership Agreement, issued by the U.S. Navy. The Treatability Study will utilize resources from UCB, LBNL, and LLNL. The report will be reviewed by the U.S. Navy, regulatory agencies, and Restoration Advisory Board.
<b>Identify Decision</b>	The Treatability Study will provide an estimate of the occurrence and rate of intrinsic bioremediation at Sites 3 and 13. The results will ultimately be utilized by the U.S. Navy and their contractor in a feasibility study or engineering estimate/cost analysis to evaluate whether the rate is sufficient to support a decision for no further action other than monitoring, whether enhanced bioremediation may be required to meet acceptable remediation schedules for reuse of Sites 3 and 13, or whether other remediation techniques should be considered for these sites.
<b>Identify Inputs</b>	The Treatability Study includes field and laboratory assays to characterize the subsurface environments at Sites 3 and 13 (Field Physical/Chemical assays); characterize the microbial populations and their ability to metabolize hydrocarbons ( Microbial Enrichment, Microcosm, and Direct Epifluorescent assays); and demonstrate that degradation due to the presence of microorganisms has occurred (Isotope assays). The methods that will be utilized are a combination of established techniques and innovative techniques developed by UCB, LLNL, and LBNL. The techniques are documented in SOPs included in Appendix A. Action levels are not appropriate for this Treatability Study because it is designed to estimate the rate and occurrence of intrinsic bioremediation. The results will be used by the U.S. Navy in selecting an appropriate remedial action for Sites 3 and 13.
<b>Define Boundaries</b>	Sampling during the Preliminary Screening phase will identify the existing limits of contamination in the area where the Treatability Study will be implemented. Modeling will be used to extrapolate the rate of degradation for each site. During the Background Level and Contaminant Area Characterization locations in the expected background and contaminated areas will be sampled. Seasonal effects on the level of subsurface biological activity will be evaluated through two sets of periodic monitoring.

**Table 5-1**  
**Summary of Data Quality Objectives for Intrinsic Bioremediation Treatability Study**

<b>Develop Decision Rule</b>	If evidence demonstrates that intrinsic remediation is occurring at Sites 3 or 13 and is likely to reduce chemicals of concern to acceptable levels within a reasonable time frame, then intrinsic remediation will be included in the feasibility studies for these sites. The strength of the evidence will be based on several independent measures of intrinsic remediation, including modeling to integrate site data and study results to estimate rates of intrinsic remediation of hydrocarbon constituents. The modeling will include sensitivity analyses to evaluate the applicability of the data from the assays in supporting the decision that intrinsic remediation is occurring at acceptable rates at the sites.
<b>Specify Limits on Decision Error</b>	The SOPs provided in Appendix A specify detection limits and acceptable levels of data accuracy for each assay that will be conducted. The assays provide complementary data which will reduce the potential for false positives and negatives. Field blanks will be assayed to establish that field contamination of samples has not occurred.
<b>Optimize the Design</b>	Prior to collection of soil gas, groundwater, and soil samples for characterization by each assay, it is essential to establish the current boundary of contamination at each site. The Preliminary Screening phase of this Treatability Study includes sampling to optimize the sampling locations for the Background Level and Contaminant Area Characterization phase. Pilot borings will be drilled at each location and sampled during the Background Level and Contaminant Area Characterization phase to identify the subsurface lithology and depth to groundwater.

**Table 5-2**  
**Frequency of Laboratory Replicate Samples**

Assay	SOP	Frequency of Replicates
Microbial Enrichment	24.1	triplicate
Microcosm Assay	24.2	duplicate
Direct Epifluorescent Microscopy	24.3	triplicate
Stable Isotope Ratio Monitoring	24.4	quadruplicate
<sup>14</sup> C Radio Isotope Tracking	24.4	quadruplicate
Field Physical/Chemical Assays	24.5	on site analysis

**Table 5-3**  
**Required Containers, Preservatives, and Holding Times for Field and Laboratory Assays**

<b>Sample Type</b>	<b>Container Characteristics</b>	<b>Field Storage Requirements</b>	<b>Sample Storage Requirements</b>	<b>Maximum Storage Time Prior to Analysis</b>
<b>Soil</b>	Sterilized brass tubes and caps	Store in plastic bag in cooler	Store in cool dry conditions	72 hours
<b>Water</b>	Sterilized brown bottles	Double bag and place in cooler	Store in cool dark, dry conditions	72 hours
<b>Soil gas</b>	Tedlar bags	Double bag, place in cooler with ice	Store in cool dark conditions	72 hours

**Table 5-4**  
**Required Containers, Preservation Methods, and Holding Times for Waste Samples**

<b>Analysis</b>	<b>Container for Water Samples</b>	<b>Preservation</b>	<b>Maximum Holding Time</b>
Total petroleum hydrocarbons as gasoline	2-40 mL glass vial	< 4°C, pH<2 HCl	14 days
Total petroleum hydrocarbons as diesel	1 liter amber glass	< 4°C	14 days
Total recoverable petroleum hydrocarbons	1 liter amber glass	< 4°C, pH<2 HCl	28 days
Volatile organic compounds	2-40 mL glass vial	< 4°C, pH<2 HCl	14 days
Poly nuclear aromatic hydrocarbons	1 L. amb. glass	< 4°C	7/40 days <sup>1</sup>
Organochlorine pesticides	1 L amb. glass	< 4°C, pH 5-9	40 days
Lead	250 mL plastic	pH<2 HNO <sub>3</sub>	6 months
pH	125 mL plastic	none required	a.s.a.p.

Note: All soil samples will be collected in precleaned stainless steel or brass tubes

# **APPENDIX A**

## **STANDARD OPERATING PROCEDURES**

## SOP 24.1

### Microbial Enrichment

#### 1. PURPOSE

This Standard Operating Procedures (SOP) establishes the method and responsibilities associated with microbial enrichment assays to be performed on soil samples at NAS Alameda Sites 3 and 13. These assays will be used for laboratory confirmation of microbial potential for contaminant biodegradation. The procedures described in this section have been specifically developed for application to NAS Alameda Sites 3 and 13.

#### 2. OBJECTIVES

The objective of these tests is the characterization of subsurface microbial populations. Classical microbial enumeration methods will be used for laboratory confirmation of biological activity in the contamination zones and to obtain biological site assessment data. Enhanced numbers of microorganisms in enrichments from the contaminated area strengthens the case for in situ bioremediation and provides a comparison for field confirmation results.

The distribution of microorganisms in sediments will be evaluated. Specifically we will enumerate heterotrophic bacteria, Actinomycetes, fungi, and protozoa in soil samples. Bacteria, Actinomycetes and fungi are known hydrocarbon degraders. Protozoa are predators in subsurface environments. Protozoan enumeration provides indirect evidence of in situ biodegradation because large a protozoan population reflects a high bacterial growth rate (Madsen et al., 1991), which in turn may reflect active bioremediation.

Heterotrophic bacteria will be enumerated by spread-plated techniques developed by Ghiorse and Balkwill (1985). The method of Sinclair and Ghiorse (1987, 1989) will be used for viable protozoa counts. The bacterial food source for viable protozoa will be nongrowing *Enterobacter aerogenes* harvested from 1-day-old, half-strength Trypticase soy agar plates (BBL Microbiology Systems). Fungi and Actinomycetes will be quantified by enrichment techniques using selective medias. Each of the above methods is completely described in this SOP.

### **3. REFERENCES**

Ghiorse WC and Balkwill DL (1983) Enumeration and morphological characterization of bacteria indigenous to subsurface environments. *Dev Ind Microbiol* 24:213-224.

Handbook of Microbiological Media, Atlas & Parks, 1993, CRC Press, Ann Arbor.

Sinclair JL and WC Ghiorse (1989) Distribution of aerobic bacteria, protozoa, algae, and fungi in deep subsurface sediments. *Geomicrobiol J* 7:15-31.

Sinclair J.L. and W.C. Ghiorse (1987) Distribution of protozoa in subsurface sediments of a pristine groundwater study site in Oklahoma. *Appl and Environ Microbiol* 53:1157-1163.

Sinclair J.L., D.H. Kampbell, M.L. Cook, J.T. Wilson (1993): Protozoa in subsurface sediments from sites contaminated with aviation gasoline or jet fuel. *Appl Environ Microbiol* 59:467-472.

Standard methods for the examination of water and wastewater, 1989, 17th ed. Ed. LS Clesceri, AE Greenberg, RR Trussell.

### **4. SPECIALIZED EQUIPMENT AND MATERIALS**

Specialized equipment to be used with the microbial enrichment assays requires various medias (1% PTYG agar, Sabourauds Dextrose agars-Emmons, 1:2 Trypticase soy agar and Actinomycetes agar), incubators, autoclave and shaker table.

### **5. EXPERIMENTAL PROCEDURES**

All protocols used for enrichment plating will follow strict adherence to aseptic technique. An overview of the procedures are presented below.

Heterotrophic bacteria will be enumerated by spread-plated techniques as follows (Ghiorse and Balkwill, 1985):

- Ten grams of sediment will be mixed with sterile 0.1% sodium pyrophosphate-10H<sub>2</sub>O, diluted to 100 ml volume, and placed on a shaker table at 160 rpm for 15 min.

- Aqueous suspensions of sediment samples will be serially diluted with sterile 0.1% sodium pyrophosphate-10H<sub>2</sub>O (pH 7).
- Aliquots of dilutions will be spread-plated in triplicate on 1% PTYG agar plates.
- Plates will be incubated at 22±2°C and colonies counted at 14 d and 28 d. The resulting microbial colonies will be scored with an automatic plate counter (New Brunswick, Biotrans III) or visually counted. Results will be reported as colony forming units per gram of dry weight soil, (CFU/gdw).

Fungi will be enumerated using the following methods (Sinclair JL and WC Ghiorse (1989), Standard methods for the examination of water and wastewater, 1989, 17th ed.:

- Undiluted sediment (1g) will be distributed on the surface of three agar plates containing 47 g Sabourauds Dextrose agar-Emmons (BBL # 11589) in 1 L deionized water autoclaved 30 min at 212 °C @ 15 psi, pH 6.9 ± 0.2.
- Incubate plates at room temperature. Care will be taken to prevent laboratory contamination of fungi spores.
- The number of fungal colonies or outgrowths from the distributed sediment particles will be visually counted after 7, 14 and 28 d.

Protozoa will be enumerated using the methods of Sinclair and Ghiorse (1987) and Sinclair et al. (1993):

- Set up 5 tubes per dilution, three dilutions per sample. Use 15 mL sterile test tubes.
- Mix sediment/buffer slurries on a magnetic stirrer and pipette 10 mL into 5 test tubes.
- Add a loopful of *Enterobacter aerogenes* (ATCC 13048) harvested from 1-day old, half-strength Trypticase into test tubes, mix to suspend cells.
- Cultures will be incubated at room temperature (21±2°C) in indirect daylight.
- To determine the presence of protozoa and distinguish the types present, samples will be removed aseptically from the tubes and examined in a Zeiss standard phase-contrast microscope under a 20x objective lens. If no protozoa are present after 1 month of incubation the tube will be counted as negative.

- MPN and appropriate dilution factors will be used to calculate the MPN x [grams dry weight]<sup>-1</sup> from the number of positive endpoint dilution. The lower limit of detection of the method is MPN 0.2 protozoa x (g dry wt)<sup>-1</sup>; standard error of the MPN counts is estimated to be 50% (Sinclair and Ghiorse 1987).

Actinomycete counts will be performed using the following procedure:

- Prepare Actinomycetes culture medium according to methods in the Handbook of Microbiological Media. Add 5.0 g of glycerol (Bacto glycerol, Difco 0282-17-0, 500 g). Autoclave for 15 min at 15 psi pressure-121°C. Distribute into tubes or 10 cm plates.
- Prepare controls: One mL antifungal antibiotic (1 mg/mL distilled water) cycloheximide (Sigma C7698), plus 2 mL sample (standard dilution), autoclaved 15 min at 121°C.
- Inoculate by mixing ten grams of sediment with sterile 0.1% sodium pyrophosphate-10H<sub>2</sub>O. Dilute to 100 mL volume, and place on a shaker table at 160 RPMs for 15 minutes.
- Serially dilute sediment samples with sterile 0.1% sodium pyrophosphate-10H<sub>2</sub>O (pH 7).
- Spread-plate diluted samples in triplicate on Actinomycetes culture plates. The resulting colonies will be scored with an automatic plate counter (New Brunswick, Biotrans III) or visually counted.
- Invert and incubate at 25°C, count colonies at 14 and 28 d.

## 6. ANALYTICAL METHODS

The scientific background for determining microbial populations in environmental samples using enrichment methods are documented in the given references.

EXPECTED ACCURACY FOR SOIL MICROBIAL COUNTS

MEASURE	UNITS	LEVEL OF DETECTION	RANGE	PRECISION
Heterotrophic plate count	CFU/g dry wt soil	>100	100 to 10 <sup>7</sup>	±10%
Fungi count	propagules/g dry wt soil	>1	100 to 10 <sup>7</sup>	±10%
Protozoa count	protozoa/g dry wt soil	>1	1 to 10 <sup>6</sup>	SE 50%
Actinomycetes count	CFU/g dry wt soil	>100	100 to 10 <sup>7</sup>	±10%

SE- standard error

EQUIPMENT	TYPE & FREQUENCY	ACCURACY	PRECISION
Incubators			
Microscopes	Check lamps, focus, alignment prior to each use.	Within 10%	±1 unit
Refrigerator	Temperature checked daily.	Within 10%	±5%
Balance	Standard weights weekly, document in log book.	Within 10%	±5%
Pipettes	Check volume by weight weekly.	SD<1%	±0.1 mL

Microscopy will be performed by operators trained by the principal investigators. Media and environmental controls will be incorporated into the daily routine analysis. Calibration of microscopes and grids will be checked according to methods specified by the manufacture.

Table 1. Surveillance of equipment

<u>Item</u>	<u>Conditions</u>	<u>Frequency</u>
Ventilation	Direction flow; changes per hour; temperature 23-29 °C	Semiannual
Temperature-controlled devices	Upper and lowers limits; power failure	Daily
Autoclaves	Sterilize spores	Weekly
Safety cabinets	Air flow and configuration	Semiannual
Microscopes	Inspection and cleaning	Semiannual
Balances	Inspection and certification	Annual

## 7. DATA MANAGEMENT, ANALYSIS AND INTERPRETATION

All procedures and data will be documented in bound laboratory notebooks and Excel spreadsheets. Recorded data will be transferred into computer files within a month of collection. Data in computer files will be checked by the person entering and by another investigator to ensure accuracy of entry. Microbial counts will be recorded as cells/mL or cells/ g dry wt sediment.

Microbial distributions are not necessarily symmetrical. Most statistical techniques assume symmetrical distribution, and it is necessary to convert skewed data to symmetrical distribution results. Data will be analyzed using the ANOVA program. A P value of <0.05 is considered necessary to establish a statistically significant difference between the control and the distributed sample for each of the parameters measured.

## 8. HEALTH AND SAFETY PLAN

Lawrence Livermore National Laboratory's (LLNL) environmental, safety, and health policy is that operations must be planned and performed safely, with full consideration for the protection

of employees, the public, and the environment. In addition to observing LLNL policies contained in the Health & Safety Manual (M-010) and Environmental Protection Handbook, LLNL employees will comply with applicable federal, state, and local regulations as stated in the Health & Safety Manual and Environmental Protection Handbook, and this Facility Safety Procedure (360, 360.01 and addendum 360-2).

All personnel involved in the drilling and sampling activities are required to have their Superfund Amendments and Reauthorization Act/Occupational Safety and Health Administration (SARA/OSHA) 40-h training (and the SARA/OSHA 8-h yearly refresher course). Investigators in the field must also be respirator fitted and wear appropriate safety shoes, glasses, hard-hats, Tyvek suits (when necessary) and gloves while sampling. Team members must be current in training courses HS-4050 and EP-0006 as stipulated in the Operational Safety Procedures, OSP 406.2.

All microbiological testing is covered under FSP-360. Groundwater testing and treatability tests are covered under the LLNL Operational Safety Procedure 377.03.

## **9. RESIDUALS MANAGEMENT**

Under Federal Treatability Study Sample Exemption Rule, collection of hazardous wastes for purposes of conducting treatability studies are conditionally exempt from generator and transporter requirements (40 CFR parts 262 and 263). No hazardous waste will be used or generated to perform the enriched microbial analysis discussed above.

All LLNL personnel participating are certified in LLNL Hazardous Waste Generation (EP0006) and disposal procedures.

## **10. RECORDS**

Records generated as a result of implementation of this SOP will be controlled and maintained in the project record files.

## **SOP 24.2**

### **Microcosm Assay**

#### **1. PURPOSE**

This Standard Operating Procedure (SOP) establishes the method and responsibilities associated with the performance of the flask microcosm experiments. It outlines specific procedures including experimental protocols, quality control, data analysis and reporting. The flask microcosm experiments will be used for laboratory confirmation of microbial potential for contaminant biodegradation. The procedures described in this section have been specifically developed for application to NAS Alameda sites 3 and 13.

#### **2. OBJECTIVES**

The objectives of the flask microcosm experiments are to determine whether intrinsic aerobic biodegradation of petroleum hydrocarbons has the potential to occur at Sites 3 and 13 of the NAS Alameda and whether this process by itself can reduce petroleum hydrocarbons to target levels within a practical time frame. The experiments will be conducted in a controlled and closed environment to give results under conditions similar to the field situation at the time of sampling. It is not meant to simulate the transient subsurface environments where conditions such as temperature, moisture, and energy fluxes are changing.

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U.S. EPA. Guide for Conducting Treatability Studies Under CERCLA biodegradation remedy selection - quick reference fact sheet. EPA/540/R-93/519b, Office of Emergency and Remedial Response Hazardous Site Control Division OS-220, U.S. EPA, 1993.

#### **4. SPECIALIZED EQUIPMENT AND MATERIALS**

The specialized equipment to be used in the microcosm study includes: bench-scale structures for the microcosms similar to those described in [4], Perkin-Elmer's 8500 Gas Chromatography/Ion Trap Detector Mass Spectrometry (GC/MS) equipped with Tekmar LSC2 Automatic Purge and Trap System, Perkin-Elmer's Autosystem Gas Chromatography/Photo Ionization

Detector/Flame Ionization Detector (GC/PID/FID) equipped with Automatic Thermal Desorption System, Fisons' 3560 Simultaneous Inductively Coupled Plasma (ICP) Atomic Emission Spectrometry, Perkin-Elmer's 3100 and 3030 Atomic Absorption (AA) Spectrophotometry, Dionex Ion Chromatography/High Performance Liquid Chromatography (IC/HPLC), and LKB WALLAC 1219 RACKBETA Liquid Scintillation (LS) Counter. The GC/PID/FID will be used to determine the amount of total petroleum hydrocarbons (TPHs) present in the soil sample at the beginning and at the end of the experiment using the GC/PID/FID.; ICP and AA the concentration of 25 elements (cations)<sup>1</sup>; the IC/HPLC for the oxidation state of metal iron and the concentration of 7 anions<sup>2</sup>, and the LS Counter for the radioactivity of <sup>14</sup>C-labeled compounds. The GC/MS will be used for speciation when necessary.

To quantify the kinetics of biodegradation of the petroleum hydrocarbons in soil, <sup>14</sup>C-labeled toluene and phenanthrene will be used as chemical markers to represent the labile fractions of the petroleum products. The LS Counter will be used to measure the amount of <sup>14</sup>C-labeled toluene and phenanthrene being converted to <sup>14</sup>CO<sub>2</sub> over time.

## 5. EXPERIMENTAL PROCEDURES

The flask microcosm protocol will be similar to the aseptic and experimental techniques described in *Holman et al.* [1995]. All flask microcosm experiments will start within 24 hours upon arrival of the soil sample (Note: each sample must be accompanied by its chain-of-custody record) and will be carried out at 21°C ± 1°C on a shaker in the dark for six weeks. Table 1 summarizes the experimental design for the flask microcosm studies.

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<sup>1</sup> The 25 metals are Aluminum, Arsenic, Barium, Beryllium, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Lithium, Magnesium, Manganese, Nickel, Phosphorus, Potassium, Silicon, Silver, Sodium, Strontium, Sulfur, Thallium, Vanadium, and Zinc.

<sup>2</sup> The 7 anions are Chloride, Fluoride, Sulfate, Nitrate, Nitrite, Phosphate, Bromide.

### **5.1 SUMMARY OF MICROCOSM EXPERIMENTAL PROCEDURE**

1. Upon arrival, a subsample of the soil core and a groundwater sample will be given to LBNL's Environmental Measurement Laboratory to measure the initial concentration of TPHs in soil and groundwater. The rest will be used in the microcosm experiment.
2. Charge the test microcosm flask and its duplicate with 50 grams of soil. Go to step 4. Charge the control flask and its duplicate with 55 grams of soil.
3. Seal the control flasks and autoclave at 18 psi and 210°C for 20 minute intervals for up to two consecutive days.
4. Remove 5 gram of soil from each control flask to test for microbial growth using standard aseptic soil microbiology techniques. Repeat steps 2-3 if growth is observed.
5. Spike the microcosm flasks with 1 ml filter-sterile water that is saturated with <sup>14</sup>C-labeled organic and unlabeled organic.
6. Samples for analytical chemistry and <sup>14</sup>CO<sub>2</sub> production will be taken from all test and control microcosm flasks at each scheduled sampling event. There will be one sampling event for inorganic analytical chemistry (25 metals, oxidation state of iron): day 0. For organic analytical chemistry, there will be two sampling events: day 0 and day 42. For <sup>14</sup>C, the sampling events will be determined by the shape of the <sup>14</sup>CO<sub>2</sub> production curves from the experiments as the study progresses.
7. Microcosm headspace samples will also be monitored for stable isotopes to provide verification data for field isotopic measurements.

## **6. ANALYTICAL METHODS**

The chemical methods for determining the 25 elements and 7 anions are from the EPA Test methods SW-846 [7]. The method for determining the oxidation state of iron is from an in-house research method derived from [3], and TPHs is from [1]. Table 2 presents the methods and detection limits (MDLs) for the different analytical methods used in this study. All analysis will be performed by the certified Environmental Measurement Laboratory at LBNL, and all lab procedures will follow the existing QA/QC program adopted at LBNL.

Standard aseptic soil microbiology methods will be used for checking microbial populations in sterile soil samples.

The method for determining  $^{14}\text{C}$  production from  $^{14}\text{C}$ -labeled compounds in water, sediments saturated with water, and soil are well documented [2,4].

## **7. DATA MANAGEMENT, ANALYSIS AND INTERPRETATION**

The data management, analysis and interpretation will be guided by [6,8,9,10]. All procedures and raw data will be documented in bound lab books, photographs, and computer spreadsheet output. The raw data will be transferred into computer files within one month of collection. The data in computer files will be checked by the person entering and by another investigator to ensure accuracy of entry. All analyte concentration entries will be in  $\mu\text{g}$  of analyte /kg of oven dry soil.  $^{14}\text{C}$  production will be in % of initial  $^{14}\text{C}$ -labeled organic injected into the microcosm flask.

This data will be transferred to graphic form using statistical analysis software that is part of EXCEL (MICROSOFT Inc.). Graphs will report analyte concentration with appropriate statistics and descriptors (i.e. mean, range, sample location, depth, and time). The graphically represented data will allow us to easily examine and communicate biodegradation potential in NAS Alameda soils.

## **8. RESIDUALS MANAGEMENT**

Because of the use of  $^{14}\text{C}$ -labeled organics in the microcosm experiments, mixed waste will be generated. Its disposal will be handled by the EH&S at LBNL.

**TABLE 1.**  
**EXPERIMENTAL DESIGN FOR THE FLASK MICROCOSM STUDIES FOR EACH**  
**SITE**

Microcosm Flasks	Petroleum Products	<sup>14</sup> C-Organic	In Situ Microorganisms	Total #
<b>TEST FLASKS:</b>				
T <sub>bo</sub>	-	+	+	10 <sup>a</sup>
T <sub>bd</sub>	-	+	+	10 <sup>a</sup>
T <sub>po</sub>	+	+	+	20 <sup>a</sup>
T <sub>pd</sub>	+	+	+	20 <sup>a</sup>
<b>CONTROL FLASKS</b>				
C <sub>bo</sub>	-	+	Sterile	10 <sup>b</sup>
C <sub>po</sub>	+	+	Sterile	20 <sup>b</sup>
<b>TOTAL:</b>				90

T<sub>bo</sub> : nonsterile microcosm flask containing background (“clean”) soil and <sup>14</sup>C-organic

T<sub>bd</sub> : duplicate nonsterile microcosm flask containing background (“clean”) soil and <sup>14</sup>C-organic

T<sub>po</sub> : nonsterile microcosm flask containing petroleum contaminated soil and <sup>14</sup>C-organic

T<sub>pd</sub> : duplicate nonsterile microcosm flask containing petroleum contaminated soil and <sup>14</sup>C-organic

C<sub>bo</sub> : sterile microcosm flask containing background (“clean”) soil and <sup>14</sup>C-organic

C<sub>po</sub> : sterile microcosm flask containing petroleum contaminated soil and <sup>14</sup>C-organic

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<sup>a</sup> Chemical Analysis

<sup>b</sup> Chemical Analysis and colony forming unit counting

**TABLE 2. METHOD DETECTION LIMITS (MDL) FOR CHEMISTRY MEASUREMENT  
OF ANALYTES IN SOIL<sup>1</sup>**

Analyte	Method (EPA #)	Units	Detection Limit	MDL
Aluminum	6010	µg/Kg	500	n/a
Arsenic	7061/7062	µg/Kg	100	n/a
Barium	6010	µg/Kg	100	21
Beryllium	6010	µg/Kg	100	28
Cadmium	6010	µg/Kg	100	72
Calcium	6010	µg/Kg	500	n/a
Chromium	6010	µg/Kg	250	120
Cobalt	6010	µg/Kg	100	43
Copper	6010	µg/Kg	100	19
Iron	6010	µg/Kg	100	31
Lead	6010	µg/Kg	500	285
Lithium	6010	µg/Kg	100	n/a
Magnesium	6010	µg/Kg	500	n/a
Manganese	6010	µg/Kg	100	47
Mercury	7470	µg/Kg	0.2	n/a
Nickel	6010	µg/Kg	250	174
Phosphorus	6010	mg/Kg	10	n/a
Potassium	6010	µg/Kg	5000	n/a
Selenium	7741	µg/Kg	100	n/a
Silicon	6010	µg/Kg	5000	n/a
Silver	6010	µg/Kg	250	133

Sodium	6010	µg/Kg	5000	n/a
Strontium	6010	µg/Kg	100	24
Sulfur	6010	mg/Kg	10	n/a
Thallium	6010	µg/Kg	250	176
Vanadium	6010	µg/Kg	100	16
Zinc	6010	µg/Kg	250	129

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<sup>1</sup> As of July 20, 1995.

**TABLE 3.**  
**METHOD DETECTION LIMITS (MDL) FOR CHEMISTRY MEASUREMENT OF**  
**ANALYTES IN SOIL<sup>1</sup>**

Analyte	Method (EPA #)	Units	Detection Limit	MDL
Oxidation State of Iron	IC <sup>2</sup>	mg/Kg	10	N.D.
Chloride	9056	µg/Kg	10	N.D.
Fluoride	9056	µg/Kg	10	N.D.
Sulfate	9056	µg/Kg	15	N.D.
Nitrate	9056	µg/Kg	10	N.D.
Nitrite	9056	µg/Kg	10	N.D.
Phosphate	9056	µg/Kg	10	N.D.
Bromide	9056	µg/Kg	10	N.D.
TPHs	M8015	µg/Kg	N.D.	N.D.
<sup>14</sup> CO <sub>2</sub>	Holman et al.	CPM	94.5%	529

CPM: Counts Per Minute

MDL: Method Detection Limits

ND: Not Determined Yet

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<sup>1</sup> As of July 20, 1995.

<sup>2</sup> In-house Research Method

## SOP 24.3

### Direct Epifluorescent Microscopy

#### 1. PURPOSE

This Standard Operating Procedure (SOP) establishes the methods and responsibilities associated with direct epifluorescent microscopy to be performed on soil samples at ANAS sites 3 and 13. These assays will be used for field verification of contaminant biodegradation. The procedures described in this section have been specifically developed for application to ANAS sites 3 and 13.

#### 2. OBJECTIVES

The objectives of these tests are the quantification and characterization of microbial activity within background and contaminated subsurface zones. These objectives will be achieved by using a mixture of established and innovative methods of direct epifluorescent microscopy to investigate the number of total and active microbial cells present on subsurface material.

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#### 4. SPECIALIZED EQUIPMENT AND MATERIALS

Specialized equipment to be used with direct epifluorescent microscopy includes: Olympus BH2 Microscope with high power Mercury-Xenon UV lamp and polarizing filters, 25 mm Gelman Sciences polysulfone filter funnels and Technical Instruments 1K X 1K pixel resolution image analyzer with computer support. Fluorescent stains specific for different microbial proteins, DNA, and/or RNA will be used to determine total biomass in subsurface samples.

Well tested fluorochrome stains will be used as biomarkers of three major fractions that comprise the soil microbial biomass: Fluorescein Isothiocyanate (FITC) - to determine total bacterial biomass; Calcofluor M2R - to determine total fungal biomass; and Schaffer-Fulton spore stains will be used to estimate the quantities of spores present in environmental samples.

In addition to the total microbial population, an important parameter for assessing *in situ* bioremediation is determining the viable or active fraction of that population. To visually determine microbial activity *in situ*, redox dyes (viability stains) that serve as non-specific substrates for microbial respiration will be employed to stain active biomass within soil samples. We will use a new redox dye ( 5-cyano-2,3-ditolyl tetrazolium chloride, CTC) in conjunction with the above fluorescent stains to provide for direct microscopic confirmation of active and inactive fractions of the soil bacterial biomass and fluorescein diacetate in conjunction with Calcofluor M2R White for

active and inactive fungi. Together with subsurface field physical/chemical assays, these quantitative microbial investigations will be correlated with laboratory microcosms, microbial enrichments and isotopic results as integrated evidence that biotransformation potential demonstrated in the laboratory is realized in the field.

## **5. EXPERIMENTAL PROCEDURES**

All protocols used for direct epifluorescent microscopy will follow strict adherence to aseptic technique. An overview of the direct microscopy procedures is presented below:

### ***5.1 PROCEDURES FOR DETERMINING TOTAL and ACTIVE BIOMASS IN SEDIMENTS AND SUBSURFACE SOILS***

#### ***5.1.2 Method Summary***

Sediments and subsurface soils will be examined with direct microscopy to determine the total and active fungal and bacterial biomass. First, a representative sample will be acquired from the field observing accepted aseptic sampling procedures. Next, the sample will be serially diluted and an appropriate dilution chosen for microscopy. Selective biological stains will then be applied to the soil dilutions; these stains emit fluorescent colors and bind only to organic microbial biomass thus allowing cells to be differentiated from inorganic soil solids. Based on their color, the stains provide for identification of fungal and bacterial biomass as well as active and inactive cells. Once stained, soil dilutions will be passed through membrane filters. The solids (biomass and soil solids) retained on the filter surface will be washed and the filters mounted on microscope slides. The filter surfaces will then be viewed under high magnification (x 1100) with ultraviolet illumination and the stained bacteria and fungi measured and counted.

#### ***5.1.3 Sample Preparation And Handling***

Since microbial activity and community structure responds to changing conditions, soil samples will be processed as soon as possible after collection. Since samples will be processed within 24 - 48 hr. of collection, soil samples will be kept at the *in situ* temperature. Samples which cannot be processed within 48 hr. will be kept between 4 - 8 C. According to Ingram and coworkers (Oregon State University, Soil Science Department), soil from warm, mesic areas should be processed within 3 days of removal from the field, while soil from dry or cool sites can be kept for seven days without significant change in activity. Soil samples will not be frozen.

Augers (split-spoon) used for retrieving subsurface samples will be free of solids, washed with a disinfectant and cleaned with high pressure steam prior to sampling. Samples will be collected in

washed and autoclaved brass casing and contained with sterilized endcaps. All laboratory transfers and dilutions will be in accordance with aseptic technique in laminar flow “bio-hoods”.

Sample cores will be opened within a laminar hood and all solids in contact with the endcaps discarded. In Erlenmeyer flasks, dilute approximately 25 grams of soil in 250 mL phosphate buffer (pH 7.2) containing 0.1% sodium pyrophosphate. Shake the flasks at 160 rpm for several hours (room temperature). With sterile forceps remove any litter, large rocks or stones.

Determine the soil moisture content and the total suspended solids concentration of the soil dilution according to the standard methods in “Methods of Soil Analyses Part 1: Physical and Mineralogical Methods 2nd Edition” Soil Science Society of America, Madison, WI, 1986.

Using the following enumeration protocol, determine the optimum dilution (aliquot quantity) that results in maximum biomass estimates for a particular soil. The optimum aliquot volume usually falls between 5 uL and 500 uL of a 10:1 (w/w) soil dilution. The detection limits of this method are determined by (i) the size of the actual microbial population, (ii) the ratio of the soil solids to microbial biomass, and (iii) the shading factors and filterability of the soil as determined by grain size distribution.

## ***5.2 LABORATORY STAINING PROTOCOLS***

### ***5.2.1 Protocol for Determining Total and Active Bacterial Biomass in Sediments and Subsurface Soils Using CTC / DTAF Staining***

To determine total and active bacterial numbers using a dehydrogenase activity stain (CTC) and a cell wall stain (DTAF). This method sequentially stains activity and cell walls without fixation. Total set up time is approximately 10 hours.

**Solution 1 CTC for detection of cellular dehydrogenase activity (modified from Rodriguez, 1992):**

- 5 mL PBS pH 7.2 ( prepared from SIGMA # P-4417)

(685 mM NaCl; 13.5 mM KCl; 50 mM Na - phosphate)

- 0.5 mL CTC stock solution (Polysciences Waring, PA) (15 mg/mL in Sterile DI)

{final incubation concentration between 1- 5 mM recommended -we found the highest CTC activity at 1.5 mM CTC in many pure cultures trials)

- 1.0 mL Filter Sterilized Distilled Water (1 ml of a surrogate substrate can be added)

The above stock solutions are stable for at least 60 days; filter sterilize and store stock solutions in the dark between 4 - 8 C. (Others substrates and higher substrate concentrations can be added for activity staining).

Beginning with CTC, sequentially draw each component volume into a sterile 10 mL syringe - final volume 7.0 mL. Vortex syringe and mix well. Aseptically transfer working volume of 0.5 mL to 2.0 mL sterile microcentrifuge tubes. Aseptically introduce sample to CTC solution in microcentrifuge tubes (prepare dilution series of soil or other bacterial suspension before CTC staining). Mix well (with pipette tip or vortex). Incubate inoculated microcentrifuge tubes at 35 C with agitation for at least 4 hours (8 is better).

### **Solution 2 - to stain cell wall with DTAF (modified from Bloem, 1995)**

(make fresh for each use)

- 10 mL PBS pH 9.0

(final concentration: 0.05 M Na<sub>2</sub>PO<sub>4</sub> + 0.85% NaCl)

- 2 mg DTAF mixed isomer (molecular probes # D-16) Stains proteins in cell wall.

The above stock solution is stable for at least 60 days; filter sterilize and store stock solutions in the dark between 4 - 8 C. The DTAF mixture however, must be made fresh (4 hrs). Remove the plunger of a 10 mL sterile syringe and place 2 mg of DTAF in the barrel. Slowly and CAREFULLY, replace the plunger and push the fluorochrome powder to the front of the barrel without discharging any solid fluorochrome mass through the needle. Draw the PBS (pH 9) into the syringe and dissolve DTAF. Vortex syringe and mix well for several minutes (note: not all of the fluorochrome will dissolve). Without losing liquid volume, replace the syringe needle with a disposable 0.2 μ syringe filter (Whatman polypropylene 13 mM dia. #6782 - 1302). Aseptically decant 1.25 mL of filtered fluorochrome staining solution to each of the above microcentrifuge tubes following CTC incubation. The final reagent volume in each of the microcentrifuge tubes will be 1.75 mL. Mix well (with pipette tip or vortex). Stain in the dark for 30 minutes.

Filter the stained contents of the microcentrifuge tubes through black polycarbonate membrane filters (Poretics Corp; filter diameter, 25 mM dia.; pore diameter 0.22 μM) supported by a silver membrane diffuser (Poretics Corp; filter diameter, 25 mM dia.; pore diameter 5.0 μM). The filters should be thread sealed in a 50 mL capacity autoclavable polysulfone filter funnel assembly (Gelman sciences; effective filtration area 2.86 cm<sup>2</sup>). All filtration should be carried out under a vacuum of no greater than 103.5 kPa.

Wash the retained bacteria with filter sterilized reagents as follows:

Prevent the filter and funnel assembly from draining and completely immerse the retained solids with pH 9.0 phosphate buffer (reagent listed above). Let stand in the dark for 20 minutes. Drain under vacuum. Repeat (this) pH 9 phosphate buffer washing step.

Finally, rinse the filter quickly with sterile filtered water. Pull air through until the filter surface is dry.

Add several drops of TRIS buffered glycerol (1:1 v/v) containing 2% 1,4 diazobicyclo [2,2,2] octane (to retard quenching of the fluorescent signal) to the dried filter under vacuum (making sure to adjust the pH of the mounting solution to 8.6 with glacial acetic acid prior to application to optimize the fluorescence of the DTAF conjugate).

Under vacuum, pull the mountant through and lay the mountant wetted filter on a clean glass slide taking care not to tear the membrane. Add a very small drop of mountant (< 50 µL) to the filter surface and place a cover slip over the drop. Take care to flatten the filter on the slide and avoid entraining air bubbles beneath the coverslip.

View the mounted filters under UV illumination fitted with polarizing filters: 380 nM Exciter filter and 510 nM Barrier filter. View immediately. DTAF stains lime green and Formazan (CTC) stains brilliant red (intracellular). Cells that retain the green fluorescein as well as the red Formazan precipitates are active; those that stain only green are considered inactive.

Count the stained bacteria in a minimum of 10 randomly chosen fields under x1100 magnification. Choose an acceptable level for variation - (we use 0.3 to 0.4 = Coef. Variation). Shading factors are not considered in the count.

$$\# \text{ of cells / Volume} = [ (\# \times A_f) / (V \times a) ] \times DF$$

Where:

# = average count per field

$A_f$  = effective filtration area (sq. cm)

a = area of microscopic field

V = volume of dilution applied

DF = dilution factor

Normalize count to the total suspended solids of the soil dilutions applied.

### ***5.2.2 Protocol for Determining Total and Active Bacterial Biomass in Sediments and Subsurface Soils Using CTC / FITC Staining***

To determine total and active bacteria using a dehydrogenase activity stain CTC and a cell wall stain FITC. This method sequentially stains activity and cell walls without fixation. Total set up time is approximately 10 hours.

#### **Solution 1 CTC for detection of cellular dehydrogenase activity (modified from Rodriguez, 1992):**

- 5 mL PBS pH 7.2 ( prepared from SIGMA # P-4417)  
(685 mM NaCl; 13.5 mM KCl; 50 mM Na - phosphate)
- 0.5 mL CTC stock solution (Polysciences Waring, PA) (15 mg/mL in Sterile DI)  
{final incubation concentration 5 mM (between 5 - 10 mM recommended for soil)}
- 1.0 mL Filter Sterilized Distilled Water (1 ml of a surrogate substrate can be added)

The above stock solutions are stable for at least 60 days; filter sterilize and store stock solutions in the dark between 4 - 8 C. (Others substrates can be added for activity staining).

Beginning with CTC, sequentially draw each component volume into a sterile 10 mL syringe - final volume 7.0 mL. Vortex syringe and mix well. Aseptically transfer working volume of 0.7 mL to 2.0 mL sterile microcentrifuge tubes. Aseptically introduce sample to CTC solution in microcentrifuge tubes (prepare dilution series of soil or other bacterial suspension before CTC staining). Mix well (with pipette tip or vortex). Incubate inoculated microcentrifuge tubes at 35 C with agitation for at least 4 hours (8 is better).

#### **Solution 2 - to stain cell wall with FITC (modified from Schmidt and Paul, 1982).**

(make fresh for each use)

- 5.5 mL Saline solution stock solution (8.5 g/L NaCl and 0.2 g/L KCl)
- 1.25 mL Carbonate buffer stock solution pH 9.6 (1.59 g/L Na<sub>2</sub>CO<sub>3</sub>; 2.93 g/L NaHCO<sub>3</sub>)
- 5 mg FITC isomer 1 (SIGMA # F- 7250) Stains proteins in cell wall.

The above stock solutions are also stable for at least 60 days; filter sterilize and store stock solutions in the dark between 4 - 8 C. Remove the plunger of a 10 mL sterile syringe and place 5 mg of FITC or DTAF in the barrel. Slowly and CAREFULLY, replace the plunger and push the fluorochrome powder to the front of the barrel without discharging any solid fluorochrome mass

through the needle. Beginning with the carbonate buffer, sequentially draw the component volumes into syringe and dissolve FITC (or DTAF). Vortex syringe and mix well for several minutes (note: not all of the fluorochrome will dissolve). Without losing liquid volume, replace the syringe needle with a disposable 0.2  $\mu$  syringe filter (Whatman polypropylene 13 mM dia. #6782 - 1302). Aseptically decant 0.7 mL of filtered fluorochrome staining solution to each of the above microcentrifuge tubes following CTC incubation. The final reagent volume in each of the microcentrifuge tubes will be 1.4 mL. Mix well (with pipette tip or vortex). Stain in the dark for 10 to 20 minutes.

Filter the stained contents of the microcentrifuge tubes through black polycarbonate membrane filters (Poretics Corp; filter diameter, 25 mM dia.; pore diameter 0.22  $\mu$ M) supported by a silver membrane diffuser (Poretics Corp; filter diameter, 25 mM dia.; pore diameter 5.0  $\mu$ M). The filters should be thread sealed in a 50 mL capacity autoclavable polysulfone filter funnel assembly (Gelman sciences; effective filtration area 2.86 cm<sup>2</sup>). All filtration should be carried out under a vacuum of no greater than 103.5 kPa.

Wash the retained bacteria with filter sterilized reagents as follows:

Prevent the filter and funnel assembly from draining and completely immerse the retained solids with pH 9.6 carbonate buffer (reagent listed above). Let stand in the dark for 5 minutes. Drain under vacuum.

(OPTIONAL STEP - BASED ON BACKGROUND FLOUORESCENCE) Wash the filter surface with several milliliters of 5% sodium pyrophosphate under vacuum. Washing time varies per soil (< 5 minutes).

Add several drops of TRIS buffered glycerol (1:1 v/v) containing 2% 1,4 diazobicyclo [2,2,2] octane (to retard quenching of the fluorescent signal) to the dried filter under vacuum (making sure to adjust the pH of the mounting solution to 8.6 with glacial acetic acid prior to application to optimize the fluorescence of the DTAF conjugate).

Under vacuum, pull the mountant through and lay the mountant wetted filter on a clean glass slide taking care not to tear the membrane. Add a very small drop of mountant (< 50  $\mu$ L) to the filter surface and place a cover slip over the drop. Take care to flatten the filter on the slide and avoid entraining air bubbles beneath the coverslip.

View the mounted filters under UV illumination fitted with polarizing filters: 380 nM Exciter filter and 510 nM Barrier filter. View immediately. FITC stains lime green and Formazan (CTC) stains brilliant red (intracellular). Cells that retain the green fluorescien as well as the red Formazan precipitates are active; those that stain only green are considered inactive.

Count the stained bacteria in a minimum of 10 randomly chosen fields under x1100 magnification. Choose an acceptable level for variation - (we use 0.3 to 0.4 = Coef. Variation). Shading factors are not considered in the count.

$$\# \text{ of cells / Volume} = [ (\# \times A_f) / (V \times a) ] \times \text{DF}$$

Where:

# = average count per field

$A_f$  = effective filtration area (sq. cm)

a = area of microscopic field

V = volume of dilution applied

DF = dilution factor

Normalize count to the total suspended solids of the soil dilutions applied.

### ***5.2.3 Protocol for Determining Total Fungal Biomass in Sediments and Subsurface Soils***

To determine total fungal biomass using a cell wall stain specific for fungal biomass (Calcofluor M2R). This method stains fungal cell walls without fixation. Total set up time is approximately 26 hours.

**Solution 1 for detection of fungal and yeast biomass and spores - modified from "Methods of Soil Analyses Part 2: Chemical and Microbiological properties 2nd Edition" Soil Science Society of America, Madison, WI, 1994.**

- 5 mL Ringers Solution (2.25 g/L NaCl; 0.11 g/L KCl; 0.12 g/L  $\text{CaCl}_2$ ; 0.05 g/L  $\text{NaHCO}_3$ )

- Calcofluor M2R White (Molecular Probes Inc.)

The stock Ringers solutions is not stable; filter sterilize and store in the dark for not more than 7 days. Aseptically transfer Ringers solution volume of 1.5 mL into 2.0 mL sterile microcentrifuge tubes. Add 5  $\mu\text{L}$  of calcofluor M2R white to each microcentrifuge tube. Mix well (with pipette tip or vortex) and add sample. Incubate inoculated microcentrifuge tubes with agitation for 24 hours at room temperature.

Filter the stained contents of the microcentrifuge tubes through black polycarbonate membrane filters (Poretics Corp; filter diameter, 25 mM dia.; pore diameter 0.8  $\mu\text{M}$  or 1.0  $\mu\text{M}$ ) supported by a silver membrane diffuser (Poretics Corp; filter diameter, 25 mM dia.; pore diameter 5.0  $\mu\text{M}$ ).

The filters should be thread sealed in a 50 mL capacity autoclavable polysulfone filter funnel

assembly (Gelman sciences; effective filtration area 2.86 cm<sup>2</sup>). All filtration should be carried out under a vacuum of no greater than 103.5 kPa.

Wash the retained bacteria with filter sterilized distilled water:

Pull vacuum until the membrane appears dry. Still under vacuum, add several drops of 1:1 glycerol as a mountant and lay the mountant wetted filter on a clean glass slide taking care not to tear the membrane. Add a very small drop of mountant (< 50 µL) to the filter surface and place a cover slip over the drop. Take care to flatten the filter on the slide and avoid entraining air bubbles beneath the coverslip.

View the mounted filters under UV illumination fitted with polarizing filters: BP365/10 nM exciter filter, FT390 chromatic mirror, and LP 395 nM cutoff barrier filter (DAPI filter set). View immediately. Calcofluor M2R stains brilliant blue-white.

Using the calibrated eyepiece graticule, estimate the length and width of the fungal hyphae in the field of view.

Count the stained fungi and yeast a minimum of 10 randomly chosen fields under x1100 magnification. Choose an acceptable level for variation - 0.3 to 0.4 = Coef. Variation. Shading factors are not considered in the count.

$$\# \text{ of cells} / \text{Volume} = [ (\# \times A_r) / (V \times a) ] \times \text{DF}$$

Where:

# = average count per field

A<sub>r</sub> = effective filtration area (sq. cm)

a = area of microscopic field

V = volume of dilution applied

DF = dilution factor

Normalize count to the total suspended solids of the soil dilutions applied.

#### ***5.2.4 Protocols for Determining Active Fungal Biomass in Sediments and Subsurface Soils Using Fluorescein Diacetate or Calcein Am.***

To determine active fungal biomass using an esterase activity stain (fluorescein diacetate or Calcein AM). This method stains fungal cell walls without fixation. Total set up time is approximately 2 hours.

**Solution 1 for detection of active fungal biomass - modified from [Stamatiadis, 1990; Ingham and Klein, 1984]**

-1 mL Fluorescein Diacetate Stock Solution: 2 mg /mL in reagent grade Acetone

- 9 mL Phosphate buffered saline pH 7.2 (.prepared from SIGMA # P-4417)

(138 mM NaCl; 2.7mM KCl; 10 mM Na - phosphate)

The above stock solutions are stable for at least 30 days; however they require different storage conditions. Filter sterilize and store FDA stock solutions in the freezer. Store sterile PBS at room temperature.

Beginning with FDA, sequentially draw each component volume into a sterile 10 mL syringe - final volume 10.0 mL. Vortex syringe and mix well. Aseptically transfer working volume of 1.5 mL into to 2.0 mL sterile microcentrifuge tubes. Aseptically introduce sample to FDA solution in microcentrifuge tubes (prepare dilution series of soil or other bacterial suspension before FDA staining). Mix well (with pipette tip or vortex). Incubate inoculated microcentrifuge tubes at room temperature with agitation for not more than 3 minutes. Staining with FDA for more than 3 minutes can facilitate cleaved fluorescein to be eliminated from extremely active cells. Since fluorescein elimination becomes an ever increasing background fluorescence problem, the slides must be viewed immediately after staining.

Filter the stained contents of the microcentrifuge tubes through black polycarbonate membrane filters (Poretics Corp; filter diameter, 25 mm dia.; pore diameter 0.8  $\mu$ M or 1.0  $\mu$ M) supported by a silver membrane diffuser (Poretics Corp; filter diameter, 25 mm dia.; pore diameter 5.0  $\mu$ M).

The filters should be thread sealed in a 50 mL capacity autoclavable polysulfone filter funnel assembly (Gelman sciences; effective filtration area 2.86 cm<sup>2</sup>). All filtration should be carried out under a vacuum of no greater than 103.5 kPa.

Wash the retained bacteria with filter sterilized TRIS or PBS adjusted to pH 8 to 8.5:

Add several drops of TRIS buffered glycerol (1:1 v/v) containing 2% 1,4 diazobicyclo [2,2,2] octane (to retard quenching of the fluorescent signal) to the dried filter under vacuum (making sure to adjust the pH of the mounting solution to 8.6 with glacial acetic acid prior to application to optimize the fluorescence of the fluorescein).

Pull vacuum until the membrane appears dry. Still under vacuum, add several drops of 1:1 glycerol as a mountant and lay the mountant wetted filter on a clean glass slide taking care not to tear the membrane. Add a very small drop of mountant (< 50  $\mu$ L) to the filter surface and place a cover slip over the drop. Take care to flatten the filter on the slide and avoid entraining air bubbles beneath the coverslip.

View the mounted filters under UV illumination fitted with polarizing filters: 380 nM Exciter filter and 510 nM Barrier filter. View immediately. Fluorescein stains lime green and Formazan (CTC) stains brilliant red (intracellular). Hyphae that retain the green fluorescein are active.

Using the calibrated eyepiece graticule, estimate the length and width of the fungal hyphae in the field of view.

Count (and/or measure hyphal length) the stained fungi and yeast in a minimum of 10 randomly chosen fields under x1100 magnification. Choose an acceptable level for variation - (0.3 to 0.4 = Coef. Variation). Shading factors are not considered in the count.

$$\# \text{ of cells} / \text{Volume} = [ (\# \times A_r) / (V \times a) ] \times \text{DF}$$

Where:

# = average count per field

$A_r$  = effective filtration area (sq. cm)

a = area of microscopic field

V = volume of dilution applied

DF = dilution factor

Normalize count to the total suspended solids of the soil dilutions applied.

## 6. TREATABILITY TEST PLAN

Replicate samples of subsurface material representing a range of saturation and contaminant concentrations at each candidate site will be analyzed for abundance and distribution of bacteria and fungi using the above stated procedures. A total of six sampling locations will be used at each site to investigate the subsurface bacterial activity. Of these sampling locations, two will be placed away from any known contamination in order to differentiate between natural background microbial activity and activity resulting from microbial transformation of subsurface contaminants. Each sampling location will consist of a borehole and several soil gas probes. Aseptically collected soil core samples will be obtained from depths that represent the shallow soil, the vadose zone, the capillary fringe zone, a shallow water table depth and median water table depth. Cores within the zone of contamination will be taken from the area of highest concentrations moving down gradient. Using accepted regression techniques (Standard Methods for Soil Analyses Pt.2 2nd Ed.:

Microbiological and Biochemical Analyses (1995)), active and inactive fractions of soil microbial biomass will be estimated from direct microscopic counts. A paired t-test, at a 90% confidence level, will be applied to determine significant differences between biomass estimates from contaminated and pristine subsurface samples.

## 7. ANALYTICAL METHODS

The scientific background and current methods for determining microbial populations in environmental samples using direct microscopy are documented in the references. Previous studies have used staining and direct epifluorescence microscopy to estimate microbial numbers in soils, biological sludges, sediments, and natural waters (Schmidt, 1974; Hernandez *et al.*, 1994; Stayer and Tiedje, 1978; and Hobbie, 1977). Quantitative microbial staining and viability assays, described in the literature, have been recently modified by Hernandez and Alvarez-Cohen (1994) and tested at the University of California at Berkeley for the express purpose of assessing intrinsic and enhanced bioremediation. The experiments performed to optimize visual soil microbial biomass estimates have been modified from epifluorescence - polycarbonate membrane filtration techniques introduced by Hobbie *et al.* (1977). As presented in the experimental procedures section, the modified membrane filtration technique involves filtering diluted soil sample mixtures with specifically targeted stains so that stained microorganisms caught on the filter surface can be counted with an ultraviolet microscope. The following tables present the level of accuracy expected from the direct microscopic assays, and the accuracy of associated equipment. Expected detection limits are on the order of  $1 \times 10^6$  bacteria per gram dry weight.

### EXPECTED ACCURACY FOR SOIL MICROBIAL BIOMASS MEASUREMENTS

MEASURE	UNITS	EXPECTED RANGE	ACCURACY	PRECISION
Active/total bacterial biomass	$\mu\text{g g}^{-1}$ dry soil	Between 0.001 and $10 \mu\text{g g}^{-1}$ dry soil	Within 20%	Least significant Digit, (LSD) = $1 \mu\text{g}$
Active/total fungal biomass	$\mu\text{g g}^{-1}$ dry soil	Between 0.01 and $10 \mu\text{g g}^{-1}$ dry soil	Within 25 %	LSD = $10 \mu\text{g}$

### EXPECTED ACCURACY AND MAINTENANCE FOR ASSOCIATED EQUIPMENT

EQUIPMENT	TYPE AND FREQUENCY	ACCURACY	PRECISION
Phase contrast\ DIC microscope	Check lamps, focus, alignment after each use. Calibrate micrometer when moved.	Within 10 %	+/- one unit

Refrigerator	Temperature checked daily.	Within 10%	+/- 5%
Balance	Standard weights weekly, document in log book.	Within 10%	+/- 5%
Pipettes	Check volume with graduated cylinder/balance weekly.	SD< 1%	+/- 0.1 ml

For quality control, laboratory microscope operators will be trained by UC Berkeley principal investigators; microscopy will be routinely performed by the same operators. On each hundredth sample, operators will gather and compare morphological criteria. Routine laboratory controls will make certain that bacteria and fungi do not grow in sterile waters, buffers or agars. Calibrations of microscopes and grids will be made whenever the lenses are changed, or the microscope is cleaned.

## 8. DATA MANAGEMENT, ANALYSIS AND INTERPRETATION

All procedures and raw data will be clearly documented in bound notebooks, photographs and computer spreadsheets. Recorded data will be transferred into computer files within one month of collection. Data in computer files will be checked by the person entering and by another investigator to ensure accuracy of entry. Microbial counts will be determined with units of # or mass per/mass of soil. Raw data will be tabulated as depicted below. This data will be transferred to graphical form using statistical analysis software (SYSTAT Inc., Evanston ILL). Graphs will report microbial counts with appropriate statistics and descriptors (i.e. mean, variance, sample location, depth and time). Examples of how raw data will be tabulated are presented below.

EXAMPLE TABLE FOR TOTAL AND ACTIVE FUNGAL BIOMASS DATA

Sample #	Sample Designator	Dilution Factor	# Total/Active units per field			# fields observed	Diameter of hyphae

EXAMPLE TABLE FOR TOTAL AND ACTIVE BACTERIAL DATA

Sample #	Sample Designator	Dilution Factor	# Total/Active units per field			# of fields observed	Diameter of bacteria

## **9. RESIDUALS MANAGEMENT**

Under Federal Treatability Study Sample Exemption Rule, collection of hazardous wastes for purposes of conducting treatability studies are conditionally exempt from generator and transporter requirements. (40 CFR parts 262 and 263). No hazardous waste will be used or generated to perform the direct microscopic soil analysis discussed above.

## **10. RECORDS**

Records generated as a result of implementation of this SOP will be controlled and maintained in the project record files.

## SOP 24.4

### Isotope Monitoring

#### 1. PURPOSE

This Standard Operating Procedures (SOP) establishes the method for using isotopic measurements of soil gas and groundwater samples to verify and quantify *in situ* microbial activity at NAS ALAMEDA sites 3 and 13. These assays will be used for field verification of contaminant biodegradation. The procedures described in this section have been specifically developed for application to NAS ALAMEDA Sites 3 and 13.

#### 2. OBJECTIVES

Microbial degradation of petroleum hydrocarbons results in metabolic byproducts (e.g., soil gas  $\text{CO}_2$ ,  $\text{CH}_4$ ; groundwater  $\text{HCO}_3^-$ ,  $\text{NO}_3^-$ ) that will be used to monitor *in situ* bioremediation activity. However, microorganisms may also degrade substrates in the subsurface besides contaminants (e.g., natural soil organic matter). Further, there are other potential generation sources of these compounds besides subsurface microbial activity (e.g., root respiration, dissolution of carbonates, atmospheric contamination). The procedure described here utilizes isotopic measurements of these soil gas and groundwater compounds to differentiate components derived from degradation of hydrocarbons from those resulting from other sources. This is accomplished by measuring the isotopic compositions of potential sources for these compounds and comparing these to the isotopic compositions of the produced soil gas and groundwater species.

There are two sets of isotope data that will be collected for this work. Stable isotope measurements compare the ratios of the major stable isotopes of an element within a compound. The isotope ratios of a compound are determined by the isotope ratios of the reactants that produced the compound and the fractionating effects associated with the process whereby the compound was formed. Once the compounds have been produced, the stable isotope ratios remain essentially constant (especially at low temperatures). The other type of isotopic measurement that will be made is radiocarbon (or  $^{14}\text{C}$ ) abundances.  $^{14}\text{C}$  is a radioactive isotope of carbon produced by interaction of cosmic rays with the upper atmosphere. Carbonaceous compounds formed from atmospheric  $\text{CO}_2$  (e.g. plants), will have a  $^{14}\text{C}$  content in equilibrium with atmospheric  $\text{CO}_2$ . When exchange with the atmosphere ceases, the  $^{14}\text{C}$  will decay according to its half life (5730 years). Since organic contaminants are manufactured from fossil carbon sources, any byproducts of microbial metabolism of these compounds will be contain no  $^{14}\text{C}$ . Because of the different factors controlling their compositions, the combination of measurements of stable carbon isotope ratios ( $^{13}\text{C}/^{12}\text{C}$ ) and  $^{14}\text{C}$  abundances provide a powerful tool for identifying the source of

microbial byproducts (e.g., soil gas CO<sub>2</sub> or groundwater HCO<sub>3</sub><sup>-</sup>), and the determining the processes by which they were produced.

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## **4. SPECIALIZED EQUIPMENT AND MATERIALS**

### ***4.1 STABLE ISOTOPE MEASUREMENTS -***

The type of highly precise isotope ratio measurements necessary for this work require a gas source isotope ratio mass spectrometer. The instrument that will be used for this study is the Prism Series II mass spectrometer (manufactured by VG Isotech, a division of Fisons Instruments) of the Center for Isotope Geochemistry (CIG) at Lawrence Berkeley National Laboratory. This machine is capable of measuring variations in the stable isotope ratios of carbon, nitrogen and oxygen of less 1 part in 10,000 or 0.1% and of hydrogen to within 1 part in 1,000 or 1%. The Prism is also fitted with automated systems for measuring the carbon and

oxygen isotope ratios of carbonates, the oxygen isotope ratios of water samples, and the hydrogen, carbon, nitrogen, and oxygen isotope ratios of gas samples, all of which will be used for this work. In addition to the mass spectrometer and its automated prep systems, a variety of techniques for preparation and purification of gas samples for isotope measurements will be used (see Appendices). These techniques require custom glass vacuum lines designed for the specific procedures (i.e., an in-line CuO furnace for combusting hydrocarbon compounds or flow-through traps for separating H<sub>2</sub>O, CO<sub>2</sub> and N<sub>2</sub>). For some of the procedures, specialized glassware is also required (i.e., fritted flow-through caustic traps for removing CO<sub>2</sub> from soil gas samples or attachments for the vacuum lines to allow injection of water samples into pyrex tubes with zinc metal for reducing the water to H<sub>2</sub> gas). All of this equipment is currently available at CIG.

#### ***4.2 RADIOCARBON MEASUREMENTS***

<sup>14</sup>C measurements on CO<sub>2</sub> isolated from soil gas, contaminant vapors, dissolved carbonates and other materials sampled in this study will be performed at the Lawrence Livermore National Laboratory, Center for Accelerator Mass Spectrometry (CAMS). The CAMS instrument is a multi-use accelerator based on an FN Tandem Electrostatic (Van de Graaff) Accelerator. Established research applications of the instrument include the use of <sup>10</sup>Be, <sup>14</sup>C and <sup>36</sup>Cl isotopes in geophysics, both as tracers and as chronometers (Davis, et al. 1990). As much as half of the facility's operating time is devoted to analysis of biomedical and environmental samples (Creek, et al. 1994, Vogel and Turteltaub, 1992).

CAMS sample preparation, measurement, and analysis techniques follow common practices within the radiocarbon and AMS communities. These are documented extensively in the journal *Radiocarbon* and in proceedings of International AMS Conferences published in the journal *Nuclear Instruments and Methods*. Chemical pretreatment and conversion of samples to CO<sub>2</sub> will be carried out by the principal investigators, as described below. CAMS personnel will be responsible for conversion of CO<sub>2</sub> to graphite using procedures documented in Vogel, Nelson, and Southon, (1987) and Loyd, Vogel, and Trumbore, (1991). CAMS requires that any laboratory submitting CO<sub>2</sub> samples for analysis shall also provide a suitable number of CO<sub>2</sub> blanks prepared from appropriate materials. Data analysis algorithms are based on those described in Stuiver and Polach (1977) and Donahue et al. (1990).

## 5. ANALYTICAL UNITS

### 5.1 STABLE ISOTOPE RATIOS

Stable isotope ratios are measured relative to internationally-accepted standards. Values are reported using the per mil notation, whereby an isotope ratio of one per mil (denoted by ‰) indicates that the isotope ratio of a material is one part per thousand greater than that of the reference standard. For carbon isotopes, the ratio of <sup>13</sup>C to <sup>12</sup>C (approximately equal to 0.011) is expressed relative to Peedee belemnite (VPDB), where:

$$d^{13}\text{C}_{\text{VPDB}} = \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}} - (^{13}\text{C}/^{12}\text{C})_{\text{VPDB}}}{(^{13}\text{C}/^{12}\text{C})_{\text{VPDB}}} \times 1000$$

### 5.2 RADIOCARBON

Owing to the extensive use of radiocarbon (<sup>14</sup>C) contents of samples for archaeological and other applications, several different reporting units are encountered in the literature, including fraction of modern carbon (F), D<sup>14</sup>C, and conventional <sup>14</sup>C age (Stuiver and Polach, 1977); due to the nature of the present investigation, we will rely on the unit F. The values are determined in AMS by measuring the <sup>14</sup>C/<sup>13</sup>C ratio in a sample and comparing that value with one derived from standards, typically NIST traceable oxalic acid samples. The ratios of standards and samples are further corrected against the <sup>13</sup>C/<sup>12</sup>C ratios of the VPDB as described above. Instrument background, contamination during sample preparation, and sample contamination *in situ* all potentially contribute to the apparent activity of a sample; the former two sources are explicitly addressed by routine comparison of sample data with results from analysis of standards and background samples (<sup>14</sup>C-free coal; backgrounds are scaled relative to sample size). Analyses of the contributions of these various sources are presented with sample preparation techniques in Vogel et al. (1984, 1987); corrections follow the method described by Donahue et al. (1990).

## 6. EXPERIMENTAL PROCEDURES

### 6.1 STABLE ISOTOPE MEASUREMENTS

Stable isotope ratios are measured using a gas-source, isotope ratio mass spectrometer. In brief, a stream of gas is admitted into the ion source of the mass spectrometer where the gas molecules are ionized by being bombarded with an electron beam. The resulting ions are accelerated down the flight tube of the mass spectrometer through a strong magnetic field which deflects the ion beam. Gas ions of differing mass (e.g., <sup>15</sup>N<sup>14</sup>N versus <sup>14</sup>N<sub>2</sub>) are deflected by different amounts, causing them to be separated into distinct beams. The intensities of the beams are measured by Faraday

cups appropriately positioned at the far end of the flight tube. The ratios of the intensities of these ion beams are used to determine the isotope ratios of the gas. In order to correct for shifts in the isotope ratios produced by the effects such as ionization, capillary flow in the inlet, etc., the beam ratios of the sample gas are compared to those of a known standard gas. As a result of this technique, stable isotope compositions are reported as deviations from the isotope ratio of the standard.

## **6.2 RADIOCARBON ANALYSES**

Radiocarbon analyses will be done by accelerator mass spectrometry at CAMS. Aliquots of CO<sub>2</sub> produced for stable isotope analyses are converted to solid graphite by heating in a hydrogen atmosphere in the presence of a powdered (200 mesh) iron or cobalt catalyst. This supported material is pressed into an aluminum target that is mounted in a sample wheel at the ion source. A cesium-sputter ion source converts a portion of the sample into a negative-ion beam that is directed into the accelerator by a low energy mass spectrometer that isolates a small range of mass/charge ratio particles. The negative ions are accelerated by positive potential, until they reach the center of the machine, where they encounter a thin carbon foil or a confined diffuse gas, which strips them of valence electrons, and dissociates any remaining molecular fragments. The resulting positive (<sup>13</sup>C<sup>4+</sup> and <sup>14</sup>C<sup>4+</sup>) ions are accelerated towards ground potential. They are then focused and directed into a high energy mass spectrometer, where <sup>13</sup>C<sup>4+</sup> are separated for counting in a stable isotope Faraday cup. <sup>14</sup>C<sup>4+</sup> ions are further filtered, and ultimately counted in a gas ionization detector. Graphite samples are held in a 60 -sample wheel (typically 45 unknowns, 15 standards and backgrounds) for an eight hour cycle.

## **6.3 SAMPLE ANALYSES**

A wide variety of analytical techniques have been developed to convert samples into gases that can be used to measure stable isotope ratios. Those procedures that will be used for this project are outlined in the analytical techniques section of this SOP and are described in detail in a series of appendices. The types of samples to be collected and analyzed, along with the rationale for those analyses, are outlined below:

- I. Soil Samples - To determine isotopic signature of potential substrates for microbial activity.
  1.  $\delta^{13}\text{C}$ ,  $^{14}\text{C}$ ,  $\delta^{15}\text{N}$  of organic matter in soils.
- II. Marine Shellfish - To determine the isotopic signature of soil carbonates.
  1.  $\delta^{13}\text{C}$ ,  $^{14}\text{C}$ ,  $\delta^{18}\text{O}$  of carbonate shells.
- III. Contaminants - Where available, to measure the initial isotopic composition of petroleum hydrocarbon contaminants.
  1.  $\delta^{13}\text{C}$ ,  $^{14}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta\text{D}$  of hydrocarbon compounds.

IV. Soil Gas Samples - To determine the contribution of byproducts of microbial metabolism of contaminants.

1.  $d^{13}C$ ,  $^{14}C$  of soil gas  $CO_2$ .
2.  $d^{13}C$ ,  $^{14}C$ ,  $dD$  of soil gas  $CH_4$ .
3.  $d^{13}C$ ,  $dD$  of soil gas VOCs.

V. Groundwater Samples - To determine the contribution of byproducts of microbial metabolism of contaminants and to trace input from different water sources (e.g., rainwater, municipal water, bay water).

1.  $d^{13}C$ ,  $^{14}C$  of dissolved inorganic carbon compounds (DIC).
2.  $d^{15}N$ ,  $d^{18}O$  of groundwater nitrate.
3.  $dD$ ,  $d^{18}O$  of groundwater.

VI.  $CO_2$  from microcosm experiments - To determine fractionating effects of microbial metabolic processes on stable isotope ratios of  $CO_2$ .

1.  $d^{13}C$  of  $CO_2$  produced during degradation experiments.

## 7. TREATABILITY TEST PLAN

### 7.1 PRELIMINARY SCREENING PHASE

The Preliminary Screening phase of sampling at both Site 3 and Site 13 will be conducted to provide data to aid in selecting sampling locations for the Background Level and Contaminant Area Characterization phase. Work for this task is aimed at making a cursory identification of the levels and types of microbial activity at the each site.

- I. Collect shallow soil cores ( $\leq 2$  m total depth, 2.5 cm diameter) with a hand coring tool. Two cores will be collected from contaminated areas and one from an adjacent clean area. Each core will be photographed and logged using the standard core log form (Appendix 24.4 A) noting changes in color, soil composition, contaminant content, etc. Samples from each core will be analyzed for the following:
  - A.  $d^{13}C$ ,  $d^{15}N$  of soil organic matter - Four samples from approximately equal spacing within each soil core will be analyzed. The exact location of the samples will be modified to reflect major changes in soil type, distribution of contaminants, and saturated versus vadose zone.
  - B.  $d^{13}C$ ,  $d^{18}O$  of fossil marine carbonate shells - Where available, two samples of shells from each core will be analyzed.
  - C.  $^{14}C$  of soil organic matter and shells - Three or four of the above samples will be analyzed for  $^{14}C$  content in order to determine the background signatures. Samples to be analyzed will be determined after the stable isotope analyses are complete, in order to resolve ambiguities in the stable isotope data.

II. Soil gas and groundwater samples will be taken from adjacent to the each of the core sites (prior to collection of soil cores) and at three other intermediate locations. At each sample site, 1/4" stainless steel tubes will be driven to three depths; one shallow ( $\leq 50$  cm depth), one just above the water table, and, where possible, one into the saturated zone. Approximately 1 liter of soil gas will be collected in Tedlar bags using a peristaltic pump at flow rate of  $\leq 100$  cc/minute after purging at least 3 tube volumes of gas. Approximately 100 ml of groundwater will also be collected with a peristaltic pump after purging 3 tube volumes of water. Where groundwater nitrate will be analyzed, additional water will be pumped and passed through anion columns in the field. Both groundwater samples and anion columns will be tightly capped and stored at  $< 4^{\circ}\text{C}$  until analyses can be completed. The following analyses will be made of these samples:

A. Soil Gas samples (all analyses will be completed within 3 days of sampling)

1.  $\text{d}^{13}\text{C}$  of soil gas  $\text{CO}_2$  - Will be measured for all samples.
2.  $\text{d}^{13}\text{C}$ ,  $\text{dD}$  of methane and/or VOCs - These will be analyzed in samples containing  $> 1000$  ppm methane or total VOCs (as determined by GC-MS and GC analyses).
3.  $^{14}\text{C}$  of soil gas  $\text{CO}_2$ ,  $\text{CH}_4$  - Four to six of the above samples will be analyzed for  $^{14}\text{C}$  content after stable isotope analyses are complete, in order to resolve ambiguities in the stable isotope data.

B. Groundwater samples

1. Dissolved oxygen, pH measurements and nitrate contents of all groundwater samples will be analyzed in the field (following methods outlined in SOP 24.5 - Field Physical/Chemical Assays).
2.  $\text{d}^{13}\text{C}$  of DIC - These analyses will be completed on all groundwater samples collected.
3.  $\text{d}^{18}\text{O}$ ,  $\text{dD}$  of groundwater - These analyses will be completed on all groundwater samples collected.
4.  $\text{d}^{15}\text{N}$ ,  $\text{d}^{18}\text{O}$  of nitrate - These analyses will be completed on all groundwater samples containing  $> 5$  mg per liter.
5.  $^{14}\text{C}$  of DIC - Two or three of the above samples will be analyzed for  $^{14}\text{C}$  content. Samples to be analyzed will be determined after the stable isotope analyses are complete, in order to resolve ambiguities in the stable isotope data.

## ***7.2 BACKGROUND LEVEL AND CONTAMINANT AREA CHARACTERIZATION PHASE***

At both Sites 3 and 13, soil gas and groundwater samples will be collected from probes installed adjacent to each boring installed for the Background Level and Contaminant Area Characterization phase. Soil gas samples will be collected from three depths within the vadose zone at each location and groundwater samples will be collected from two depths within the saturated zone at each location. Intermediate locations will also be sampled. During installation of sampling probes at 3

of these locations, a more detailed set of soil gas/groundwater samples (~12 intervals at each location) will be collected for diffusion calculations.

- I. From each drill core, soil samples from the intervals being studied for microbial activity will be collected for isotopic analysis. Samples will be analyzed for the following:
  - A.  $\delta^{13}\text{C}$  of soil organic matter - Every sample (and in any samples of petroleum hydrocarbons that might be available).
  - B.  $\delta^{15}\text{N}$  of soil organic matter - Every other sample (and in any samples of petroleum hydrocarbons that might be available).
  - C.  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  of fossil marine carbonate shells - Where available, up to 3 samples per core (if no shells are found within the sampled intervals, shells from other sections of the cores will be analyzed).
  - D.  $^{14}\text{C}$  of soil organic matter and shells - Ten to fifteen of the above samples will be analyzed for  $^{14}\text{C}$  content in order to determine the background signatures. Samples to be analyzed will be determined after the stable isotope analyses are complete, in order to resolve ambiguities in the stable isotope data.
2. Soil gas and groundwater samples will be collected following the methods described in Section 7.1:
  - A. Soil Gas samples (all analyses will be completed within 3 days of sampling)
    1.  $\delta^{13}\text{C}$  of soil gas  $\text{CO}_2$  - Will be measured for all samples.
    2.  $\delta^{13}\text{C}$ ,  $\delta\text{D}$  of methane and/or VOCs - Up to 20 samples of methane and VOCs separated from soil gas samples will be analyzed (after abundances have been determined by GC-MS and GC analyses).
    3.  $^{14}\text{C}$  of soil gas  $\text{CO}_2$ ,  $\text{CH}_4$  - In order to resolve ambiguities in the stable isotope data, ten to fifteen of the above samples will be analyzed for  $^{14}\text{C}$  content after stable isotope analyses are complete.
  - B. Groundwater samples
    1. Dissolved oxygen, pH measurements and nitrate contents of all groundwater samples will be analyzed in the field (following methods outlined in SOP 24.5 - Field Physical/Chemical Assays).
    2.  $\delta^{13}\text{C}$  of DIC - Every sample.
    3.  $\delta^{18}\text{O}$ ,  $\delta\text{D}$  of groundwater - Every sample.
    4.  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$  of nitrate - Will be analyzed in 30-50% of groundwater samples.
    5.  $^{14}\text{C}$  of DIC - Ten to fifteen of the above samples will be analyzed for  $^{14}\text{C}$  content. Samples to be analyzed will be determined after the stable isotope analyses are complete.

### **7.3. PERIODIC SAMPLING PHASE**

To determine the effects of seasonal variations in weather (changing temperature, moisture levels, groundwater level, etc.) on subsurface microbial activity, two additional sets of soil gas and groundwater samples will be collected from each site. Ideally, when all three phases of sampling are finished (Preliminary Screening, Background Level and Contaminant Area Characterization and Periodic Sampling), there will be at least one set collected prior to the rainy season, one at the height of the rainy season (preferably within a few days of a major rain event) and one after the rainy season has ended but before the groundwater has begun dropping significantly. Samples for this phase will be collected from existing sampling probes installed during the Background Level and Contaminant Area Characterization phase).

1. Sampling methods will be the same as those described in Preliminary Screening Phase, Section 7.1. For each sampling period, the following analyses will be made:
  - A. Soil Gas samples (all analyses will be completed within 3 days of sampling)
    1.  $\delta^{13}\text{C}$  of soil gas  $\text{CO}_2$  - Will be measured for all samples.
    2.  $\delta^{13}\text{C}$ ,  $\delta\text{D}$  of methane and/or VOCs - Up to ten samples of methane and VOCs separated from soil gas samples will be analyzed (after abundances have been determined by GC-MS and GC analyses).
    3.  $^{14}\text{C}$  of soil gas  $\text{CO}_2$ ,  $\text{CH}_4$  - In order to resolve ambiguities in the stable isotope data, approximately five of the above samples will be analyzed for  $^{14}\text{C}$  content after stable isotope analyses are complete.
  - B. Groundwater samples
    1. Dissolved oxygen, pH measurements and nitrate contents of all groundwater samples will be analyzed in the field (following methods outlined in SOP 24.5 - Field Physical/Chemical Assays).
    2.  $\delta^{13}\text{C}$  of DIC - Every sample.
    3.  $\delta^{18}\text{O}$ ,  $\delta\text{D}$  of groundwater - Every sample.
    4.  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$  of nitrate - Will be analyzed in 30-50% of groundwater samples.
    5.  $^{14}\text{C}$  of DIC - Approximately five of the above samples will be analyzed for  $^{14}\text{C}$  content. Samples to be analyzed will be determined after the stable isotope analyses are complete.

### **7.4 MICROCOSM EXPERIMENTS**

To determine the magnitude of fractionation effects caused by microbial metabolism of hydrocarbons, the  $\delta^{13}\text{C}$  ratios of  $\text{CO}_2$  samples collected from microcosm experiments will be analyzed. For each site, the carbon isotope ratios from 4 sets of microcosm experiments will be monitored.

- I.  $\delta^{13}\text{C}$  of organic carbon in samples - One for each microcosm experiment.

- II.  $d^{13}C$ ,  $dD$  of substrate hydrocarbons - Where available.
- III.  $d^{13}C$  of  $CO_2$  produced during experiments - 3 samples per week throughout the duration of the experiments.
- IV.  $d^{13}C$  of organic carbon remaining in microcosm at the end of the experiments - One for each microcosm experiment.

## **8. ANALYTICAL METHODS**

The analytical methods that will be used to make the isotopic measurements required for this project are summarized below. Detailed descriptions of the precise methods used in our laboratories (including references to published techniques) are contained in Appendices 24.4 B through 24.4 K.

EXPECTED ACCURACY ISOTOPE MEASUREMENTS

MEASURE	UNITS	EXPECTED RANGE	ACCURACY	PRECISION
$^{13}\text{C}/^{12}\text{C}$ ratios of organic compounds. <sup>1</sup>	per mil (‰) relative to VPDB	0 to -40‰	±0.1‰	±0.1‰
D/H ratio of organic compounds. <sup>1</sup>	‰ relative to VSMOW	0 to -400‰	±3‰	±3‰
$^{15}\text{N}/^{14}\text{N}$ ratios of organic matter <sup>2</sup>	‰ relative to air	+10 to -15‰	±0.2‰	±0.2‰
$^{13}\text{C}/^{12}\text{C}$ ratios of carbonate minerals <sup>3</sup>	‰ relative to VPDB	+2 to -5‰	±0.1‰	±0.1‰
$^{18}\text{O}/^{16}\text{O}$ ratios of carbonate minerals <sup>3</sup>	‰ relative to VPDB	0 to -5‰	±0.1‰	±0.1‰
$^{13}\text{C}/^{12}\text{C}$ ratios soil gas $\text{CO}_2$ <sup>4</sup>	‰ relative to VPDB	-10 to -35‰	±0.2‰	±0.2‰
$^{13}\text{C}/^{12}\text{C}$ ratios hydrocarbon gases ( $\text{CH}_4$ , VOCs) <sup>5</sup>	‰ relative to VPDB	0 to -100‰	±0.2‰	±0.2‰
D/H ratios hydrocarbon gases <sup>5</sup>	‰ relative to VSMOW	0 to -400‰	±3‰	±3‰
$^{13}\text{C}/^{12}\text{C}$ ratios of groundwater DIC <sup>6</sup>	‰ relative to VPDB	+10 to -25‰	±0.1‰	±0.1‰
$^{15}\text{N}/^{14}\text{N}$ ratios of groundwater nitrate <sup>7</sup>	‰ relative to air	+10 to -25‰	±0.2‰	±0.2‰
$^{18}\text{O}/^{16}\text{O}$ ratios of groundwater nitrate <sup>7</sup>	‰ relative to VSMOW	+20 to 0‰	±0.5‰	±0.5‰
D/H ratios of groundwater <sup>8</sup>	‰ relative to VSMOW	0 to -90‰	±2‰	±2‰
$^{18}\text{O}/^{16}\text{O}$ ratios of groundwater <sup>9</sup>	‰ relative to VSMOW	0 to -13‰	±0.1‰	±0.1‰
$^{14}\text{C}$ contents <sup>10</sup>	% of modern $^{14}\text{C}$ (F or pmc)	0 to 1.15 pmc (F)	±1‰	±1‰

<sup>1</sup> See Appendix 24.4 B - "Reaction and Extraction Instructions for  $\text{d}^{13}\text{C}$  and  $\text{dD}$  Analyses of Organic Compounds."

<sup>2</sup> See Appendix 24.4 C - "Extraction of Nitrogen from Organic Compounds for  $\text{d}^{15}\text{N}$  Analyses."

- 3 See Appendix 24.4 D - "d<sup>13</sup>C and d<sup>18</sup>O Analyses of Carbonate Samples Using the ISOCARB System."
- 4 See Appendix 24.4 E - "Extraction of CO<sub>2</sub> from Soil Gas Samples for d<sup>13</sup>C Analyses."
- 5 See Appendix 24.4 F - "Separation and Preparation of Hydrocarbon Gases for d<sup>13</sup>C and dD Analyses."
- 6 See Appendix 24.4 G - "Extraction of Dissolved Inorganic Carbon Compounds (DIC) from Water Samples for d<sup>13</sup>C Analyses."
- 7 See Appendix 24.4 H - "Extraction of Nitrate from Water for d<sup>15</sup>N and d<sup>18</sup>O Analyses."
- 8 See Appendix 24.4 I - "Conversion of Water to H<sub>2</sub> Gas for D/H Analyses."
- 9 See Appendix 24.4 J - "Analysis of the d<sup>18</sup>O Values of Water Samples Using the ISOPREP 18."
- 10 See Appendix 24.4 K - "CAMS practices standard data".

#### EXPECTED ACCURACY AND MAINTENANCE FOR ASSOCIATED EQUIPMENT

EQUIPMENT	TYPE AND FREQUENCY	ACCURACY	PRECISION
Mass spectrometer	Analyze the isotopic ratios of known gas standards with all data runs.	d <sup>13</sup> C, d <sup>15</sup> N and d <sup>18</sup> O: ±0.1‰ dD: ±1‰	d <sup>13</sup> C, d <sup>15</sup> N: ±0.02‰ d <sup>18</sup> O: ±0.05‰ dD: ±0.3‰
Gas flow meter	Calibrate against bubble flow meter monthly; manufacturer calibration yearly.	±3%	±3%
Pressure transducers	Zero and set span to atmospheric pressure daily.	± 1 torr	± 1 torr

## 9. DATA MANAGEMENT, ANALYSIS AND INTERPRETATION

Procedures and raw data for all field work, sample preparation and isotopic analyses will be clearly documented in bound notebooks. Pertinent data for different sample sets (soil, soil gas and groundwater) will be transferred onto data sheets, examples of which are contained in Appendix 24.4 L. Both electronic and paper copies of these data sheets will be maintained.

Data will be entered into computer spreadsheets (Microsoft Excel), plotted onto plane maps of the sites and contoured, when appropriate. Where significant correlations exist between different factors (e.g., %CO<sub>2</sub> and d<sup>13</sup>C of soil gas CO<sub>2</sub>, F-fraction of modern <sup>14</sup>C), graphical representations of these data sets will be produced. These graphs will aid in distinguishing sources

of soil gas and groundwater compounds and determining what processes produced them (e.g., aerobic biodegradation of hydrocarbons, dissolution of marine carbonate shells).

## **10. RESIDUALS MANAGEMENT**

Under Federal Treatability Study Sample Exemption Rule, collection of hazardous wastes for purposes of conducting treatability studies are conditionally exempt from generator and transporter requirements. (40 CFR parts 262 and 263). Hazardous wastes generated through sample preparation procedures (e.g., NaOH solutions used to trap CO<sub>2</sub> from gas samples, phosphoric acid used to dissolve carbonates) will be disposed of according to standard procedures already in place at the CIG and at CAMS.

## **11. RECORDS**

Records generated as a result of implementation of this SOP will be controlled and maintained in the project record files.



## APPENDIX 24.4 B

### Reaction and Extraction Instructions for $d^{13}C/dD$ Analyses of Organic Compounds

#### A. Reaction of organic material to produce $CO_2/H_2O$ :

1. Weigh out enough sample to produce 80 to 100 mmoles of  $CO_2$  gas (~2.5 mg of pure organic compounds).
2. For samples containing carbonate minerals, leach sample with dilute HCL (10%). Rinse thoroughly with de-ionized water and dry.
3. Load 500 mg copper oxide (CuO) into a 9mm vycor tube. Add sample using a thin paper funnel. Add 300mg of granular copper (Cu). Evacuate tube on vacuum line and seal using a hot flame. Shake tube to mix sample powder, Cu and CuO.
4. Place sample tubes in stainless steel tubes on rack in the muffle furnace. React samples for 2 hours at  $900^{\circ}C$  and cool  $50^{\circ}C/hr$ .

#### B. Extraction of $CO_2$ gas:

1. Prepare 2 methanol slushes for flow-through traps on extraction line. The slush for cold trap 1 (containing glass beads) should be maintained between  $-80$  and  $-90^{\circ}C$ . The second slush will be used intermittently on cold trap 2 and should be kept between  $-60$  and  $-70^{\circ}C$ .
2. Score sample tubes lightly and place in tube cracker. Attach to vacuum line and pump down.
3. After line is evacuated, isolate manifold from pump, crack sample tube and expand the sample gas into manifold.
4. **Slowly** (to avoid rush of gas which can cause glass beads to break line), open valve to first cold trap (cooled with  $-90^{\circ}C$  methanol slush). Wait ~2 minutes and expand sample into second cold trap cooled with liquid  $N_2$  (LN). Once the pressure on the Varian gauge stabilizes, pump away the non-condensable gases.
5. Isolate trap 2 and manometer finger. Replace LN on trap 2 with  $70^{\circ}C$  methanol slush and freeze manometer finger with LN. Allow  $CO_2$  to freeze into manometer finger. Isolate manometer finger and drop LN trap. Record pressure reading (in torr).
6. Place partially filled dewar of LN on sample collection tube. Isolate manifold and allow  $CO_2$  sample to transfer from manometer finger into the sample collection tube. Once the Varian

gauge has stopped falling, top off LN, pump away non-condensibles, isolate collection tube and seal with torch.

7. Drop slush from trap 2 and use the heat gun to remove water frozen in the trap. Remove water in trap 1 every 4-8 samples.

C. Extraction of H<sub>2</sub>O\*:

1. To analyze the dD value of water released during combustion, remove methanol slushes on both traps and transfer water into 6 mm O.D. pyrex tube containing zinc (~50 mg per ml of water expected).
2. Seal tube and react at 500°C for 20 minutes to convert water into H<sub>2</sub> gas (see Appendix 24.4.8.9).

\* Most organic matter contains exchangeable hydrogen and should not be analyzed following this technique. This method is only useful for analyzing the dD of petroleum hydrocarbon compounds.

## APPENDIX 24.4 C

### Extraction of Nitrogen from Organic Compounds for $d^{15}\text{N}$ Analyses

1. Weigh out enough sample to produce at least 20 mmoles of  $\text{N}_2$  gas.
2. Load 2 g copper oxide ( $\text{CuO}$ ) into a 9mm vycor tube. Add sample and 150-300 mg calcium oxide ( $\text{CaO}$ ) using a thin paper funnel. Add 2 g more of  $\text{CuO}$  followed by 3g of granular copper ( $\text{Cu}$ ). Evacuate tube on vacuum line and seal using a hot flame. Shake tube to mix sample powder,  $\text{CaO}$ ,  $\text{CuO}$  and  $\text{Cu}$ .
3. Place sample tubes in stainless steel tubes on rack in the muffle furnace. React samples for 2 hours at  $900^\circ\text{C}$  and cool  $50^\circ\text{C/hr}$ .
4. Samples can be released directly into the mass spectrometer without further purification. Score tubes at end opposite  $\text{CaO}$  powder and load into a flex-tube cracker. Attach to the Multiport, pump down, manually crack tube and expand sample gas into the mass spectrometer.

#### References:

Kendall, C. and Grim, E., 1990, Combustion tube method for measurement of nitrogen isotope ratios using calcium oxide for total removal of carbon dioxide and water: Analytical Chemistry, vol. 62, p. 526-529.

Stump, R., and Frazer, J., 1972, Analytical Chimica Acta, v. 60, p. 277-285.

## APPENDIX 24.4 D

### **d<sup>13</sup>C and d<sup>18</sup>O Analyses of Carbonate Samples Using the ISOCARB System\***

1. Change prep system on mass spectrometer to carbonate carousel (using AC command). **Before changing from other prep systems, be sure to close all valves, including the toggle switch to the water equilibrator.**
2. Place magnetic stir bar in inner compartment of carbonate reaction vessel and add 5 ml 100% phosphoric acid. To load onto carousel, close manual valve to auxiliary roughing pump (down position), open P4 valve and toggle switch to capillary inlet. Exchange empty reaction vessel on carousel for vessel with acid and stir bar (check to make sure O-ring is clean and seats properly; no twisting). Close capillary inlet and open toggle switch to capillary bypass. When the reading on Pirani 3 drops below  $8e-2$ , open manual valve and close valve to capillary bypass. Attach the water lines from the constant temperature bath to the reaction vessel (water into lower tube of reaction vessel and out of upper tube). Fill water reservoir in constant temperature bath with de-ionized water to ~5 cm below the top (do not fill too high or water will overflow when it warms up). Turn on water bath. When temperature reaches ~60°C (it should be set to 90°C), turn on magnetic stir bar (MS command). Pump on phosphoric acid at 90°C for at least two hours prior to beginning run.
3. Load carbonate powders into sample boats and place them in the numbered holder. For relatively pure carbonates, fill sample boat ~1/4 full (equals approximately 1 mg of sample). Include at least 6 and preferably 9 standards per run (2-3 at the beginning, 2-3 in the middle and 2-3 at the end of the set). These standards are used to correct the data for run-to-run fluctuations such as slight changes in the temperature of the water bath. If the approximate isotopic compositions of the samples are known, use a standard with a similar composition.
4. To load sample boats into the carousel, remove the screws to the top plate of the carousel, close the manual valve to auxiliary roughing pump (down position), open P4 valve and toggle switch to capillary inlet. Remove the top plate and load the samples into the numbered holes (beginning with #2), being careful not to spill any sample powder. Before loading, check to make sure that sample hole #1 is positioned slightly past the opening to the acid bath **and** that the computer is indicating that the position of the carousel is #1. When finished, put the top back on the carousel and loosely tighten down the screws. Close capillary inlet and open toggle switch to capillary bypass (**it is very important to remember this step, or your sample powders will get sucked out of the boats into the prep system**). When the reading on Pirani 3 drops below  $8e-2$ , open manual valve and close valve to capillary bypass.

5. Place a large stainless steel dewar with chilled methanol on the cold trap, insert the probe to the Cryocooler and turn on the Cryocooler (allow at least 30 minutes for the temperature of the methanol to stabilize at  $-90^{\circ}\text{C}$ ).
6. Enter sample run data into the computer. Be sure to change the sample position for the first sample from 1 to 2. Also, be sure that the carbonate parameter file is loaded (command SFS 1 followed by command SFL). If the parameter file is changed, check that the source is still properly tuned.
7. Fill the large liquid nitrogen (LN) dewar to the top and insert the tubes to the cold fingers. Be sure that there is no water/ice blocking the tubes. Begin the auto run (command AG).
8. After the run, turn off the Cryocooler (**do not move the arm until it has had a chance to warm up**), turn off the water bath, remove the reaction vessel from the line and replace it with an empty vessel (following procedure outlined in 2). Wrap water trap with heat tape and pump out while heating to  $\sim 140^{\circ}\text{C}$ . Set the carousel back to position 1 (MPGT 1).

Reference:

McCrea, J.M., 1950, On the isotope chemistry of carbonates and a paleotemperature scale: Jour. of Chem. Physics, v. 18, p. 849-857.

- \* The ISOCARB system can only be used for relatively pure samples ( $\geq 20\%$  carbonate) of reactive carbonates (e.g., calcite, aragonite). Unreactive carbonates (e.g., dolomite, siderite) need to be reacted for longer time periods and do not produce complete yields (leading to contamination of later samples reacted in a common acid bath).

## APPENDIX 24.4 E

### Extraction of CO<sub>2</sub> from Soil Gas Samples for d<sup>13</sup>C Analyses

#### A. Trapping CO<sub>2</sub> gas from samples:

1. Mix 15 ml of de-ionized water with 5 ml of 50% w/w NaOH solution in fritted flow-through caustic traps and seal traps.
2. After making required measurements of compositions of soil gas samples, slowly (~50cc/min) pump sample out of Tedlar bag, through a caustic trap and into a second Tedlar bag with a peristaltic pump.

#### B. Evolving CO<sub>2</sub> gas:

1. Add 3 ml 100% phosphoric acid to 40 ml reaction tubes and cap with a septa from a 7 ml Vacutainer (lightly grease septa with silicon grease).
2. Pump out reaction tubes using 22 gauge needles attached to vacuum line. Heat phosphoric acid with heat gun until it boils and continue to pump on acid for ~1 hour.
3. Inject enough solution from caustic traps to produce at least 100mmoles of CO<sub>2</sub> into the reaction tubes. Be careful not to add too much caustic solution so as to overpressure the reaction vessels (~1200 mmoles) or the septa will pop out of the tube.

#### C. Extraction of CO<sub>2</sub> gas:

1. Prepare 2 methanol slushes for flow-through traps on extraction line. The slush for cold trap 1 (containing glass beads) should be maintained between -80 and -90°C. The second slush will be used intermittently on cold trap 2 and should be kept between -60 and -70°C.
2. Load reaction vessels on line (embed needle deep enough into septa to seal needle, but not so deep that needle pushes through septa) and pump down.
3. After line is pumped down, close valves to manifold. Push the needle through the septa of the sample to be analyzed and open the valve to the manifold (with the valves to the water trap and to the vacuum pump closed). Gently heat the sample with a heat gun until the water begins to boil.
4. After letting the sample cool and the CO<sub>2</sub> equilibrate (~2 minutes), close the valve to the sample and **slowly** (to avoid rush of gas which can cause glass beads to break line) open the

valve to water trap (cooled with  $-90^{\circ}\text{C}$  methanol slush). Wait  $\sim 2$  minutes and expand the sample into second cold trap cooled with liquid  $\text{N}_2$  (LN). Once the pressure on the Varian gauge stabilizes, pump away the non-condensable gases.

5. Isolate trap 2 and manometer finger. Replace LN on trap 2 with  $70^{\circ}\text{C}$  methanol slush and freeze manometer finger with LN. Allow  $\text{CO}_2$  to freeze into manometer finger. Isolate manometer finger and drop LN trap. Record pressure reading (in torr).
6. Place partially filled dewar of LN on sample collection tube. Isolate manifold and allow  $\text{CO}_2$  sample to transfer from manometer finger into the sample collection tube. Once the Varian gauge has stopped falling, top off LN, pump away non-condensibles, isolate collection tube and seal with torch.
7. Drop slush from trap 2 and use the heat gun to remove water frozen in the trap. Remove water in trap 1 every 4-8 samples. Before loading new samples, check to be sure that liquid is in the needle or the tubes to the sample manifold.

## APPENDIX 24.4 F

### Separation and Preparation of Hydrocarbon Gases for $d^{13}C$ and $dD$ Analyses

#### A. Separation and combustion of methane:

1. After making required measurements of gas compositions, strip  $CO_2$  from sample by passing through NaOH solution (see Appendix 24.4.8.5).
2. Place dewars with liquid nitrogen (LN) on all five double U-traps on the gas line. Slowly pass sample through the first set of double U-traps (to remove water and other hydrocarbon compounds) and on through the CuO furnace (at  $\sim 900^\circ C$ ). Pump away residual gas.
3. When pressure has stopped falling (with the CuO furnace at  $900^\circ C$  it will never drop back to baseline), isolate the CuO furnace section of the line, place a methanol slush (at  $\leq -60^\circ C$ ) on the double U-trap before the furnace, open the bypass valve and allow pressure to settle again.
4. Close valve between CuO furnace and last two double U-traps. Close valve after manometer. Isolate trap 2 and manometer finger. Replace LN on double U-traps with methanol slushes and freeze manometer finger with LN. Allow  $CO_2$  to freeze into manometer finger. Isolate manometer finger and drop LN trap. Record pressure reading (in torr).
5. Place partially filled dewar of LN on sample collection tube. Isolate manifold and allow  $CO_2$  sample to transfer from manometer finger into the sample collection tube. Once the Varian gauge has stopped falling, top off LN, pump away non-condensibles, isolate collection tube and seal with torch.
6. Thaw double U-trap before CuO furnace and transfer water through bypass section into double U-traps after the furnace (while pumping). When pressure stabilizes, close valve between CuO furnace and last two double U-traps. Close valve to vacuum line. Thaw traps and transfer water into 6 mm sample tube with zinc for conversion of water to  $H_2$  gas (see Appendix 24.4.8.9).

#### B. Combustion of remaining hydrocarbons:

1. Replace LN on first two double U-traps with methanol slushes (at  $\geq -5^\circ C$ ; the higher temperature is to thaw all remaining hydrocarbons while retaining water). Place another slush on the trap before the CuO furnace and LN on the traps after the furnace. Pump sample through furnace.
2. When pressure has stabilized, repeat steps 3 through 6 above to collect  $CO_2$  and  $H_2O$  resulting from combustion of hydrocarbons.

## APPENDIX 24.4 G

### Extraction of Dissolved Inorganic Carbon Compounds (DIC) from Water Samples for $d^{13}C$ Analyses

#### A. Extraction of DIC from water:

1. Add 3 ml 100% phosphoric acid to 40 ml reaction tubes and cap with a septa from a 7 ml Vacutainer (lightly grease septa with silicon grease).
2. Pump out reaction tubes using 22 gauge needles attached to vacuum line. Heat phosphoric acid with heat gun until it boils and continue to pump on acid for ~1 hour.
3. Inject enough sample into the reaction tubes to produce 100mmoles of  $CO_2$  (generally at least 10 ml).

#### B. Extraction of $CO_2$ gas:

1. Prepare 2 methanol slushes for flow-through traps on extraction line. The slush for cold trap 1 (containing glass beads) should be maintained between  $-80$  and  $-90^\circ C$ . The second slush will be used intermittently on cold trap 2 and should be kept between  $-60$  and  $-70^\circ C$ .
2. Load reaction vessels on line (embed needle deep enough into septa in order to seal needle, but not deep enough that needle pushes through septa) and pump down.
3. After line is pumped down, close valves to manifold. Push the needle through the septa of the sample to be analyzed and open the valve to the manifold (with the valves to the water trap and to the vacuum pump closed). Gently heat the sample with a heat gun until the water begins to boil.
4. After letting the sample cool and the  $CO_2$  equilibrate (~2 minutes), close the valve to the sample and **slowly** (to avoid rush of gas which can cause glass beads to break line) open the valve to water trap (cooled with  $-90^\circ C$  methanol slush). Wait ~2 minutes and expand the sample into second cold trap cooled with liquid  $N_2$  (LN). Once the pressure on the Varian gauge stabilizes, pump away the non-condensable gases.
5. Isolate trap 2 and manometer finger. Replace LN on trap 2 with  $70^\circ C$  methanol slush and freeze manometer finger with LN. Allow  $CO_2$  to freeze into manometer finger. Isolate manometer finger and drop LN trap. Record pressure reading (in torr).

6. Place partially filled dewar of LN on sample collection tube. Isolate manifold and allow CO<sub>2</sub> sample to transfer from manometer finger into the sample collection tube. Once the Varian gauge has stopped falling, top off LN, pump away non-condensibles, isolate collection tube and seal with torch.
7. Drop slush from trap 2 and use the heat gun to remove water frozen in the trap. Remove water in trap 1 every 4-8 samples. Before loading new samples, check to be sure that liquid is in the needle or the tubes to the sample manifold.

References:

Graber, E. R., and Aharon, P., 1991, An improved microextraction technique for measuring dissolved inorganic carbon (DIC), d<sup>13</sup>C<sub>DIC</sub> and d<sup>18</sup>O<sub>H<sub>2</sub>O</sub> from milliliter-size water samples: Chem. Geol. (Isotope Geoscience Sect.), v. 94, p. 137-144.

## APPENDIX 24.4 H

### Extraction of Nitrate from Water for $d^{15}\text{N}$ and $d^{18}\text{O}$ Analyses

#### A. Resin-column preparation:

1. Suspend resin in a column and settle. Gravity drip 2 ml of 1.25 M  $\text{CaCl}_2$  through column, followed by 5 rinses of 2 ml deionized water. Keep column saturated with 0.5 ml of water and cap both ends.

#### B. Sample collection:

1. Determine nitrate ( $\text{NO}_3^-$ ) concentration.
2. Filter samples through 0.45m filter and drip filtered samples through columns at rate  $<1$  liter/hour until  $\sim 200$  mmol of nitrate has been loaded onto a column. Refrigerate column until ready to strip.

#### C. Stripping columns:

1. Gravity drip 3 ml of 3M HCl through the column 5 times. Blow excess HCl through the column in between the 3 ml increments.

#### D. Sample preparation:

1. Neutralize samples with  $\text{Ag}_2\text{O}$  until pH paper reads 5.5-6.0.
2. Filter liquid through a 0.45m filter into a 100 ml, tri-cornered plastic beaker. Rinse the  $\text{AgCl}$  with 15 ml de-ionized water.
3. Split sample for separate  $d^{18}\text{O}$  and  $d^{15}\text{N}$  analyses.

#### E. Continued preparation for $d^{15}\text{N}$ analyses:

1. Freeze beakers in liquid nitrogen (LN) and cover with parafilm. Punch a few holes in the edges of the parafilm and freeze dry the samples.
2. Dissolve the freeze dried solids in 2 ml deionized water and pipette into 9mm Vycor tubes. Freeze solution in the tubes using a methanol slush. Cover tubes with a piece of Kimwipe held with a rubber band and freeze dry again.
3. Add 3.5 g of copper oxide ( $\text{CuO}$ ), 350 mg calcium oxide ( $\text{CaO}$ ) and 2.5 g copper metal ( $\text{Cu}$ ) to the Vycor sample tubes. Pump down on the tubes for 1 hour and seal with a hot torch.
4. Reaction: place sample tubes in stainless steel tubes on a stainless steel rack in the muffle furnace. React samples for 2 hours at  $850^\circ\text{C}$  and cool  $50^\circ\text{C}/\text{hour}$ .

F. Continued preparation for  $d^{18}O$  analyses:

1. Take the remaining portion of each sample split in step 8.D.3. and add an excess of  $BaCl_2$  to precipitate sulphate, phosphate and Ag.
2. Filter solution through 0.45m filter. Pass the filtrate through a Bio-Rad AG-50 X8, 100-200 mesh cation column and collect nitrate. Rinse with 1 ml of deionized water 3 times.
3. Neutralize  $HNO_3$  solution with 1 g of  $Ag_2O$ . Filter solution and then agitate with 0.4g activated carbon for 20 minutes.
4. Filter out activated carbon with 0.2m nylon filter. Freeze solution, freeze dry, redissolve solids in 2 ml of deionized water, pipette solution into a 9mm Vycor reaction tube and freeze dry again.
5. Add 4-5 mg of ground spectrographic graphite to the reaction tube. Evacuate the tube and seal with a hot torch.
6. Reaction: place sample tubes in stainless steel tubes on a stainless steel rack in the muffle furnace. Heat samples to  $850^{\circ}C$  and immediately cool at  $50^{\circ}C/hour$ .

**APPENDIX 24.4 I**  
**Conversion of H<sub>2</sub>O to H<sub>2</sub> gas for D/H Analyses**

**A. Sample Preparation:**

1. Load 50mg of zinc into 6 mm O.D. pyrex tubes (annealed at 500°C and stored in a drying oven).
2. Insert tubes into #7 Ace fittings by placing the O-ring ~1 cm from the top of the tube. Then slide the tube into the black nylon fitting and screw into the threaded glass fitting on the manifold and tighten. Pump out all tubes.
3. Heat the zinc with a heat gun about 1 minute until a silvery halo of zinc vapor condenses on the lower part of the tube. Pump on tubes for at least 1 hour.
4. Loosen the black nylon fitting and carefully slide the tube up until it reaches the top of the glass fitting, just below the red & white septa. Pump down tubes for an extra 5 minutes if they leaked to the atmosphere.
5. Rinse a 10 ml syringe 3 times with the sample to be injected. Draw up ~3 ml of sample, then draw in air until a bubble appears above the needle and the total volume of water in the syringe can be measured. Record the amount of water in the D/H logbook. Wipe needle dry.
6. Close valve between glass fitting and the manifold. Insert the needle through the septa and into the sample tube inside the glass fitting. Depress plunger. Leaving the needle in the tube, place a dewar of liquid nitrogen (LN) on the bottom 2.5 cm of the tube, just covering the zinc. Heat the glass fitting, making sure that any water frozen in the needle is removed. "Chase" the water down the tube with the heat gun until all the water is frozen into the bottom of the sample tube. Remove the syringe and check for water trapped in the needle.
7. After injecting and heating 4 samples, raise the level of the LN to the top of all 4 dewars. Isolate the manifold and open the valve between the manifold and the sample tube. Record the non-condensables in the logbook. Pump away noncondensables and use gas torch to seal tube.

**B. Reaction:**

1. Preheat muffle furnace to 500°C. Place sample tubes in aluminum rack and put the rack in the furnace for 15-20 minutes.

Reference:

Venneman, T.W., and O'Neil, J. R., 1993, A simple and inexpensive method of hydrogen isotope and water analyses of minerals and rocks based on zinc reagent: Chem. Geol. (Isotope Geoscience Sect.), v. 103, p. 227-234.

## APPENDIX 24.4 J

### Analysis of the $d^{18}O$ values of water samples on the ISOPREP 18

1. Pipette 2 ml of sample into an equilibrator flask. Load standards in ports AA, BA, AL, and BL and unknowns in the remaining 20 ports by tightening cajon fittings onto the neck of the equilibrator flasks.
2. Evacuate flasks by opening all the ports until the Pirani guage reads  $<0.5$  mbars.
3. Close all ports and evacuate the air remaining in the sample bank. Open all ports, switch over the three way bank-selection valve and open  $CO_2$  valve to release  $CO_2$  into each equilibrator flask. Wait one minute.
4. Close the  $CO_2$  valve, close all ports, and pump away  $CO_2$  remaining in the pipework.
5. Place plexiglass cover over the equilibrator flasks, turn on heater to regulate temperature at  $25^\circ C$  and begin to shake flasks.
6. Place a large stainless steel dewar with chilled methanol on the cold trap, insert the probe to the Cryocooler and turn on the Cryocooler (in order for the temperature of the methanol to stabilize at  $-90^\circ C$ , do this at least 30 minutes prior to beginning a run).
7. If the mass spectrometer is on a different prep system, change to the equilibrator (using AC command). **Before changing from other prep systems, be sure to close all valves.** Enter sample run data into the computer. Start the run (command AG). When the computer asks for delay until beginning sample run, enter time in hours remaining before the samples should be equilibrated. Samples should equilibrate for at least 5 hours (if the mass spectrometer is being used for other analyses, equilibration can be started before entering the sample run data by using the control panel on the front of the ISOPREP 18). **Be sure the toggle switch to the inlet system of the mass spectrometer is open before the run starts!!**

## APPENDIX 24.4 K

### CAMS PRACTICES STANDARD DATA

CENTER FOR ACCELERATOR MASS SPECTROMETRY (CAMS)  
PO BOX 808, L-397  
LAWRENCE LIVERMORE NATIONAL LABORATORY  
LIVERMORE, CA 94551-9900

GEOSCIENCES RADIOCARBON PROGRAM  
Isotope Geochemist: Michael Kashgarian  
Telephone (510) 422-3703, fax (510) 423-7884

#### PROCEDURES

CAMS sample preparation, measurement, and analysis techniques follow common practices within the radiocarbon and AMS communities. These are documented extensively in the journal *Radiocarbon* and in proceedings of International AMS Conferences published in the journal *Nuclear Instruments and Methods*. For collaborative work with Mark Conrad and Paul Daley, chemical pretreatment and conversion of samples to CO<sub>2</sub> will be carried out by them. CAMS will be responsible for conversion of CO<sub>2</sub> to graphite using procedures documented in Vogel, Nelson, and Southon, *Radiocarbon* 29 (1987) 323-333 and Loyd, Vogel, and Trumbore, *Radiocarbon* 33 (1991) 297-301. CAMS requires that any laboratory submitting CO<sub>2</sub> samples for analysis shall also provide a suitable number of CO<sub>2</sub> blanks prepared from appropriate materials. The CAMS spectrometer is described in Davis et al, *Nucl. Instr. and Meth.* B52 (1990) 269-272. Data analysis algorithms are based on those described in Stuiver and Polach, *Radiocarbon* 19 (1977) 355-363 and Donahue, Linick, and Jull, *Radiocarbon* 32 (1990) 135-142.

#### TRAINING

Setup and tuning of the AMS analytical equipment is carried out only by the chief analyst, Dr. John R. Southon, or an assigned deputy. Data acquisition runs under computer control, with monitoring by a knowledgeable individual. Data analysis is carried out by the isotope geochemist or an assigned deputy. Training for spectrometer setup and operation and for data analysis is carried out by a trainee operating the system under supervision until the chief analyst is satisfied that the trainee has acquired a sufficiently comprehensive understanding of the measurement and analysis system.

## CONTROL OF TEST AND MEASUREMENT EQUIPMENT

Setup and tuning of the analytical equipment is carried out only by trained personnel, and measurements are undertaken only when the analyst in charge is satisfied that the hardware and software are operating normally.

All radiocarbon measurements are made relative to standards (NBS Oxalic Acid) provided by NIST. Internationally recognized secondary standards which are traceable to NBS Oxalic are measured before and with every group of unknowns, together with blanks; and no measurements on unknowns are undertaken until the analyst is satisfied with the accuracy of the measurements on the secondary standards. Secondary standards include other NBS standards and materials from previous radiocarbon intercalibration studies.

Results from secondary standards and from blanks provide the verification for the hardware, software, and procedures for the entire sample preparation, measurement, and analysis process.

## MATERIAL IDENTIFICATION AND CONTROL

Samples entering the laboratory are assigned a unique identification number which is used to track each sample through the preparation, measurement, and analysis process. These ID numbers are cross-referenced with submitter's own ID numbers and are recorded by computer and on hard copy.

## ANALYTICAL RECORDS

Hard copy and computer files from data acquisition and analysis are stored along with the final analysis reports. Any departures from normal conditions, are documented in the record keeping system and in the final reports. Data analysis is performed by the chief analyst or a knowledgeable individual trained to his satisfaction.

## RECORDS DISPOSITION

Standard CAMS procedure is to fax the final report to the submitter. Hard copy or computer data files from the acquisition and analysis are also available for distribution to knowledgeable users.

## TECHNICAL REVIEW

For work performed for this study, analytical results will be reviewed by the isotope geochemist and the principal investigators.

**APPENDIX 24.4 L - DATA SHEETS FOR ISOTOPE ANALYSES**

**Soil Sample Data**

Site #: \_\_\_\_\_ Location: \_\_\_\_\_ Date Sampled: \_\_\_\_\_

**Organic Carbon**

Lab #	Depth (cm)	Date	Analyst	Wt. (mg)	Yield (torr)	Wt.% C	d <sup>13</sup> C (‰)	<sup>14</sup> C (pmc)

**Carbonates**

Lab #	Depth (cm)	Date	Analyst	Wt. (mg)	d <sup>13</sup> C (‰)	d <sup>18</sup> O (‰)	<sup>14</sup> C (pmc)

**Nitrogen**

Lab #	Depth (cm)	Date	Analyst	Wt. (mg)	d <sup>15</sup> N (‰)

### Soil Gas Sample Data

Site #: \_\_\_\_\_ Location: \_\_\_\_\_ Date Sampled: \_\_\_\_\_

Sample Size (liters): \_\_\_\_\_

#### Gas Composition

Lab #	Depth (cm)	Date	Analyst	N <sub>2</sub> (%)	O <sub>2</sub> (%)	Ar (%)	CO <sub>2</sub> (%)	CH <sub>4</sub> (%)	N <sub>2</sub> O (%)

#### CO<sub>2</sub>

Lab #	Depth (cm)	Date	Analyst	Vol. (ml)	Yield (torr)	CO <sub>2</sub> (%)	d <sup>13</sup> C (‰)	<sup>14</sup> C (pmc)

#### CH<sub>4</sub>

Lab #	Depth (cm)	Date	Analyst	Yield (torr)	CO <sub>2</sub> (%)	d <sup>13</sup> C (‰)	dD (‰)	<sup>14</sup> C (pmc)

#### VOCs

Lab #	Depth (cm)	Date	Analyst	Yield (torr)	CO <sub>2</sub> (%)	d <sup>13</sup> C (‰)	dD (‰)

## Groundwater Sample Data

Site #: \_\_\_\_\_ Location: \_\_\_\_\_ Date Sampled: \_\_\_\_\_

Sample Size (liters): \_\_\_\_\_

### Water Chemistry

Lab #	Depth (cm)	Date	Analyst	pH	DO ( $\frac{\text{mmol}}{\text{liter}}$ )	NO <sub>3</sub> <sup>-</sup> ( $\frac{\text{mmol}}{\text{liter}}$ )

### DIC

Lab #	Depth (cm)	Date	Analyst	Yield (torr)	DIC ( $\frac{\text{mmol}}{\text{liter}}$ )	d <sup>13</sup> C (‰)	<sup>14</sup> C (pmc)

### NO<sub>3</sub><sup>-</sup>

Lab #	Depth (cm)	Date	Analyst	d <sup>15</sup> N (‰)	d <sup>18</sup> O (‰)

### d<sup>18</sup>O of Water

Lab #	Depth (cm)	Date	Analyst	d <sup>18</sup> O (‰)

### dD of Water

Lab #	Depth (cm)	Date	Analyst	dD (‰)

## SOP 24.5

### FIELD PHYSICAL / CHEMICAL ASSAYS

#### 1. PURPOSE

This Standard Operating Procedures (SOP) establishes the method and responsibilities associated with classical physical/chemistry assays and oxidation/reduction measurements.

#### 2. OBJECTIVES

The objective of these tests is the characterization of subsurface physical/chemical conditions germane to microbial activity.

These assays will result in general site information regarding environmental conditions for microbial activity. Data gathered is essential in the determination of necessary and sufficient conditions for *in situ* bioremediation at ANAS sites 3 and 13. An important parameter for assessing biological activity is the measurement of reduction-oxidation (redox) indicators such as oxygen, sulfide, ferrous iron, ammonia concentrations and pH values.

##### 2.1 METHODS SUMMARY

Ground water samples will be analyzed in the field for immediate detection of a number of biologically relevant inorganic species by the following methods. Oxygen analysis using the Winkler method utilizes treatment with manganous sulfate and alkaline iodide-azide reagent to form an orange-brown precipitate. Upon acidification of the sample, this floc reacts with iodide to produce free iodine as  $I_2$  in proportion to the oxygen concentration. The iodine is titrated with sodium thiosulfate to a starch end point. Sulfide is analyzed by the U.S.E.P.A. method 376.2. Hydrogen sulfide and acid-soluble metal sulfides react with N,N-dimethyl-p-phenylenediamine oxalate to form methylene blue. The intensity of the blue color is proportional to the sulfide concentration. The ferrous iron ( $Fe^{2+}$ ) analysis will be performed using the 1,10 phenanthroline indicator which reacts with ferrous iron in the sample to form an orange color in proportion to the iron concentration. Ferric iron does not interfere. The ferric iron ( $Fe^{3+}$ ) concentration will be determined by subtracting the ferrous iron concentration from the results of a total iron test.

Analysis of ammonia is based on the salicylate method. Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-

aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green colored solution.

pH will be measured using the electrometric pH measurement which determines the activity of the hydrogen ions by potentiometric measurement using a standard hydrogen electrode and a reference electrode.

### 3. REFERENCES

*Standard methods for the examination of water and wastewater*, 1989, 17th ed. Ed. LS Clesceri, AE Greenberg, RR Trussell.

*Methods of Soil Analysis: Physical and Mineralogical methods*. A.L. Page (ed.) Agron. Monogr. 2nd Edition. ASA and SSSA, Madison, WI.

### 4. SPECIALIZED EQUIPMENT AND MATERIALS

A Hach portable DR/700 colorimeter with interchangeable filter modules will be used for field site measurements.

### 5. EXPERIMENTAL PROCEDURES

#### 5.1 DISSOLVED OXYGEN ASSAY USING THE AZIDE MODIFICATION OF THE WINKLER METHOD

##### Method

Using the Hach test kit, the following procedures apply:

- Using a peristaltic (for screening tests) or submersible pump, collect a water sample in a clean 300 mL BOD bottle.
- Add the contents of one manganous sulfate powder pillow and one alkaline iodide-azide reagent powder pillow.
- Immediately insert the stopper so air is not trapped in the bottle, and invert several times to mix.
- Wait until the floc in the solution has settled. Again invert the bottle several times and wait until the floc has settled.

- Remove the stopper and add the contents of one sulfamic acid powder pillow. Replace the stopper without trapping air in the bottle and invert several times to mix.
- Use 200 mL of sample and 0.200 thiosulfate titration cartridge.
- Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator body.
- Hold the digital titrator with the cartridge tip pointing up. Turn the delivery knob to eject air and a few drops of titrant. Reset the counter to zero and wipe the tip.
- Use a graduated cylinder to measure the sample volume. Transfer the sample into a 250 mL Erlenmeyer flask.
- Place the delivery tube tip into the solution and swirl the flask while titrating with sodium thiosulfate to a pale yellow color.
- Add two dropperfuls of starch indicator solution and swirl to mix.
- Continue the titration to a colorless end point. Calculate and record results as mg/L dissolved oxygen.

## ***5.2 FERROUS IRON (0 TO 5.00 MG/L) 1,10 PHENANTHROLINE METHOD USING HACH TEST KIT***

### Method

Using the Hach DR/700 colorimeter and Hach test kit:

- Install module 50.01 in a DR/700.
- Press: i/o. The display will show 500 nm and module number 50.01.
- After 2 seconds, the display will show a program number, concentration units and the zero prompt.
- Fill a 25 mL cell to the 25 mL line with sample.
- Add the contents of one ferrous iron reagent powder pillow to the sample cell. Cap and invert to mix.
- Wait 3 minutes.
- Fill a 25 mL cell to the 25 mL line with sample. Cap.
- Place the blank in the cell holder.
- Press: zero.
- Place the prepared sample in the cell holder.
- Press: read.

**5.3 SULFIDE (0 to 0.600 mg/L S<sup>2-</sup>) USEPA METHOD 376.2. USING THE HACH TEST KIT ADAPTED FROM STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER**

Using the Hach DR/700 colorimeter and Hach test kit, install a 61.01 module. Follow procedures described below:

- Fill a 25 mL sample cell to the 25 mL line with the sample.
- Fill another 25 mL cell to the 25 mL line with deionized water
- Add 1.0 mL of sulfide 1 reagent to each cell. Swirl to mix.
- Add 1.0 mL of sulfide 2 reagent to each cell and cap. Immediately swirl to mix.
- Wait 5 minutes.
- Place the blank in the cell holder. Blank or zero the spectrometer.
- Immediately place the prepared sample into the cell holder and record the result in mg/L sulfide.

**5.4 AMMONIA (0 TO 2.50 MG/L NH<sub>3</sub>-N) SALICYLATE METHOD USING THE HACH TEST KIT**

This procedure is conducted in a chemical hood. Using the Hach DR/700 colorimeter and Hach test kit the following procedures apply.

- Install a 61.01 module.
- Remove the caps from 2 AmVer Diluent Reagent vials, Add 2 mL of sample to one vial. Add 2 mL of ammonia-free water to another.
- Using a funnel, add the contents of one Ammonia Salicylate Reagent Powder Pillow for 5 mL sample to each vial.
- Using a funnel, add the contents of one Ammonia Cyanurate Reagent Powder Pillow for 5 mL sample to each vial.
- Cap the vials tightly and shake thoroughly to dissolve the powder.
- Wait 20 minutes.
- Fully insert the COD Vial Adapter into the cell holder with the tabs in the square slot.
- Clean the outside of both vials with a towel. Place the blank into the vial adapter with the Hach logo facing the keypad. Zero the spectrometer. Place the sample into the vial adapter with the Hach logo facing the keypad and read and record result. Multiply the value by 1.22 and record as mg/L NH<sub>3</sub>-N.

- Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis. If chlorine is known to be present, add one drop of 0.1 N sodium thiosulfate for each 0.3 mg/L Cl<sub>2</sub> in a one liter sample. Preserve the sample by reducing the pH to 2 or less with Hydrochloric acid (at least 2 mL). Store at 4°C or less. Preserved samples may be stored up to 28 days. Warm samples to room temperature. Correct the test result for volume additions.

### 5.5 pH

Method 45000-H+ Standard Methods for the Examination of Water and Wastewater 17th ed., page 4-195, pages 4-94 to 4-101:

- Calibrate the electrode system against standard buffer solutions of known pH. Because buffer solutions may deteriorate as a result of mold growth or contamination, prepare fresh as needed for accurate work. Use three standards pH 4.01, 7.00 and 10.00 adjusted for temperature.
- Instrument calibration: follow manufacturer's instruction for pH meter and for storage and propitiation of electrodes for use.
- Sample analysis: Establish equilibrium between electrodes and sample by stirring sample to insure homogeneity; stir gently to minimize carbon dioxide entrainment. For buffered samples or those of high ionic strength, condition electrodes by dipping them into the sample for 1 min. Blot the electrode dry and immerse it in a fresh portion of the same sample. Read the pH units.

Precision is approximately  $\pm 0.02$  pH unit and an accuracy of  $\pm 0.1$  pH unit.

## 6. ANALYTICAL METHODS

The scientific background and current methods for the above physical and chemical determinations are well documented.

### EXPECTED ACCURACY AND MAINTENANCE FOR ASSOCIATED EQUIPMENT

#### Accuracy check-Standard solution method : Dissolved Oxygen

An iodate-iodide standard solution equivalent to 10 mg/L dissolved oxygen is used to check the strength of the sodium thiosulfate titrant.

#### Accuracy check-standard solution method : Ferrous Iron

Prepare a ferrous iron stock solution (100 mg/L Fe<sup>2+</sup>) by dissolving 0.7022 grams of ferrous ammonium sulfate, hexahydrate, in deionized water. Dilute to 1 liter. Prepare immediately before

use. Dilute 1.0 mL of this solution to 100 mL with deionized water to make 1.0 mL standard solution. Prepare this immediately before use.

#### Accuracy check : Sulfide

Sulfide standard solutions are very unstable and should be prepared from sodium sulfate and standardized as described in Standard Methods for the Examination of Water and Wastewater 17th ed., page 4-195. Limit of detection is 0.010 mg/L S<sup>2-</sup> (adapted from Analytical Chemistry, 1980, 52, 2242-2249).

#### Accuracy check-standard solution method: Ammonia

To check accuracy, use a 1.0 mg/L Ammonium Nitrogen standard solution listed under optional reagents. Or, dilute 1 mL of solution from a Voluette Ampoule Standard for ammonium to 50 mL with deionized water.

Table 1. Surveillance of equipment

<u>Item</u>	<u>Conditions</u>	<u>Frequency</u>
pH meter	Inspection and calibration	Daily
Colorimeter	Inspection	Daily
<u>Balances</u>	<u>Inspection and certification</u>	<u>Annual</u>

## **7. DATA MANAGEMENT, ANALYSIS AND INTERPRETATION**

All procedures and data will be documented in bound laboratory notebooks and Excel spreadsheets. Recorded data will be transferred into computer files within a month of collection. Data in computer files will be checked by the person entering and by another investigator to ensure accuracy of entry.

## **8. HEALTH AND SAFETY PLAN**

Lawrence Livermore National Laboratory's (LLNL) environmental, safety, and health policy is that operations must be planned and performed safely, with full consideration for the protection of employees, the public, and the environment. In addition to observing LLNL policies contained in

the Health & Safety Manual (M-010) and Environmental Protection Handbook, LLNL employees will comply with applicable federal, state, and local regulations as stated in the Health & Safety Manual and Environmental Protection Handbook, and this Facility Safety Procedure (360, 360.01 and addendum 360-2).

All personnel involved in the drilling and sampling activities are required to have their Superfund Amendments and Reauthorization Act/Occupational Safety and Health Administration (SARA/OSHA) 40-h training (and the SARA/OSHA 8-h yearly refresher course). Investigators in the field must also be respirator fitted and wear appropriate safety shoes, glasses, hard-hats, Tyvek suits (when necessary) and gloves while sampling. Team members must be current in training courses HS-4050 and EP-0006 as stipulated in the Operational Safety Procedures, OSP 406.2.

All microbiological testing is covered under FSP-360. Groundwater testing and treatability tests are covered under the LLNL Operational Safety Procedure 377.03.

## **9. RESIDUALS MANAGEMENT**

Under Federal Treatability Study Sample Exemption Rule, collection of hazardous wastes for purposes of conducting treatability studies are conditionally exempt from generator and transporter requirements (40 CFR parts 262 and 263). No hazardous waste will be used or generated to perform the direct and enriched microscopic microbial analysis discussed above.

All LLNL personnel participating are certified in LLNL Hazardous Waste Generation (EP0006) and disposal procedures.

## **10. RECORDS**

Records generated as a result of implementation of this SOP will be controlled and maintained in the project record files.

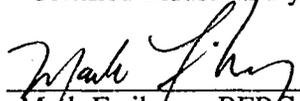
## **APPENDIX B**

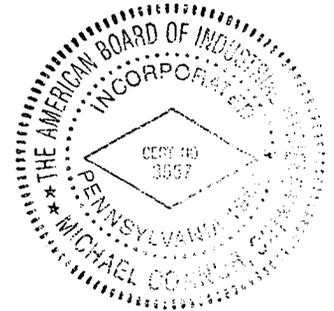
# **PROJECT HEALTH AND SAFETY PLAN**

**PROJECT HEALTH AND SAFETY PLAN**  
for  
**INTRINSIC BIOREMEDIATION TREATABILITY STUDY**  
**NAVAL AIR STATION ALAMEDA**  
**Fast Track Environmental Cleanup**  
**Contract Number N62474-94-D-7430**

Prepared For  
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University of California Berkeley

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Attachment 2	Safety Meeting Sign-Off Sheet
Attachment 3	Accident/Incident Report Form
Appendix A	Site Safety Procedures

## ABBREVIATIONS AND ACRONYMS

(Sheet 1 of 3)

AC	-	alternating current
ACGIH	-	American Conference of Governmental Industrial Hygienists
APR	-	air-purifying respirator
ALARA		as low as reasonably achievable
ANSI	-	American National Standards Institute
ATG	-	Allied Technology Group, Inc.
ATSDR		Agency for Toxic Substance and Disease Registry
BERC	-	Berkeley Environmental Restoration Center
CAL/OSHA		California Occupational Safety and Health Administration
CCR	-	California Code of Regulations
CFR	-	Code of Federal Regulations
CGI	-	Combustible gas indicator
CH <sub>4</sub>	-	methane
CIH	-	Certified Industrial Hygienist
CPR	-	cardiopulmonary resuscitation
CPT	-	cone penetrometer testing
CRZ	-	Contamination Reduction Zone
CSE	-	Certified Safety Executive
dBA	-	decibels, measured on the A-weighted scale
DRI	-	direct-reading instrument
ECM	-	Erythema chronic migraine
EPA	-	U.S. Environmental Protection Agency
eV	-	electronvolt
EZ	-	exclusion zone
°F	-	degrees Fahrenheit

## ABBREVIATIONS AND ACRONYMS

(Sheet 2 of 3)

FID	-	flame ionization detector
HEPA	-	High efficiency particulate air
HSP	-	Health and safety plan (site-specific)
HP	-	Health Physics
IDLH	-	immediately dangerous to life or health
IP	-	ionization potential
LEL	-	lower explosive limit
LEPC	-	Local Emergency Planning Committee
MSHA	-	United States Mine Safety and Health Administration
mg/ m <sup>3</sup>	-	milligrams per cubic meter of air
NAS	-	Naval Air Station
NH <sub>3</sub>	-	ammonia
NIOSH	-	National Institute for Occupational Safety and Health
NOSC	-	Navy On-Scene Coordinator
NOSCDR	-	Navy On-Scene Commander
O <sub>2</sub>	-	oxygen
OM	-	O <sub>2</sub> meter
OSHA	-	United States Occupational Safety and Health Administration
OVA	-	organic vapor analyzer
PCB	-	polychlorinated biphenyls
PDS	-	personnel decontamination station
PEL	-	OSHA or CAL/OSHA Permissible Exposure Limit
PI	-	Principal Investigator
PID	-	ultraviolet photoionization detector
PM	-	ATG's Project Manager
PPE	-	Personal protective equipment
ppm	-	parts per million

## ABBREVIATIONS AND ACRONYMS

(Sheet 1 of 3)

ROICC -	Resident Officer in Charge of Construction
SHSO -	Site Health and Safety Officer
SSP -	standard safety procedure
SS -	Site Superintendent
SZ -	Support zone
TLV -	Threshold Limit Value
TWA -	time-weighted average
UCB -	University of California - Berkeley
VOC -	Volatile organic compounds



## 1.0 INTRODUCTION

This Health and Safety Plan (HSP) has been prepared by Allied Technology Group, Inc., (ATG) as subcontractor to the Berkeley Environmental Restoration Center (BERC), University of California Berkeley (UCB) to support the intrinsic bioremediation treatability study authorized by Engineering Field Activity West, Naval Facilities Engineering Command, Contract Number N62474-94-D-7430, Delivery Order 5. The study will evaluate whether an intrinsic process, bioremediation, is capable of degrading the chemicals found in the soil and groundwater at Sites 3 and 13 of Naval Air Station (NAS) Alameda.

Work shall be conducted in accordance with the Treatability Study Work Plan, Intrinsic Bioremediation, Sites 3 and 13, BERC Health and Safety Plan (Program) and the requirements of this HSP. A copy of each document will be available on-site during field activities. ATG will manage the field work.

This HSP is intended to protect UCB, Lawrence Livermore National Laboratory (LLNL), Lawrence Berkeley National Laboratory (LBNL), and ATG employees as well as ATG's subcontractors performing the work. This HSP has also been prepared to assure that requirements promulgated in 29 Code of Federal Regulations (CFR) 1910.120(b)(4) Site-Specific Safety and Health Plan, and in Title 8 of the California Administrative Code, Section 5192(b)(1) are met. The BERC Health and Safety Plan (Program) describes health and safety procedures for all BERC projects. This document describes the health and safety procedures that will be instituted for all field activities associated with the intrinsic bioremediation treatability study to be conducted at Sites 3 and 13 of NAS Alameda. This HSP references standard safety procedures described in Section 12 of the BERC Health and Safety Plan (Program); a copy of which will be available on-site. The more relevant site standard safety procedures to this project are included in Appendix A to this HSP.

This HSP will be provided to all on-site personnel including UCB, LLNL, LBNL, ATG and subcontractor personnel and site visitors participating in any field activities associated with this work. The Site Health and Safety Officer (SHSO), with the support of ATG's Project Manager (PM), will be responsible for enforcement of the HSP for all work activities at Sites 3 and 13. Failure to follow site-specific HSP procedures will result disciplinary action that may, at a maximum, include dismissal from the BERC project work sites.

The ATG Certified Industrial Hygienist (CIH) and the BERC Program CIH, shall be responsible for resolving health and safety related issues and disputes in consultation with ATG's PM and the SHSO.

## **2.0 PROJECT PERSONNEL RESPONSIBILITIES**

This HSP covers all field personnel working at Sites 3 and 13, including subcontractors and visitors. The ATG PM and the SHSO will be responsible for implementation and enforcement of the health and safety provisions of this HSP. Their duties are described in this section along with the duties of other project personnel. Duties for project personnel are more specifically describe in Section 2 of the BERC Health and Safety Plan (Program). Each on-site personnel will be required to sign the Health and Safety Plan Acceptance form provided in Attachment 1.

### **2.1 BERC PRINCIPAL INVESTIGATOR**

Dr. Lisa Alvarez-Cohen is the BERC Principal Investigator. She is responsible for reviewing proposed activities and safety precautions at Sites 3 and 13.

### **2.2 UCB PROGRAM CERTIFIED INDUSTRIAL HYGIENIST**

Mark Freiberg is the UCB Program Certified Industrial Hygienist (CIH). He has the overall responsibility for the health and safety activities at BERC project work site at Site 13. He reviewed and approved this HSP. No changes may be made to this HSP without his written approval.

### **2.3 ATG PROJECT MANAGER**

Mary McDonald, R.G., is ATG's Project Manager (PM). With the assistance of the Site Superintendent (SS), he is responsible for the job-related health and safety of site personnel and managing the risks associated with project equipment and facilities.

### **2.4 ATG CERTIFIED INDUSTRIAL HYGIENIST**

Mr. Michael Connor, CIH, CSP, is ATG's CIH. Mr. Connor is responsible for developing, establishing, and coordinating the implementation of health and safety policies and procedures for ATG Program activities on BERC projects managed by ATG.

ATG's CIH will be the first point of contact for field personnel. ATG's CIH will communicate with UCB's Program CIH on all matters relating to health and safety activities at Site 13 and any decision made regarding health and safety activities. Accident/Incident reports will be sent to ATG's CIH with a copy furnished to UCB's Program CIH.

## **2.5 SITE HEALTH AND SAFETY OFFICER**

The SHSO is the primary enforcement authority on hazardous waste project sites, as delegated by the responsible ATG PM, for the policies and provisions of this HSP, UCB's Health and Safety Program, and ATG's Health and Safety Program. The SHSO for this project is Mr. Lawrence Chiu, P.E. During drilling activities, the alternate SHSO shall be Ms. Mary McDonald, R.G.; her responsibilities will be the same as the SHSO.

## **2.6 SITE SUPERINTENDENT**

Mr. Chiu is the Site Superintendent (SS) and will direct all field activities including emergency response operations. He will ensure necessary preparation and coordination for all site operations, including health and safety. Mr. Chiu will be assisted by the Project Geologist, Ms. Mary McDonald, R.G., during drilling activities. Her responsibilities will be the same as the SS.

## **2.7 SUBCONTRACTOR MANAGEMENT AND PERSONNEL**

Each subcontractor responsible for workers required to enter a hazardous waste site exclusion zone shall comply with the requirements of 29CFR1910.120, 8CCR5192, the requirements of the BERC Health and Safety Plan (Program) and this HSP. Copies of the BERC Health and Safety Plan (Program) and this HSP will be submitted to the subcontractor by ATG. These documents are not intended to supersede or replace the subcontractor's own illness and injury prevention program as required by 8CCR 3203. The subcontractor will be required to implement a medical surveillance program and employee training. Responsibilities of subcontractor management and personnel are described in additional detail in Section 2.5 of the BERC Health and Safety Plan (Program).

## **2.8 FIELD PERSONNEL**

The responsibilities described for all personnel are in addition to those described in Section 2.6 of the BERC Health and Safety Plan (Program). Health and safety precautions are of paramount importance during on-site activities at all hazardous waste sites. Despite thorough preparation, field personnel may not have complete knowledge of site conditions, and it is impossible to anticipate every health and safety hazard that could arise. Therefore, the personnel should use common sense, experience, and the best professional judgment at all times.

All field personnel shall:

- Read and comply with this HSP.

- Practice reasonable health maintenance procedures—the employee should realize that some personal habits, such as alcohol consumption, smoking, or controlled substance abuse, heighten the risks and deleterious effects resulting from exposure to contaminants and may create a hazard to the health and safety of fellow workers. Therefore, working at a hazardous waste site while under the influence of alcohol or controlled substances is strictly forbidden.
- Know and observe any and all medical restrictions placed on their own activities (such as corrective lenses or lifting limitations) and inform the SHSO of these restrictions.
- Enforce the “buddy system” in accordance with 29 CFR 1910.120(a)(3), “Definitions” (organizing field personnel into work groups such that each employee is designated to be observed by at least one other employee in the work group) for all on-site working exclusion zones.
- Use safety equipment in accordance with training received and written instructions.
- Inspect safety equipment before each work shift to determine whether it is in good condition and proper working order.
- Look for health and safety hazards and report them to the ATG PM and the SHSO for corrective action.
- Maintain a high level of safety awareness—when in doubt, follow the safest course of action.
- Meet all training and refresher training requirements and medical surveillance requirements.
- Refrain from activities that would create additional hazards during field work including smoking, eating, chewing tobacco or gum, drinking, or using cosmetics.
- Notify the ATG CIH or the SHSO of known or suspected pregnancy and then refrain from participation in hazardous waste site field activities.
- Report all injuries, suspected chemical or physical hazard exposures, and exposure symptoms to the SHSO as soon as possible.

## **2.9 VISITORS**

Site visitors are also required to adhere to this HSP. Either the ATG Project Manager or the SHSO shall brief site visitors on site health and safety hazards when they first arrive on site. In general, site visitors will not be allowed access to contaminated areas (exclusion zones) unless they have demonstrated compliance with medical surveillance and training requirements. The responsibilities of visitors to Sites 3 and 13 are further described in Section 2.7 of the BERC Health and Safety Plan (Program).

## **3.0 PROJECT HAZARD ANALYSIS AND CONTROL PROGRAM**

The following sections describe the site location, provide descriptions of Site 3 and Site 13 of NAS Alameda, describe tasks and objectives of the treatability study, and discuss the hazards associated with the treatability study.

### **3.1 SITE LOCATION**

Site 3 and Site 13 are located at NAS Alameda. A site map is shown on Figure 1. NAS Alameda is located on the western end of Alameda Island. The base, rectangular in shape, is approximately 2 miles long and 1 mile wide, and occupies 2,634 acres. Approximately 1,526 acres of NAS Alameda are land and 1,108 acres are bay. Much of the land now occupied by NAS Alameda was once covered by waters of San Francisco Bay or was tidal flats.

### **3.2 SITE DESCRIPTIONS**

#### **3.2.1 Site 3**

Site 3 consists of an abandoned fuel storage area located in an inner island of Atlantic Avenue, approximately 200 feet west of the East Gate. The two-acre site previously contained four partially buried concrete tanks lined with carbonyl and one partially buried steel tank. Each tank previous contained 115/145 aviation gasoline (AVGAS) and had a nominal capacity of 100,000 gallons.

In 1975 it was discovered that three of the concrete tanks had leaked and in 1978 the fourth concrete tank was found to be leaking. Based on tank inventories, NAS Supply Fuels Branch personnel estimated that approximately 365,000 gallons of AVGAS may have escaped from the fuel storage area in the 1960s and early 1970s.

### **3.2.2 Site 13**

Site 13 consists of an abandoned oil refinery which operated between 1879 and 1903. Refinery wastes and asphaltene residues were dumped at the site during the 24-year history of the oil refinery. The oil refinery consisted of pump and lubricating houses, stills, two laboratories and agitators, as well as approximately 19 above-ground iron oil storage tanks, six underground iron storage tanks, and a storage area containing drums of oil. Site 13 consists of approximately 30 acres located in the southeast corner of NAS Alameda.

## **3.3 TASK DESCRIPTION AND OBJECTIVES**

### **3.3.1 Summary**

The purpose of this project is to conduct a treatability study to evaluate whether an intrinsic process, bioremediation, is capable of degrading contaminants found in the soil and groundwater at Sites 3 and 13 of NAS Alameda. Table 1 lists the known contaminants at Site 3. Table 2 lists the same information for Site 13. The field work will involve approximately 8 days of drilling (either with hand augers or a drill rig) to characterize surface conditions at the site. This will require the collection of soil and groundwater samples.

### **3.3.2 Field Methods And Equipment**

The treatability study at Sites 3 and 13 of NAS Alameda, will require the collection and analysis of soil, soil gas, and groundwater samples during the preliminary screening phase and during the background level and contaminant area characterization phases.

#### *Soil Boring Methods*

Borings for soil sample collection will be drilled using a truck-mounted drilling rig equipped with six or eight-inch outside diameter hollow-stem augers and a portable, hydraulically driven soil coring system as described in Section 4.2.2 of the Work Plan. In addition, a hand held auger will be used to install some borings to a total depth of 6 feet as described in Section 4.1.3 of the Work Plan. This depth is approximately 1 foot below the anticipated depth of the water table and will allow the collection of soil samples from the vadose zone, capillary fringe, and from below the water table.

#### *Soil Sampling Methods*

Soil samples collected during hand augering will be collected with a hand coring tool as described in Section 4.1.4 of the Work Plan. Soil samples for the background level and contaminant area

characterization phases will be collected in a split spoon sampler as described in Section 4.2.3 of the Work Plan.

#### *Soil Gas Sampling Methods*

Various 1/4-inch stainless steel tubes will be driven directly into the soil to various depths to allow collection of soil gas samples from within the unsaturated zone and the capillary fringe. Sampling methods are described in Section 4.4 of the Work Plan.

#### *Groundwater Sampling Methods*

Groundwater samples will be collected from borings drilled during the background level and contaminant area characterization phases using a submersible pump as described in Section 4.2.4 of the Work Plan. Groundwater samples will also be collected during each phase of the treatability study by driving 1/4-inch steel tubing directly into the soil and pumping with a peristaltic pump as described in Section 4.5 of the Work Plan.

### **3.4 HAZARD EVALUATION**

The work associated with the treatability study essentially will consist of hand augering and drilling to collect soil and groundwater samples. As such, the hazards will consist of chemical hazards associated with handling contaminated soil and groundwater; physical hazards associated with working on and around a drill rig while wearing personal protective equipment (e.g. utilities, heavy equipment use, noise, heat stress) and with general field work (e.g. electrical, and slip, trip and fall hazards); and biological hazards associated with flora and fauna on site. The following sections discuss these site specific hazards.

#### **3.4.1 Chemical Hazards**

Tables 1 and 2 list the contaminants found at Sites 3 and 13 respectively. These contaminants can be classified as volatile organic compounds (VOCs), semivolatile organic compounds (SVOCs), metals and pesticides. The chemical hazard associated with these contaminants is proportional to worker exposure by inhalation, skin contact or ingestion. This hazard is potentially greatest for work in the exclusion zone access to which shall be restricted to authorized, properly trained and properly equipped personnel only.

The presence of VOCs means that there is potential inhalation exposure as workers come into contact with air during drilling and sampling work. Actual exposures are anticipated to be below applicable federal and California PELs because they would originate from small point sources (i.e.

borings) and because known contamination levels are relatively low. However, worker exposures will be monitored through direct reading methods as described in Section 10 of this HSP to ensure adequate employee protection. This HSP makes provisions for respiratory protection should air monitoring results so warrant.

Although the some VOCs known to be present on site are flammable, the fire and explosion hazard is not anticipated to be significant because flammable concentrations are well above the Action Levels described in Section 10.6 of this HSP. Nonetheless, there will be periodic monitoring of the work area with a combustible gas indicator. Fires hazards will be controlled by following standard safety procedure 12.16 of the BERC Health and Safety Plan (Program). This standard safety procedure is included in Appendix A to this HSP.

The SVOCs are not expected to be an inhalation hazard since by definition they have low vapor pressures. However, inhalation exposure is possible should there be visible dust generated during field activities. In general, this does not occur during drilling. However, as discussed in Section 9 of this HSP, there are provisions to monitor airborne dust concentrations, and for dust suppression, if necessary.

As with SVOCs, neither metals nor pesticides are anticipated to represent significant airborne hazards. Section 3.2.1 and Table 4 of the BERC Health and Safety Plan (Program) discuss how even with visible dust, it is unlikely that the PELs for site metals will be exceeded. This same analysis would apply to pesticides which are present in very low concentrations.

By contrast, dermal exposure to site contaminants is more likely than inhalation. However, the use of personal protective equipment as described in Section 8 of this HSP will minimize this hazard. Ocular exposure shall be minimized through the use of appropriate eye protection, and through dust mitigation.

Ingestion exposure to site contaminants is also expected to be low because eating, drinking and smoking is forbidden in the exclusion zone, and because personal decontamination is required when leaving the exclusion zone.

### **3.4.2 Physical Hazards**

This section discusses potential physical hazards associated with implementation of the Treatability Study. Standard operating procedures for each potential hazard (from the BERC Health and Safety Plan (Program)) are included as an appendix to this HSP.

#### **3.4.2.1 Utilities**

Work on the site may be in proximity to above ground and underground utilities. ATG's Project Manager shall conduct a utility survey to identify this potential hazard before field work begins. The survey shall be coordinated with appropriate Navy or base personnel. ATG's Project Manager and the SHSO shall coordinate the necessary arrangements to either disconnect or de-energize power lines wherever possible. Section 11.3 of this HSP describes standard safety procedures when working around utilities.

#### **3.4.2.2 Drilling Operations**

Work on and around a drill rig involves a number of physical hazards associated with rotating machinery, pinch points and overhead loads. These hazards shall be minimized by using qualified drill rig operators, by guarding all nip and pinch points, by daily inspection of the equipment before it is used and by the use of hard hats. Either the ATG PM or the SHSO shall review these hazards with field personnel during tailgate safety meetings. The Standard Safety Procedure for drilling operations is contained in Section 12.4 of the BEREC Health and Safety Plan (Program); this standard safety procedure shall be reviewed during the site specific health and safety training.

#### **3.4.2.3 Noise**

Elevated noise levels are anticipated in areas of drilling operations where other heavy equipment also may be in operation. The use of hearing protection will be required during operations associated with noise levels in excess of 85 dBA (decibels measured on the A-weighted scale). Noise levels shall be measured with a Type 2 sound level meter (as defined in ANSI S1.4) whenever there is reason to suspect levels exceed 85 dBA. All work will be done in compliance with the requirements 29 CFR 1926.52, "Occupational Noise Exposure," and Cal-OSHA Article 105. BEREC field staff or ATG workers may request the SHSO to perform a noise survey, as needed. Section 12.14 of the BEREC Health and Safety Plan (Program) contains the hearing conservation program for the project.

#### **3.4.2.4 Heat Stress**

Heat stress is a potential hazard during warm months due to physical exertion associated with construction activities while wearing personal protective clothing. When ambient temperatures reach 70 degrees Fahrenheit (o F) and workers are wearing personal protective clothing, work-rest cycles will be scheduled on a regular basis and liquids with electrolytes will be available to replenish body fluids. The PM and SHSO shall establish the work rest cycles as necessary. This

shall be discussed during tailgate safety meetings. Because the incidence of heat stress depends upon a variety of factors, all workers, even those not wearing PPE, will be observed and instructed to report any symptoms of heat stress. Section 12.15 of the BERC Health and Safety Plan (Program) contains the standard safety procedures for handling heat stress related hazards. It shall be reviewed during the site specific training. Cold stress is not anticipated to be a hazard for this project.

#### ***3.4.2.5 Hazardous Energy***

Electrical hazards other than utilities as discussed in Section 4.5.1 are not expected during the treatability study. Should field work require the use of electrical or mechanical equipment that could expose workers to shock, crush, a pinch hazards, the procedures specified in Section 12.6 of the BERC Health and Safety Plan (Program) will be followed.

#### ***3.4.2.6 Slip, Trip, and Fall Hazards***

Slip, trip, and fall hazards may be present at Sites 3 and 13. Such hazards will be identified and reviewed by the SHSO at the daily health and safety meeting before field work begins. Slip, trip, and fall hazards are discussed in Section 12.17 of the BERC Health and Safety Plan (Program).

#### ***3.4.2.7 Biological Hazards***

Neither Site 3 nor Site 13 have flora and fauna that would present a biological hazard. However, the ATG Project Manager or the SHSO shall inspect the site before field work begins to ensure the absence of such hazards. This shall be discussed during the site specific training. Section 12.24 of the BERC Health and Safety Plan (Program) contains the standard safety procedures for biological hazards.

## **4.0 TRAINING REQUIREMENTS**

Work in the exclusion zones of the project will require completion of a 40 hour hazardous waste class. Site supervisors shall also have completed an appropriate 8 hour supervisor course. This general training shall be updated annually. These training requirements are described in detail in Section 4 of the BERC Health and Safety Plan (Program). Additionally, all site personnel shall receive site specific training on the contents and requirements of this HSP. The site specific training shall include the following:

- Site roles and responsibilities
- Site hazards
- Site control procedures

- Site personal protective equipment
- Site decontamination procedures
- Site specific standard safety procedures
  - 12.4 Drilling
  - 12.6 Control of Hazardous Energy
  - 12.14 Noise
  - 12.15 Heat Stress
  - 12.16 Fire Prevention
  - 12.17 Slip, trip and fall
  - 12.24 Flora and Fauna

Emergency preparedness

- Communications
- Location of emergency facility
- Evacuation/muster points

Personnel without site specific training shall not be permitted into the exclusion zone. Visitors to the site will receive site orientation and training to include an overview of the site hazards and site hazard controls.

Furthermore, the SHSO or the ATG PM shall conduct daily tailgate safety meetings to review anticipated hazards before field activities begin each day. Attachment 2 is a Safety Meeting Sign-off sheet.

## **5.0 MEDICAL SURVEILLANCE**

All BEREC, ATG, and sub-contractor personnel that will be working either with contaminated materials or in an exclusion zone shall participate in an annual medical surveillance program in accordance with federal and state requirements. A California licensed and certified physician shall clear workers to wear respiratory devices and protective clothing as required.

Field personnel shall receive pre-placement examinations and annual re-evaluations to update clearances. Employees leaving their positions shall also be provided with an exit physical, if they have not had an annual re-examination in the last six months. Documentation of examinations shall be provided to the Navy five working days prior to the Preconstruction Meeting.

The SHSO shall communicate medical restrictions to the affected employee whose work tasks shall

be revised to be consistent with terms of the restrictions. Medical surveillance requirements for field personnel are further described in Section 5 of the BERC Health and Safety Plan (Program).

## **6.0 SITE CONTROL**

Site control procedures are specified in Section 6 of the BERC Health and Safety Plan (Program), and include establishment of the exclusion zone, contamination reduction zone, support zone, and site security. The exclusion zone (EZ) will extend at least 10 feet from all drilling and sampling activities and be marked by cones, barricade tapes or other equivalent methods. The EZ is the area of the site where equipment and contamination of personnel occurs or could occur. Personal protective equipment as described in Section 8 of this HSP shall be worn in the EZ. Visitors will not be permitted to enter the EZ without the authorization of the SHSO. The EZ shall be terminated when boreholes have been backfilled. The ATG PM or SHSO will also establish a site log-in, log-out procedure for work in the EZ.

The contamination reduction zone (CRZ) will be immediately adjacent to and upwind from the EZ and will also be marked by cones. The CRZ shall be used to clean contaminated tools and equipment and to remove personal protective equipment.

The support zone (SZ) will be situated in a clean, uncontaminated area outside the CRZ, where exposure to either hazardous materials or conditions is minimal. No contaminated equipment, samples, or personnel are permitted in the SZ.

## **7.0 PERSONAL PROTECTIVE EQUIPMENT**

The initial level of personal protection for field work will be EPA Level D. Figure 2 shows a Field Activities Equipment List of all personal protective equipment and monitoring equipment required for work at Sites 3 and 13 at NAS Alameda. The PPE required in the exclusion zone will consist of the following modified level D ensemble:

- Hard hat
- Safety glasses with side shields
- Polyethylene coated Tyvek suits
- Nitrile gloves with surgical inner gloves
- PVC or neoprene boots with steel toe and shank

This ensemble will be upgraded to Level C if air monitoring results so indicate. The respiratory protection to be used shall consist of full face air purifying respirators fitted with organic vapor/

HEPA combination filters. Respirator use shall be consistent with Section 7.5 of the Health and Safety Plan (Program).

## 8.0 DECONTAMINATION AND DISPOSAL

### 8.1 DECONTAMINATION PROCEDURES

The following decontamination stations are recommended by for modified Level D protection decontamination:

- 1) Segregated equipment drop. Drop equipment used on site (hand tools, monitoring equipment and sampling containers, radios, clipboards, etc.) on plastic drop cloths or in different containers with plastic liners.

Equipment: various size containers  
plastic liners  
plastic drop cloths

- 2) Boot cover, outer glove, and outer garment wash and rinse. Scrub outer boot covers, outer gloves, and outer garment with decontamination solution or detergent water. Rinse off decontamination solution or detergent water using copious amounts of water. Repeat as many times as necessary.

Equipment: containers (20-30 gallons)  
decontamination solution or detergent water  
pressurized spray unit  
containers (30-60 gallons)  
water  
5 to 6 long-handle, soft-bristle scrub brushes

- 3) Removal station for boot covers and outer gloves. Remove duct tape around boots and gloves and deposit in container with plastic liner.

Equipment: containers (30-50 gallons)  
plastic liners  
bench or stool

- 4) Removal station for outer garment. With assistance of a helper, remove protective garment and deposit it in container with plastic liner.

Equipment: containers (30-50 gallons)  
bench or stool  
plastic liner

- 5) Hand and face wash and rinse. Wash hands and face.

Equipment: water  
soap  
tables  
wash basins or buckets

## 8.2 DISPOSAL PROCEDURES

Used but clean disposable protective clothing will be double-bagged and drummed for disposal as ordinary waste. Disposable sampling tools and visibly contaminated protective equipment shall be double bagged and disposed of appropriately based on analytical results from soil or groundwater samples. Wash and rinse waters will be drummed and disposed of based on analytical results. Drill cuttings will be containerized and analyzed. Disposal of cuttings will be also be based on analytical results.

## 8.3 DECONTAMINATION DURING MEDICAL EMERGENCIES

If prompt life-saving first aid or medical treatment is required, decontamination procedures will be omitted as needed. Protective clothing and equipment shall be cut away before transportation to the emergency facility. On-site personnel will accompany contaminated victims to the medical facility to advise on matters involving decontamination. This is not anticipated to be a significant issue since treatability study field work is not expected to be associated with significant exposure to contaminants. Section 8.4 of the Health and Safety Plan (Program) provides additional details on emergency decontamination procedures.

## 9.0 EXPOSURE MONITORING

The purpose of exposure monitoring is to ensure that personnel are adequately protected and to verify that site chemical hazards have been properly evaluated. The exposure monitoring necessary

during the field work component of the treatability study shall consist of use of a photoionization detector (PID) fitted with a 10.2 eV bulb, a combustible gas indicator (CGI) and an MIE PDM-3 MINIRAM aerosol monitor will be used to determine exposures to particulate matter. This equipment shall be used by individuals trained on the equipment's use and limitations. The equipment shall be calibrated in accordance with the manufacturer's instructions. The ATG Project Manager or the SHSO are responsible for ensuring the necessary monitoring is conducted.

The PID shall be used to evaluate potential exposures to VOCs. This will require periodic monitoring of boreholes. The results should be noted in the boring log. When VOCs are detected at the borehole, worker breathing zones should be monitored. Respirators as described in Section 8 of this HSP shall be donned when the results exceed the criteria shown in Section 9.1. Respirator use may be discontinued when results fall below those criteria. The use of respiratory protection should be noted in the daily work log as should the location of the elevated results. Repeated need for respiratory protection shall be discussed with the ATG CIH to determine whether additional monitoring is required.

The CGI shall be used to verify that there are no flammable gases present at ignitable concentrations. As discussed in Section 4 of this HSP, such concentrations are not anticipated since they would have already been detected by PID monitoring. Nonetheless, the work areas and boreholes shall be monitored periodically to ensure there is no fire or explosion hazard associated with the site contaminants.

The MIE PDM-3 MINIRAM aerosol monitor shall be used to evaluate airborne dust exposures should site activities generate visible dust. Note that if they do, dust control measures shall be implemented. This shall include wet methods.

## 9.1 ACTION LEVELS

This section describes the criteria against which air monitoring results shall be evaluated.

### 9.1.1 Total Organic Vapors

Normal Background Levels	=	Level D protection
Above Background Levels		
>0 to 5 ppm	=	Level C protection
>5 to 500 ppm	=	Level B protection
>500 ppm	=	Stop work; evacuate site; call ATG

## CIH

These levels are as measured in worker breathing zones and as sustained for a period of five consecutive minutes. Respiratory protection may be downgraded when results fall below the specified criteria for five consecutive minutes. Note that Level B protection is improbable for the treatability study

### 9.1.2 Total Particulate Matter

< 5 mg/M <sup>3</sup>	=	Level D
5 to 10 mg/M <sup>3</sup>	=	Level C, respirators equipped with HEPA filters

### 9.1.3 Oxygen Content

20.8%	=	Normal background level
20.8 to 19.5%	=	Continue investigation with continuous air monitoring
<19.5 %	=	Stop work; call ATG CIH

### 9.1.4 Explosive Atmospheres

0 to 10% LEL	=	Normal background levels
> 10% LEL	=	Stop work; evacuate site; call ATG CIH

## 10.0 EMERGENCY RESPONSE

In the event of a site emergency, the SHSO will initiate emergency response procedures including evacuation if appropriate. Emergency supplies listed in Figure 2 and a cellular phone will be available in the field van. For major emergencies, the SHSO will evacuate the site and contact the appropriate base or off-site emergency responders. Major emergencies would consist of serious chemical related injury or fatality, or sustained elevated air monitoring results (more than 500 ppm by PID, or 10% LEL by CGI). For minor emergencies, the SHSO will be the first responder. Emergency routes to the East Gate are shown on Figure 3.

## 10.1 INJURY AND ILLNESS

### 10.1.1 General First Aid

- Dermal Exposure** Wash with soap immediately and rinse with copious amounts of clean water. Watch for signs of skin irritation. Seek medical attention at first signs of irritation.
- Inhalation** Move victim to fresh air. Give artificial respiration if necessary, unless otherwise indicated. Observe victim for signs of shock. Seek medical attention immediately.
- Ingestion** CALL POISON CONTROL CENTER. Seek immediate medical attention. If possible, a sample of ingested material will be collected and transported to hospital with victim.

### 10.1.2 Hospital And Evacuation Route

Site personnel will conduct a run to the nearest hospital before field work begins. The purpose of this is to familiarize personnel with the route to the hospital, and to notify the hospital of the planned site activities and potential medical needs. A hospital route map is shown as Figure 4 to this site-specific HSP.

## 10.2 FIRES AND EXPLOSIONS

As discussed in Section 3 of this HSP, fires and explosions are not anticipated. However, the potential for such shall be monitored with a CGI.

## 10.3 SPILLS

The field activities will involve relatively small quantities of contaminated materials which shall be properly packaged before removal from the exclusion zone. In the unlikely event of spills or container breakage, the spilled materials shall be promptly cleaned up with shovels and brooms. The wastes shall then be packaged for disposal.

## 10.4 EMERGENCY RESOURCES

Before work begins at each site, contact will be made with local authorities and emergency services to establish communication channels during an event of emergency and to familiarize the project personnel with the communication procedure and services. Pertinent emergency information will be included at the daily tailgate safety meetings.

From a base telephone (found in all government offices)

On base calls: Dial "3" plus the last 4 digits of an on-base number

Off base calls (local): Dial "9" plus the 7 digit number

Off base calls (long distance): Dial "91" plus the area code and 7 digit number,

***Public Agencies***

Fire (Base)	(510) 263-4300
(Alameda City)	911 or (510) 522-2423
Ambulance	(510) 263-4444
Police (Alameda City)	911 or (510) 522-2423
OSHA	(415) 744-6670
Cal/OSHA (Oakland)	(510) 568-8602

***Key Project and BEREC Personnel***

RPM, EFA West (Ken Spielman)	(415) 244-2539
Principal Investigator	(510) 643-5969 (510) 643-1300
Program CIH (Mark Freiberg)	(510) 643-8676 pager (510) 430-5038
ATG CIH (Michael Connors)	(415) 252-0778 pager (415) 245-4501
Project Manager (Mary McDonald)	(510) 652-1164 home (510) 843-8535
Project Superintendent (Lawrence Chiu)	(510) 490-3008 pager (800) 690-3573
Site Health and Safety Officer (Lawrence Chiu)	(510) 490-3008 pager (800) 690-3573
Occupational Health Physician (Thomas Gamsky, MD.)	(510) 643-7116
Navy Contact [Resident Officer in Charge of Construction (ROICC)] (Wayne Coffey)	(510) 302-3354
Base Health and Safety Office	(510) 263-3395
Agency for Toxic Substance and Disease	(404) 639-0615

Registry (ATSDR)	
Navy On-Scene Coordinator (NOSC)	(510) 263-3276
Navy On-Scene Commander (NOSCDR)	(510) 263-3003
Local Emergency Planning Committee (LEPC)	(510) 263-3050
Regional Poison Control Center	(510) 476-6600

### **Medical Care Facilities**

Hospital Name:	Alameda Hospital
Hospital Address:	2070 Clinton Avenue Alameda, CA
Alameda Hospital Telephone:	(510) 522-3700
Alameda Emergency Room	(510) 523-4357
Base Medical:	(510) 263-4444

### ***Directions to Alameda Hospital:***

Exit the NAS site through the East Gate (Figure 4). Cross Main Street, continue east on Atlantic Avenue. Turn right onto Webster Street. Turn left onto Central Avenue. 90 degree right turn onto Sherman Street. Turn left onto Clinton Avenue. Hospital is on the right hand side between Chestnut Street and Willow Street. Emergency room entrance is on the western side of the hospital.

### **Regulatory Agencies:**

California State Office of Emergency Service	(510) 646-5908
Fish and Game	(800) 952-5400
Regional Water Board	(510) 464-1255
CHEMTREC	(800) 424-9300
Poison Control Center	(800) 356-3129
Underground Services Alert	(800) 642-2444
Base Hot Work Permits	(510) 263-3279

## **10.5 ACCIDENT/INCIDENT REPORTING**

Accidents and incidents shall be reported on the form contained in Attachment 3, in accordance with UCB's Health and Safety Program and ATG's Health and Safety Program. The accident/incident form will be forwarded to ATG's CIH, then to BERC's Program CIH. A copy will be provided to EFA contracting officer. Any employee who experiences a lost time injury or chemical exposure will not be allowed to perform site work until released by an occupational

medical physician.

## **11.0 STANDARD SAFETY PROCEDURES**

This section describes certain standard safety procedures relevant to the field work associated with the treatability study. This section is not intended to supersede safety procedures described in the BERC Health and Safety Plan (Program). Particularly relevant to this HSP are the following site safety procedures:

- 12.4 Drilling
- 12.6 Control of Hazardous Energy
- 12.14 Noise
- 12.15 Heat Stress
- 12.16 Fire Prevention
- 12.17 Slip, trip and fall
- 12.24 Flora and Fauna

The site safety procedures have been included in Appendix A for reference purposes.

### **11.1 SITE ENTRY PROCEDURES**

All field personnel shall attend a site orientation meeting before field work starts at the site. Thereafter, an on-site health and safety meeting will be held at the beginning of each work day to discuss pertinent health and safety issues. Attachment 2 contains a Safety Meeting Sign-Off Sheet for persons who attended the meeting.

### **11.2 SITE SECURITY**

All equipment, when not in operation, shall be left in a safe and secured condition (e.g., wheels blocked and buckets on the ground. Motorized equipment shall be locked so that it cannot be used by unauthorized personnel.

### **11.3 WORK WITH UTILITIES**

The following practices shall be observed when working in areas with underground and overhead utilities are as follows:

- The utility locations shall be communicated to all site workers during the initial daily health and safety meeting. Utilities will be marked or access otherwise restricted to avoid the risk of accidental contact.

- Overhead or above-ground electric lines should be considered “live” or “active” until a reliable source, such as base electrician or personnel from the relevant operating company, has documented them to be otherwise.
- Clearance will be adequate for the movement of vehicles and for the operation of construction equipment.
- Drill rigs or vehicle superstructures will be erected at least 20 from overhead electrical lines until the line is de-energized, grounded, or shielded and a competent electrician has certified that arcing cannot occur between the work place and superstructure.
- Overhead transmission and distribution lines will be carried on towers and poles that provide safe clearance over roadways and structures.
- Workers will be instructed to use care in working under or around utilities, to avoid hot surfaces, loud noises, pressurized gases or air, leaking pipelines, discharging steam or hot liquids and must work to prevent accidental contact with breakage.

The following clearances will be maintained between equipment and energized power lines:

<u>Voltage</u>	<u>Working Clearance</u>	<u>Equipment Clearance</u>
Less than 50 kV	10 feet	4 feet
50 to less than 345 kV	10 feet, plus 4 inches per each extra kV	10 feet
345 to 750 kV	10 feet, plus 4 inches per each extra kV	16 feet

## 11.4 RECORD KEEPING

The health and safety record keeping requirements are an important component of UCB Health and Safety Program and ATG's Health and Safety Program. The following list highlights the record keeping requirements for BERC field staff and ATG's employees and site-specific activities. The items will be retained by ATG for 30 years after each covered employee has ended employment with BERC or ATG, respectively.

- Medical surveillance results for each of BERC's or ATG's employees
- Names, addresses, and phone numbers of examining and consulting physicians and clinics
- A copy of respirator fit test results
- A copy of training certificates for initial 40 hours of project work site training, SHSO

training, 8 hours of supervisor's health and safety training, 8 hours of annual refresher training, CPR and first aid training, and any other training received

- A copy of employee CPR and Red Cross certificates

The following records will be retained by ATG for 30 years after close-out of Delivery Order 05:

- Copies of UCB's and ATG's Health and Safety Programs
- Records of site visits by ATG's employees and subcontractors
- A copy of pages from logbooks on field calibration of health and safety monitoring equipment for air sampling and other field issues related to health and safety.
- All health and safety survey reports
- Accident/incident Notification Reports

**Table 1**  
**Site 3 Contaminants**  
**Alameda NAS**

<b>VOC</b>	<b>Max. Conc.</b>	<b>Min. Conc.</b>	<b>Unit</b>	<b>Depth(ft) Max. Conc.</b>
Acetone	0.2	ND	mg/kg	5-5.5
Toluene	0.13	ND	mg/kg	5-5.5

<b>SVOC</b>	<b>Max. Conc.</b>	<b>Min. Conc.</b>	<b>Unit</b>	<b>Depth(ft)</b>
Benzo(g,h,i)perylene	1.6	ND	mg/kg	10.5-11
Pyrene	3.1	ND	mg/kg	10.5-11

<b>Metals</b>	<b>Max. Conc.</b>	<b>Min. Conc.</b>	<b>Unit</b>	<b>Depth(ft)</b>
Barium	0.58	0.33	mg/L	*
Chromium	0.25	0.12	mg/L	*
Cobalt	0.05	ND	mg/L	*
Copper	0.1	0.044	mg/kg	*
Manganese	2.7	1.8	mg/L	*
Nickel	0.24	0.15	mg/L	*
Selenium	0.054	ND	mg/L	*
Vanadium	0.22	0.11	mg/L	*
Zinc	0.19	0.11	mg/L	*

Table 2  
Site 13 Contaminants  
Alameda NAS

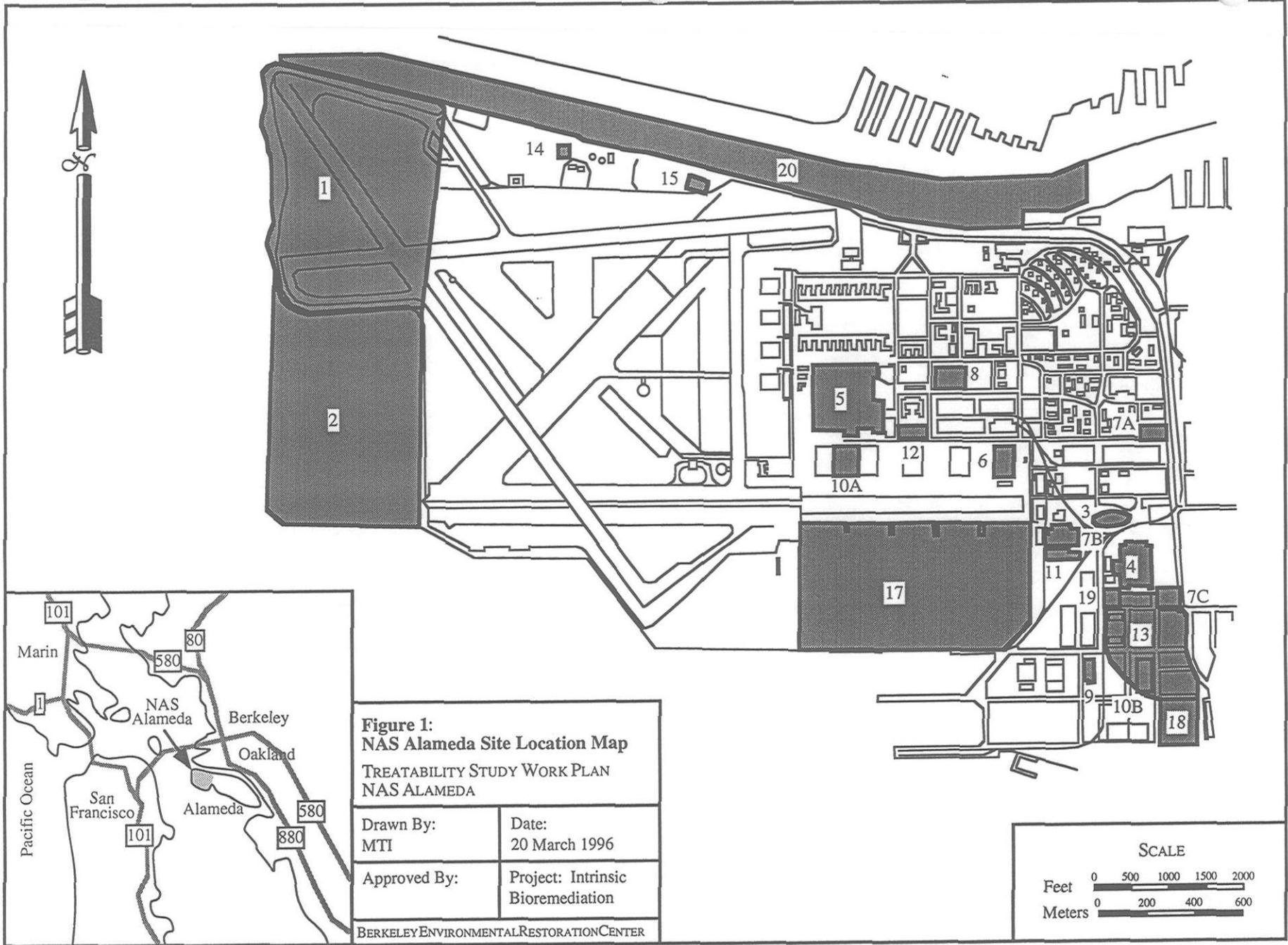
VOC	Max. Conc.	Min. Conc.	Unit	Depth(ft) of Max. Conc.
Benzene	1	ND	mg/kg	6.5-7
1,2-Dichloroethene	0.005	ND	mg/kg	11-11.5
Methylene Chloride	0.16	ND	mg/kg	11-11.5
Methyl Ethyl Ketone	0.005	ND	mg/kg	7-7.5
Toluene	1.6	ND	mg/kg	2-2.5
Xylene	4.1	ND	mg/kg	11-11.5

SVOC	Max. Conc.	Min. Conc.	Unit	Depth(ft) of Max. Conc.
Anthracene	0.1	ND	mg/kg	11-11.5
1,2-Benzanthracene	0.39	ND	mg/kg	11-11.5
Benzo(a)pyrene	0.52	ND	mg/kg	12-12.5
Benz(e)acephenanthrylene	1.1	ND	mg/kg	11-11.5
Benzo(g,h,i)perylene	1.4	ND	mg/kg	11-11.5
Benzo(k)fluoranthene	0.51	ND	mg/kg	12-12.5
Chrysene	2.3	ND	mg/kg	0.5-1
Ethylbenzene	1.8	ND	mg/kg	11-11.5
Fluorene	0.79	ND	mg/kg	11-11.5
Fluoranthene	0.8	ND	mg/kg	11-11.5
Indeno(1,2,3-cd)pyrene	0.69	ND	mg/kg	11-11.5
2-Methylnaphthalene	17	ND	mg/kg	11-11.5
Naphthalene	5.4	ND	mg/kg	11-11.5
N-Nitrosodiphenylamine	2.7	ND	mg/kg	11-11.5
Pentachlorophenol	1	ND	mg/kg	4-4.5
Phenanthrene	1.8	ND	mg/kg	14-14.5
Pyrene	1.9	ND	mg/kg	11-11.5

**Table 2 (Cont'd)**  
**Site 13 Contaminants**  
**Alameda NAS**

<b>Metals</b>	<b>Max. Conc.</b>	<b>Min. Conc.</b>	<b>Unit</b>	<b>Depth(ft) of Max. Conc.</b>
Arsenic	0.077	ND	mg/L	*
Barium	1.9	0.37	mg/L	*
Beryllium	0.0084	0.0054	mg/L	*
Chromium	1.1	0.13	mg/L	*
Cobalt	0.25	0.14	mg/L	*
Copper	0.32	0.042	mg/L	*
Lead	0.18	0.054	mg/L	*
Manganese	12	2.7	mg/L	*
Nickel	1.7	0.19	mg/L	*
Selenium	0.18	0.097	mg/L	*
Vanadium	0.76	0.11	mg/L	*
Zinc	0.86	0.12	mg/L	*

<b>Pesticides</b>	<b>Max. Conc.</b>	<b>Min. Conc.</b>	<b>Unit</b>	<b>Depth(ft) of Max. Conc.</b>
Beta-BHC	0.0035	ND	mg/kg	10.5-11
4,4'-DDD	0.014	0.0045	mg/kg	13.5-14
4,4'-DDE	0.035	0.0037	mg/kg	12.5-13
4,4'-DDT	0.16	ND	mg/kg	0.5-1
Heptachlor Epoxide	0.0054	ND	mg/kg	0.5-1
Toxaphene	2.5	0.4	mg/kg	10.5-11



**Figure 1:**  
**NAS Alameda Site Location Map**  
 TREATABILITY STUDY WORK PLAN  
 NAS ALAMEDA

Drawn By: MTI	Date: 20 March 1996
Approved By:	Project: Intrinsic Bioremediation
BERKELEY ENVIRONMENTAL RESTORATION CENTER	

SCALE	
Feet	0 500 1000 1500 2000
Meters	0 200 400 600

## FIGURE 2

### FIELD ACTIVITIES EQUIPMENT LIST

The following equipment shall be available on site during field work associated with the Treatability Study at Sites 3 and 13.

#### Personal Protective Equipment:

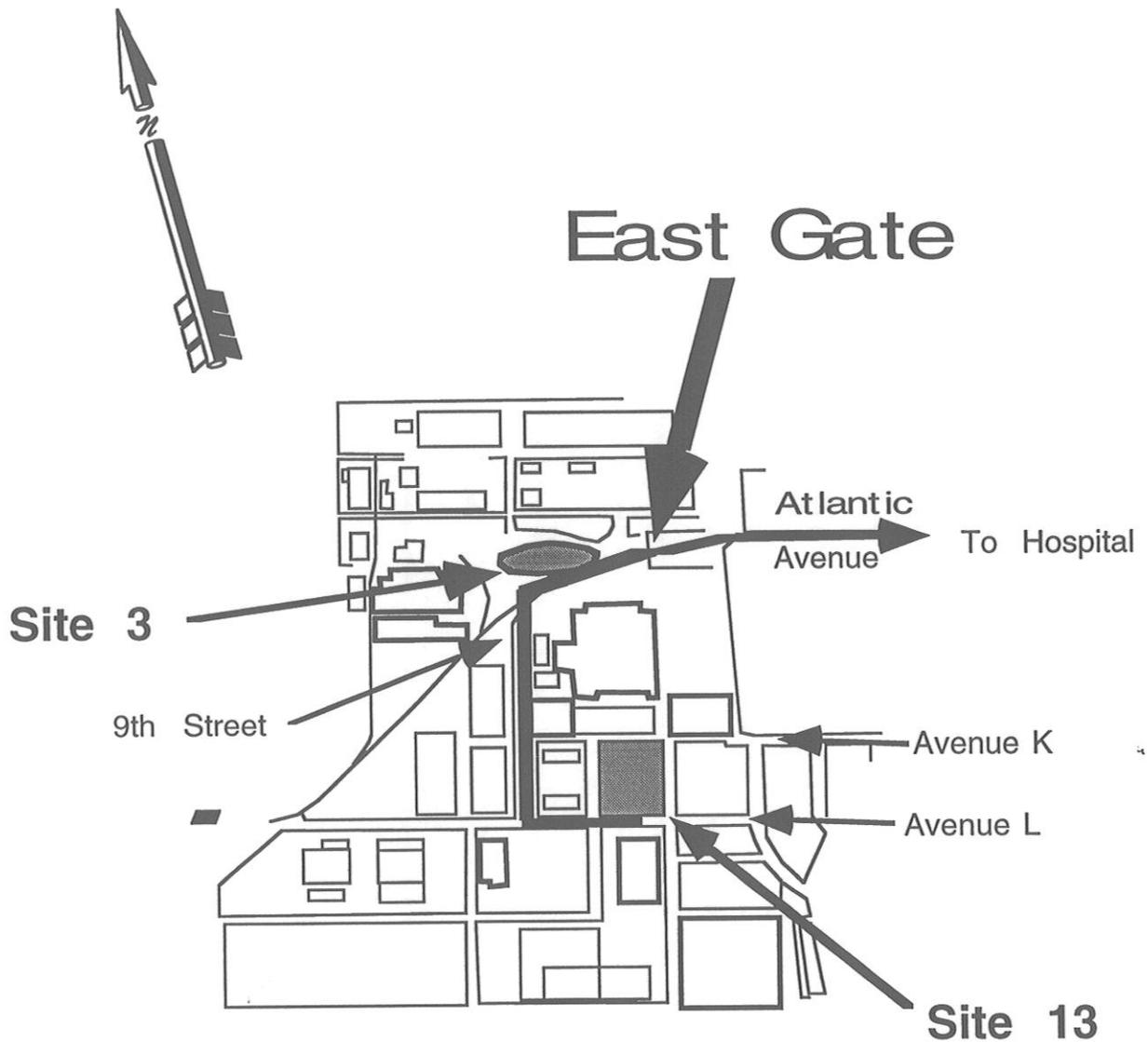
- Air purifying respirators  
(full face, organic vapor cartridges with aerosol/particulate filter). See Section 10 regarding use conditions.
- Safety glasses with side shields.
- Hard hats
- Polyethylene coated Tyvek
- Latex inner gloves
- Nitrile outer gloves
- PVC or neoprene boots with steel shanks

#### Air Monitoring Equipment:

- Photoionization detector (10.2 eV Lamp)
- Oxygen deficiency/ combustible gas indicator
- Mini ram aerosol monitor

#### Miscellaneous:

- First aid kit
- Eyewash and/or shower
- Adequate water supply, soap, towels
- Fire extinguisher
- Flash light



## Evacuate Through East Gate

### From Site 3 to East Gate

1. Proceed a few hundred feet east on Atlantic Avenue to guard house.

### From Site 13 to East Gate

1. Proceed west on Avenue L 1 block to 9th Street.
2. Turn right on 9th Street and proceed 3 blocks north to Atlantic Avenue.
3. Turn right on Atlantic Avenue and proceed a few hundred feet east to guard house.

**Figure 3**  
**Emergency Route to East Gate**

TREATABILITY STUDY WORK PLAN  
NAS ALAMEDA

Drawn By: WJS      Date: 9 February 1996

Approved By:      Project: Intrinsic  
Bioremediation

BERKELEY ENVIRONMENTAL RESTORATION CENTER

# Emergency Hospital Route

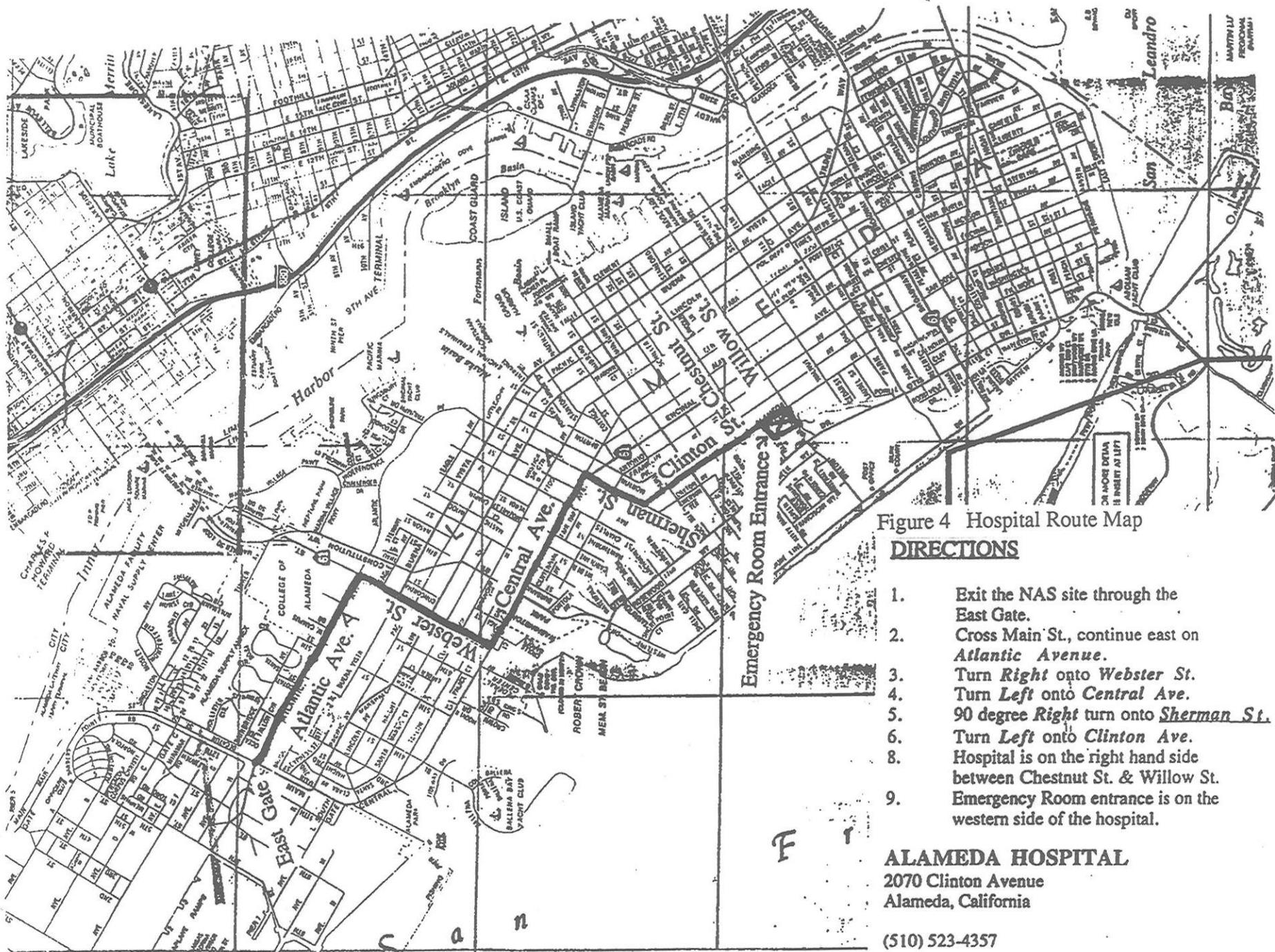


Figure 4 Hospital Route Map

## DIRECTIONS

1. Exit the NAS site through the East Gate.
2. Cross Main St., continue east on Atlantic Avenue.
3. Turn Right onto Webster St.
4. Turn Left onto Central Ave.
5. 90 degree Right turn onto Sherman St.
6. Turn Left onto Clinton Ave.
7. Hospital is on the right hand side between Chestnut St. & Willow St.
8. Emergency Room entrance is on the western side of the hospital.

**ALAMEDA HOSPITAL**  
2070 Clinton Avenue  
Alameda, California

(510) 523-4357

**ATTACHMENT 1**  
**HEALTH AND SAFETY PLAN ACCEPTANCE FORM**

**INSTRUCTIONS:** This form is to be completed by each person prior to beginning work at Site 3 and 13 of Naval Air Station Alameda. THIS FORM IS TO BE RETURNED TO THE ATG CIH.

Project No. \_\_\_\_\_

Location \_\_\_\_\_

By my signature below, I acknowledge that I have read and understand the contents of the Health and Safety Plan (HSP) for this project. I agree to perform my work in accordance with the requirements of the HSP

---

Signature

---

Print Name

---

Company

---

Address

---

Telephone Number

---

Date

**ATTACHMENT 2  
SAFETY MEETING SIGN-OFF SHEET**

THIS FORM IS TO BE RETURNED TO THE ATG CIH.

Meeting Held by: \_\_\_\_\_ Date: \_\_\_\_\_

Project No.: \_\_\_\_\_ Site/Facility: \_\_\_\_\_

**ITEMS DISCUSSED:**

**Hazard Evaluation:**

Toxic Vapors	Yes	No	_____
Explosion	Yes	No	_____
O <sub>2</sub> Depletion	Yes	No	_____
Physical Hazards	Yes	No	_____

Personal Protection to be Worn and Equipment to be Used:	Yes	No	_____
Decontamination Procedures:	Yes	No	_____

Other: \_\_\_\_\_

---

**EMERGENCY INFORMATION**

First Aid	Yes	No	_____
Hospital Route	Yes	No	_____
Poison Control Center	Yes	No	_____

**Project Team Member Signatures**

**Date**

Site Supervisor:

\_\_\_\_\_

\_\_\_\_\_

Site Health and Safety Officer:

\_\_\_\_\_

\_\_\_\_\_

Subcontractor Health and Safety  
Representative:

\_\_\_\_\_

\_\_\_\_\_

**ATTACHMENT 3**  
**ACCIDENT/INCIDENT REPORT FORM**  
(Sheet 1 of 2)

THIS FORM IS TO BE RETURNED TO THE ATG CIH.

FIELD TEAM LEADER'S REPORT OF ACCIDENT/INCIDENT  
(USE FOR ON-SITE ACCIDENTS OR EXPOSURES ONLY)

To: ATG Program CIH

From: \_\_\_\_\_

Telephone Number: \_\_\_\_ / \_\_\_\_ - \_\_\_\_\_

Name of Injured/Ill Employee:

\_\_\_\_\_

Date of Accident/Incident: \_\_\_\_\_

Time of Accident/Incident: \_\_\_\_\_

Exact Location of Accident/Incident:

\_\_\_\_\_

Description of Accident/Incident:

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Nature Of Illness or Injury and Part Of Body Involved:

\_\_\_\_\_

\_\_\_\_\_

Probable Disability (check one)

\_\_\_\_\_ Fatal

\_\_\_\_\_ Lost work days (No. of days:\_\_\_\_)

\_\_\_\_\_ Restricted activity (No. of days:\_\_\_\_)

**ATTACHMENT 3**  
**ACCIDENT/INCIDENT REPORT FORM**  
(Sheet 2 of 2)

\_\_\_\_\_ No lost work days  
\_\_\_\_\_ First aid only

Action(s) Taken by Reporting Unit:

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Corrective Action That Remains to be Taken (By whom and by when):

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Name of Project Manager: \_\_\_\_\_

Signature: \_\_\_\_\_

Date: \_\_\_\_\_

Name of Site Supervisor: \_\_\_\_\_

Signature: \_\_\_\_\_

Date: \_\_\_\_\_

## APPENDIX A STANDARD SAFETY PROCEDURES

This appendix contains the more relevant standard safety procedures described in Section 12 of the BERC Health and Safety Plan (Program) which is required to be maintained on site during field work. This appendix has been included for references purposes only. The standard safety procedures included in this appendix are numbered as they appear in the BERC Health and Safety Plan (Program) to minimize confusion.

### 12.3 VEHICLE TRAFFIC

The project worksite is located within an active military base with both industrial and personal vehicle traffic nearby. Work in such areas presents a risk of being stuck by a vehicle. Collisions between vehicles are also possible.

Vehicle operators will check carefully for nearby traffic before proceeding at a cautious pace on facility roadways. Unless otherwise marked, speeds should be held to 15 mph or less while on site.

Care should be taken to ensure that trucks, equipment and materials are placed in a manner that keeps obstruction of local traffic to a minimum. During work activities, it may become necessary to move equipment in order to accommodate traffic and site activities.

Workers on foot should not wander into the active roadways. If work in active traffic areas is required, workers will wear bright orange safety vests, and the work area will be marked with lighted barricades, cones or flags to warn traffic.

Where traffic control is necessary, base representatives will be contacted to ensure minimal disruption of base activities. When the base cannot provide traffic control officers, project workers will do so using high visibility road vests, hand-held stop signs and traffic cones.

### 12.4 DRILLING

Drilling is associated with a number of potential hazards which include underground utilities, overhead power lines, rotating machinery and pinch points. All operations involving powered drilling rigs will follow generally accepted drilling practices. One person will be assigned as Lead Driller who shall be responsible for operating the drilling rig safely. Additional personnel will assist as needed under the direction of the Lead Driller.

The following procedures shall be observed when drilling:

- Determine the presence of underground utilities and relocate drilling as necessary to avoid contact. This will require calling the Underground Service Alert at least 48 hours ahead of time. A qualified subcontractor will also make a utility survey of each drilling point. Nearby utilities shall be marked on the ground; and
- Drill rigs must maintain at least a 20-foot clearance from any overhead lines at all times. While the rig is being positioned and readied for use, the operator must be completely within the operator's area and no one else shall be permitted to touch the rig until it has been secured; and
- All drill rigs or stationary equipment shall be chocked or blocked and the parking brake set to prevent accidental movement; and
- The drilling equipment when in use shall be inspected weekly. Guards shall be kept in place at all times except when servicing equipment; and
- An "Exclusion Zone" will be established around the drilling rig using barricade tape physical barrier; and
- All operators and crew members will be familiar with the rig operations and will have received practical training; and
- Equipment and tools shall be decontaminated as specified in the HSP; and
- Hard hats are required when working within the drilling rig work zone; and
- No loose fitting clothing, jewelry, or free long hair is permitted near the drilling rig or moving machinery parts; and
- Hands and loose clothing must be kept away from moving parts of the machinery; and
- Drilling must cease immediately if combustible gas concentrations greater than 10% of the LEL are detected at the borehole; and
- A first aid kit and fire extinguisher will be available at all times; and
- The off-going driller will inform the oncoming driller of any special hazards or ongoing work that may affect the safety of the crew; and
- If lubrication fittings are not accessible with guards in place, machinery must be stopped for oil and greasing; and

- Rigging equipment for material handling should be checked prior to use on each shift and as often as necessary to ensure it is safe. Defective rigging shall be removed from service immediately; and
- The area around the derrick ladder must be kept clear to provide unimpeded access to the ladder; and
- Work areas and walkways must not be obstructed; and
- The work area around the borehole shall be kept free of obstructions, and free of undue accumulation of oil, water, ice or circulating fluids; and
- No drilling will occur during impending electrical storms or tornadoes, or when rain, ice, snow, or wind conditions create undue potential hazards; and
- One worker shall not lift auger flights by himself or attempt to carry equipment or materials of excessive weight; and
- The driller will not attempt to reach a well or borehole location in a manner that compromises the safety of the rig or crew; and
- All well or borehole locations will be inspected by the drill crew to ensure that a stable surface exists; and
- The drill rig will be properly blocked and leveled prior to raising the mast; and
- The drill rig shall be driven or moved only after the mast has been lowered; and
- The leveling jacks shall not be raised until the derrick is lowered; and
- Appropriate exposure monitoring shall be conducted as specified in Section 9.1 when working in contaminated areas. Additional PPE may be required during these conditions.

Soil cuttings from the drilling processes will be placed in DOT-approved containers, sealed, and stored in a secured area. The containers will be properly labeled and documented. BERC shall manifest and properly dispose of all contaminated liquids and soil cuttings to a Resource Conservation and Recovery Act (RCRA) permitted treatment, storage and disposal facility.

#### **12.4.1 Hoisting Operations**

The following procedures shall be followed during hoisting operations:

- Drillers must never engage the rotary clutch without watching the rotary table, and ensuring it is clear of personnel and equipment; and
- Unless the drawworks is equipped with an automatic feed control, the brake must not be left unattended, without first being tied down; and
- Drill pipe or casing must not be picked up suddenly; and
- Drill pipe must not be hoisted until the driller is sure that the pipe is latched in the elevator, or the derrickman has signaled that he may safely hoist the pipe; and
- During instances of unusual loading of the derrick or mast, such as when making an unusually hard pull, only the driller may be on the rig floor, and no one may be on the rig or derrick; and
- The brakes on the drawworks of every drilling rig must be tested by each driller, when he comes on shift to determine whether they are in good order. The brakes must be thoroughly inspected by a competent individual each week; and
- A hoisting line with a load imposed must not be permitted to be in direct contact with any derrick member or stationary equipment, unless it has been specifically designed for line contact; and
- Workers must never stand near the boring whenever any wire line device is being run; and
- Hoisting control stations must be kept clean and controls labeled as to their function; and
- Personnel are forbidden to ride traveling blocks or elevators unless catlines are used.

### **12.4.2 Catline Operations**

The following procedures shall be followed during catline operations:

- Only experienced workers will be allowed to operate the cathead controls. The kill switch must be clearly labeled and operational before use of the catline; and
- The cathead area must be kept free of obstructions and entanglements; and
- The operator must not use more wraps than necessary to pick up the load. More than one layer of wrapping is not permitted; and

- Personnel must not stand near, step over, or go under a cable or catline under tension; and
- Employees rigging loads on catlines must:
  - keep out from under the load; and
  - keep fingers and feet where they will not be crushed; and
  - be sure to signal clearly when the load is being picked; and
  - use standard visual signals only and not depend on shouting to coworkers, and
  - make sure the load is properly rigged, since a sudden jerk in the catline will shift or drop the load.

### **12.4.3 Pipe Handling**

The following procedures shall be followed during pipe handling operations:

- Pipe must be loaded and unloaded, layer by layer, with the bottom layer pinned or blocked securely on all four corners. Each successive layer must be effectively blocked or chocked; and
- Workers must not be permitted on top of the load during loading, unloading, or transferring of pipe or rolling stock; and
- Employees shall stand clear of rolling pipe and shall not stop rolling pipe or casing; and
- Slip handles must be used to lift and move slips. Employees must not be permitted to kick slips into position; and
- When pipe is being hoisted, personnel must not stand where the bottom end of the pipe could whip and strike them; and
- Pipe stored in racks, catwalks or on flatbed trucks must be chocked to prevent rolling.

### **12.4.5 Derrick Operations**

The following procedures shall be followed during derrick operations:

- The derrick climber must be used whenever climbing the derrick. Personnel on the derrick must be tied off, or otherwise protected from falling when working in an unguarded elevated position; and

- All stands of pipe and drill collars racked in a derrick must be secured with rope or otherwise adequately secured; and
- Tools, derrick parts, or materials of any kind shall not be thrown from the derrick; and
- The elevators must be properly clamped onto all pipe joints prior to the driller engaging the load.

#### **12.4.6 Making and Breaking Joints**

The following procedures will be followed when making and breaking joints:

- Tongs shall be used for the initial making up and breaking of the joint. The rotary table shall not be used for the initial breaking of a joint; and
- Employees making or breaking joints shall not be permitted to stand within the arc of the tong handles when the tong pull line is in tension. Employees shall handle the tongs only by the appropriate handles; and
- Employees shall be trained in the safe use of spinning chains. Spinning chains must not be handled near the rotary table while it is in motion.

### **12.6 CONTROL OF HAZARDOUS ENERGY**

The use or maintenance of electrical and mechanical equipment can expose workers to shock hazards, and crushing or pinch hazards. Lockout and tagout procedures are required whenever there is potential exposure to hazardous energy from equipment activation.

#### **12.6.1 GENERAL LOCKOUT/TAGOUT REQUIREMENTS**

Lockout and tagout procedures are required during maintenance of power tools or equipment, during valve changeouts and other work on hazardous waste or materials lines. Other tasks may also require lockout and tagout procedures if nearby equipment or material transfer lines could harm employees. Examples of lockout/tagout tags are shown in Appendix D, *Forms*. The requirements of lockout and tagout include:

- Locks and tags are to be used when a machine, equipment or piping system is capable of being locked out. Tags alone are allowed only when the equipment will not accept locks; and
- Authorized padlocks shall be assigned to each authorized employee. Each group's lock will be individually keyed and the shift supervisor shall maintain the master keys; and

- All new equipment installed must be designed to accept a lockout device; and
- Where multiple items must be locked out, a group lock box must be used; and
- Where multiple locks must be placed on an item, a multiple lock hasp must be used; and
- Only the protected employee may remove a personal lock. When the employee is no longer present and the lock must be removed, only that employee's immediate supervisor may remove the lock and tag, and only after ensuring that the employee is out of harm's way; and
- All locks must be accompanied by a tag indicating the name of the employee applying the lock, the date the lock was applied, equipment name or number, the reason for the lockout and a warning against the potential hazard of activation; and
- A legend must be displayed warning against activation and stating that the lock and tag may be removed only by authorized personnel; and
- Tags must be single-use, hand-attachable, legible and designed to withstand the environment where they are in use. Tags must be self-locking and non-releasable with a minimum unlocking strength of 50 pounds; and
- A "Lockout Log" shall be maintained by the site supervisor as part of the HSP; and
- The SPM or PS is responsible for informing the client of the lockout/tagout procedure to be used at the jobsite. This must be documented on FADLs; and
- Subcontractors are to use BERCC's lockout/tagout procedures. Their own procedure may be used only after it has been reviewed and approved by the Program CIH; and
- If the client has their own lockout/tagout requirements, these shall be implemented only after BERCC's requirements have been met; and
- The SPM and PS shall assure that locks, hasps and other equipment and site specific training are provided; and
- Lockout or tagout of utilities or systems that will impact NAS Alameda operations will be coordinated with the RPM, ROICC or PWC as appropriate; and
- Tools found to be defective shall be tagged "Defective" and removed from service.

Lockout/tagout procedures are not required when work is conducted on equipment where an employee has direct control over the cord(s) or plug(s) connected to the associated equipment.

### **12.6.2 Lockout/Tagout Checklist**

Where lockout/tagout procedures are required, the following steps shall be followed:

- Check the equipment file for specific lockout/tagout procedures; and
- Determine the requirements for lockout. Identify each energy source to the equipment; and
- Conduct a survey to locate and identify all isolation devices that apply to the equipment; and
- Use the equipment's type-specific procedures if applicable. Complete the "Lockout/Tagout Log" for logging all data, and return it to the supervisor. See Appendix D; and
- Shut off the energy source(s) to the affected equipment; and
- Affix lock(s) and tag(s) to each energy source controlling the device; and
- Identify work on process lines or vessels and determine isolation requirements; and
- Blind, blank, disconnect or double-valve and vent all hazardous materials lines (including steam). Identify isolation points with tags; and
- When a tag only is used because the equipment can't be locked out, remove fuses, block machine, etc. ; and
- Relieve all stored energy (e.g., capacitor banks, springs, compressed air, hydraulic and steam) ; and
- Verify that isolation of energy has occurred by attempting to activate equipment at the on/off switch and return the control switch to the off position before proceeding.

Before returning any equipment to service following lockout and tagout, the following procedures are required:

- Ensure that all nonessential items (e.g., tools and cleaning rags) are removed from the equipment; and

- Ensure that equipment components are intact; and
- Check the work area to ensure that all employees are safely positioned or removed from the area; and
- Notify all affected employees and site supervisor before re-energizing the equipment; and
- Remove the lockout/tagout device; and
- Re-energize the equipment or open valves and restore flow in process line and place it back into service.

Where equipment must be locked out for longer than one work shift, the individual lock(s) of the outgoing shift working on equipment will be removed and replaced by the on-coming shift's individual lock(s). The authorized employees of the on-coming shift must inspect and "try" the system to ensure de-energization. The site supervisor shall re-audit the system as necessary.

## **12.14 NOISE**

Some project equipment may result in noise exposures in excess of 85 dBA. The SHSO shall monitor noise exposures as discussed in Section 9.1.5 of this HSP. Employees exposed to more than 85 dBA shall participate in a hearing conservation Program which shall include annual audiometric testing, annual training and the use of hearing protection.

Whenever possible, low noise output will be selected for this project. Otherwise, engineering controls (enclosures, increased distance etc.) shall be used to minimize worker noise exposure. Criteria for determining when hearing protection is needed shall be specified in the HSP.

Exposure to sound levels above 85 dBA can cause temporary impairment of hearing. Prolonged and repeated exposure to sound levels above 85 dBA can cause permanent hearing damage. The risk and severity of hearing loss increases with the intensity and duration of the exposure. In addition to damaging hearing, noise can impair voice communication, thereby increasing the risk of incidents.

The SHSO shall evaluate the need for double hearing protection as appropriate in response to increase exposure to sound levels, especially those above 85 dBA.

## 12.15 HEAT STRESS

### 12.15.1 Adverse Heat Effects

The use of PPE (PPE) can put site personnel at considerable risk of heat stress and heat related illnesses because of reduced cooling. Heat related illnesses range from transient heat fatigue to heat stroke and death. Contributory factors include environmental conditions, clothing, work load, and individual susceptibility and acclimatization. Physical fitness, diet, alcohol/drug use, sleeping habits, acclimation, genetics, medical condition, age and weight also play a role in heat stress.

**Heat Cramps.** Heat cramps are caused by heavy sweating and inadequate electrolyte replacement. Signs and symptoms include muscle spasms and pain in the hands, feet and abdomen.

**Heat Exhaustion.** Heat exhaustion occurs from increased stress on various body organs. Signs and symptoms include:

- Pale, cool, moist skin;
- Heavy sweating;
- Dizziness, nausea; or
- Fainting.

**Heat Stroke.** Heat stroke is the most serious form of heat stress and should always be treated as a medical emergency. The body's temperature regulation system fails, and body temperature rises rapidly to critical levels. Immediate action must be taken to cool the body before serious injury or death occurs. Signs and symptoms of heat stroke include:

- Skin that is red, hot, and either dry or moist from perspiration
- Lack of, or reduced perspiration;
- Body temperature of 104° F or higher
- Nausea;
- Dizziness and confusion;
- Strong, rapid pulse and/or
- Coma.

**Sunburn.** Operations will require BERC and subcontractor employees to work outside during daylight hours, typically seven to nine hours per day. Under these conditions, workers are at risk for developing sunburn on unprotected skin.

Sunburn is a burn to the skin caused by overexposure to ultra-violet light (a component of sunshine). The symptoms of exposure are not usually apparent until two to four hours after initial exposure. Depending upon the severity of the exposure the symptoms can range from reddening of the skin accompanied by mild discomfort to painful deep burns and blisters. Although light-haired, fair-skinned, blue-eyed personnel are at the greatest risk of sunburn, all complexion types can develop sunburn if the exposure is long and intense enough.

Sunscreen products with sun protection factor ratings of 15 or higher will be available to project personnel. Areas of primary concern include; nose, cheeks, ears and the back of the neck. Sunscreen will be applied as necessary and reapplied in accordance with recommendations from the manufacturer.

### **12.15.2 Heat Stress Prevention**

The best way to manage heat stress is to prevent it. Preventive measures include:

- Site workers will be encouraged to drink plenty of water or electrolyte replacement fluids (e.g. Gatorade) throughout the day; and
- On-site drinking water will be kept cool to encourage personnel to drink frequently; and
- All personnel will be trained on the hazards and symptoms of heat stroke, heat exhaustion and heat cramps; and
- All employees shall be informed of the importance of adequate rest, acclimation and proper diet in the prevention of heat stress disorders; and
- The PS and SHSO shall adjust work & rest schedules to provide adequate rest periods for cooling down. Cooling breaks shall take place in shaded rest areas. Personal protective clothing shall be removed during cooling breaks. No other tasks shall be assigned during these breaks; and
- Workers shall be instructed to limit their intake of alcohol during off-hours and beverages containing caffeine because of their diuretic effects; and
- Employees shall be instructed to monitor themselves and coworkers (as described in Section 11) for signs of heat stress and to take additional breaks as necessary.

### 12.15.3 Heat Stress Monitoring Program

Daily temperature maxima and minima on-site shall be recorded. BERC health and safety policy shall be to prevent heat stress wherever possible. Work requiring the use of personal protective clothing will be scheduled to cooler parts of the day. Ambient temperatures shall be recorded to evaluate heat stress potential. When temperatures exceed 70°F, the PS and SHSO shall evaluate re-scheduling of work which requires the use of protective clothing, and re-assignment of tasks to minimize use of protective clothing. If work with protective clothing cannot be re-scheduled, additional monitoring shall be conducted with a WBGT heat stress monitor. The results will be used to establish work rest regimes based on Table 12 (in Section 9.1.6). This table will be adjusted by a correction factor of -6°C when Tyvek suits are used. Additionally, employees will be reminded of the preventive procedures discussed in Section 12.15.2.

If necessary, a biological monitoring Program shall be implemented. This shall be discussed in HSP. The biological monitoring Program shall consist of:

- Heart rate. Count the radial pulse during a 30-second period as early as possible in the rest period; and
  - If the heart rate exceeds 110 beats per minute at the beginning of the rest period, shorten the next work cycle by one-third and keep the rest period the same; and
  - If the heart rate still exceeds 110 beats per minute at the next rest period, shorten the following work cycle by one-third.
- Oral temperature. Use a clinical thermometer (3 minutes under the tongue) or similar device to measure the oral temperature at the end of the work period (before drinking) ; and
  - If oral temperature exceeds 99.6 °F (37.6 °C), shorten the next work cycle by one-third without changing the rest period. ; and
  - If oral temperature still exceeds 99.6 °F (37.6 °C) at the beginning of the next rest period, shorten the following work cycle by one-third; and
  - Do not permit a worker to wear a semipermeable or impermeable garment when the workers' oral temperature exceeds 100.6 °F (38.1 °C).

The frequency of biological heat stress monitoring shall be as follows:

- Every 2 hours if adjusted temperature (TA) is less than 77.5 °F; or

- Every 90 minutes if TA is between 77.5 °F and 82.5 °F; or
- Every hour if TA is between 82.5 °F and 87.5 °F; or
- Every 30 minutes if TA is between 87.5 °F and 90 °F.

Adjusted temperature (TA) is the product of ambient temperature and a sunshine factor (expressed as a percent) times a constant of 13.

No work requiring PPE shall be allowed where TA exceeds 90 °F.

### **12.15.4 Heat Stress Management**

Individuals with symptoms of heat stress shall notify the PS and shall immediately halt field activities and be treated for heat stress as follows:

- Remove affected person and lie them down in a cool, shaded area or air-conditioned room and elevate their feet. Abbreviated decontamination procedures may be followed (see section 7.4 for decontamination guidelines during medical emergencies); and
- Loosen or remove as much clothing as possible; and
- Apply wet towels or fine mist to assist in lowering the body temperature. Never ice down to avoid further physiological shock; and
- If victim is conscious, encourage the intake of replacement fluid; and
- Evaluate victim for heat stroke or unconsciousness. Obtain emergency assistance as necessary

Cases of heat stress shall be recorded in the OSHA 200 log.

### **12.16 FIRE PREVENTION**

Project field work has the potential for fire either from dried vegetation or from the presence or use of flammable liquids or gases. The following fire prevention practices shall be in effect during the project:

- Smoking or open flames are prohibited except in designated smoking areas; and
- Vehicles and equipment will not be left idling or parked in or around dried vegetation where catalytic converters may ignite it. Equipment and vehicles shall stay on the paved areas wherever possible; and

All flammable liquids will be stored in Underwriters Laboratory (UL) or Factory Mutual (FM) approved storage cabinets. Small quantities of most flammable liquids (five gallons or less) may be stored in work areas, or carried in vehicles, providing those materials will be used that day and will be contained in a safety can or other approved container. Class IA flammable liquids should be limited to two gallons in an approved safety can. Any flammable wastes will be stored or disposed of in metal containers, clearly marked as containing flammable materials. Storage of combustible materials, in work areas, will be kept to a minimum; and

Portable dry-chemical fire extinguishers must be provided to each project site as follows:

MINIMUM RATING	REQUIRED LOCATION
1A, 5BC	Each Company Owned or Leased Vehicle
2A, 10BC	Each Fuel-dispensing Vehicle
3A, 40BC	Solvent Storage Areas

- Portable fire extinguishers shall be maintained on site and shall be inspected monthly, and serviced at least annually by a person licensed or registered by the State Fire Marshal; and
- Only UL approved three-wire electrical extension cords rated for hard or extra-hard usage may be used where temporary power is necessary. Only double insulated or grounded electrical power tools may be used; and
- A BERCC Hot Work Permit must be completed and posted prior to any hot work on site, including hot work performed by subcontractors. The Base Fire Department must be contacted to determine if other permits are required prior to hot work; and
- In case of a fire on the site, the PS or the SHSO will assess the situation and determine the proper response. BERCC will also call the Base Fire Department at the number listed in Section 12.7 and notify the ROICC immediately. Only BERCC personnel trained in the use of extinguishers may attempt to put out the fire with available equipment, if safe to do so. If these trained employees do not wish to make the attempt, they are to evacuate also. Persons without fire extinguisher training shall evacuate the area.

## 12.17 SLIP, TRIP AND FALL HAZARDS

Poor housekeeping results may result in slip, trip and fall hazards which can cause serious injuries, including fractures, contusions, and lacerations. The following measures shall be taken to

minimize slip trip and fall hazards:

- Maintain all stairways, passageways, gangways, and accesses free of materials, supplies, and obstructions at all times.
- Loose or light material shall not be stored or left on roofs or floors that are not closed in, unless safely secured.
- Tools, materials, extension cords, hoses, or debris shall not be placed where they may cause tripping or other hazards.
- Tools, materials, and equipment subject to displacement or falling shall be adequately secured.
- Empty bags having contained lime, cement, and other dust-producing material shall be removed and properly disposed of immediately.
- Scrap lumber and debris shall be cleared from work areas and accesses.
- Daily work area inspections for adequate housekeeping. Prompt correction of hazards identified

## 12.24 FLORA AND FAUNA

Poisonous or stinging insects, spiders and snakes may be a concern for project personnel during sampling and other site activities. Disease vectors, such as ticks, may also be present. Poison oak or other noxious flora, which can cause severe skin irritation on contact, may be present on project sites. There may also be thistles and other thorny weeds.

Site workers shall inspect protected areas (e.g., boreholes, pits, storage areas and portable toilets) before either reaching into them or entering them. Stinging insects and their nests shall be avoided wherever possible and workers shall wear long pants and gloves for protection.

When tick exposure is anticipated, employees shall work in buddy teams and shall check each other as frequently as possible for ticks. After returning from the field, buddies shall check each other carefully for ticks. Particular attention shall be paid to the head and neck (adult ticks) and lower extremities (nymphs and larvae). The following procedures shall be used for tick removal:

- Using tweezers, grasp the tick behind the mouthparts (head) and slowly but firmly remove the tick. Do not attempt field removal of any remaining body parts as this should be done by medical personnel; and

- Do not use any chemical agents such as alcohol, or petroleum oils, hot match heads or other similar methods; and
- Ticks should not be handled or smashed with fingers as disease inoculation by such action is possible; and
- Clean the wound and apply an antiseptic; and
- Monitor tick bites and be alert for a rash or other symptoms that may develop up to eight weeks following bite. Save the tick for identification.

### *Insect Bites*

DO NOT cut the site of the bite to suck out venom. Lie the victim down and keep them calm. Maintain affected areas below the heart. Ice may be applied to the area of the bite, but make sure that there is not direct skin contact with the ice. Use a towel for insulation to prevent freezing the skin. DO NOT use a tourniquet or constricting band on the affected limb. Get the victim to medical attention by calling the emergency number for the ambulance as listed in Section 10.7.

# **APPENDIX C**

## **CURRICULUM VITAE**

## LISA ALVAREZ-COHEN

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### EDUCATION:

- Ph.D., Environmental Engineering and Science** April 1991  
*Stanford University, Palo Alto, CA*  
*Thesis Research: Cometabolic Biotransformation of Trichloroethylene and Chloroform by Methanotrophs: Experimental Studies and Modeling of Toxicity and Sorption Effects*
- M.S., Environmental Engineering and Science** June 1985  
*Stanford University, Palo Alto, CA*
- B.A., Engineering and Applied Science** June 1984  
*Harvard University, Cambridge, MA*

### EMPLOYMENT HISTORY:

- Assistant Professor, Environmental Engineering** 7/91 - present  
*Civil Engineering Dept., University of California, Berkeley, CA*  
Teaching duties include graduate and undergraduate courses on hazardous and industrial waste management, environmental microbiology, and introduction to environmental engineering. Research projects involve biotransformation of hazardous contaminants including complex hydrocarbon mixtures, liquid and gas-phase halogenated solvents, and polynuclear aromatics; thermally enhanced aquifer remediation; and methods for evaluating in situ bioremediation.
- Faculty Associate, Energy and Environment Division** 8/92 - present  
*Lawrence Berkeley Laboratory, Berkeley, CA*
- President's Post-Doctoral Research Fellow** 1/91 - 12/91  
*University of California, Berkeley, CA*
- Research Assistant** 1/86 - 12/90  
*Stanford University, Palo Alto, CA*
- Teaching Assistant** 9/88 - 3/89  
*Stanford University, Palo Alto, CA*
- Environmental Engineer** 6/85 - 9/85  
*Brown and Caldwell Consulting Engineers, Pleasant Hill, CA*
- Laboratory Assistant** 10/83 - 8/84  
*Harvard University, Atmospheric Chemistry Lab., Cambridge, MA*
- Research Intern** 6/83 - 9/83  
*Brookhaven National Laboratory, Upton, NY*

### PROFESSIONAL AFFILIATIONS:

Association of Environmental Engineering Professors, Water Environment Federation,  
American Society for Microbiology, American Chemical Society, Sigma Xi

## HONORS AND AWARDS:

- W. M. Keck Foundation Award for Engineering Teaching Excellence 1994
- National Science Foundation Young Investigator Award 1994
- Advisor to one undergraduate and two graduate student winners of 1994 Water Environment Federation Student Paper Competition and graduate winner of the 1994 American Society of Civil Engineers Student Essay Competition. 1994
- Voted "Most Entertaining Lecturer" by Berkeley Undergraduate Chapter of the American Society of Civil Engineers 1993
- Department of Energy Environmental Restoration and Waste Management Junior Faculty Award 1992-1994
- Association of Environmental Engineering Professors Doctoral Thesis Award 1992
- University of California President's Post-Doctoral Fellowship 1991
- Switzer Foundation Graduate Scholarship 1989-1991
- AWWA Larson Aquatic Research Support Scholarship 1989
- American Chemical Society Graduate Student Award in Environmental Chemistry 1989
- National Science Foundation Predoctoral Fellowship 1985-1988

## FUNDED RESEARCH:

- A Comparison of Product Toxicity from the Cometabolic Transformation of Halogenated Organics by Several Monooxygenase Cultures*, Department of Energy, 9/92-9/94.
- Thermally Enhanced Remediation of Subsurface Contamination* (with Kent Udell in Mechanical Engineering, Tad Patzek in Material Science) NIEHS, 4/92-3/96.
- Biotransformation of Gas-Phase Halogenated Solvents in Unsaturated Porous Media* (with James Hunt in Civil Engineering, Mary Firestone in Soil Science) NIEHS, 4/92-3/96.
- In Situ Reduction of Acid Rock Drainage* (with James Hunt in Civil Engineering, Fiona Doyle in Mineral Science) UC Toxics Substances Research, 8/94-7/95.
- Microbial Degradation of Hazardous Contaminant Mixtures* National Science Foundation, 9/94-8/99.
- Evaluating In Situ Bioremediation* Chevron Research and Technology Co., 10/94-11/96.
- Biological Transformation of Polynuclear Aromatic Hydrocarbons* Chevron Research and Technology Co., 10/94-2/97.
- Field Evaluation of Intrinsic Bioremediation at Alameda Naval Air Station*, U.S. Dept. of Navy, 10/95-2/97.

## PROFESSIONAL ACTIVITIES:

- Member of The Committee on USGS Water Resources Research, National Research Council/ National Academy of Sciences, Water Science and Technology Board (1993-1996).
- Member of The Committee on *In Situ* Bioremediation, National Research Council/ National Academy of Sciences, Water Science and Technology Board (1992-1993).
- Co-convener of Seminar Session on Innovations in Biological Treatment of Wastes for 1994 American Society for Microbiology Annual Meeting.
- Interviewer for Switzer Foundation Environmental Scholarships (1992).
- Peer Review for Archival Journals: *Environmental Science and Technology*, *Biodegradation*, *Water Research*, *Biotechnology and Bioengineering*, *Water Environment Research*, *Canadian Journal of Microbiology*, *Hazardous Waste and Hazardous Materials*
- Proposal Review for: National Science Foundation: Molecular and Cellular Biosciences, Bioengineering and Environmental Systems; Department of Defense Environmental Security Program; University of California Water Resources Center; Office of Solid Waste Research, University of Illinois at Urbana-Champaign.

## PATENT:

- #5,139,682 -- Zeolite Enhanced Organic Biotransformation, with P. L. McCarty.

## PUBLICATIONS:

- Chu, K. H., and L. Alvarez-Cohen. 1995. "TCE Degradation by Methane Oxidizing Cultures Grown with Various Nitrogen Sources", *Water Environment Research*, in press.
- Chang, H-L., and L. Alvarez-Cohen. 1995. "A Model for the Cometabolic Degradation of Chlorinated Organics" *Environmental Science and Technology*, 29(9):2357-2367.
- Chang, H-L., and L. Alvarez-Cohen. 1995. "Transformation Capacities of Chlorinated Organics by Mixed Cultures Enriched on Methane, Propane, Toluene or Phenol", *Biotechnology and Bioengineering*, 45:440-449.
- Aitken, M. D., P. E. Heck, L. Alvarez-Cohen, S. J. Grimberg, and W. T. Stringfellow. 1994. "Activated Sludge and Other Aerobic Suspended Culture Processes", *Water Environment Research*, 66:325-336.
- Alvarez-Cohen, L. 1993. "Engineering Challenges Associated with the Application Of *In Situ* Bioremediation" in *In Situ Bioremediation: When Does it Work?*, p. 136-152. National Academy Press, Washington DC.
- Committee on In Situ Bioremediation (LA-C committee member) 1993. *In Situ Bioremediation: When Does it Work?*, National Academy Press, Washington DC.
- Alvarez-Cohen, L., P. L. McCarty, and P. V. Roberts. 1993. "Sorption of Trichloroethylene onto a Zeolite Accompanied by Methanotrophic Biotransformation", *Environmental Science and Technology*, 27(10):2141-2148.
- Aitken, M. D., P. E. Heck, L. Alvarez-Cohen, S. J. Grimberg, and W. T. Stringfellow. 1993. "Activated Sludge", *Water Environment Research*, 65:324-336.
- Alvarez-Cohen, L. 1993. "Application of Methanotrophic Oxidations for the Bioremediation of Chlorinated Organics" in *Microbial Growth on C<sub>1</sub> Compounds*, p. 337-350. J. C. Murrell and D. P Kelly eds., Intercept Ltd., Hampshire England.
- Alvarez-Cohen, L., P. L. McCarty, E. Boulygina, R. S. Hanson, G. A. Brusseau, and H. C. Tsien. 1992. "Characterization of a Methane-Utilizing Bacterium from a Bacterial Consortium that Rapidly Degrades Trichloroethylene and Chloroform", *Applied and Environmental Microbiology*, 58(6):1886-1893.
- Alvarez-Cohen, L., and P. L. McCarty. 1991. "Effects of Toxicity, Aeration, and Reductant Supply on Trichloroethylene Transformation by a Mixed Methanotrophic Culture", *Applied and Environmental Microbiology*, 57(1):228-235.
- Alvarez-Cohen, L., and P. L. McCarty. 1991. "A Cometabolic Biotransformation Model for Halogenated Aliphatic Compounds Exhibiting Product Toxicity", *Environmental Science and Technology*, 25(8):1381-1387.
- Alvarez-Cohen, L., and P. L. McCarty. 1991. "Two-Stage Dispersed-Growth Treatment of Halogenated Aliphatic Compounds by Cometabolism", *Environmental Science and Technology*, 25(8):1387-1393.

Alvarez-Cohen, L., and P. L. McCarty. 1991. "Product Toxicity and Cometabolic Modeling of Chloroform and Trichloroethylene Transformation by Methanotrophic Resting Cells", *Applied and Environmental Microbiology*, 57(4):1031-1037.

Criddle, C. S., L. M. Alvarez, and P. L. McCarty. 1991. "Microbiological Processes in Porous Media", in *Transport Processes in Porous Media*, p. 639-691. J. Bear and M.Y. Corapcioglu, eds., Kluwer Academic Publishers, 1991.

#### PRESENTATIONS, PROCEEDINGS, REPORTS:

Chang, H-L., and L. Alvarez-Cohen. 1995. "Modeling Cometabolic Biodegradation of Chlorinated Organics" American Chemical Society Annual Meeting, Atlanta Georgia.

Alvarez-Cohen, L. 1995. "Biological Destruction of Chlorinated Organics", Annual Meeting of American Society for Microbiology, Washington DC. *Invited Lecture*.

Alvarez-Cohen, L. 1995. "Aerobic Degradation of Hazardous Contaminants", University of North Carolina, Chapel Hill. *Invited Lecture*.

Chu, K. H., J. Vernalia, and L. Alvarez-Cohen. 1995. "Evaluation Of Nitrogen Sources For Bioremediation Of Trichloroethylene In Unsaturated Porous Media" Proceedings of International Symposium on In Situ and On-Site Bioreclamation, San Diego, CA.

Deeb, R., and L. Alvarez-Cohen. 1994. "Thermally Enhanced Bioremediation of a Gasoline Contaminated Aquifer Using Toluene Oxidizing Bacteria" Annual Meeting of the American Society of Civil Engineers, Boulder Colorado. First Place Winner ASCE Graduate Student Essay Competition.

Avila, G., and L. Alvarez-Cohen. 1994. "Biodegradation of Trichloroethylene in a Two-Stage Reactor" Proc. Water Environment Federation Annual Meeting, Chicago Illinois. First Place Winner WEF Undergraduate Student Paper Competition.

Chang, H-L., and L. Alvarez-Cohen. 1994. "Modeling Product Toxicity and Competitive Inhibition of Cometabolic Degradation of Chlorinated Organics" Proc. Water Environment Federation Annual Meeting, Chicago Illinois. Third Place Winner Ph.D. category WEF Student Paper Competition.

Deeb, R., and L. Alvarez-Cohen. 1994. "Biodegradation of BTEX Compounds: Temperature and Mixture Effects" Proc. Water Environment Federation Annual Meeting, Chicago Illinois. Second Place Winner M.S. category WEF Student Paper Competition.

Chang, H-L., and L. Alvarez-Cohen. 1994. "Biological Treatment of Chlorinated Organics by Four Aerobic Cultures" Annual Meeting of the American Institute of Chemical Engineers, San Francisco, CA.

Chu, K. H., and L. Alvarez-Cohen. 1994. "The Effects of Nitrogen Sources on TCE Degradation, Energy Storage, and Growth of Methane Oxidizing Bacteria" Annual Meeting of the American Institute of Chemical Engineers, San Francisco, CA.

Alvarez-Cohen, L. 1993. "Biological Aspects of *In Situ* Remediation" Proceedings of the Nineteenth Biennial Groundwater Conference, Sacramento, CA. *Invited Lecture and Paper*

Alvarez-Cohen, L. 1992. "Applications of Bioremediation Processes Involving Methanotrophs" 7th International Symposium on Microbial Growth on C<sub>1</sub>-Compounds, Warwick, England. *Invited Lecture and Paper*

Alvarez-Cohen, L. 1992. "Basic Principles of Bioremediation Processes with Emphasis on In-situ Applications", International Solar Energy Conference, American Society of Mechanical Engineering, Maui, Hawaii. *Invited Lecture*

Alvarez-Cohen, L., and P. L. McCarty, 1991. "Optimization of a Two-Stage Reactor Design for the Cometabolic Transformation of Halogenated Organics Alone and in Mixtures", Water Pollution Control Federation Annual Conference, Toronto, Canada.

Alvarez-Cohen, L., and P.L. McCarty, 1991. "Product toxicity of Chloroform and Trichloroethylene Transformation by Methanotrophic Resting Cells", Annual Meeting of American Society for Microbiology, Dallas, Texas.

Tsien, H. C., L. Alvarez-Cohen, P. L. McCarty, and R. S. Hanson. 1991. "Use of Soluble Methane Monooxygenase Component B Gene Probe for the Detection of Trichloroethylene Degrading Methanotrophs", Annual Meeting of American Society for Microbiology, Dallas, Texas.

Alvarez, L. M., and P. L. McCarty, 1989. "The Cometabolic Transformation of Trichloroethylene by a Methanotrophic Consortia", Annual Meeting of American Society for Microbiology, New Orleans, La.

Alvarez, L.M., P.L. McCarty and P.V. Roberts, 1989. "Sorption and Biotransformation in the Presence of Aquifer and Synthetic Solids", International Symposium on Processes Governing Movement and Fate of Contaminants in the Subsurface Environment, IAWPRC Stanford, CA.

Alvarez, L.M., P.L. McCarty and P.V. Roberts, 1989. "The Effects of Sorption on the Biotransformation Rate of TCE by Methanotrophs --Experiments with a Synthetic Zeolite", Water Pollution Control Federation Annual Conference, San Francisco, CA.

Lipfert, F. W., L. R. Dupuis and L. M. Alvarez. 1984. "Urban and Local Source Effects on Precipitation Chemistry", Brookhaven National Laboratory Technical Report.

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## **EDUCATION**

**Ph.D. in Geology** (Harvard University, March 1990)

Thesis: The relation of propylitic alteration and O<sup>18</sup>-depletion patterns to Ag/Au vein deposits in the Tayoltita mining district of Durango, Mexico.

**A.M. in Geology** (Dartmouth College, June 1982)

Thesis: Variations within the layering of the Skaergaard intrusion, East Greenland.

**B.A. in Geology** (Pomona College, May 1979)

Thesis: The petrology of a portion of the San Dimas Experimental Forest, San Gabriel Mountains, southern California.

## **PROFESSIONAL EXPERIENCE**

**Geological Scientist** (Lawrence Berkeley National Laboratory; 5/95-present): Monitoring subsurface bacterial activity with stable isotopes, paleoclimatic patterns in California and stable isotope systematics of clay minerals.

**Geologist Postdoctoral Fellow** (Lawrence Berkeley National Laboratory; 5/92- 5/95): Stable isotope evidence for subsurface bacterial activity, groundwater hydrology and water-rock interaction in geothermal systems.

**Research Associate** (Dartmouth College; 10/90-5/92): Research on fluid-rock interaction in a variety of geologic environments.

**Consulting geologist** (Meridian Gold Company; 4/88-12/88, 3/90-5/90): Evaluation of gold property in the Mother Lode of California.

**Teaching Fellow** (Harvard University; 1984-1987): Introductory mineralogy and summer field camp.

**Exploration Geologist** (Anaconda Minerals Company; 6/80-10/80, 3/81-7/81, 6/82-8/83): Evaluation of properties in the United States and Mexico.

**Teaching Fellow** (Dartmouth College; 1979-1982): Optical mineralogy, igneous and metamorphic petrology, structural geology and field methods.

**Summer Geologist** (Noranda Exploration, Inc.; 5/79-8/79): Reconnaissance mapping of volcanic rocks in Arizona to evaluate mineral potential.

**Professional Affiliations:** Geological Society of America, Mineralogic Society of America, American Geophysical Union, Society of Economic Geologists, Clay Minerals Society.

## PUBLICATIONS

### Journal Articles

- Chamberlain, C.P., and Conrad, M.E., 1991, The relative permeabilities of quartzites and schists at mid-crustal levels: *Geophys. Res. Lett.*, v. 18, p. 959-962.
- Chamberlain, C.P., and Conrad, M.E., 1991, Oxygen isotope zoning in garnet: *Science*, v. 254, p. 403-406.
- Chamberlain, C.P., and Conrad, M.E., 1993, Oxygen-isotope zoning in garnet: A record of volatile transport: *Geochim. et Cosmochim. Acta*, v. 57, p. 2613-2630.
- Conrad, M.E., and Naslund, H.R., 1989, Modally-graded rhythmic layering in the Skaergaard intrusion: *J. Petr.*, v. 30, p. 251-269.
- Conrad, M.E., Petersen, U., and O'Neil, J.R., 1992, Evolution of an Au-Ag producing hydrothermal system: The Tayoltita mine, Durango, Mexico: *Econ. Geol.*, v. 87, p. 1451-1474.
- Conrad, M.E., and Chamberlain, C.P., 1992, Laser-based, *in situ* measurements of fine-scale variations in the  $\delta^{18}\text{O}$  values of hydrothermal quartz: *Geology*, v. 20, p. 812-816.
- Conrad, M.E., O'Neil, J.R., and Petersen, U., 1995, The relation between widespread  $^{18}\text{O}$ -depletion patterns and precious metal mineralization in the Tayoltita mine of Durango, Mexico: *Econ. Geol.*, v. 90, p. 322-342.
- Macfarlane, A.W., Prol-Ledesma, R., and Conrad, M.E., 1994, Isotope and fluid inclusion studies of the geological and hydrothermal evolution of the Hualgayoc district, northern Peru: *International Geology Review*, v. 36, p. 645-677.
- Ingram, B.L., Conrad, M.E., and Ingle, J.C., in press, Stable isotope variations in estuarine waters: Relation to salinity and freshwater inflow: *Geochim. et Cosmochim. Acta*.
- Thomas, D.M., Paillet, F.L., and Conrad, M.E., in review, Hydrogeology of the HSDP borehole KP-1, Part II: Ground-Water geochemistry and regional flow patterns: *Jour. of Geophys. Research*.

### Recent Abstracts

- Conrad, M.E., and Thomas, D.M., 1995, Fluid mixing, boiling and water-rock interaction in the East Rift Zone of Kilauea volcano, Hawaii: *Geol. Soc. Am., Abst. with Prog.*, v. 25, no. 6, p. A203.
- Conrad, M.E., Daley, P.F., Fischer, M.F., Buchanan, B.B., and Leighton, T., 1995, Carbon isotope evidence for subsurface bacterial activity at the Naval Air Station, Alameda, California. *EOS, Trans., Am. Geophys. Union*, v. 76, no. 17, p. S119.
- Conrad, M.E., Karasaki, K., and Freifeld, B., 1994, The use of deuterium-enriched water as a tracer for hydrologic tests: Abstracts of the Eighth International Conference on Geochronology, Cosmochronology and Isotope Geology, U.S. Geological Survey Circular 1107, p. 66.
- Conrad, M.E., Leighton, T., and Buchanan, B.B., 1994, Carbon isotope fractionation by *Bacillus subtilis*. *Geological Society of America Abstracts with Programs*, v. 26, p. A-510.

Curriculum vitae: Paul Freeman Daley, B.S., M.S., Ph.D.

Home Address:  
293 Casper Place  
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Business Address:  
University of California  
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Born: August 7, 1952  
Married: to Debra T. Santa Maria, 1986  
Children: Jozefa Maria Daley, age 9 years  
Michael Elliott Daley, age 6 years

Education:

Univ. California at Davis B.S. Environmental Toxicology 1974  
Univ. California at Davis M.S. Entomology 1977  
Univ. of California at Berkeley Ph.D. Entomology 1981

Postgraduate Work Experience:

Lawrence Livermore National Laboratory; Environmental Scientist, Environmental Restoration Division. November 1988-Present.

Innovative Technologies Team Leader for the 'Site 300' Groundwater Remediation Program; design and operational analysis of soil and ground water remediation systems; automated process monitoring systems. Development of: indwelling fiber optic sensors for volatile chlorinated hydrocarbons; synthetic atmosphere plant growth systems for experimental animal diet production; methods for characterizing microbial breakdown of fuels by fingerprinting carbon isotope composition of soil CO<sub>2</sub>.

Lawrence Livermore National Laboratory; Postdoctoral Fellow, Environmental Scientist, Environmental Sciences Division. April 1984-October 1988.

Field studies of adaptation of alfalfa and Ponderosa pine to high CO<sub>2</sub> atmospheres. Quantitative video imaging of chlorophyll fluorescence for 'visualization' of photosynthesis. Supervisor: Dr. Joseph Shinn

Postdoctoral Research Fellow; Université Laval, Québec, P.Q., Canada. Dec 1981-March 1984. Analysis of physiological and economic impact of leafmining Diptera on photosynthesis and growth of alfalfa, modification of host wound healing responses by leafminers, impacts of defoliators and aphids on photosynthesis in cereals. Supervisor: Dr. Jeremy N. McNeil

Graduate Research Assistant; U.C. Berkeley. July 1977-June 1981. Physiological studies of alfalfa weevil damage in alfalfa; crop modelling. Supervisor: Dr. Andrew P. Gutierrez.

## Publications:

Angel, S.M., P.F. Daley, and T. Kulp 1987. *In situ* detection of organic chemicals. Lawrence Livermore National Laboratory, Livermore, CA, UCID-21206-43.

Angel, S.M., P.F. Daley, and T. Kulp 1987. Optical chemical sensors for environmental monitoring. in: *Proceedings of the Symposium on Chemical Sensors (Electrochemical Society, Inc.)*, 87: 484.

Angel, S.M., P.F. Daley, K.C. Langry, R. Albert, T.J. Kulp, and I. Camins 1987. Quarterly Technical Report (February 1, 1987 to April 30, 1987), *The Feasibility of Using Fiber Optics for Monitoring Groundwater Contaminants VI. Mechanistic Evaluation of the Fujiwara Reaction for Detection of Organic Chlorides*, Lawrence Livermore National Laboratory, Livermore, CA, UCID-19774, Vol. VI.

Angel, S.M., K.C. Langry, T.J. Kulp, P.F. Daley, and D.J. Bishop 1988. *In situ* detection of organic molecules. Lawrence Livermore National Laboratory, Livermore, CA, UCRL-21081.

Angel, S.M., P.F. Daley, and F.P. Milanovich. 1991. A fiber-optic sensor for monitoring TCE. in: *Energy and Technology Review*, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-JC-52000-91-7/8, p. 54.

Blystone, P.G., M.D. Johnson, W.R. Haag, and P.F. Daley. 1991. Advanced ultraviolet flashlamps for the destruction of organic contaminants in air. *Proc. ACS Natl. Mtg.* Oct 3, 1991, Atlanta GA.

Daley, P.F. 1992. Automated monitoring of a soil remediation system. *Scientific Computing and Automation.* 8: 6: 23-28.

Daley, P.F. 1995. Chlorophyll fluorescence analysis and imaging in plant stress and disease. *Can. J. Plant Path.* 17: 167-173.

Daley, P.F., K. Raschke, J.T. Ball, and J.A. Berry. 1989. Topography of photosynthetic activity of leaves obtained from video images of chlorophyll fluorescence. *Plant Physiology* 90: 1233-1238.

Daley, P.F., J.T. Ball, J.A. Berry, J. Patzke, and K. Raschke. 1990. Visualizing photosynthesis through processing of chlorophyll fluorescence images. *Biomedical Image Processing*, Alan C. Bovik, William E. Higgins, Eds., *Proc. SPIE* 1245: 243-249.

Daley, P.F., B.W. Colston, Jr., S.B. Brown, K. Langry and F.P. Milanovich. 1992. Fiber optic sensor for continuous monitoring of chlorinated solvents in the vadose zone and in groundwater: Field test results. *Proc. SPIE National Mtg.*, September 1991, Austin, TX. In Press.

- Daley, P.F., C.F. Cloutier, and J.N. McNeil. 1984. A canopy porometer for photosynthesis studies in field crops. *Can. J. Bot.* 62:290-295.
- Daley, P.F. and J.N. McNeil. 1987. Photosynthesis and dry matter partitioning in alfalfa attacked by the alfalfa blotch leafminer (*Agromyza frontella* (Rond.)). *Can. J. Plant Sci.* 67: 433-443.
- Daley, P.F., K.A. Surano, and J.H. Shinn. 1988. Long-term exposure of alfalfa (*Medicago sativa* L.) to elevated atmospheric carbon dioxide. I. Photosynthesis, yield, and growth analysis. Lawrence Livermore National Laboratory, Livermore, CA, UCRL-98576.
- Fried, J.S., K.A. Surano, P.F. Daley, J.H. Shinn, and P. Anderson. 1986. Biomass production and nutrient responses of Ponderosa pine to long-term elevated CO<sub>2</sub> concentrations. *Proc. Ninth N. Am. For. Biol. Workshop.* Okla. State Univ. Press, Stillwater, pp 11-18.
- Houpis, J.L.J., K.A. Surano, P.F. Daley, and J.H. Shinn. 1986. Growth and morphology of *Pinus ponderosa* seedlings exposed to long-term elevated atmospheric carbon dioxide concentrations. *Proc. Ninth N. Am. For. Biol. Workshop.* Okla. State Univ. Press, Stillwater, pp 19-26.
- Langry, K., S.M. Angel, and P.F. Daley. 1988. Problems confronting the design and implementation of invasive fiber optic sensors. SPIE (Society of Photo-Optical Instrumentation Engineers) Proceedings, 906.
- Milanovich, F., P. Daley, D. Garvis, and S.M. Klainer. 1986. The feasibility of using fiber optics for monitoring groundwater contaminants. IV. Laboratory and preliminary field test results using organic chloride FOCS and dedicated portable instrumentation. Lawrence Livermore National Laboratory, UCID-19774.
- Milanovich, F., P. Daley, S.M. Klainer, and L. Eccles. 1986. Remote detection of organochlorides with a fiber optic based sensor. II. A dedicated portable fluorimeter. *Analytical Instruments* 15: 347-358.
- Milanovich, F.P., P.F. Daley, S.M. Angel, K.C. Langry, W. Colston, Jr., and S.B. Brown. 1991. A fiber-optic sensor for the continuous monitoring of chlorinated hydrocarbons. in: Environmental Technology Program Annual Report, FY 90, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-LR-105199, pp. 16-20.
- Milanovich, F.P., P.F. Daley, K. Langry, B.W. Colston Jr., S.B. Brown, and S.M. Angel. 1991. A fiberoptic sensor for the continuous monitoring of chlorinated hydrocarbons. *Proc. Second International Conference for Field Screening Methods*

for Hazardous Wastes and Toxic Chemicals (EPA) Feb. 12-14 1991, Las Vegas, NV. pp 43-48 (awarded "Outstanding Technical Contribution").

Milanovich, F.P., P.F. Daley, K.C. Langry, W.W. Colston, S.B. Brown and L. Burgess. 1992. Redesigned fiber-optic sensor for continuous monitoring of chlorinated solvents in the vadose zone and in groundwater. in: Environmental Technology Program Annual Report FY 91, J.L. Yow, Program Leader, Lawrence Livermore National Laboratory, Livermore, CA, UCRL-LR-105199-91, pp. 23-25.

Osmond, C.B., J.A. Berry, S. Balachandran, P.F. Daley, and R.C. Hodgson. 1990. Potential consequences of virus infection for shade-sun acclimation in leaves. *Botanica Acta*. 103:226-229.

Surano, K.A. P.F. Daley, J.L.J. Houppis, J.H. Shinn, J.A. Helms, R.J. Palassou, and M.P. Costella. 1986. Effects of long-term elevated CO<sub>2</sub> concentrations on *Pinus ponderosa*. *Tree Physiology* 2: 243-259.

Hoi-Ying N. Holman  
Earth Sciences Division  
Lawrence Berkeley National Laboratory

## EXPERIENCE

11/1994 - present: Staff scientist, Earth Sciences Division, Lawrence Berkeley National Laboratory

Investigating factors that control the biodegradation of organic hydrocarbons in both the vadose and saturated zones.

Developing an experimental protocol to examine factors that affect the bioavailability of ingested organic hydrocarbons from soil to human.

Successfully trained new chemists to take over the certified Environmental Measurement Laboratory and to analyze water and soil samples from sites contaminated with volatile organic compounds (VOCs).

1/1989 - 11/1994: Staff scientist, Earth Sciences Division, Lawrence Berkeley National Laboratory

Investigated microbial transformation of petroleum hydrocarbons in vadose zone.

Conducted theoretical and experimental studies on the dissolution and transport of volatile NAPL compounds in transient subsurface environment.

Established and managed a certified Environmental Measurement Laboratory to analyze water and soil samples from contaminated sites.

Supervised and managed the organic chemistry laboratory

Supervised chemists to perform analytical works in the laboratory.

Supervised field sampling teams to obtain VOC samples that meet EPA's requirements.

1/1986 - 1/1989: Postdoctoral fellow, Earth Sciences Division, Lawrence Berkeley Laboratory

Designed experiments to investigate the dissolution of NAPLs in subsurface environment.

Developed mathematical models to predict the dissolution of NAPLs in groundwater.

Applied numerical models to study the migration of Radon in soil.

9/1981 - 12/1985 : Research assistant, Division of Sanitary and Environmental Engineering, UC Berkeley

Performed theoretical and experimental studies of particle coagulation in an aquatic environment, which involved (1) the design and construction of a microcomputer-controlled laser system to measure the rate of particle coagulation in fluid flow, and (2) development of software/hardware to automate data collection and signal processing.

## EDUCATION

Ph.D. Civil Engineering, University of California, Berkeley, CA, 1986

Major : Sanitary and Environmental Engineering

Minors: (1) Chemistry and Chemical Engineering, (2) Statistics

M.S. Atmospheric Sciences, San Jose State University, CA, 1980

Major : Air Pollution

Minor : Mathematics

B.S. Earth Sciences, Chinese University of Hong Kong, 1978

## PUBLICATION ACTIVITIES

Holman, H.-Y. N. and I. Javandel, 1996. Dissolution potential of SWSCs from a LNAPL pool.

- Accepted for publication, Journal of water resources research.

Holman, H.-Y., and Y.W. Tsang, 1995. Effects of soil moisture on biodegradation of petroleum hydrocarbons. In In situ aeration: air sparging, bioventing and related remediation processes, 323-332, Battelle Press, Richland.

Holman, H.-Y., Tsang, Y.W., and V.A. Wolff, 1995. Effect of moisture on mineralization of petroleum hydrocarbons in silt loam, Technical Report LBNL#-36993, Lawrence Berkeley National Laboratory, Berkeley, CA.

Holman, H.-Y. N. , Javandel, I., and G. Moridis, 1992. Effects of water table fluctuations on the development of an aqueous hydrocarbon plume: A numerical and experimental study. Technical Report LBNL#-32777, Lawrence Berkeley National Laboratory, Berkeley, CA.

Javandel, I., Falta, R.W., and H.-Y. Holman, 1990. Recent developments in transport and fate of nonaqueous phase liquids in the subsurface environment, Iranian Journal of Science and Technology, 14(2/3): 269-287.

Narasimhan, T.N., Tsang, Y.W. and H.-Y. Holman, 1990. On the potential importance of transient air flow in advective radon entry into buildings, Geophysical Research Letters, 17(6): 821-824.

## CURRICULUM VITAE

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### Education:

B.S. in Civil and Environmental Engineering, University of California, Irvine, 1972  
M.S. in Environmental Engineering, Stanford University, 1973  
Ph.D. in Environmental Engineering Science, California Institute of Technology, 1980

### Employment:

6/73-9/75 Environmental Engineer, Hydrocomp Inc., Palo Alto, California.  
7/80-6/87 Assistant Professor, Department of Civil Engineering, UC Berkeley  
7/87-6/94 Associate Professor, Department of Civil Engineering, UC Berkeley  
7/94-present Professor, Department of Civil and Environmental Engineering, UC Berkeley  
7/89-6/92 Vice Chair, Academic Affairs, Department of Civil Engineering, U.C. Berkeley

### Recent Professional Activities:

Registered Civil Engineer in California  
Department of Energy Q Clearance  
Diplomate of the American Academy of Environmental Engineering  
Science Advisory Committee, Environmental Protection Agency Great Lakes - Mid-Atlantic Hazardous Substance Research Center, 1991-1995  
National Water Research Institute, Research Advisory Board, 1993-present  
National Research Council, Marine Board Committee on Contaminated Marine Sediments, 1993-1996  
University of California Water Resources Center, Coordinating Board, 1994-1997

### Recent University Activities:

Group Leader of Environmental Engineering Program in the Department of Civil and Environmental Engineering, 1993-present  
Elected member of the Berkeley Divisional Council, University of California Academic Senate, 1995-1997

### Honors:

1991 Editors' Citation for Excellence in Refereeing - Water Resources Research, American Geophysical Union.  
1991 Outstanding Doctoral Thesis Award to advisee Jil T. Geller, Association of Environmental Engineering Professors.

Current Research Projects:

- Transport and Transformation of Volatile Organic Solvents in Unsaturated Soils, National Institute of Environmental Health Sciences 1992-1996 (with M. K. Firestone and L. Alvarez-Cohen).
- Aggregation of Petroleum Hydrocarbons with Particles in Urban Runoff and Estuarine Waters, Interagency Ecological Study Program, San Francisco Bay-Delta, 1993-1996.
- Subsurface Noble Gas Transport at the Nevada Test Site, Los Alamos National Laboratory, 1993-1996.
- In-Situ Reduction of Acid Rock Drainage, University of California Toxic Substances Teaching and Research Program, 1994-1996 (with L. Alvarez-Cohen and F. M. Doyle).
- Microbial Degradation of Petroleum Hydrocarbons in Unsaturated Soils: The Mechanistic Importance of Water Potential and the Exopolymer Matrix, U. S. Environmental Protection Agency, 1994-1996 (with M. Firestone).
- Additional Sampling and Analysis: Sediment Characterization and Treatability Study at Naval Air Station Alameda, California, U. S. Navy, 1996-1997 (with N. Sitar and scientists at LBNL and LLNL)

Patent:

- K. S. Udell, N. Sitar, J. R. Hunt, and L. D. Stewart (1991) Process for in situ decontamination of subsurface soil and groundwater, U. S. Patent No. 5,018,576.

Refereed Publications:

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January 18, 1996

1996 Curriculum Vitae  
Paula Krauter

**Present Position:**

Environmental Scientist  
Lawrence Livermore National Laboratory  
Environmental Restoration Division, L-619  
7000 East Ave.  
Livermore, CA 94550  
(510) 422-0429

**Citizenship:** USA

**Professional Memberships:**

American Society for Microbiology  
Society of Industrial Microbiologist  
Genetic and Environmental Toxicology Association  
Society of Toxicology of Northern California

**Previous Research Experience:**

I have been an LLNL employee since 1987. In the Environmental Sciences Division I studied cytogenetic anomalies in blood cells of *Rana catesbeiana* as a new model of aquatic organisms for studying in vivo metabolism and genotoxicity of environmental contaminants. In 1990 I joined the Environmental Restoration Division to assess subsurface microorganisms. During the past five years I have quantified the microorganisms in water and sediment, interpreted microbial characterization data, and study the influences of man-made environmental changes on the indigenous microorganisms.

**Distinctions:**

Recipient of 1994 Laboratory Directed Research and Development grant (\$245,000).  
Graduated Cum Laud (California State University at Hayward, 1986).

**General Research Interests:**

Microbiology, bioremediation, biodiversity, ground water remediation

**Patent:**

Water Treatment Process for Heavy Metal Removal Using *Saccharomyces Cerevisiae*. Inventors: Paula Krauter and Gordon Krauter. In progress, LLNL Docket No. IL-9272.

**Publications:**

Krauter P. W., D. MacQueen and R. Martinelli (1996) Effect of subsurface thermal remediation on the microbial populations at a gasoline spill site. UCRL-JC-115979, submitted to J. Microbial Ecology.

Krauter P., MacQueen D., Horn J., Bishop D. (1996) Effect of Subsurface Electrical Heating and Steam Injection on the Indigenous Microbial Community. UCRL -JC-122299 in submission to Spectrum 1996.

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Mehlhorn R. J. , I. Fry and P. Krauter (1995) Analysis of the reduction of hexavalent chromium by *Saccharomyces cerevisiae*. UCRL-JC-119558.

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Krauter P.W., Anderson S.L., Harrison F.L. (1987) Radiation-induced micronuclei in peripheral erythrocytes of *Rana Catesbeiana*: an aquatic animal model for in vivo genotoxicity studies. Environ Mol Mutagen 10:285-296.

Krauter P., F. Harrison, S. Anderson, and J. Knezovich (1986) Micronuclei Frequency in Peripheral Erythrocytes of Tadpoles Exposed to Direct- and Indirect-Acting Mutagens. In the proceedings of Genet. Environ. Toxicol. Assoc. North. California, Menlo Park, December 4, 1986. UCRL-95631.

Knezovich J. P., P.W. Krauter, M.P. Lawton, and F.L. Harrison, "The Metabolism and Genotoxicity of Aromatic Amines in Aquatic Organisms," Department of Energy Contractors Meeting, Monterey, CA June 24-26, 1987. UCRL-97122.

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Krauter P., F. Harrison, S. Anderson, and J. Knezovich (1986) Micronuclei Frequency in Peripheral Erythrocytes of Tadpoles Exposed to Direct- and Indirect-Acting Mutagens," in the 1986 GETA Bulletin.

**Education:**

Ms.Sc. California State University Hayward, 1986. Biology, Physiological Biology Option, cum laud.

B.Sc. University of California, Davis, 1976. Fermentation Science.

Currently enrolled in San Jose State University Secondary Teaching Credential Program (1996).

## **MARY LUCAS McDONALD**

### **Geologist**

#### **Education**

B.A., Geology, Carleton College, 1981.

#### **Professional Registration**

Registered Geologist, California, 1992, N o. 5506.

#### **Professional Training**

OSHA 40-hour Health and Safety

#### **Experience**

Ms. McDonald is the E2 project manager for the underground storage tank removal and building demolition at a MacArthur Station site for the BART Hazardous Materials Programs; and the tank removal at the Balboa Park maintenance yard and the drum disposal at the Kirkland bus yard for the San Francisco Municipal Railway.

Previously with Harding Lawson Associates, Ms. McDonald was project manager for the CERCLA investigation of Hunter's Point Annex in San Francisco. Technical tasks included the assessment of soil and groundwater at 16 identified sites within the facility; preliminary assessments of 44 sites and implementation of site inspections at two; planning of removal actions at three sites including air modeling to assess potential health risks from two of the removal actions; planning for sediment, water, and tissue sampling to assess the potential impact of the facility on San Francisco Bay; and planning of an environmental risk assessment to assess the potential risk to local biota. Responsible for primary client contact, regulatory agency contact, subcontractor overview, development and maintenance of project budget and schedule, review of technical documents, and budget and contract negotiations.

Earlier with Brown and Caldwell Consultants, Ms. McDonald was initially responsible for conducting field work and report preparation. Promoted to project manager, was responsible for project budgeting and scheduling, staff supervision, technical review, regulatory agency and client contact, and business development activities. Planned and implemented projects in accordance with Title 22, Title 23, and underground storage tank regulations. Trained in site safety practices in accordance with OSHA regulations. Experienced in field quality assurance/quality control methods. Assessed soil and groundwater contamination by petroleum products, organic solvents, pesticides, and metals at numerous sites and implemented site remediations. Projects included:

- Project hydrogeologist for the site investigation and remediation of a chemical blending and packaging plant in Santa Clara Valley. Responsible for development of the technical

approach to the investigation and remediation of site soil and groundwater containing primarily halogenated hydrocarbons. Implemented soil vapor surveys and groundwater monitoring well installation to investigate the distribution of organics in the on and off site soil and groundwater. Negotiated with the San Francisco Bay Regional Water Quality Control Board on approach to evaluate potential contribution from off site sources and on the on site groundwater remediation. Provided oversight of the implementation of a pilot scale soil venting system to remediate site soils near the above ground tank farm. Submitted application for permit to operate the full scale soil remediation.

- Project manager for the investigation and remediation of soil containing DDT at an airport in the Central Valley. Excavation and class I disposal, capping, and fixation were evaluated.
- Project geologist for the site investigation at a Los Angeles refinery for a major oil company. Developed the technical approach for the soil and groundwater quality investigation and conducted the field work. Prepared the final report presenting the methods and results of the investigation. Developed the technical approach to the investigation of a previous waste disposal site at the refinery and provided oversight for its implementation.
- Office coordinator for site investigations and remediations of underground storage tanks at 17 service stations in the San Francisco Bay Area for a major oil company. Responsible for project scheduling, development and review of cost estimates, and review of work products for consistency and technical quality. Acted as the primary client contact for scheduling and development of new work.
- Project manager for underground storage tank project at numerous service stations. Developed the technical approach and provided oversight for tank removal, site investigations, and remediation. Implemented a groundwater remediation and evaluated remedial options for the soil.
- Project manager for the soil and groundwater remediation at a former gasoline service station being developed as a motel in Santa Cruz, California. Developed groundwater treatment system for groundwater produced during normal dewatering activities.
- Project manager of a preliminary environmental assessment of 10 sand and gravel operations and one golf course in northern California. Evaluated the presence of hazardous materials and/or wastes and their potential environmental affects at each site. Reviewed property ownership records and aerial photographs to identify indications of past use, storage, or disposal of hazardous materials or wastes. Reviewed ongoing investigations at nearby sites to identify whether they could impact the sites being assessed.

# **APPENDIX D**

## **RESPONSE TO COMMENTS**



## UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

REGION 4

75 Hawthorne Street  
San Francisco, CA 94105-3901**MEMORANDUM****SUBJECT:** Review of Draft Treatability Study Work Plan, Intrinsic Bioremediation of Sites 3 and 13, NAS Alameda.**FROM:** Ned Black, Ph.D. JNPB  
Ecologist, Technical Support Section**TO:** James Ricks, Barbara Smith  
Remedial Project Managers**DATE:** 5 April 1996

This Work Plan describes techniques for a proposed assessment of intrinsic biodegradation of petroleum hydrocarbons in the subsurface at Sites 3 and 13, NAS Alameda. In general, I strongly support the research described in this Work Plan. The BEREC researchers have designed a program that is both technically innovative and straightforward. The treatability study should provide a valuable demonstration of the potential of intrinsic bioremediation.

As an overall comment, it would be better if the researchers were more rigorous about their literature citations the first time an argument or point is made. Two specific examples are:

1. The size of the protozoan population is referred to several times in the main text as an indicator of *in situ* bacterial degradative activity, yet the reference in support of this claim (Madsen *et al.*, 1991) is not mentioned until Appendix A in the SOP for Microbial Enrichment. Furthermore, the full citation for Madsen *et al.* (1991) does not appear until the SOP for Direct Epifluorescent Microscopy.
2. The background discussion text for the biological significance of measuring radio- and stable isotopes was not adequately referenced. Although neither of these examples must be addressed in a revision of this Work Plan, the BEREC investigators should take care to completely reference and justify all arguments in any future reports.

With regard to the use of redox (viability) dyes for studying the size and composition of the microbial community, the BEREC researchers should at least consider the alternative of using the techniques for analysis of fatty acid biomarkers developed by D.C. White and coworkers (e.g., Tunlid *et al.*, 1989, AEM 55:1368; Guckert *et al.*, 1985, FEMS Microbiol. Ecol. 31:147.). If the staining and microscopy techniques are sufficient to characterize the subsurface community, yet are superior for reasons such as cost or simplicity, the Work Plan should address this specifically.

**RESPONSE TO COMMENTS ON  
FEBRUARY 7, 1996 TREATABILITY STUDY WORK PLAN  
INTRINSIC BIOREMEDIATION  
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY**

This document presents the response to comments received from the United States Environmental Protection Agency (U.S. EPA). UCB's responses to the review comments are provided below. A copy of the U.S. EPA review is enclosed for convenience..

Thank you for a thoughtful, science-based review of our Work Plan.

*Point 1:* We had purposely left out many of the scientific citations from the body of the Work Plan since it had been requested that the writing target a wide variety of audiences, including those who would have little if any access to the literature. However, the references specified by the reviewer have been added to the text and we assure the reviewer and the Navy that all reports from this project will include thorough references and citations.

*Point 2:* Thank you for the suggestion of using the FA biomarker analysis developed by D.C. White as part of our project. We did consider this analysis and rejected it for the following reasons: Whereas the FAME analysis is capable of supplying information about the characterization of microbial populations within the subsurface community, it is not capable of supplying quantitative information about the numbers of total cells or the amount of cellular activity in subsurface materials. In addition, the issue of sample preservation during transportation would have had to be addressed in the use of this analysis since the laboratories that carry it out are located on the east coast. There is presently a lively debate in the bioremediation community over the sample preservation methods and their potential drawbacks. We have been able to avoid this issue entirely by choosing methods that can be applied to samples immediately and that involve analyses that are available locally.

## GENERAL COMMENTS

1. Overall, the document is well organized and the experimental design appears to be sound for determining at what rate intrinsic bioremediation of petroleum hydrocarbons is currently occurring.
2. The work plan does, however, need to contain more specific objectives to evaluate the usefulness of the data. The results of this study should support the following feasibility study evaluation criteria:
  - Overall protection of human health and the environment
  - Compliance with applicable or relevant and appropriate requirements
  - Long-term effectiveness and permanence
  - Reduction of toxicity, mobility, and volume through treatment
  - Short-term effectiveness
  - Implementability
  - Cost
  - State acceptance
  - Community acceptance

Only with information on these criteria will the treatability study be useful in accelerating the feasibility study and ultimately in cleaning up the site.

For example, for the first criterion (overall protection of human health and the environment), this study should generate graph showing the predicted change in concentrations of the chemicals of concern (COC) over time so the regulators can see if intrinsic bioremediation can reduce the COCs to levels acceptable to them within an acceptable time frame. This objective is eluded to in several sections but it should be a specific objective of the study. Also, the cost of monitoring intrinsic bioremediation for the time period required to reduce the COCs below acceptable levels should be included as an objective so it can be evaluated against other types of remediation.

3. A significant data base exists on bioremediation, particularly for petroleum hydrocarbons. A brief summary of a literature search should be presented on rates of intrinsic bioremediation so the reader can get a feel for the degradation rates that can be expected.
4. Three overlapping approaches are proposed in the work plan to evaluate the occurrence and rate of intrinsic bioremediation of petroleum hydrocarbons: loss of hydrocarbons in the bioactive area, laboratory confirmation of microbial potential, and field confirmation of microbial activity. The work plan should also state that other physicochemical mechanisms besides intrinsic bioremediation may also be reducing the concentrations of petroleum hydrocarbons. These mechanisms include volatilization of the lighter fractions, dilution and adsorption.

5. The section on modeling (Section 6) is not integrated with the overall objectives of the treatability study. It is written at a level that would likely not be understood by most members of the Restoration Advisory Board and the public. In addition, it appears to be focused on groundwater modeling and does not include modeling the reduction of contaminants in soil. This section should be rewritten and integrated into the overall objectives of the study. Also, the modeling section states that groundwater flow will be assessed by preparing an overall water balance for NAS Alameda and identifying boundary conditions. This would duplicate the groundwater modeling effort to be performed by PRC for the Navy. Rates of intrinsic bioremediation or other constants obtained from this effort would be useful in the PRC modeling effort.

#### PAGE-SPECIFIC COMMENTS

**Section 1.2, page 2, first paragraph:** The text states that the work plan will evaluate the process of intrinsic bioremediation as a remedial alternative for reducing the hydrocarbon levels in the soil and the groundwater at Sites 3 and 13.

A percent reduction in hydrocarbon concentration should be included in the objectives. *Guide for Conducting Treatability Studies Under CERCLA: Biodegradation Remedy Selection*, (U.S. Environmental Protection Agency, EPA/540/R-93/519a, August 1993) recommends demonstrating that the most resistant COCs meet cleanup standards under test conditions for a remedy selection treatability study. The work plan should identify the chemicals expected to be at the site that are the most difficult to biodegrade (and pose a risk to human health or the environment) that will be evaluated during the study and a target percent reduction in concentration to reduce risk.

Also, the objectives of the modeling presented in Section 6 should be included in the overall objectives of the work plan.

**Section 2.2.3, page 10, second paragraph:** The text states that only copper and magnesium exceeded typical levels found in naturally occurring soil samples. The text should include arsenic since it was also found at concentrations that exceeded typical levels found in naturally occurring soil samples.

Also, it should be noted that the "background levels" for metals have not been identified yet.

**Section 3.1, page 23, last paragraph:** Here and in sections that follow, the term "background" is used to refer to those areas that are not contaminated. The term "background" should not be used in this instance since it has regulatory implications. A better choice might be non-contaminated areas.

**Section 4.1, page 30, first paragraph:** Figure 2-5 is cited for showing the planned locations of the soil borings at Site 3. The figure does not indicate where the soil will be sampled. This should be

Mr. Spielman  
March 5, 1996  
Page 4

corrected.

**Table 5-1, page 2, first paragraph:** The text states that, if evidence shows intrinsic bioremediation is occurring at Sites 3 or 13, then intrinsic remediation will be included as part of the remedial action plan. This is not necessarily true. If the demonstrated rate of intrinsic bioremediation is such that it shows promise for remediating the COCs in a "reasonable" time frame and there is enough information to evaluate it, it will be included in the feasibility study.

If you have any questions regarding these review comments, please call me at (206) 587-4685.

Sincerely,

Jerry Shuster, P.E.  
Project Engineer

cc: Susan Willoughby, PRC  
Project File

**RESPONSE TO COMMENTS ON  
FEBRUARY 7, 1996 TREATABILITY STUDY WORK PLAN  
INTRINSIC BIOREMEDIATION  
PRC ENVIRONMENTAL MANAGEMENT, INC.**

This document presents the response to comments received from PRC Environmental Management, Inc. UCB's responses to the review comments are provided below. A copy of the PRC review is enclosed for convenience. The numbers indicated correspond to the numbers used in PRC's letter.

**GENERAL COMMENTS**

1. Thank you.

2. The treatability study to be performed at Sites 3 and 13 will provide data to evaluate the occurrence and rate of intrinsic bioremediation at these sites. The data can be used by the feasibility study contractor to rate the performance of intrinsic bioremediation against other potential remedial responses. It is beyond the scope of Delivery Order 5 to estimate costs associated with long term monitoring that would be performed until the concentrations of chemicals of concern are reduced to acceptable levels. Again, this can be completed by the feasibility study contractor as part of a feasibility study or engineering evaluation/cost analysis. Section 1.2 of the Work Plan has been changed to include the evaluation criteria that will be used by the feasibility study contractor to evaluate intrinsic bioremediation in comparison to other potential remedial technologies for Sites 3 and 13. The modeling approach will generate plots showing the change in concentrations of the chemicals of concern over time as requested by the reviewer.

3. During negotiation of Delivery Order 5, the U.S. Navy requested UCB not to include a literature search in this Work Plan. A literature search will be included in the final report prepared at the completion of the treatability study.

4. Section 1 of the Work Plan has been revised to identify other mechanisms that could be responsible for loss of contaminants and to identify assays that will be conducted to demonstrate the degree to which intrinsic bioremediation is likely responsible for contaminant loss at Sites 3 and 13.

5. *First point:* The modeling section was written as a separate section at the request of the Navy (Ken Spielman, personal communication). In the opinion of the Work Plan authors, this request was quite logical and served to increase the readability of the Work Plan because it differentiates data that are experimentally collected measurements from data that will be obtained or computed by analysis and interpretation (i.e. modeling).

*Second point:* The modeling section has been substantially rewritten to simplify the language and to clear up the misconceptions pointed out in the review. For example, the modeling approach does not focus only on groundwater modeling to the exclusion of soil contaminants, it in fact factors in partitioning of contaminants between all possible phases (solid, liquid, gas) and specifically deals with reduction of contaminants in soil. This section should now be clearly understandable to a wide range of audiences including the Navy, PRC and the Restoration Advisory Board.

*Third point:* We have no desire to duplicate groundwater modeling efforts being performed by PRC. On the contrary, we will be happy to incorporate the results of PRC's work into our modeling approach. We have recently become aware of the document PRC document "Protocol for Contaminant Fate and Transport Modeling, Technical Memorandum" dated March 29, 1996 which outlines the modeling projects that PRC will undertake with respect to NAS Alameda, and

look forward to receiving their results from the relevant studies. We in turn will be happy to provide PRC with all of the bioremediation data that is collected and developed as part of this study.

## **PAGE SPECIFIC COMMENTS**

### **Section 1.2, Page 2, first paragraph:**

It would not be appropriate to include a “percent reduction of hydrocarbons” in the objectives. First of all, percent reduction from what? It is not known what the initial hydrocarbon concentrations were at either of these sites. Second of all, percentages are meaningless from a regulatory point of view when concentration ranges cover orders of magnitude. Regulators generally rely on concentration limits. Further, our objectives (as stated clearly in the text) are to evaluate the occurrence and rate of bioremediation, and by means of the modeling effort will provide predictions of future concentrations of soil and groundwater hydrocarbons.

With respect to identifying the most biologically resistant chemicals of concern at the sites, due to the lack of information about the current degradation of chemicals at the sites, it is not yet possible to identify these chemicals. This topic will be addressed in the reports following the data collection and analysis of this project.

The modeling objectives are included in the overall objectives of the Work Plan (paragraph 6, section 1.2).

**Section 2.2.3, Page 10, second paragraph:** The data available to UCB do not indicate that arsenic exceeded naturally occurring levels. If arsenic is present at elevated concentration, we would be happy to include the data. The presence of arsenic should not affect this treatability study.

### **Section 3.1, Page 23, last paragraph:**

UCB is not convinced that a completely uncontaminated area can be found at either Site 3 or 13. We are more comfortable with the term “background” and have not changed the terminology in the Work Plan.

### **Section 4.1, page 30, first paragraph:**

The figure referred to should have been Figure 2-4. The appropriate change has been made in the text.

### **Table 5-1, page 2, first paragraph:**

Table 5-1 has been revised to state that intrinsic bioremediation will be included in the feasibility study if the treatability study demonstrates that intrinsic bioremediation is likely to reduce chemicals of concern to acceptable levels within a reasonable time frame.

14 Mar 96

## MEMORANDUM

From: Code 09KRE

To: Ken Spielman, Code 1831.4

Subj: TREATABILITY STUDY WORK PLAN, INTRINIC BIOREMEDIATION, SITES 3 AND 13, NAS ALAMEDA, CONTRACT NO. N62474-94-D-7420, DELIVERY ORDER 005, SITE SPECIFIC HEALTH AND SAFETY PLAN REVIEW COMMENTS

Ref: (a) Your Review Memorandum dtd 12 Feb 96  
(b) BERC Delivery Order 005 Submittal: Treatability Study Work Plan and the Allied Technology Group, Inc (ATG) Project Site Specific Health and Safety Plan dtd 9 Feb 96  
(c) 29 CFR 1910.120 and 29 CFR 1926.65

1. As requested by reference (a), reference (b) was reviewed for compliance with reference (c) safety considerations. Final acceptance of the project HSP by this office is dependent upon the health considerations review by Gilbert Nickelson. The proposed project Site Specific Health and Safety Plan was found acceptable for safety with the following exceptions:

a. Sec. 1. Introduction. Add a statement that a copy of the ATG Health and Safety Program document and applicable SOPs will be available on-site. Subject document is referenced in the SHSP as a source of guidance information.

b. Sec. 3.7. Subcontractors. Will ATG be reviewing the subcontractors' HASPs and/or SOPs for adequacy? Add a review statement.

c. Sec. 4.4.1, Para. 1, Sentence 1. Check the suitability of the word "Usually."

d. Sec. 4.4.5. Site-specific field operation controls to effect worker safety and health are not directly addressed (i.e., use of heavy equipment, overhead and underground utility involvement, dust generation control). Recommend that rather than state that the control of a potential hazard will be in compliance with the referenced Federal OSHA regulation, it is preferable that procedures or measures (SOPs) to effect safety be provided or a reference made to the applicable controlling ATG Safety and Health Program document section and/or SOP. Proposed HSP information can be provided in tabular form. Health and safety considerations related to sampling collection is not addressed in the HASP (PPE is addressed in the SOPs).

e. Sec. 6.5. Recommend adding a statement that any employee who experiences a lost time injury or a chemical exposure will not be allowed to perform site work until released by the occupational medical physician.

Subj: TREATABILITY STUDY WORK PLAN, INTRINSIC BIOREMEDIATION, SITES 3 AND 13, NAS ALAMEDA, CONTRACT NO. N62474-94-D-7420, DELIVERY ORDER 005, SITE SPECIFIC HEALTH AND SAFETY PLAN REVIEW COMMENTS

f. Sec. 11. Emergency Response Plan. The following minimum requirements are not identified in the SSHP and this section: (a) Site person responsible for initiating emergency support activities; (b) Location of emergency supplies (i.e., first aid kit, eye wash, fire extinguisher); (c) site evacuation procedures; (d) site communications system (i.e., hand signals, cellular phone); and (e) who will be responsible for responding - trained ATG personnel or outside support forces.

g. Sec. 11.4 Add the name and telephone number of the EFA West POC.

h. Sec. 11.5. Add that a copy of the accident/incident report will be provided to the EFA West contracting officer.

2. Please request Allied Technology Group, Inc. to review and address above review comments. Recommended changes should be clearly identified. This may be done in several ways: by submitting revised pages with reasons for the changes noted, by the use of strikeout and underline, by the use of shading and italics, or by cover letter stating how each comment has been addressed.

3. Any questions or comments, please contact the undersigned at (415) 244-2955.

REGINA ENG

Copy to: 09K: Chron, Contract (w/ref b), 1825GN, RE

**RESPONSE TO COMMENTS ON  
FEBRUARY 7, 1996 TREATABILITY STUDY WORK PLAN  
INTRINSIC BIOREMEDIATION  
REGINA ENG, EFA WEST**

This document presents the response to comments received from Regina Eng of EFA West on the Health and Safety Plan (HSP). UCB's responses to the review comments are provided below. A copy of the EFA West review is enclosed for convenience. The numbers correspond to the numbers provided in the comment letter. In addition to changes made in response to these comments, the HSP was revised to parallel the BERC Health and Safety Plan (Program).

1.a. See section 1

1.b. This section has been changed to require subcontractors to comply with the requirements of the UCB HSP for this project.

1.c. This section has been changed to reflect site specific hazards anticipated during the treatability studies.

1.d. The revised HSP addresses site specific hazards and appropriate standard safety procedures contained in the BERC Health and Safety Plan (Program).

1.e. See Section 10.5

1.f. See Section 10

1.g. See Section 10.4

1.h. See Section 10.5

**MEMORANDUM**  
**Job Order No. 96B63BIR**  
**3 April, 1996**

**RPM Mr. Kenneth H. Spielman**  
**Code 18314**

**From:** Gilbert Nickelson, Jr., Code 18253

**To:** Kenneth H. Spielman, Code 18314

**Via:** Raymond E. Ramos, Code 1825 *RER 4/3/96*

**Subject:** TREATABILITY STUDY WORK PLAN, INTRINIC BIOREMEDIATION, SITES 3 AND 13, NAS ALAMEDA, CONTRACT NO. N62474-94-D-7420, DELIVERY ORDER 005, SITE SPECIFIC HEALTH AND SAFETY PLAN REVIEW COMMENTS

- References:**
- (a) Treatability Study Work Plan, and the Allied Technology Group, Inc (ATG) Project Site Specific Health and Safety Plan, BERC Delivery Order 005 Submittal.
  - (b) HASP Review Memorandum, Code 09K, Regina Eng's review and comments of BERC Delivery Order 005, NAS Alameda, Contract NO. N62474-94-D-7420.
  - (c) 29 Code of Federal Regulations (CFR) 1910.120 (Hazardous Waste Operations and Emergency Response)
  - (d) 29 Code of Federal Regulations (CFR) 1926.65 Subpart D
  - (e) Navy/Marine Corps Installation Restoration Manual (February 1992)
  - (f) American Conference of Governmental Industrial Hygienist (ACGIH) 1995-1996

1. The subject document, reference (a) was prepared for ENGFLDACT WEST by the BERC Management Office at the University of California at Berkeley, and is dated 7 February, 1996. I have also reviewed Code 09K, Regina Eng's comments, reference (b). I compared the Health and Safety Plan (HASP) to federal requirements under the Occupational Safety and Health Administration (OSHA) regulations, and to the Department of the Navy requirements under the "Navy/Marine Corps Installation Restoration Manual" (see references (c), (d), (e), and (f), above). If there are any questions regarding my comments, please contact me at (415)-244-2577, DSN 494-2577. My comments are provided as follows:

(a) Page 7, Section 8.0 "Health and Safety Plan"

Comments: This section describes that Personal Protective Equipment (PPE) that should be used by employees involved in drilling and sampling activities. Sentence 2, paragraph 2, list PPE, and states " wear appropriate safety shoes, glasses, hard-hats"...

Sentence 1, paragraph 2, " states," (SARA/OSHA) 40-hr training...etc."

Recommendations: Change sentence structure to read, "safety shoes, safety glasses with side shield, hard hat..." for clarity. Please change sentence 1 to read (OSHA) 29 CFR 1926(e)(6), and 29 CFR 1910.120(e)(4)(ii)(B).

(b) Page 3, Section 3.5 addresses "ATG Site Supervisor"

Comment: Sentence 3 states that Mr. Chiu (ATG Site Supervisor), will be assisted by Ms Mary McDonald, R.G., during drilling activities.

Recommendation: Define the responsibilities of Ms McDonald, is she the designated alternate for the site?

(c) Page 24, Section 6.2.3 addresses " Post-Exposure Examination"

Comment: Sentence 2, paragraph 1 states " In rare instances, for example during prolonged field work...."

Recommendation: Revise the sentence to read " For example in rare instances,...etc."

(d) Page 28, Section 7.1 addresses " Exclusion Zone"

Comment: Sentence 1, paragraph 3 states "The level of protection required in the EZ varies according to the work task...."

Recommendation: A clear rational for upgrade/downgrade of PPE must be established.

(e) Page 39, Section 10.2 addresses "Combustible Gas Indicator"

Comment: Sentence 1, paragraph 2 states " ...combination CGI and OM (calibrated for a specific instrument calibration gas).

Recommendation: I recommend inserting the term "Span," to read,(" instrument span calibration gas)."

(f) Page 41, Section 10.3 addresses "Photoionization Detector"

Comment: Bullet No. 7, states that " The PID will not respond to methane (CH4).

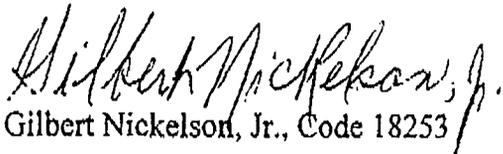
Recommendation: Define what method and type of instrumentation that will be used to detect methane gas.

2. Revise the document reference (a) to include the information that has been requested in this review, and if data is located in other documents, then clearly indicate its location.

3. Page one, "Title Page"

Comment: The title page was not signed and dated by the responsible personnel in the Approval and Review section.

Recommendation: I recommend that in the future all HASP submitted to EFA- West be signed and dated by personnel listed in the Review and Approval Section. HASP's will be returned for appropriate signatures without review in the future. The signatures indicate the HASP has been reviewed prior to submittal.

  
Gilbert Nickelson, Jr., Code 18253

Industrial Hygienist

**RESPONSE TO COMMENTS ON  
FEBRUARY 7, 1996 TREATABILITY STUDY WORK PLAN  
INTRINSIC BIOREMEDIATION  
GILBERT NICKELSON, JR., EFA WEST**

This document presents the response to comments received from Gilbert Nickelson of EFA West on the Health and Safety Plan (HSP). UCB's responses to the review comments are provided below. A copy of the EFA West review is enclosed for convenience. The numbers correspond to the numbers provided in the comment letter. In addition, the HSP was revised to parallel the BERK Health and Safety Plan (Program).

1.a. We could not locate the referenced section.

1.b. See Sections 2.5 and 2.6.

1.c. This section was deleted from the HSP and the appropriate section of the BERK Health and Safety Plan (Program) is referenced.

1.d. Criteria for selecting appropriate PPE are addressed in Section 9.1, Action Levels.

1.e. Procedures for calibrating the combustible gas indicator (CGI) were deleted from the HSP. The CGI will be calibrated according to the manufacturers recommendations.

1.f. Details regarding the PID were deleted from this site specific HSP. The PID is discussed in the BERK Health and Safety Plan (Program). Methane is not expected to be of concern at Sites 3 and 13.

STATE OF CALIFORNIA

PETE WILSON, Governor

**CALIFORNIA REGIONAL WATER QUALITY CONTROL BOARD**  
**SAN FRANCISCO BAY REGION**  
2101 WEBSTER STREET, SUITE 500  
OAKLAND 94612

Phone: (510) 286-1255  
FAX: (510) 286-1530  
BBS: (510) 286-0404



April 29, 1996  
File No: 2199.9285 (GK)

Mr. Ken Spielman  
EFA-WEST  
Naval Facilities Engineering Command  
900 Commodore Drive  
San Bruno, California 94066-5006

**SUBJECT: COMMENTS ON THE DRAFT TREATABILITY STUDY WORK PLAN INTRINSIC BIOREMEDIATION SITES 3 AND 13, NAVAL AIR STATION ALAMEDA, dated 2/7/96**

Dear Mr. Spielman:

The following are comments based on the San Francisco Bay Regional Water Quality Control Board staff's review of the referenced document.

General Comments

1. RWQCB staff applauds the Navy's efforts in performing this type of treatability study. Studies of this type are important to provide a scientific basis for decisions on remediation of TPH. In the future, RWQCB staff hopes to use the information gathered from this study and apply it to other similar petroleum contaminated sites at ANAS, both in the IR program as well as the UST program.

Specific Comments

2. Page 4, 1st paragraph: The statement that the RWQCB has amended their Basin Plan to include non-attainment of groundwater is inaccurate. Currently, the State Water Resources Control Board is developing state wide policy addressing the containment zone policy (previously termed the non-attainment zone policy).

It is correct that the RWQCB recognizes that natural bioremediation is an acceptable remedial alternative, but only if the source has been removed.

3. Page 10, 2nd paragraph: How was background of inorganics determined, to my knowledge the calculation of background is still under discussion between the Navy and regulatory agencies.

If you have any questions or concerns, I can be reached at the San Francisco Bay Regional Water Quality Control Board at (510) 286-4267.

Post-It™ brand fax transmittal memo 7871 # of pages # 1

To: Ken Spielman	From: Gina Kathuria
Co.	Co.
Dept.	Phone #
Fax #	Fax #

Sincerely,  
*Gina Kathuria*  
Gina Kathuria, P.E.  
Project Manager

cc: Mr. Tom Lanphar, DTSC  
Mr. James Ricks, USEPA

**RESPONSE TO COMMENTS ON  
FEBRUARY 7, 1996 TREATABILITY STUDY WORK PLAN  
INTRINSIC BIOREMEDIATION  
GINA KATHURIA, RWQCB**

This document presents the response to comments received from Gina Kathuria of the RWQCB. UCB's responses to the review comments are provided below. A copy of the RWQCB letter is enclosed for convenience. The numbers below correspond to the numbers provided in the comment letter.

1. Thank you. We look forward to providing information that will be useful in your evaluation of other sites.
2. The text of Section 1.2 has been revised to include the wording suggested by the RWQCB.
3. To our knowledge, background levels of inorganics have not been established for NAS Alameda. In our discussion we have included metals which were identified at concentrations greater than naturally occurring levels, based on PRC review. However, metals concentrations should have little, if any, effect on this treatability study because the focus is on the intrinsic bioremediation of hydrocarbons.