Unit 4 Source Area In-Situ Bioremediation Treatability Study Bench-Scale Work Plan
Nevada Environmental Response Trust Site
Henderson, Nevada

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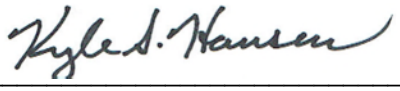
Acronyms/Abbreviations	Definition
AWF	Athens Well Field
BMI	Black Mountain Industrial
COD	chemical oxygen demand
COPC	contaminant of potential concern
DI	deionized water
DO	dissolved oxygen
FBR	fluidized bed reactor
g	gram
GAC	granular activated carbon
GWETS	groundwater extraction and treatment system
ISB	in situ bioremediation
mg/L	milligrams per liter
mL	milliliters
mVs	millivolts
NCBI	National Center for Biotechnology Information
NDEP	Nevada Division of Environmental Protection
NERT or Trust	Nevada Environmental Response Trust
Qal	Quaternary alluvial
ppb	parts per billion
ppm	parts per million
psi	pounds per square inch
Site	Nevada Environmental Response Trust Site
TDS	total dissolved solids
Tetra Tech	Tetra Tech, Inc.
Tronox	Tronox LLC
UMCf	Upper Muddy Creek formation
Unit 4	Unit 4 cell building
UNLV	University of Nevada, Las Vegas
VOC	volatile organic compound
WBZ	water-bearing zone
Work Plan	Unit 4 Source Area In-Situ Bioremediation Treatability Study Bench-Scale Work Plan
xMCF	Transitional Muddy Creek Formation

CERTIFICATION

I hereby certify that I am responsible for the services described in this document and for the preparation of this document. The services described in this document have been prepared in a manner consistent with the current standards of the profession, and to the best of my knowledge, comply with all applicable federal, state, and local statutes, regulations, and ordinances.

Description of Services Provided: Unit 4 Source Area Bioremediation Treatability Study Bench-Scale Work Plan, Nevada Environmental Response Trust Site, Henderson, Nevada



September 12, 2017

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Date

Nevada CEM Certificate Number: 2167
Nevada CEM Expiration Date: September 18, 2018

1.0 INTRODUCTION

At the direction of the Nevada Environmental Response Trust (NERT or Trust), Tetra Tech, Inc. (Tetra Tech) has prepared this Unit 4 Source Area Bioremediation Treatability Study Bench-Scale Work Plan (Work Plan) for the NERT site (Site), located in Clark County, Nevada (Figure 1). This Work Plan is being submitted to the Nevada Division of Environmental Protection (NDEP) under the Interim Consent Agreement effective February 14, 2011. This Work Plan presents the technical approach and scope of work for conducting bench-scale tests that will support the design and implementation of a treatability field study to remediate perchlorate, chlorate, and chromium (collectively referred to as chemicals of potential concern [COPCs]) at the former Unit 4 cell building (Unit 4). The details of the treatability study will be provided under separate cover in the Unit 4 Source Area In-Situ Bioremediation Treatability Study Work Plan.

1.1 PROJECT OBJECTIVES

The overall objective of the bench-scale studies (developed in cooperation with Dr. Jacimaria Batista at the University of Nevada – Las Vegas [UNLV]) is to provide data necessary for the design of the treatability study to biodegrade the COPCs in soil and groundwater at Unit 4. Unit 4 has been identified as a source for perchlorate and hexavalent chromium in groundwater plumes at the Site. Recent investigations of the subsurface at Unit 4 have identified distinct zones within the Quaternary alluvium (Qal) and the underlying, finer-grained Upper Muddy Creek Formation (UMCf) with elevated concentrations of perchlorate, chlorate, hexavalent chromium and total dissolved solids (TDS) (Tetra Tech, Inc., 2017). In-situ bioremediation of perchlorate and hexavalent chromium has been successfully demonstrated in downgradient portions of the groundwater plume through several completed and on-going treatability studies (Tech Tech, Inc., 2016a) and (Tetra Tech, Inc., 2016b). In those treatability studies, the concentrations of perchlorate and TDS have not been as high as those observed at Unit 4; therefore, it is necessary to evaluate how the high COPC and TDS concentrations will impact biodegradation prior to performing the field treatability study.

Specific objectives of the bench-scale studies are:

1) To determine the impact of concentrations of high TDS on the biodegradation kinetics of the COPCs using groundwater and soil collected from the proposed Unit 4 treatability study area.
2) To evaluate the impact of volatile organic compound (VOC) presence on the biodegradation of perchlorate, chlorate, and chromium.
3) To examine the impact of bioaugmentation, along with adding nutrients and vitamins, on the biodegradation of the COPCs.

The work plan will be implemented by Dr. Jacimaria Batista and her team at UNLV. Tetra Tech has overseen three similar bench-scale tests conducted by Dr. Batista and her team.

1.2 WORK PLAN ORGANIZATION

This Work Plan is organized as follows:

- **Introduction (Section 1.0):** Provides the primary objectives of the bench-scale tests.
- **Background (Section 2.0):** Provides an overview of bioremediation of perchlorate, a summary of previous bench-scale bioremediation treatability studies, a summary of Unit 4 characteristics, including those that may impact bioremediation.
- **Bench-Scale Study Design (Section 3.0):** Describes the conceptual design of the bench-scale microcosm and column tests.
- **Reporting (Section 4.0):** Summarizes reporting related to design and execution of the bench-scale tests.

- **Schedule (Section 5.0):** Summarizes the schedule for conducting the bench-scale tests and associated reporting.
- **References (Section 6.0):** Lists the documents referenced in this Work Plan.

2.0 BACKGROUND

Bench-scale studies and field applications of in-situ bioremediation (ISB) have previously been conducted for the Site. The field applications have focused on downgradient, lower perchlorate concentration portions of the groundwater plumes. The focus of the previous bench-scale studies was to evaluate biodegradation timescales, rate kinetics, and the rates at which the substrate adsorb and desorb to the soil matrix. The following sections summarize the site history, investigation results at Unit 4, and the potential effects high concentrations of COPCs and TDS might have on the biodegradation of the COPCs.

2.1 SITE LOCATION AND DESCRIPTION

This section provides a brief description of the Site location and history along with details regarding the Unit 4 treatability study area derived from the *Unit 4 and 5 Buildings Investigation Work Plan* (Tetra Tech, Inc., 2015), the Unit 4 and 5 Buildings Investigation First Mobilization Technical Memorandum (Tetra Tech, Inc., 2016c), and Unit 4 and 5 Buildings Investigation Second Mobilization Technical Memorandum (Tetra Tech, Inc., 2017).

The NERT Site, which was formerly owned and operated by Tronox LLC (Tronox), comprises approximately 264 acres located within the Black Mountain Industrial (BMI) Complex in an unincorporated portion of Clark County that is surrounded by the City of Henderson, Nevada. The Site has been used for industrial operations since 1942, when it was developed by the U.S. government as a magnesium plant in support of World War II operations. Following the war, various industrial activities, including the production of perchlorates, boron, and manganese compounds, continued at the BMI Complex. Former industrial and waste management practices at the Site and adjacent properties have resulted in impacts to soil, groundwater, and surface water. Tronox formerly owned and operated a portion of the Site, including the Investigation Area. In conjunction with the settlement of Tronox's bankruptcy proceeding, ownership of the Site was transferred to NERT on February 14, 2011. Tronox currently leases an approximately 114 acre portion of the Site which includes the Investigation Area, and continues to operate its chemical manufacturing business under a lease agreement with the Trust (Ramboll Environ, 2016a).

There are a total of ten unit buildings (numbered 1 through 10) aligned in a row from west to east along the southern portion of the Site (Figure 1). Each unit building consists of three structures: a chlorinator building on the north side, a cell building in the center, and substation building on the south side. Four of the unit buildings (Units 3, 4, 5, and 6) are leased from NERT by Tronox (Figure 2). The roof, above grade walls and floors of the Unit 1 and 2 cell buildings have been demolished, with the basement walls and slabs remaining intact. In addition, the eastern half of the Unit 3 cell building has been demolished. Unit buildings 7, 8, 9, and 10 are not located on property owned by the Trust.

The Unit 4 cell building is no longer used, and its above-ground structures were demolished in the mid-2000s. In 2012, Tronox retrofitted the Unit 4 substation building to house an advanced battery manufacturing process. The Unit 4 chlorinator building is currently used by Tronox, primarily for storage. The Unit 4 cell building historically contained chlorinators (furnaces) that created molten magnesium chloride by reacting magnesium oxide/carbon pellets with chlorine gas at high temperatures. Magnesium metal was then produced in banks of electrolytic cells in the cell building by electrochemical reduction of the molten magnesium chloride. From 1945 to 1989, sodium chlorate and sodium perchlorate were produced by electrolytic processes, which involved the use of sodium dichromate (hexavalent chromium) on the first floor of the Unit 4 and 5 cell buildings. The concrete basements

reportedly served as sumps to collect process liquor, spillage, and wash water, and process chemicals may have leaked to the soil through cracks in the basements of the Unit 4 and 5 cell buildings (Tetra Tech, Inc., 2015).

2.2 GEOLOGY

The Site is located near the southeast end of the Las Vegas Valley, a northwest-southeast trending structural basin that extends approximately 55 miles and includes metropolitan areas of North Las Vegas, Las Vegas, and Henderson. Locally, the ground surface slopes to the north towards the Las Vegas Wash. The Las Vegas Valley is bounded on the west by the Spring Mountains, on the north by the southern ends of the Sheep and Las Vegas Ranges, on the east by Frenchman and Sunrise Mountains, and on the south by the River Mountains and McCullough Range (Plume, 1989). The structural basin that underlies Las Vegas Valley is comprised of Precambrian crystalline rocks; Precambrian and Paleozoic carbonate rocks; Permian, Triassic, and Jurassic clastic rocks; and Miocene igneous rocks. Gravity data indicate that the deeper portions of the basin are filled with 3,000 to 5,000 feet of clastic sedimentary deposits that range in age from Miocene through Holocene (Plume, 1989).

The clastic sedimentary valley-fill deposits of Las Vegas Valley are more than 4,000 feet thick beneath Henderson (Plume, 1989), and consist of Quaternary alluvial deposits, transitional Muddy Creek Formation (xMCf), and Pleistocene UMCf. The alluvium is generally described as reddish-brown discontinuous layers of sand and gravel with minor amounts of silt, clay and caliche. The thickness of these alluvial deposits ranges from less than one foot to more than 50 feet beneath the Site (Ramboll Environ, 2016a).

The lithology encountered in the Unit 4 and 5 Buildings Investigation Area, which includes the Unit 4 treatability study area, consists of interlayered clay, silt, sand, and gravel of varying thicknesses, which is characteristic of the Qal and UMCf deposits. The Qal consists primarily of sand and silty sand while the UMCf, which underlies the Qal, consists of interbedded coarse-grained and fine-grained sediments. The contact between the base of the Qal and the top of the UMCf at Unit 4 is encountered at a depth of approximately 50 feet bgs. The upper 30-40 feet of the UMCf at Unit 4 is characterized by a higher proportion of sandy interbeds which transition into predominately fine-grained materials, including silt, sandy silt, and clayey silt. Intervals of predominantly coarse-grained water-bearing sand and/or gravel and intervals of predominantly fine-grained silt and/or clay units were identified throughout the boreholes (Tetra Tech, Inc., 2017).

2.3 HYDROLOGY AND HYDROGEOLOGICAL CONDITIONS

Surface water at the Site generally flows from south to north toward the Las Vegas Wash but does not leave the Site. During the 2011 Interim Soil Removal Action (ENVIRON, 2012), many portions of the Site were graded such that storm water would be retained on the Site. Existing roads, utility berms, and other site features were created to prevent storm water from flowing off the Site. Two main storm water retention basins, the Central Retention Basin and Northern Retention Basin, were constructed to control storm water flow and maintain storm water on the Site. The Central Retention Basin collects surface runoff from the Tronox-leased area. The Northern Retention Basin collects surface runoff water from north of the former Beta Ditch (located near the center of the Site) and accepts overflow from the Central Retention Basin.

According to previous work performed at the Site, the depth to groundwater ranges from approximately 27 to 80 feet bgs, is generally deepest in the southern portion of the Site, and becomes shallower to the north as it approaches the Las Vegas Wash. The average groundwater gradient ranges from 0.015 to 0.020 feet/foot south of the Athens Road well field (AWF) located approximately two miles north of the Investigation Area, decreasing to approximately 0.007 to 0.010 feet/foot to the north of the AWF. The direction of groundwater flow on the Site is generally north to north-northeast; to the north of the Site, the direction of groundwater flow is toward the northeast (Ramboll Environ, 2016b).

The NDEP has defined the following three water-bearing zones (WBZs) that occur within the BMI Complex:

- Shallow WBZ – The first occurrence of groundwater in the area occurs within either the alluvium or the Upper Muddy Creek Formation. Groundwater in the Shallow WBZ occurs under unconfined to partially confined conditions and is considered the "water table aquifer." At the Site, the Shallow WBZ is comprised of the saturated portions of the alluvium and the uppermost portion of the UMCf to depths of approximately 90 feet bgs (Ramboll Environ, 2016b).
- Middle WBZ – Groundwater in the Middle WBZ generally occurs between 90 and 300 feet bgs. Water-bearing units in the Middle WBZ are confined (Ramboll Environ, 2016a). Groundwater in the Middle WBZ exhibits an upward vertical gradient (Ramboll Environ, 2016b).
- Deep WBZ – Groundwater in the Deep WBZ generally occurs between 300 and 400 feet bgs. Water-bearing units in Deep WBZ are confined. Groundwater in the Deep WBZ exhibits an upward vertical gradient (Ramboll Environ, 2016b).

Environmental investigations at the Site have primarily focused on the Shallow WBZ; however investigations conducted by Northgate Environmental Management in 2009 included the installation of several monitoring wells in the Middle WBZ to characterize the vertical distribution of chemical constituents (Ramboll Environ, 2016a). In addition, the recent investigations conducted by Tetra Tech of the Unit 4 and 5 buildings included drilling over 60 soil borings and collecting discrete depth groundwater samples in multiple horizons within the Shallow and Middle WBZ (maximum depth 250 ft bgs) (Tetra Tech, Inc., 2016c and Tetra Tech, Inc., 2017).

2.4 SUMMARY OF UNIT 4 AND 5 BUILDINGS INVESTIGATION

As part of the Trust's ongoing Remedial Investigation / Feasibility Study, Tetra Tech is currently performing an environmental investigation in the area of the Unit 4 and 5 buildings as described in the Unit 4 and 5 Buildings Investigation Work Plan (Tetra Tech, Inc., 2015). The implementation of the investigation was divided into three field mobilizations. The first field mobilization was conducted in late-2015 and consisted of advancing four boreholes and collecting discrete-depth groundwater samples from the four exterior corners of the Unit 4 cell floor to obtain preliminary data to guide subsequent mobilizations. The second field mobilization was conducted between June 28, 2016 and January 3, 2017 and consisted of advancing 69 soil borings and collecting discrete-depth groundwater samples throughout the proposed investigation area in the vicinity of the Unit 4 and 5 buildings. The third field mobilization is currently ongoing and consists of advancing angled boreholes to investigate beneath the Unit 5 Building and installation of seven nested and clustered monitoring wells to confirm the discrete-depth groundwater samples (Tetra Tech, Inc., 2017). A summary of the discrete-depth groundwater sampling results from the first and second mobilizations is provided as ***Table 1.***

Table 1 Summary of Groundwater Concentrations at Unit 4 and 5 Buildings

Analyte	Quaternary Alluvium (0-35 feet bgs)	Upper Muddy Creek Formation (35-75 feet bgs)	Upper Muddy Creek Formation (75-125 feet bgs)	Upper Muddy Creek Formation (125-250 feet bgs)
Perchlorate	3.9 – 2,900	0.92 – 3,500	1.4 – 6,700	0.057 – 570
Hexavalent Chromium	0.34 – 12.0	0.002 – 42.0	0.003 – 110	0.003 – 6.1
Total Chromium	0.43 – 12.0	0.012 – 38.0	0.013 – 110	0.032 – 5.7
Chloroform	0.043 – 1.6	0.002 – 0.66	0.00097 – 7.8	0.0003 – 0.18
TDS	3,100 – 13,000	430 – 24,000	890 – 48,000	630 – 6,400

Notes:
All concentrations listed above are in mg/L – milligrams per liter
bgs – below ground surface

Based on the summary data in Table 1 it is clear that the highest COPC concentrations were observed in samples from the deeper UMCf (generally around 90-110 feet below ground surface). The highest perchlorate, chlorate and TDS concentrations were observed directly below and downgradient (north) of Unit 4 in the UMCf. Elevated concentrations of hexavalent chromium and chloroform were also observed in the Unit 4 source area. In addition to chloroform, a number of other VOCs were detected in groundwater samples (Tetra Tech, Inc., 2017).

2.5 MICROBIOLOGY AND BIODEGRADATION OF PERCHLORATE

2.5.1 Perchlorate Biodegradation

Perchlorate is the anionic component of ammonium perchlorate, a common ingredient of solid rocket fuel. Perchlorate salts are very soluble in water, (approximately 200,000 milligrams per liter [mg/L] for ammonium perchlorate and approximately 2,100,000 mg/L for sodium perchlorate) and do not adsorb very strongly to most soils. Perchlorate tends to be biologically stable under aerobic conditions or when there is a limited source of electron donor/organic carbon. In the presence of an electron donor/carbon substrate and after dissolved oxygen (DO) and nitrate have been depleted, perchlorate can act as an electron acceptor for anaerobic respiration. A variety of perchlorate-reducing bacteria have been isolated, including both strict anaerobes and facultative microbes. In the absence of more easily reduced constituents (e.g., nitrate), the first step in perchlorate biodegradation is carried out by the enzyme perchlorate reductase, wherein perchlorate is sequentially converted to chlorate and subsequently converted to chlorite. A second enzyme, chlorite dismutase, further reduces the chlorite to chloride and oxygen (ITRC, 2008).

Perchlorate-reducing microorganisms are known to be ubiquitous in the subsurface and are adaptable to a range of geochemical conditions. The key to successful groundwater treatment is the development of an understanding of the chemical, geochemical, physical, geological, and hydrogeological conditions in the subsurface and devising a prudent approach to engineer a successful remedial strategy. Certain environmental conditions, however, may make perchlorate biodegradation challenging at some locations. These conditions include high or low pH, high salinity, or the presence of other electron acceptors, such as chlorate and nitrate, that act as competitors for bacterial respiration.

Successful bioremediation of perchlorate relies on achieving and maintaining appropriate geochemical conditions that allow for sustainable biodegradation to occur. Favorable redox conditions appropriate for perchlorate biodegradation are less than 0 millivolts (mVs), preferably between 0 to -100 mVs. This range of redox is generally indicative of the following aquifer conditions: DO is depleted, nitrate is consumed, and perchlorate the next preferred electron acceptor for microbial respiration (ITRC, 2008). For the Site, it has been demonstrated from previous bench-scale testing at the UNLV that the sequence of degradation is:

chromium > nitrate > chlorate > perchlorate

2.5.2 Impact of Elevated Perchlorate Concentrations

Studies elsewhere have shown that the presence of high concentrations of COPCs and other constituents present in the groundwater at Unit 4, including perchlorate, have an impact on the degradation rates for perchlorate (USDOD, 2000; Megharaj, Ayudainayagam, & Naidu, 2003; Shananhan, Attaway, Harper, McDonald, & Smith, 1996; and Petrilli & De Flora, 1977). These constituents, therefore, may impact degradation rates for perchlorate in the source areas of the NERT site, and these impacts are briefly discussed in Section 2.5.1. One of the primary objectives of the bench-scale studies will be to evaluate to what extent biodegradation of perchlorate is impacted by the concentrations of COPCs and other constituent present in the groundwater at the Unit 4 source area in-situ bioremediation treatability study area and how to mitigate these impacts during field implementation.

The kinetics of perchlorate degradation are highly influenced by perchlorate concentrations. For pure bacterial cultures, reported perchlorate half-saturation constant (the concentration at which maximum perchlorate reduction rates are halved) has been reported to vary from 2.2 to 18 mg perchlorate/L and 0.14 to 76.6 mg perchlorate/L,

respectively for acetate and hydrogen as sole electron donors. For mixed cultures, half saturation constants have been reported to range between 0.1 to 20 mg perchlorate/L for acetate and 0.01 to 567.3 mg perchlorate/L for hydrogen as the electron acceptor. In field conditions where perchlorate reducing bacteria are a mixture of many strains, kinetics will be slowed by half when perchlorate concentrations are less than 20 mg/L (Shrestha, 2016) . Extremely high concentrations of perchlorate (greater than 5,000 to 10,000 mg/L), such as those detected within the Unit 4 treatability study area, could be inhibitory to the rate of biodegradation, but dilution to lower concentrations could reduce toxicity effects and maintain degradation rates (USDOD, 2000 and Shananhan, Attaway, Harper, McDonald, & Smith, 1996).

2.5.3 Impact of VOCs

Numerous VOCs have been detected in groundwater at the Site (Tetra Tech, Inc., 2017). Of the VOCs, chloroform is of specific concern due to possible inhibitory effects on the rate of biodegradation. A review on chloroform biodegradation (Cappelletti, Frascari, Zannoni, & Fedi, 2012) reports that both aerobic and anaerobic degradation of chloroform has been documented with anaerobic degradation having slower kinetics than aerobic degradation. The review also indicates that most chloroform degradation occurs as cometabolism, but there are reports of chloroform used as a terminal electron acceptor by bacteria, similar to how perchlorate or chlorate are degraded. One study found that chloroform degradation does not occur under denitrifying conditions (Bouwer & McCarty, 1983). Chloroform concentrations in groundwater at the Site, therefore, are expected to degrade after concentrations of nitrate in groundwater are biodegraded. A chloroform concentration of approximately 1 mg/L was found to have significant inhibitory effects in anaerobic systems (Alvarez-Cohen & McCarty, 1991) and (Yu & Smith, 2000). Chloroform concentrations as high as 7.8 mg/L were found in the UMCf in the Unit 4 building area (***Table 1***). One study, however, reported that the inhibitory effects of chloroform to bacteria can be overcome by the addition of Vitamin B-12 (Guerrero-Barajas & Field, 2005).

2.5.4 Impact of Elevated TDS Concentrations

The impact of high TDS levels on bacterial growth and biological degradation of perchlorate has been previously studied (Logan, 2001; Gingras & Batista, 2002; Kesterson, Amy, & Batista, 2005; Ahn et. al, 2009; Xiao & Roberts, 2010; Venkatesan & Batista, 2011; Xaio & Roberts, 2013; and Coates & Gu, 2006; and Park & Marchland, 2006). Logan (2001) observed a decrease in microbial growth with an increase in salinity using a pure enrichment salt tolerant culture. The growth rate decreased from 0.06/day at 3% salinity to 0.037 at 11% salinity. Growth was stopped at salinity greater than 13% (Logan, 2001). Ahn et al. (2009) observed changes in the microbial community at 3% salinity in a membrane biofilm reactor treating synthetic ion exchange brine. Gingras and Batista (2002) observed a decrease in perchlorate reduction by half when salinity was 0.5% and further decreased by more than 90% at salinity concentrations of 1 to 1.5%. Bardiya and Bae (2011) observed that salt concentrations exceeding 4% inhibited perchlorate reduction completely. The high TDS at the concentrations at Unit 4 (48,000 mg/l or 4.8%), therefore, have the potential to negatively influence perchlorate degradation kinetics.

2.5.5 Impact of Elevated Chromium Concentrations

Groundwater at Unit 4 contains up to 110 mg/L of hexavalent chromium (***Table 1***). Recent results (unpublished data) from bench-scale testing at UNLV has demonstrated that hexavalent chromium concentrations as high as 20 mg/L can be effectively biodegraded by indigenous bacteria present at the Site. It has been reported that hexavalent chromium concentrations as high as 100 mg/L are not toxic to some chromate reducing bacteria, but most chromate reducing bacteria can only tolerate hexavalent chromium concentrations between 20-50 mg/L (Megharaj, Ayudainayagam, & Naidu, 2003) and (Petrilli & De Flora, 1977). It is, therefore, possible that chromium concentrations in portions of the Unit 4 treatability study area could be high enough to cause inhibitory effects to the biodegradation rate of indigenous bacteria.

3.0 BENCH-SCALE STUDY DESIGN

Bench-scale tests, consisting of batch microcosm and flow-through column tests, will be conducted utilizing UMCf soil and groundwater collected during the Unit 4 and 5 Buildings Investigation Third Mobilization (Tetra Tech, Inc., 2017). Following baseline characterization of the groundwater and soil samples (described in Section 3.1 below), batch microcosm tests will be conducted in two phases. Phase 1 will evaluate the viability of in-situ biodegradation reactions using full-strength groundwater with and without VOCs as potential inhibitors. Phase 1 microcosms will also evaluate the impact of the addition of biomass or supernatant from the on-Site fluidized bed reactors (FBRs) as perchlorate bacteria seed material. Phase 2 microcosms will evaluate the impact of high TDS and COPC concentrations on biodegradation kinetics. After the microcosm tests are completed, flow-through column studies will be conducted to simulate the aquifer conditions at the Site, including hydraulic conductivity and groundwater velocity, and assess the impacts of mass transfer on bioremediation. An estimate of the mass transfer from fine-grained UMCf to the groundwater (and thus available for bioremediation) will be evaluated in the column studies by comparing the mass of COPCs associated with the UMCf solids before and after running the column experiments. Details of these procedures are described in the following sections.

3.1 BASELINE CHARACTERIZATION OF THE GROUNDWATER AND SOILS

Soil and groundwater will be collected for use in batch and column tests from selected groundwater wells installed during the Unit 4 and Unit 5 Investigation Third Mobilization (Tetra Tech, Inc., 2017). To obtain a homogeneous sample representative of the UMCf, equal volumes of borehole soil cuttings from each drilling horizon (i.e., every five feet) will be mixed together in sterilized plastic pans using sterile hand shovels. The soil COPC content will be determined by extraction with deionized water (DI) and analysis as per methods shown in ***Table 2***. Extraction entails adding sequential amounts of DI to the samples and extracting the water using a centrifuge. Experience with soils from the Site has shown that rinsing a 30 gram (g) soil sample with 20 milliliters (mL) of DI followed by centrifugation to extract the water, repeated three times, is sufficient to remove the bulk of the COPCs, including VOCs. Based on the elevated COPC concentrations at Unit 4, this procedure will be tested with samples from the Site and adjusted as needed. The extracted water and the groundwater samples will be analyzed for the parameters listed in ***Table 2***.

Table 2 Analytical Methods to Analyze Groundwater and Soil Leachate Samples

Parameter	Hach or EPA Method	Equipment
Chemical Oxygen Demand	8000	Spectrophotometer (Hach DR 5000)
Nitrate	10020	Test ‘N Tube™ Vials
Ammonia	10205	Spectrophotometer (DR 5000)
Perchlorate and chlorate	314.0	Ion Chromatograph (Dionex ICS-2000)
Phosphate	8048	Spectrophotometer (DR 5000)
Sulfate	8051	Spectrophotometer (DR 5000)
Total Iron	8008	Spectrophotometer (DR 5000)
Hexavalent Chromium	8023	DR-900 Hach Colorimeter
Total Nitrogen	10071	Spectrophotometer (DR 5000)
TDS	160.1	Conductivity meter
pH	8156	pH Electrode
Total Chromium	SM 3000	Thermo iCAP 6300 ICP-OES

Parameter	Hach or EPA Method	Equipment
Chloride	8225	Burette Titration
VOCs	8260B	Gas Chromatograph/Mass Spectrometer

3.2 BATCH MICROCOSMS

Batch microcosm tests will be conducted in two phases (Table 3). Phase 1 will determine the impacts of high concentrations of VOCs, particularly chloroform, on biodegradation kinetics for full-strength groundwater. Phase 2 will evaluate the impacts of high concentrations of perchlorate, chlorate, and TDS on the biodegradation kinetics. If elevated concentrations of VOCs are identified based on the results of the baseline groundwater analysis, treatment of some of the water through a column of granular activated carbon (GAC) will be implemented to eliminate the impact of VOCs (primarily chloroform) in batch and column tests. VOCs and some chromium will be removed in this process. Therefore, once the groundwater has passed through a GAC column, hexavalent chromium will be added, as necessary, to mimic the COPC composition of the UMCf groundwater prior to use in any test.

Table 3 Proposed Microcosm Test Matrix for Groundwater Collected from the Unit 4 Treatability Study Area

Sample Week	1	2	4	6	8	10	12	14	16	20	22	24
Phase 1 VOC Impacts	UMCf Groundwater											
	X	X	X	X	X	X	X	X	X	X		
	UMCf Groundwater Passed through GAC											
	X	X	X	X	X	X	X	X	X	X		
	UMCf Groundwater With Vitamin B-12											
	X	X	X	X	X	X	X	X	X	X		
	UMCf Groundwater With Biomass from FBR											
	X	X	X	X	X	X	X	X	X	X		
Phase 2 [A] Perchlorate, Chlorate, and TDS Impacts	1:1 Dilution of UMCf Groundwater to Distilled Water											
				X	X	X	X					
	1:2 Dilution of UMCf Groundwater to Distilled Water											
				X	X	X	X					
	1:3 Dilution of UMCf Groundwater to Distilled Water											
							X	X	X	X	X	X
	1:4 Dilution of UMCf Groundwater to Distilled Water											
							X	X	X	X	X	X
	Diluted UMCf Groundwater Blanks											
							1:1 [B]				1:2	1:3

[A] The results from Phase 1 will determine if Phase 2 batches will use GAC or Vitamin B-12 treated groundwater and whether FBR biomass will be added to Phase 2 batches.
[B] Indicates ratio of UMCf groundwater to distilled water.

Batch microcosm tests will be performed using blended soil and groundwater from the UMCf at Unit 4. For the microcosm tests, emulsified oil and a soluble substrate will be evaluated as the electron donor. Once baseline concentrations of perchlorate, nitrate, chromium, and chlorate are determined in the groundwater and aquifer solids, the amount of electron donor needed can be computed from stoichiometric biodegradation reactions. In-situ dissolved oxygen concentrations will be measured and included in the calculation of electron donor demand.

The baseline concentrations of groundwater nitrate and phosphate will be evaluated to determine whether there macronutrients are present in concentrations able to sustain biodegradation (based on typical bacterial cell composition of $C_5H_7O_2NP_{0.1}$). In the groundwater samples from the Units 4 and 5 investigation where nitrate was measured, the nitrate levels were low (averaging 8 mg/L). Based on the high concentration of COPCs present, nitrogen and phosphorus additions may be needed. A mixture of di-ammonium phosphate and urea will be used as a nutrient sources in the microcosm tests. Nutrient requirement calculations will assume a typical bacterial cell composition of $C_5H_7O_2NP_{0.1}$.

Each batch microcosm degradation test will include 30 g of wet UMCf soil. The soil will be transferred into autoclaved 125 ml borosilicate bottles. Depending on the moisture of the saturated zone soils, the volume of added groundwater will be adjusted to provide a consistent liquid: solid ratio. Approximately 100 mL of groundwater will be added to each bottle followed by enhancements of the calculated amount of oil,soluble carbon substrate and inorganic compounds like iron.

In some microcosms, groundwater will be mixed with biomass or supernatant from the existing FBRs currently operating at the Site. The objective of the addition of biomass or supernatant from the operating FBRs is to evaluate any decreased acclimation time and increased degradation kinetics. The amount of biomass or supernatant added will be determined by preliminary degradation tests.

An additional set of microcosms will include unaltered UMCf groundwater (i.e., with any VOCs) and Vitamin B-12 to evaluate the effectiveness of that additive. Once the microcosms containing soil, groundwater, biomass or supernatant, vitamins/nutrients, and carbon source are complete, the bottles will be closed with a butyl rubber cap and crimp-sealed with an aluminum ring. Bottles will be wrapped in aluminum foil and placed in a rotary shaker to mix at 21°C ± 1°C. At established time intervals, the bottles will be taken out of the shaker and opened. The contents of the bottles will be extracted for the COPCs using a combination of centrifugation and filtration. The bottle contents will be transferred to a refrigerated centrifuge tube and extracted. The extracted water will be placed in a labeled vial for analysis of chemical oxygen demand (COD) and COPCs. COD was selected as a surrogate analysis for total organic carbon (TOC) because COD analysis provides rapid results and eliminates the need to dilute a sample prior to analysis. In comparison, TOC analyses often require multiple sample dilutions to perform the analysis.

3.3 COLUMN STUDY

3.3.1 Evaluation of Biological Degradation Using Column Testing

The results of the microcosm tests will be used to determine the simulation conditions for the columns. The column tests will provide a greater degree of understanding of the potential issues that could arise during the full-scale implementation and potential engineering solutions. For example, the addition of a flow through column that incorporates actual aquifer materials, while simulating full-scale implementation more closely allows for the evaluation of:

- The sequence of contaminant degradation in a flow through system, a comparison of the flow through system performance with the data and observations from the batch microcosm, and projection of performance patterns for the full scale field implementation,
- A greater ability to assess the potential for secondary impacts, such as metals mobilization, if any;
- The impact of microbial activity on the permeability of the aquifer materials; and
- The projected and potential longevity of the carbon substrate.

These evaluations will be important in designing the full-scale implementation. This section describes the currently anticipated simulation conditions, which might be adjusted based on the results of the microcosm tests.

For acclimation purposes and study time, columns will start during week 6 of the microcosm tests and will first use 1:3 diluted groundwater. The groundwater dilution will be decreased based on the results of the microcosm tests. A set of columns (four) will be used to investigate biological reduction of chromium in UMCf soils. The proposed columns will be 2 inches in diameter. Two columns will start in week 6 and will use 1:3 diluted UMCf groundwater to decrease the impact of TDS and high concentrations of COPCs. The other two will use full-strength groundwater and will be introduced later, once the impact of dilution is known or if the other two columns clog. The results of the batch microcosm test will be used to determine the electron donor/carbon source to be used.

For the column tests, the soils from the UMCf collected from Unit 4 will be air dried for several days. Due to the high clay content of the UMCf soils and based on prior experience at UNLV, it will be necessary to break the dried clay clumps with a rubber hammer and screen the soil before it can be loaded to the columns. UNLV has tested and will utilize a pressure valve on the columns that, with pressures ranging from 5 to 30 pounds per square inch [psi], create enough flow through the column to conduct the tests. The packing will be performed to achieve a hydraulic conductivity close to that found at the field. At least two feet of soil will be placed in the column. Once packed, the columns will be connected to pressure pumps to saturate the columns (upflow conditions at 15 to 30 psi) with UMCf groundwater dosed with carbon source and nutrients.

Effluent flowrate will be analyzed daily. Chromium and nitrate will be measured daily. Perchlorate and chlorate will only be measured when nitrate concentrations are less than 1 mg/L, but earlier effluent samples (i.e., collected before nitrate concentrations are less than 1 mg/L) will be preserved for perchlorate analyses at a later date, if needed. Column effluent will be monitored based on the characterization performed in the baseline characterization (***Table 2***). Parameters to be monitored include chemical oxygen demand (COD), perchlorate, chlorate, nitrate, total chromium, hexavalent chromium, flowrate, and pH.

3.3.2 Microbiological Evaluation

The microbial ecology of the microcosm and column tests will be evaluated to determine which microbes are growing under the simulated field conditions. An initial, baseline test (see Table 2) will be conducted to establish the baseline population of perchlorate degrading bacteria. After eight weeks of incubation and again after twelve weeks, the contents of the sacrificed microcosms, including water and soils, will be subjected to a microbiological evaluation if evidence of biodegradation is observed. The material will be shipped to a commercial laboratory (Research and Testing Laboratories, Lubbock, Texas) for bacterial community analysis. Bacterial populations (numbers and diversity) will be evaluated using mRNA analyses. The presence and abundance of perchlorate reducing bacteria will be evaluated using primers published by Coates et al. (1999). Once the sequences are generated, the data undergo detection and removal of short, singleton, noisy and bad read sequences. The quality checked sequences will be clustered at a 4% divergence using the USEARCH clustering algorithm. The sequences will be identified using an in-house-maintained database that is derived from the National Center for Biotechnology Information (NCBI). The final result obtained will include the percentages for each organism identified down to species level.

The same procedure will be performed for the column effluent samples after week 12 and also for column soils after the experiment is completed. For both microcosm and columns, a total of eight samples will be submitted for microbiological analyses.

4.0 REPORTING

As discussed earlier in this document, this Unit 4 Source Area Bioremediation Treatability Study is being completed in two phases: the bench scale study and the field study. The Unit 4 Source Area Bioremediation Treatability Study report will include all aspects of the forthcoming treatability study work plan including the following items associated with the bench-scale studies as described herein:

- Summary of baseline soil and groundwater samples;
- Summary of microcosm results;
- Summary of column test results;
- Evaluation of the effects TDS, perchlorate, chromium, and chloroform have on perchlorate degradation kinetics;
- Evaluation of the benefits or need to add nutrients, vitamins, biomass or supernatant to enhance perchlorate degradation kinetics;
- Estimation of perchlorate degradation kinetics that are attainable in the field; and
- Evaluation of the technology's feasibility and effectiveness for scale-up to support the design of the treatability study.

During the bench-scale studies, monthly progress reports will be submitted to document the status of the tests (e.g., initiate batch tests, initiate column tests), preliminary data and any issues encountered and their resolution.

5.0 SCHEDULE

Table provides the general schedule for the primary activities and deliverables associated with implementing the bench-scale activities. This schedule is contingent upon approval of this Work Plan and Trust approval of funding/notice to proceed. The schedule may also be adjusted as necessary in coordination with other activities at the Site, such as the implementation of the third mobilization for the Unit 4 and Unit 5 Buildings Investigation.

Table 4 Preliminary Project Schedule

Task/Milestone	Estimated Start Date	Estimated Completion Date
NDEP/EPA/Stakeholder Review Work Plan	September 2017	October 2017
Finalize Work Plan	October 2017	October 2017
Conduct Bench-scale Testing	October 2017	April 2018

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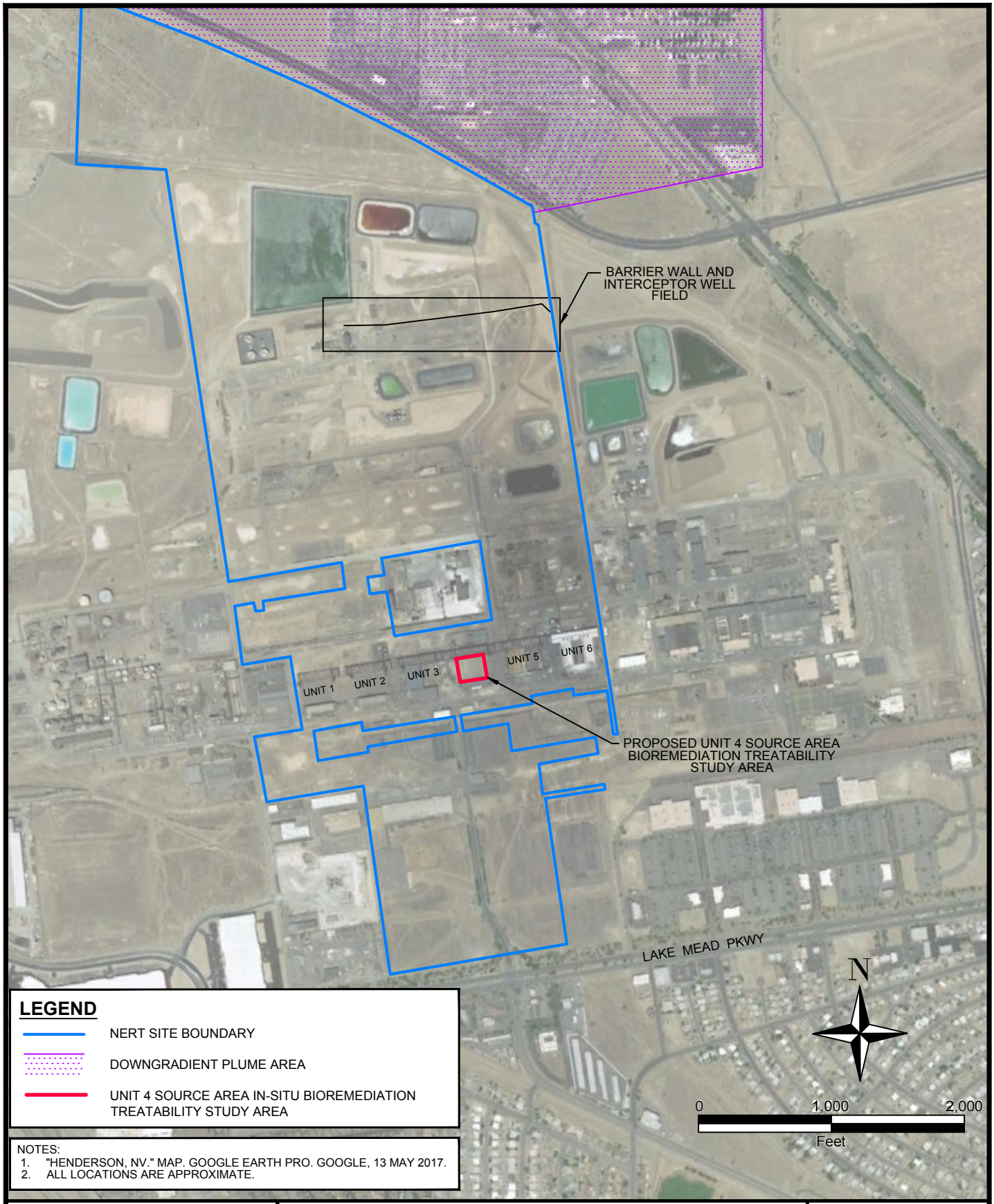
Figures



LEGEND

- NERT SITE BOUNDARY
- DOWNGRADIENT PLUME AREA
- UNIT 4 SOURCE AREA IN-SITU BIOREMEDIATION TREATABILITY STUDY AREA

NOTES:
1. "HENDERSON, NV." MAP. GOOGLE EARTH PRO. GOOGLE, 13 MAY 2017.
2. ALL LOCATIONS ARE APPROXIMATE.

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NEVADA ENVIRONMENTAL RESPONSE TRUST SITE

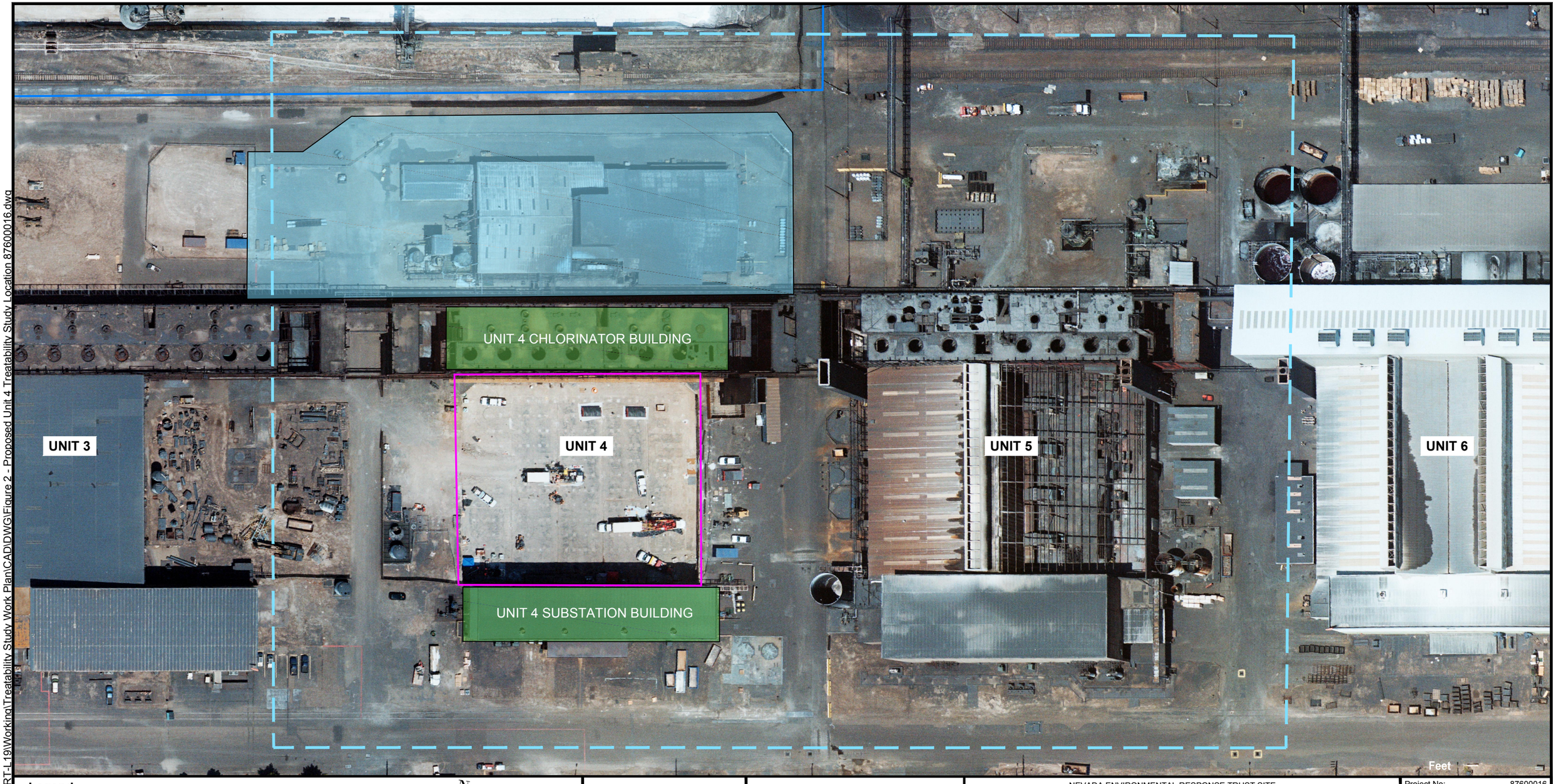
UNIT 4 SOURCE AREA IN-SITU BIOREMEDIATION TREATABILITY STUDY
BENCH-SCALE WORK PLAN

SITE LOCATION MAP

Project No:	87600016
Date:	August 18, 2017
Designed By:	PK
Figure No.	1

P:\87600016-NERT-L19\Working\Treatability Study Work Plan\CAD\DWG\Figure 1 - Site Location Map 87600016.dwg Aug 18, 2017 - 9:56am daniel.keady

P:\87600016-NERT-L19\Working\Treatability Study Work Plan\CAD\DWG\Figure 2 - Proposed Unit 4 Treatability Study Location 87600016.dwg



Legend

- UNIT 4 SOURCE AREA IN-SITU BIOREMEDIATION TREATABILITY STUDY AREA
- UNIT 4 & 5 BUILDING INVESTIGATION AREA
- NERT SITE BOUNDARY
- DEPARTMENT OF HOMELAND SECURITY RESTRICTED AREA
- EXISTING UNIT 4 BUILDING (NOT TO BE DEMOLISHED). ACTIVE TREATMENT AREA

N

0 80 160

Feet

Notes:
1. All locations are approximate.
2. Imagery Source: Aerotech Mapping, August 2016.

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NEVADA ENVIRONMENTAL RESPONSE TRUST SITE

UNIT 4 SOURCE AREA IN-SITU BIOREMEDIATION TREATABILITY STUDY
BENCH-SCALE WORK PLAN

PROPOSED UNIT 4 SOURCE AREA IN-SITU BIOREMEDIATION TREATABILITY STUDY LOCATION

Project No:	87600016
Date:	August 18, 2017
Designed By:	PK

Figure No.

2