

Dehalococcoides-containing microbial consortium (SDC-9TM) for anaerobic bioremediation

1. Dr. Robert J. Steffan, CB&I Federal Services, LLC. (formerly Shaw Environmental, Inc.)
2. Composed of anaerobic bacteria including *Dehalococcoides mccartii* in an aqueous medium.
3. MSDS and Technical Data Sheets: attached
4. Number of Field-scale Applications to Date: 650+ applications
5. Case Studies – Attached.
6. Technical Summary. The SDC-9TM culture is a pathogen-free, non-genetically altered microbial consortium capable of biologically degrading halogenated aliphatic pollutants including 1,1,1-TCA, 1,1,2-TCA, 1,1-DCA, 1,2-DCA, 1,2-DBE, TeCA, Carbon tetrachloride, Chloroform, PCE, TCE, DCE, VC, and Freon 113 (1,2-dichloro-1,2-difluoroethane), and also mixtures thereof. Molecular biological analyses of the SDC-9 culture has demonstrated that the culture has at least three closely-related strains of *Dehalococcoides* sp. bacteria which is the only bacterial genus known to degrade chlorinated ethenes completely to ethene. In addition, the culture contains other known pollutant degrading bacteria including *Desulfotobacterium* and *Desulfovibrio* strains. It has now been successfully applied more than 600 times, and 100,000 L have been delivered to sites throughout the United States. The culture has been applied commercially since 2003, and it is sold by licensed distributors under several trade names including RTB-1TM, BAC-9TM, TSI-DCTM, and BDIplusTM.

Material Safety Data Sheet

SECTION 1 – CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Product Name: DHC microbial consortium (SDC-9)

Manufacturer CB&I 17 Princess Road, Lawrenceville,
NJ 08648. Phone (609) 895-5340

CAS #: N/A (Not Applicable)

Product Use: For remediation of contaminated groundwater (environmental applications).

Material Description: Non-toxic, naturally occurring, non-pathogenic, non-genetically altered anaerobic microbes in a water-based medium.

IN CASE OF EMERGENCY CALL CHEMTREC 24 HOUR EMERGENCY RESPONSE PHONE NUMBER (800) 424-9300

SECTION 2 – COMPOSITIONS AND INFORMATION ON INGREDIENTS

| Components | % | OSHA PEL | ACGIH TLV | OTHER LIMITS |
|---------------------------|-----|-------------|--------------|-----------------|
| Non-Hazardous Ingredients | 100 | N/A | N/A | N/A |

DHC microbial consortium (SDC-9) comprised of microorganism of the genus *Dehalococcoides*, *Desulfovibrio*, and *Desulfitobacterium*, and methanogenic archebacteria.

SECTION 3 – HAZARDS IDENTIFICATION

The available data indicates no known hazards associated with exposure to this product. Nevertheless, individuals who are allergic to enzymes or other related proteins should avoid exposure and handling. Health effects associated with exposure to similar organisms are listed below.

Ingestion: Ingestion of large quantities may result in abdominal discomfort including nausea, vomiting, cramps, diarrhea, and fever.

Inhalation: Hypersensitive individuals may experience breathing difficulties after inhalation of aerosols.

Skin Absorption: May cause irritation upon prolonged contact. Hypersensitive individuals may experience allergic reactions..

Eye contact: May cause irritation unless immediately rinsed.

SECTION 4 – FIRST AID MEASURES

Ingestion: Thoroughly rinse mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Get immediate medical attention. Never give anything by mouth to an unconscious or convulsing person.

Inhalation: Get medical attention if allergic symptoms develop.

Skin Absorption: N/A

Skin Contact: Wash affected area with soap and water. Get medical attention if allergic symptoms develop.

Eye Contact: Flush eyes with plenty of water for at least 15 minutes using an eyewash fountain, if available. Get medical attention if irritation occurs.

NOTE TO PHYSICIANS: All treatments should be based on observed signs and symptoms of distress in the patient. Consideration should be given to the possibility that overexposure to materials other than this material may have occurred.

SECTION 5 – FIRE AND EXPLOSION DATA

Flammability of the Product: Non-flammable

Flash Point: N/A

Flammable Limits: N/A

Fire Hazard in Presence of Various Substances: N/A

Explosion Hazard in Presence of Various Substances: N/A

Extinguishing Media: Foam, carbon dioxide, water

Special Fire Fighting Procedures: None

Unusual Fire and Explosion Hazards: None

SECTION 6 – ACCIDENTAL RELEASE MEASURES

Reportable quantities (in lbs of EPA Hazardous Substances): N/A

No emergency results from spillage. However, spills should be cleaned up promptly. Absorb with an inert material and put the spilled material in an appropriate waste disposal container. All personnel involved in the cleanup must wear protective clothing and avoid skin contact. After clean-up, disinfect all cleaning materials and storage containers that come in contact with the spilled liquid.

SECTION 7 – HANDLING AND STORAGE

Avoid breathing breathe aerosol. Avoid contact with skin. Use personal protective equipment recommended in Section 8.

Keep containers tightly closed in a cool, well-ventilated area. The DHC microbial consortium (SDC-9) can be supplied in stainless steel kegs designed for maximum working pressure of 130 psi and equipped with pressure relief valves. The kegs are pressurized with Nitrogen up to the pressure of 15 psi. Do not exceed pressure of 15 psi during transfer of DHC microbial consortium (SDC-9) from kegs. Don't open keg if content of the keg is under pressure.

DHC microbial consortium (SDC-9) may be stored for up to 3 weeks at temperature 2-4°C without aeration. Avoid freezing.

SECTION 8 – EXPOSURE CONTROLS/PERSONAL PROTECTION

Hand Protection: Rubber, nitrile, or vinyl gloves.

Eye Protection: Safety goggles or glasses with side splash shields.

Protective Clothing: Use adequate clothing to prevent skin contact.

Respiratory Protection: N95 respirator if aerosols might be generated.

Ventilation: Provide adequate ventilation to remove odors.

Other Precautions: An eyewash station in the work area is recommended.

SECTION 9 – PHYSICAL/CHEMICAL CHARACTERISTICS

Physical state and appearance: Light greenish murky liquid. Musty odor.

Boiling Point: 100°C (water)

Specific Gravity (H₂O = 1): 0.9 - 1.1

Vapor Pressure @ 25°C: 24 mm Hg (water)

Melting Point: 0°C (water)

Vapor Density: N/A

Evaporation Rate (H₂O = 1): 0.9 - 1.1

Solubility in Water: Soluble

Water Reactive: No

pH: 6.0 - 8.0

SECTION 10 – STABILITY AND REACTIVITY DATA

Stability: Stable

Conditions to Avoid: None

Incompatibility (Materials to Avoid): Water-reactive materials

Hazardous Decomposition Byproducts: None

SECTION 11 – TOXICOLOGICAL INFORMATION

This product contains no toxic ingredients.

SDC-9 consortium has tested negative for pathogenic microorganisms such as *Bacillus cereus*, *Listeria monocytogens*, *Salmonella* sp., Fecal Coliform, Total Coliform, Yeast and Mold and *Pseudomonas* sp.

SECTION 12 – ECOLOGICAL INFORMATION

Ecotoxicity: this material will degrade in the environment.

SECTION 13 – DISPOSAL CONSIDERATIONS

Waste Disposal Method: No special disposal methods are required. The material is compatible with all known biological treatment methods. To reduce odors and permanently inactivate microorganisms, mix 100 parts (by volume) of SDC-9 consortium with 1 part (by volume) of bleach. Dispose of in accordance with local, state and federal regulations.

SECTION 14 – TRANSPORT INFORMATION

DOT Classification: N/A
Labeling: NA
Shipping Name: Not regulated

SECTION 15 – REGULATORY INFORMATION

Federal and State Regulations: N/A

SECTION 16 – OTHER INFORMATION

MSDS Code: ENV 1033
MSDS Creation Date: 10/06/2003
Last Revised: April 30, 2013.

While the information and recommendations set forth herein are believed to be accurate as of the date hereof, CB&I MAKES NO WARRANTY WITH RESPECT HERETO AND DISCLAIMS ALL LIABILITY FROM RELIANCE THEREON.



SDC-9 Technical Data

Robert J. Steffan, Ph.D.
CB&I Federal Services, LLC
(Formerly, Shaw Environmental, Inc.)



Isolated in 2002

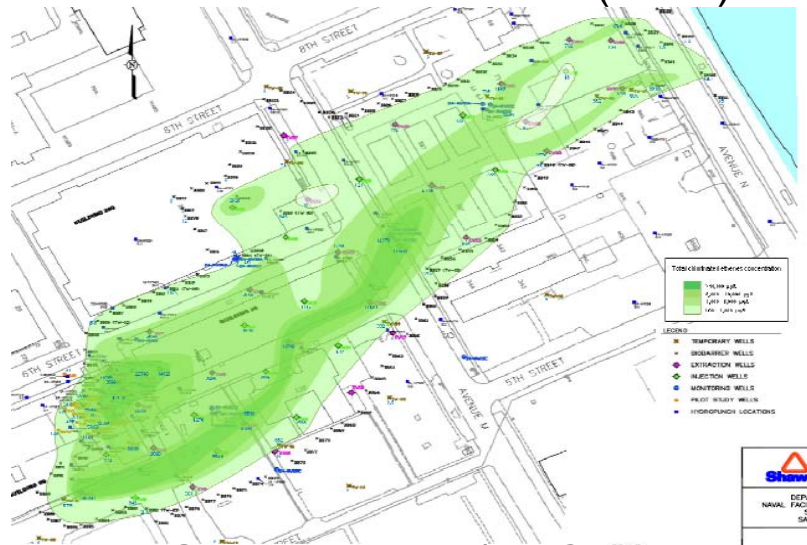
Enrichment culturing with samples from North Island Naval Station, CA Site 9

Grown exclusively on Lactate plus PCE with trace amounts of Yeast Extract

Grown under strict anaerobic conditions

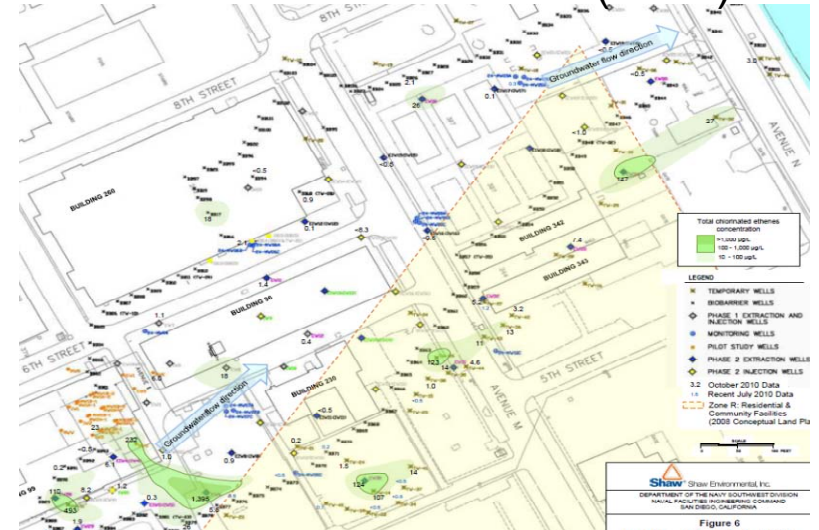
First commercial-scale application – Treasure Island, CA Site 24– October 6, 2003

Before Biotreatment (2003)



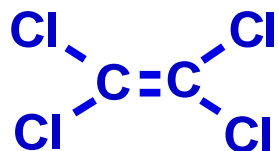
Dark Green >10 mg/L TCE

After Biotreatment (2010)

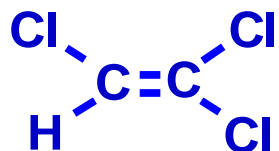




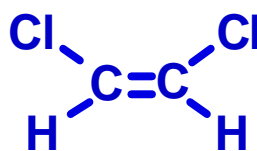
Pollutants Degraded by SDC-9™



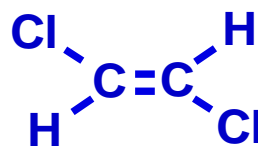
PCE



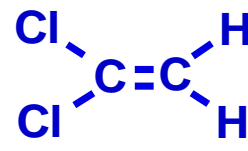
TCE



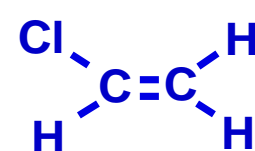
cDCE



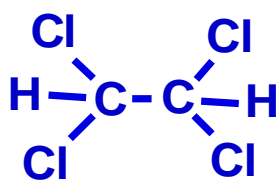
tDCE



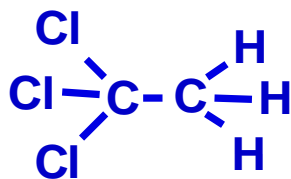
1,1-DCE



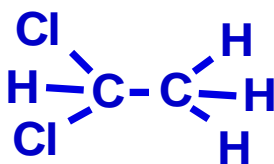
VC



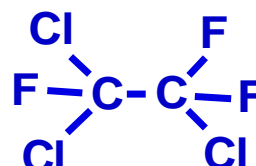
1,1,2,2-TeCA



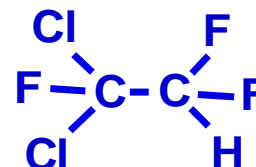
1,1,1-TCA



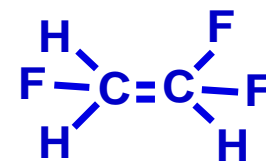
1,1-DCA



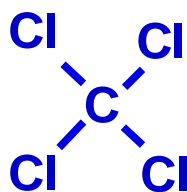
Freon 113



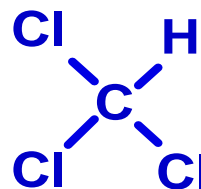
HCFC



TFE



CT



CF



SDC-9 Vendors and Trade Names



EOS Remediation, Inc.

REDOX TECH, LLC



Terra Systems



REGENESIS

Advanced Technologies for Groundwater Resources

JRW *BIOREMEDIATION* LLC

SDC-9™

SDC-9™

Bac-9™

RTB-1™

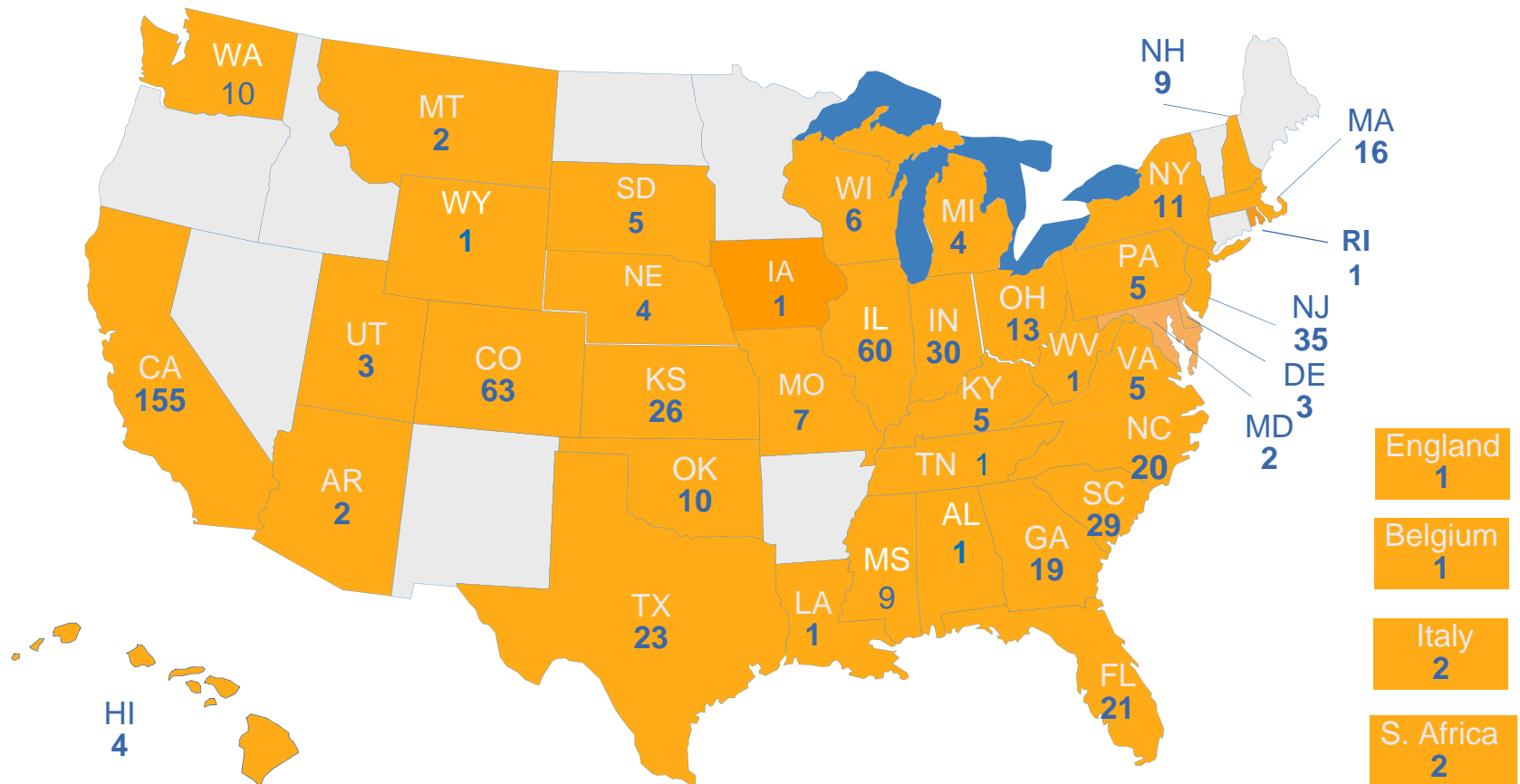
TSI-DC™

BDIplus™

SDC-9™



Bioaugmentation Culture Applications*



Includes SDC-9™,
PJKS™, and Hawaii-05™

Total Applications: 641

Total Volume Delivered: ~106,206 L

* Data represent culture deliveries as of 9/24/13 and include licensed culture distributors

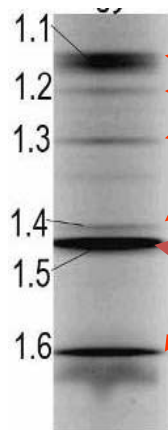


Gene Library Analysis by CDM et al.

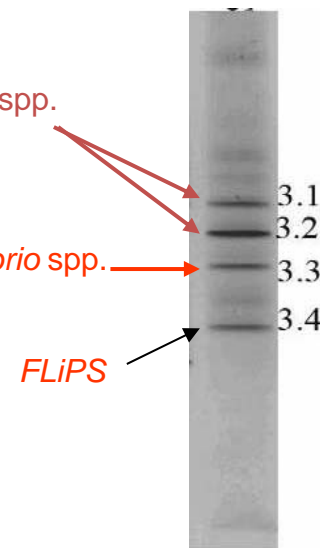
- 4 *Dehalococcoides* strains
- *vcrA* present
 - Most closely related to Strain VS *vcrA*
 - >99% sequence similarity
- *bvcA* not present

DGEE Analysis of SDC-9

1,1,1-TCA Grown



PCE Grown



Dehalococcoides sp. – Common in cVOC-contaminated groundwater – dechlorinate DCE and VC to ethene

Desulfovibrio spp. – Common groundwater microbes – reduce sulfate, may dechlorinate PCE and TCE

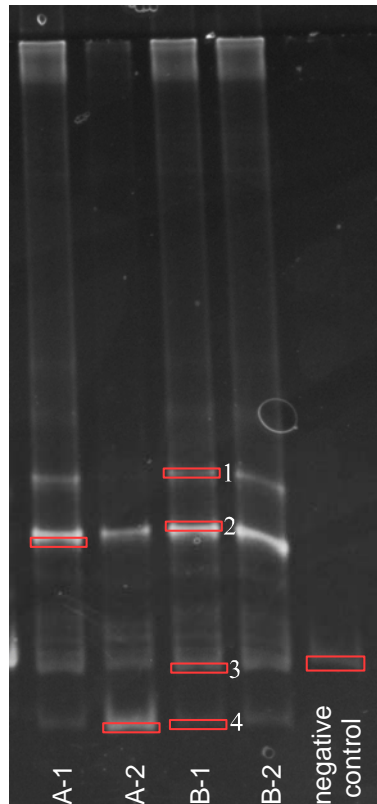
Desulfitobacterium spp. – Common groundwater microbes – ferment, may dechlorinate PCE and TCE

FLiPS – Common in DHC consortia - free living polymorphic spirochaetes- believed to ferment. Not recently detected in SDC-9

Also contains Methanogens



DGGE Analysis of Carbon Tet-Grown SDC-9



| Band | ID | E value | Identities |
|------------------|---|----------|---|
| A-1 | Uncultured bacterium | 3.00E-75 | 188/202 (93%) Community Uranium Reduction and Reoxidation |
| A-2 | Uncultured bacterium | 3.00E-84 | 172/172 (100%) Anaerobic Polychlorinated Biphenyl Dechlorinating Consortia |
| B-1, band 1 | <i>Bacteroidales</i> bacterium | 1.00E-93 | 189/189 (100%) Dehalococcoides Population Dechlorinating PCB Mixture Aroclor 1260 |
| B-1, band 2 | Same as A-1 | | |
| B-1, band 3 | Uncultured bacterium | 2.00E-97 | 196/196 (100%) Polychlorinated-dioxin-dechlorinating microbial community |
| B-1, band 4 | Same as A-2 | | |
| negative control | <i>Shigella boydii</i> , <i>E. coli</i> | 5.00E-98 | 197/197 (100%) |



SDC-9 Pathogen Analysis



Microbac Laboratories, Inc.

Baltimore Division
2101 Van Deman Street • Baltimore, MD 21224

Phone: 410-633-1800
Fax: 410-633-6553
www.microbac.com

CERTIFICATE OF ANALYSIS

SHAW ENVIRONMENTAL & INFRA.
17 PRINCESS ROAD
LAWRENCEVILLE, NJ 08648

Project: CONSORTIUM SAMPLES
Project Number: CONSORTIUM SAMPLES
Project Manager: SIMON VAINBERG

Report: 12C0784
Reported: 03/20/2012 13:37

SDC-9

12C0784-01 (Water) Sampled: 03/13/2012 00:00; Type: Not Specified

| Analyte | Result | Reporting Limit | Units | Prepared | Analyzed | Analyst | Method | Notes |
|---------|--------|--------------------|-------|----------|----------|---------|--------|-------|
|---------|--------|--------------------|-------|----------|----------|---------|--------|-------|

Microbac Laboratories, Inc., Baltimore Division

Microbiology

| | | | | | | | | |
|-----------------------|----------|-----|---------|-------------|-------------|-----|--------------|--|
| Bacillus cereus | ND | 3.0 | CFU/g | 031312 1103 | 031712 1600 | JAT | AOAC 980.31 | |
| Coliform, Total | ND | 3.0 | MPN/g | 031312 1114 | 031512 0945 | DML | FDA-BAM | |
| E. Coli | ND | 3.0 | MPN/g | 031312 1114 | 031512 0945 | DML | FDA-BAM | |
| Fecal Coliform | ND | 3.0 | MPN/g | 031312 1130 | 031512 0945 | DML | FDA-BAM | |
| Listeria monocytogens | NEGATIVE | | per 25g | 031312 1120 | 031612 0935 | JAT | AOAC 2003.12 | |
| Salmonella | NEGATIVE | | per 25g | 031312 1122 | 031512 0630 | DML | AOAC 2003.09 | |
| Yeast and Mold | ND | 10 | CFU/g | 031312 1123 | 031812 1140 | JAT | FDA-BAM | |

Microbac Laboratories, Inc., Central Pennsylvania

MICROBIOLOGY

| | | | | | | | | |
|-------------|----|----|-------|-------------|-------------|-----|-----------|--|
| Pseudomonas | ND | 10 | CFU/g | 031412 1845 | 031612 1600 | GLF | ISO 13720 | |
|-------------|----|----|-------|-------------|-------------|-----|-----------|--|

Testing performed at least annually; Data available from 2005 8

| | |
|--|---|
|  Shaw ® Shaw Environmental, Inc. | 17 Princess Road Lawrenceville, NJ 08691 (609) 895-5340 Fax (609) 895-1858 |
| <p align="center">CERTIFICATE OF QUALITY</p> <p align="center">Batch # JS70730-1 (04/04/2011)</p> | |

| Test | Results | Date | Method |
|--|----------|----------|--------------|
| DHC content of Pre-concentrated culture, copies/L | 3.90E+11 | 4/6/2011 | qPCR |
| DHC content of Concentrated culture, copies/L | 6.00E+12 | 4/6/2011 | qPCR |
| PCE dechlorination activity, mg/h per gram of dry weight | 240 | 4/4/2011 | Bottle Assay |
| cDCE dechlorination activity, mg/h per gram of dry weight | 201 | 4/4/2011 | Bottle Assay |

This certificate has been reviewed and is signed by:



Robert J. Steffan, Ph.D.
 Director,
 Biotechnology Development and Applications Group
 Telephone: (609) 895-5350

- Stedtfeld, R.D., T.M. Stedfeld, M. Kronlein, G. Seyrig, **R.J. Steffan**, A.M. Cupples, and S.A Hashsham. DNA-extraction free quantification of *Dehalococcoides* spp. in groundwater using a hand-held device. In press.
- Steffan, R. J.** and S. Vainberg. 2013. Production and handling of *Dehalococcoides* bioaugmentation cultures. pp. 89-113 in, H.F. Stroo, A. Leeson, and C.H. Ward (eds) *Bioaugmentation for Groundwater Remediation*. Springer Science+Business Media, New York..
- Stroo, H .F., D. W. Major, **R. J. Steffan**, S. S. Koenigsberg, C. H. Ward. 2013. Bioaugmentation with *Dehalococcoides*: A decision guide. pp. 117-140 in, H.F. Stroo, A. Leeson, and C.H. Ward (eds) *Bioaugmentation for Groundwater Remediation*. Springer Science+Business Media, New York..
- Aziz, C., R.Wymore, and **R. Steffan**. 2013. Bioaugmentation considerations. pp. 141-169 in, H.F. Stroo, A. Leeson, and C.H. Ward (eds) *Bioaugmentation for Groundwater Remediation*. Springer Science+Business Media, New York..
- Schaefer, C.E., D. R. Lippincott, and **R. J. Steffan**. 2010. Field-scale evaluation of bioaugmentation dosage for treating Chlorinated ethenes. Ground Water Monitor. Remediat. 30:113-124.
-
- Schaefer, C.E., R.M. Towne, S. Vainberg, J.E. McCray, and **R.J. Steffan**. 2010. Bioaugmentation for treatment of dense non-aqueous phase liquid in fractured sandstone blocks. Environ. Sci. Technol. 44:4958-4964.
- Schaefer, C. E., S. Vainberg, C. Condee, **R.J. Steffan**. 2009. Bioaugmentation for chlorinated ethenes using *Dehalococcoides* sp.: Comparison between batch and column experiments. Chemosphere 75:141-148.
- Vainberg, S., C.W. Condee, **R.J. Steffan**. 2009. Large scale production of *Dehalococcoides* sp.-containing cultures for bioaugmentation. J. Indust. Microbiol. Biotechnol. 36:1189-1197.



SDC-9™ Case Studies

Site Characteristics

- TCE from 100 – 10,000 µg/L
- Little or no cis-1,2-DCE, VC

- DO >1 mg/L
- ORP >200 mV
- pH between ~4.5 – 6.5
- GW velocity ~ 150ft/yr

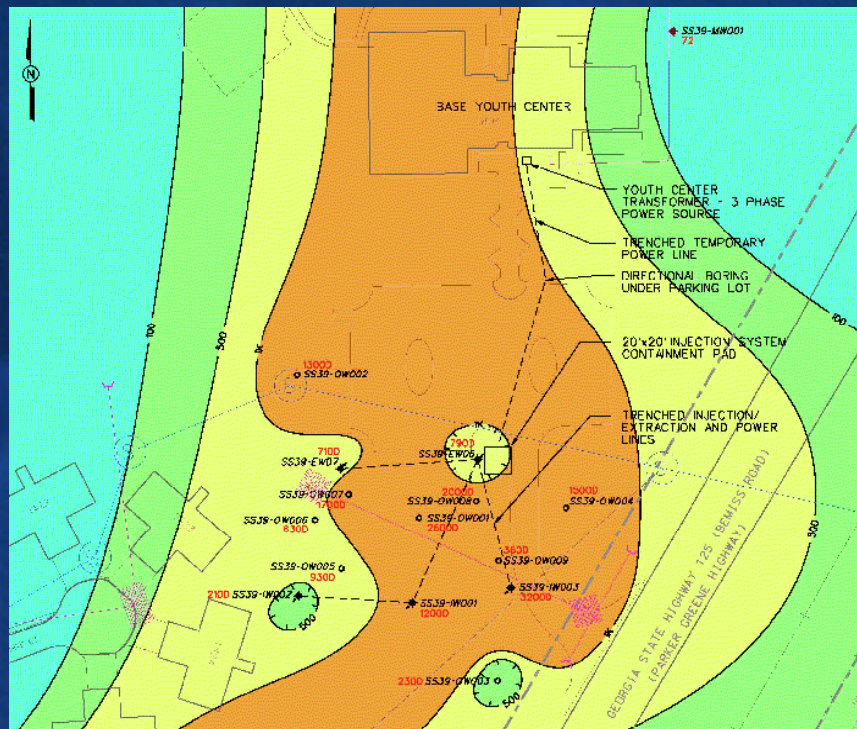
- TCE/DCE 50 – 1,000 µg/L



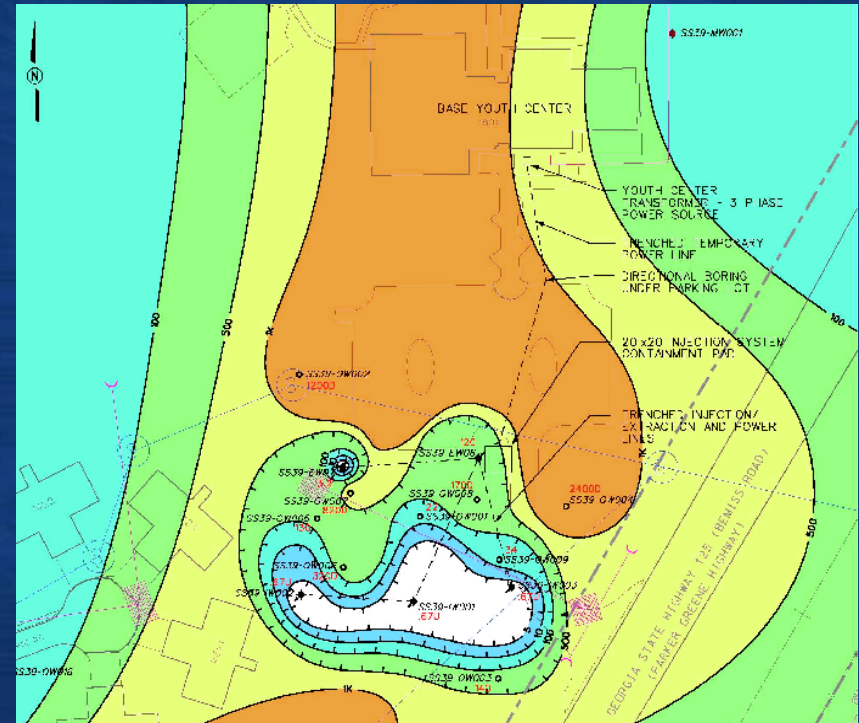
Golf Course Area

- TCE >500 ppb
- Recirculation
- Lactate
- Bioaugmentation with SDC-9

Golf Course Area (SS-39) Pilot Study Results



**TCE concentrations in DEC 03
(prior to system start-up)**



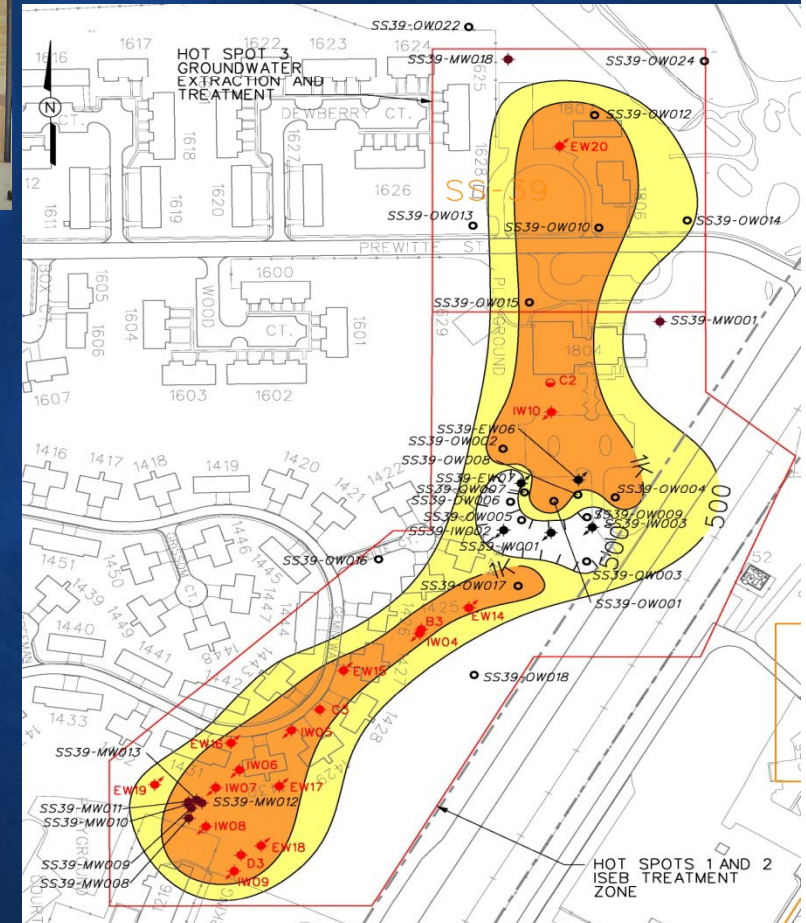
**TCE concentrations in JAN 05
(two years post system start-up)**

Golf Course Area (SS-39) Expanded Groundwater Recirculation System

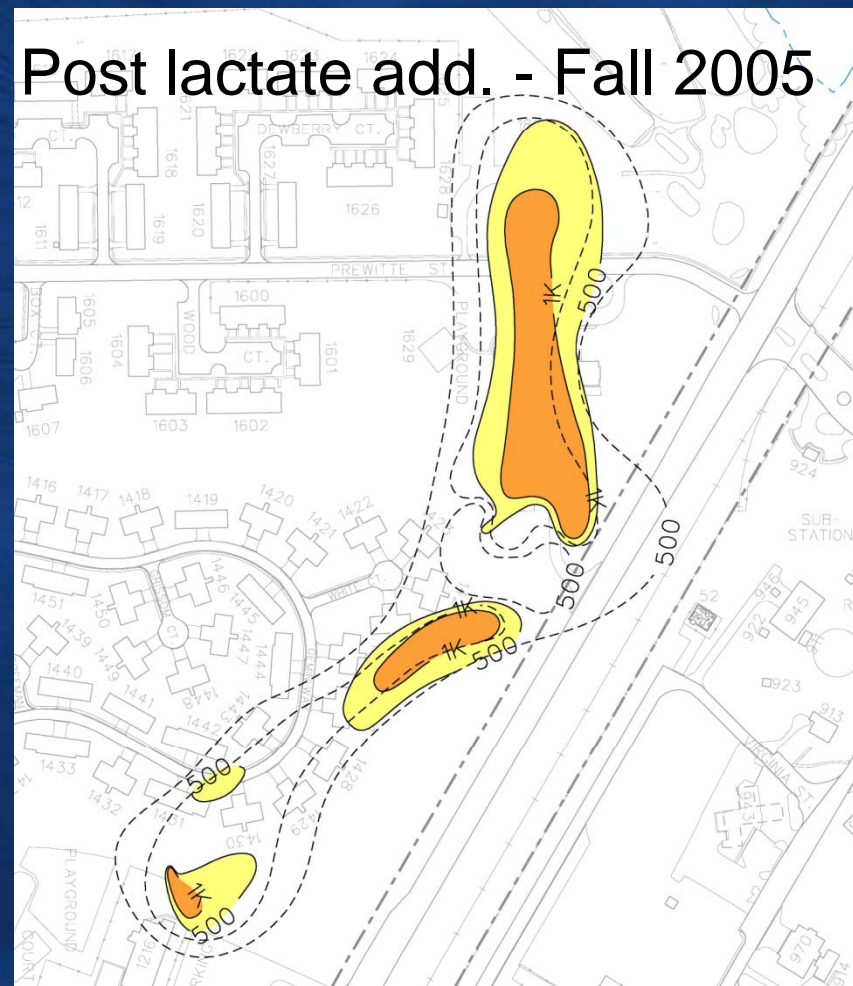
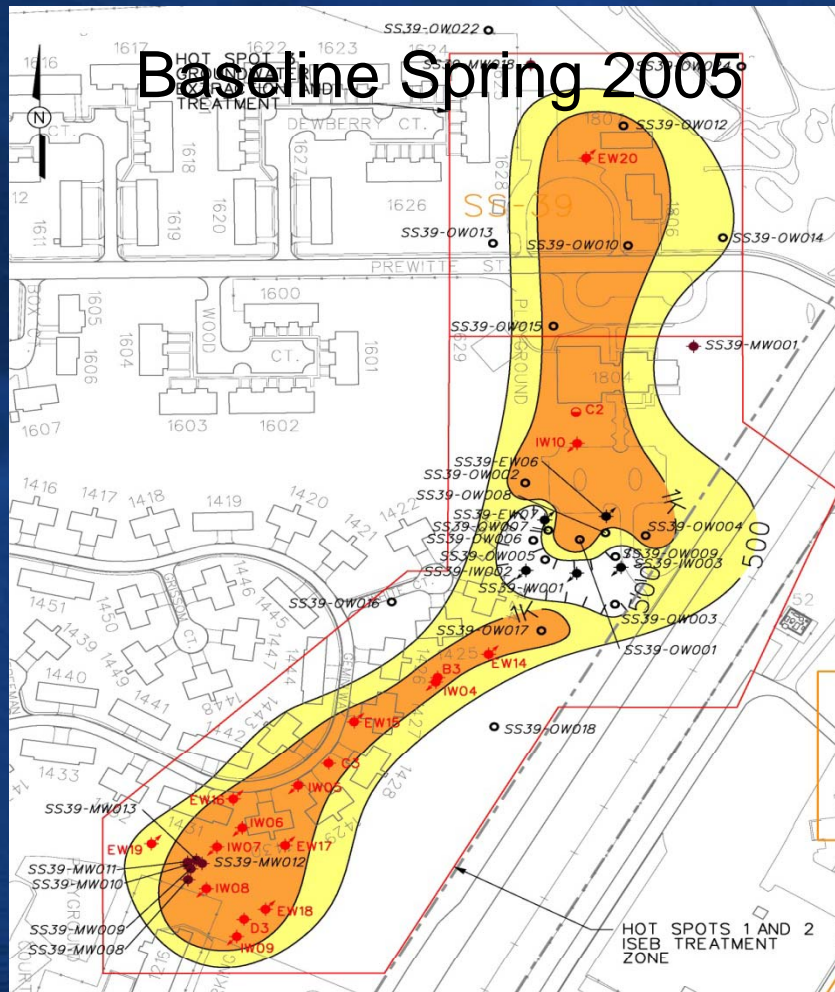


Expanded System

- TCE > 500 µg/L
- 10 Injection Wells
- 8 Extraction Wells
- Carbon Source Sodium Lactate
- Bioaugmentation SDC-9



Golf Course Area (SS-39) Results



System currently shut down – No further action required

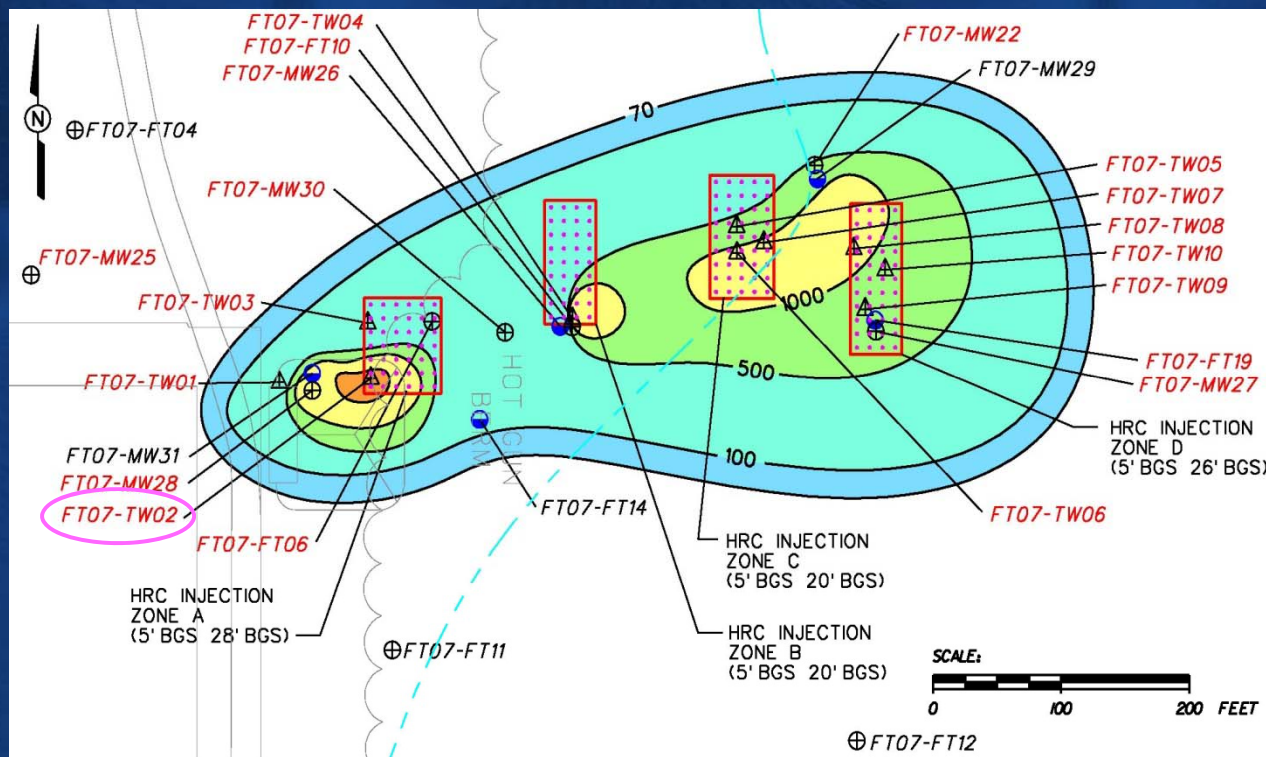
Fire Training Area

- **Passive Treatment**
- **Lactate**
- **Bioaugmentation with SDC-9**

Former Fire Training Area (FT-07)

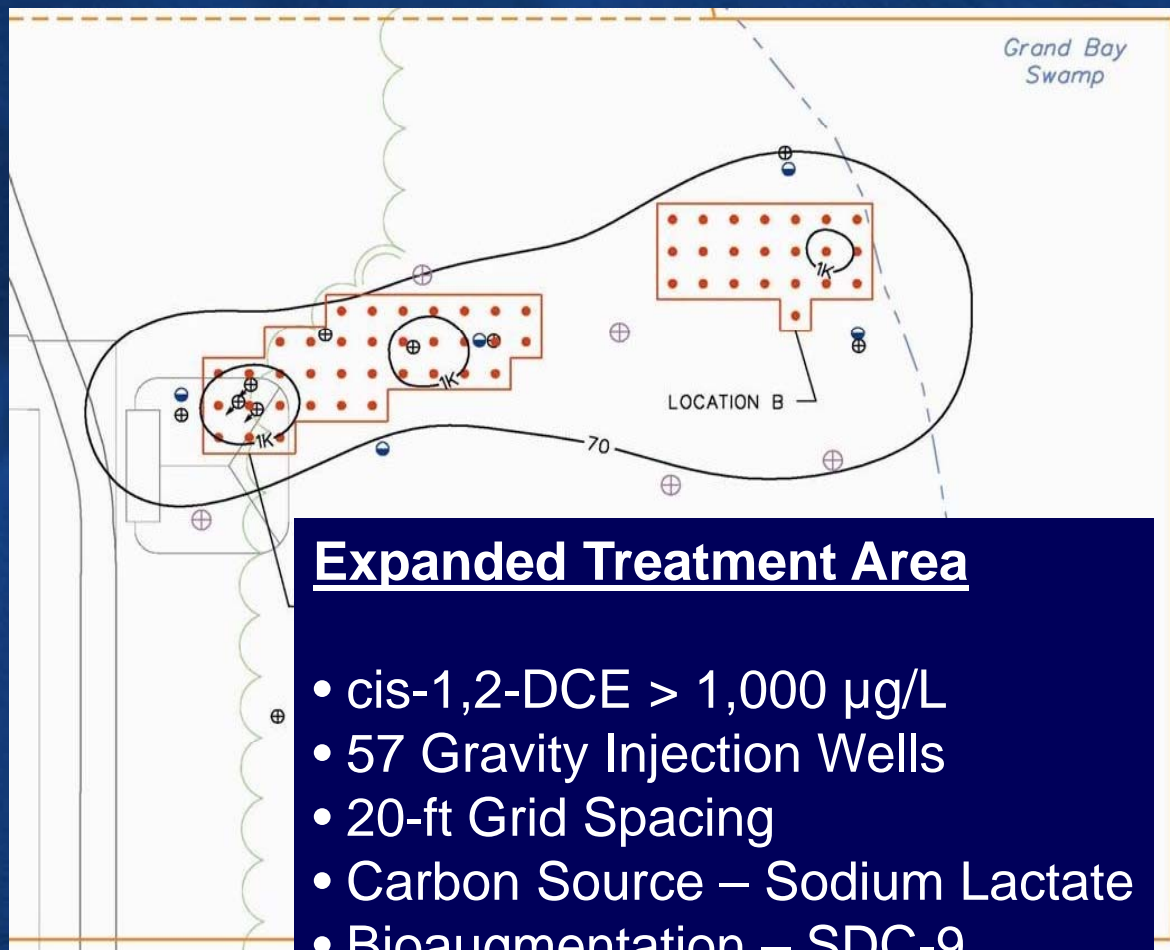
Anaerobic Bioremediation Pilot Study

A pilot study was initiated in November 2002 to evaluate direct injection and distribution via ambient groundwater flow.

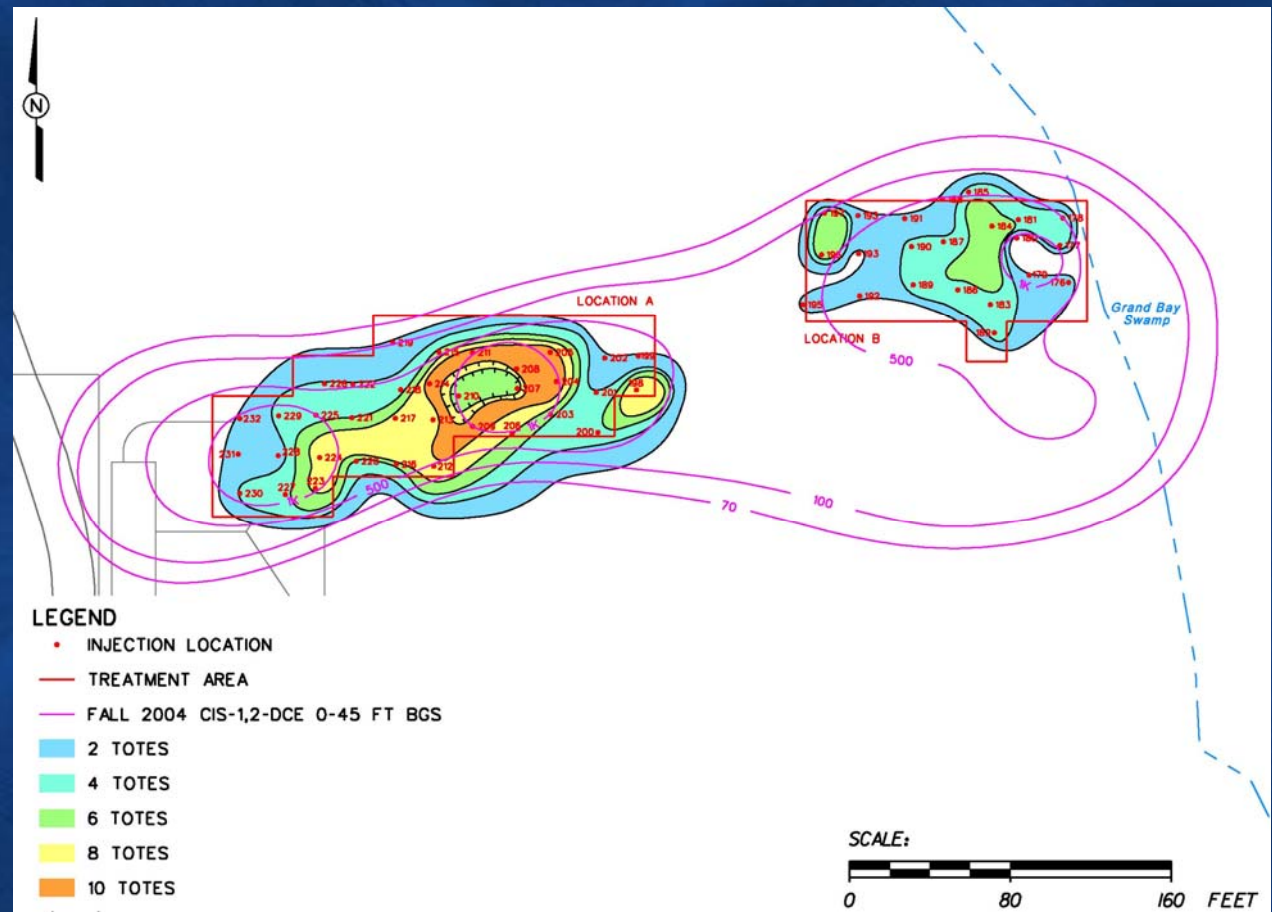


- HRC injection
November 2002
- HRC injection
December 2003
- Bioaugmentation
June 2004

Former Fire Training Area (FT-07) Expanded Passive Delivery System



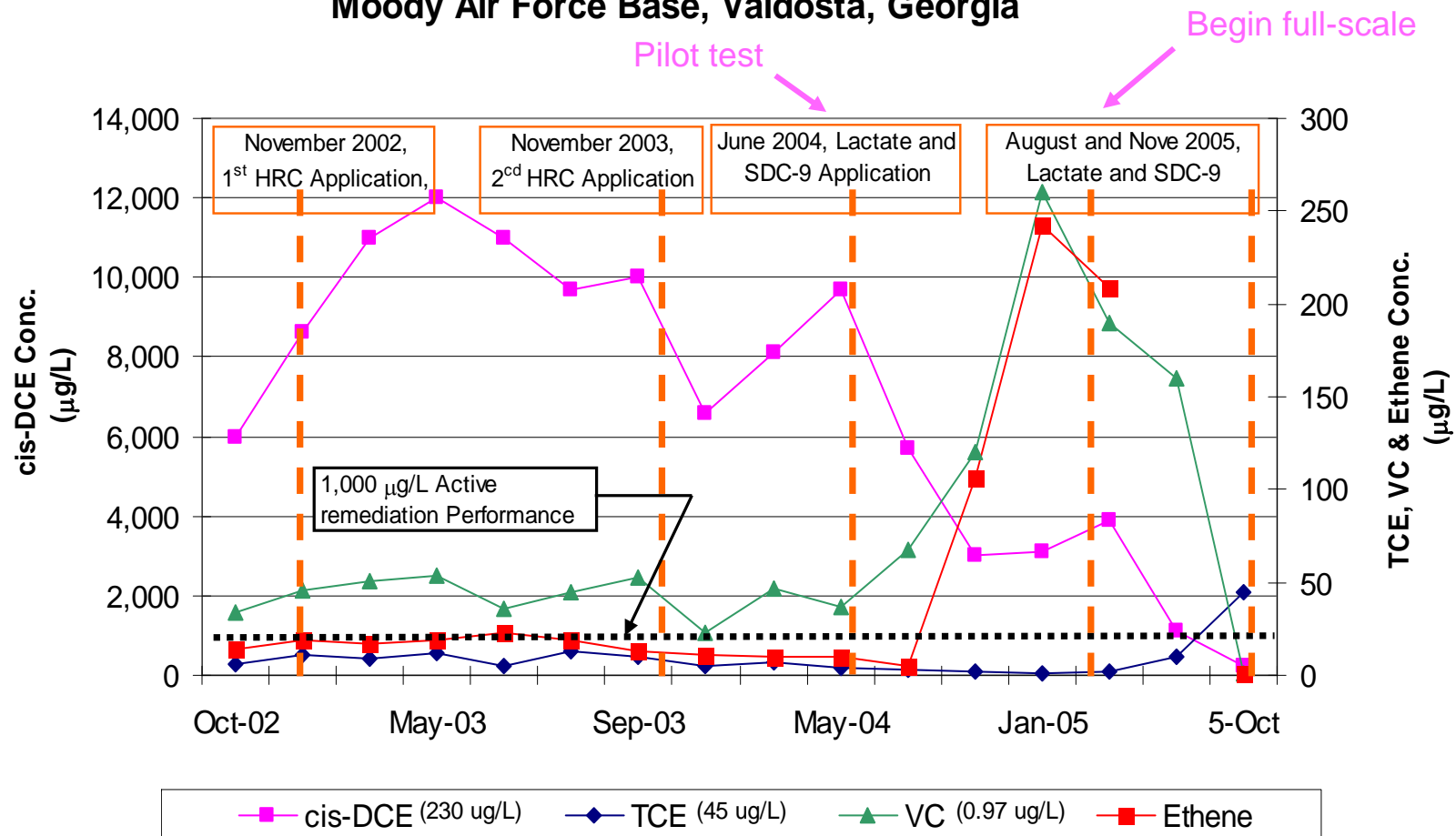
Former Fire Training Area (FT-07) Lactate Distribution via Gravity Feed



Former Fire Training Area (FT-07) Pilot Study Results

VOC and Ethene Trends in Well TW02 at FT-07

Moody Air Force Base, Valdosta, Georgia



Flight Line Storm Drain Area

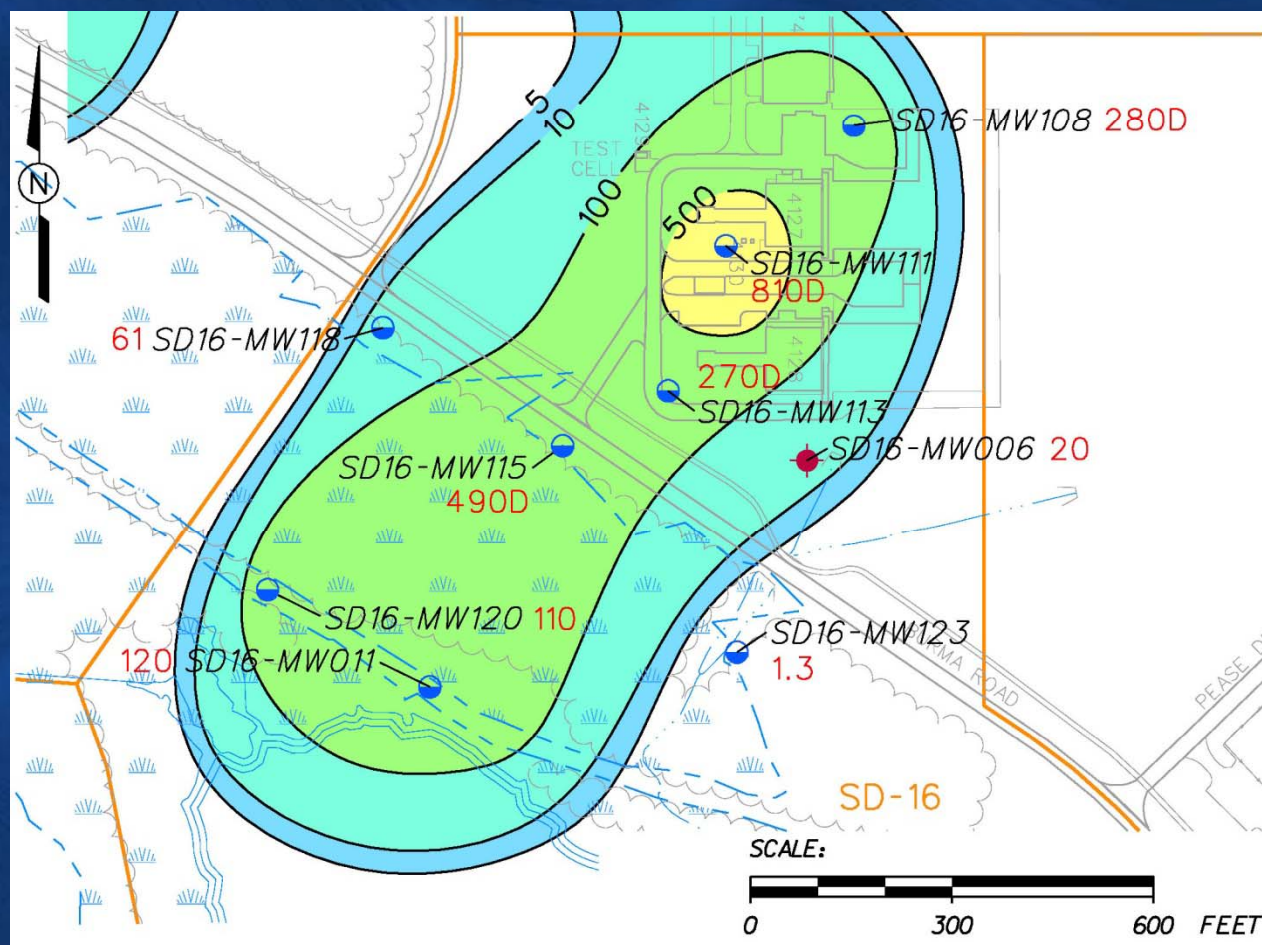
- **Passive Treatment**
- **Veg. Oil**
- **Bioaugmentation with SDC-9**

Flightline Storm Drain Outfall & Mission Lake (SD-16) Full-Scale Anaerobic Bioremediation

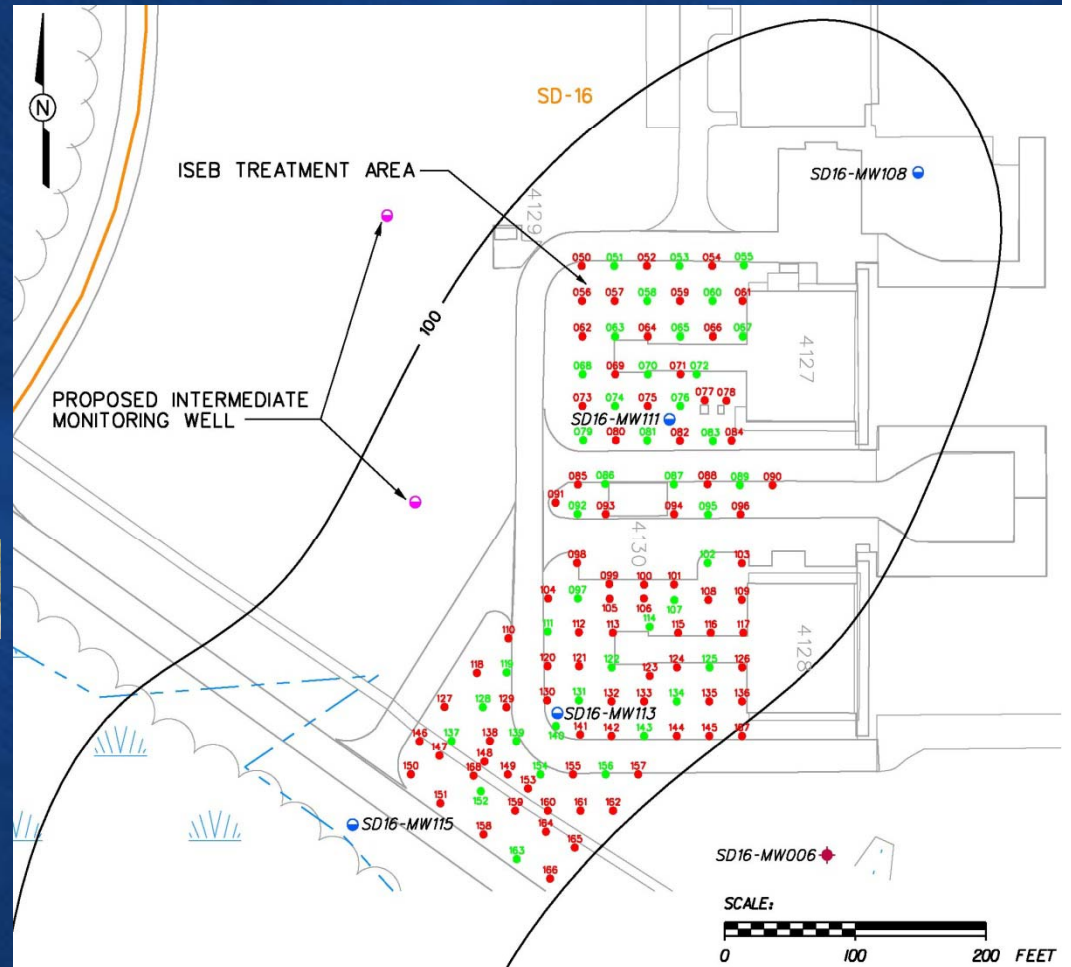
Treatment Area

- TCE > 100 µg/L
- 118 Injection Wells
- 25-ft Grid Spacing
- Carbon Source
Emulsified Oil
- pH Buffer
Sodium Bicarbonate
- Bioaugmentation
SDC-9

Track # 2900 L

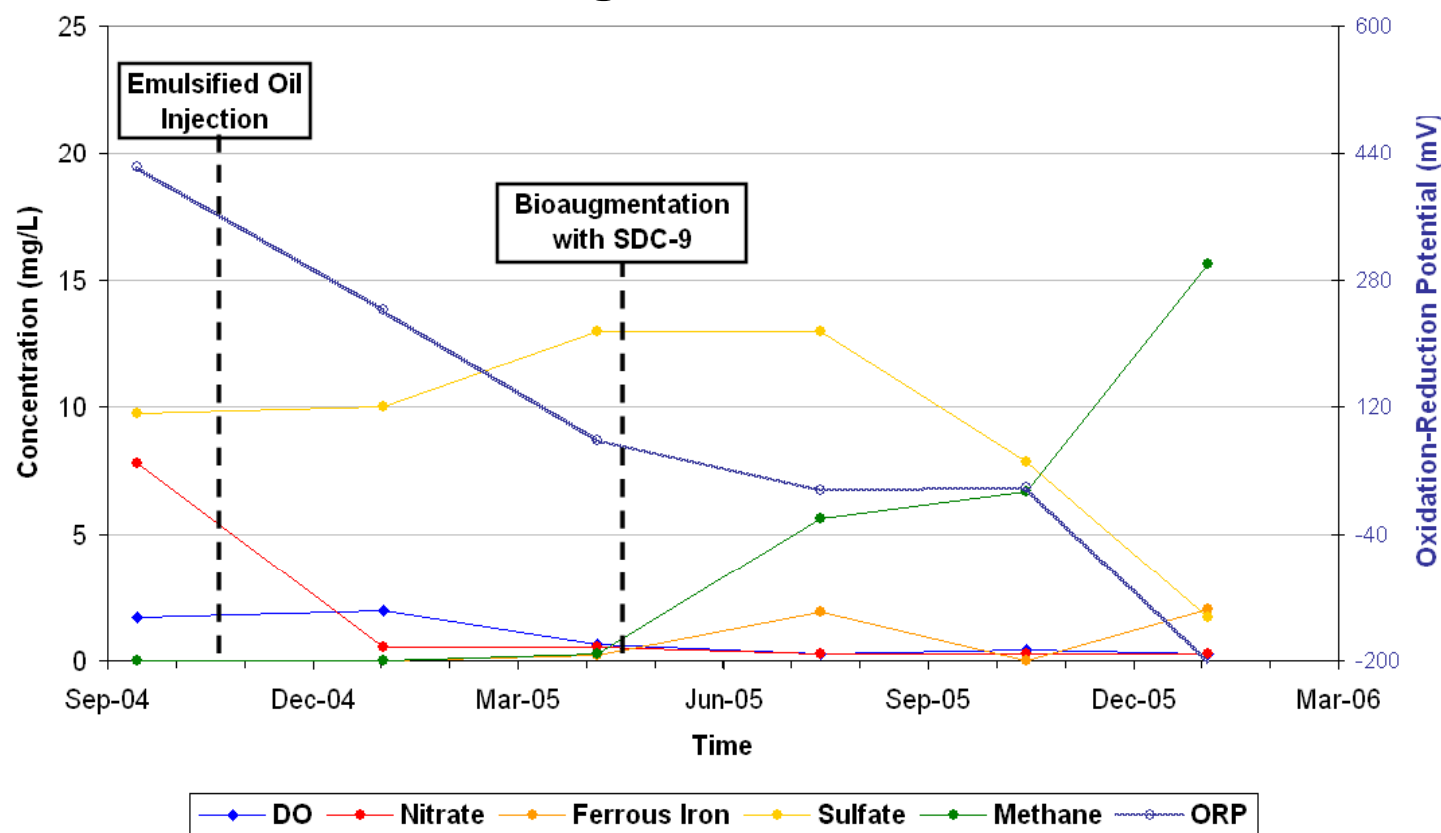


Flightline Storm Drain Outfall & Mission Lake (SD-16) Grid Application of **Emulsified Oil and Bioaugmentation**



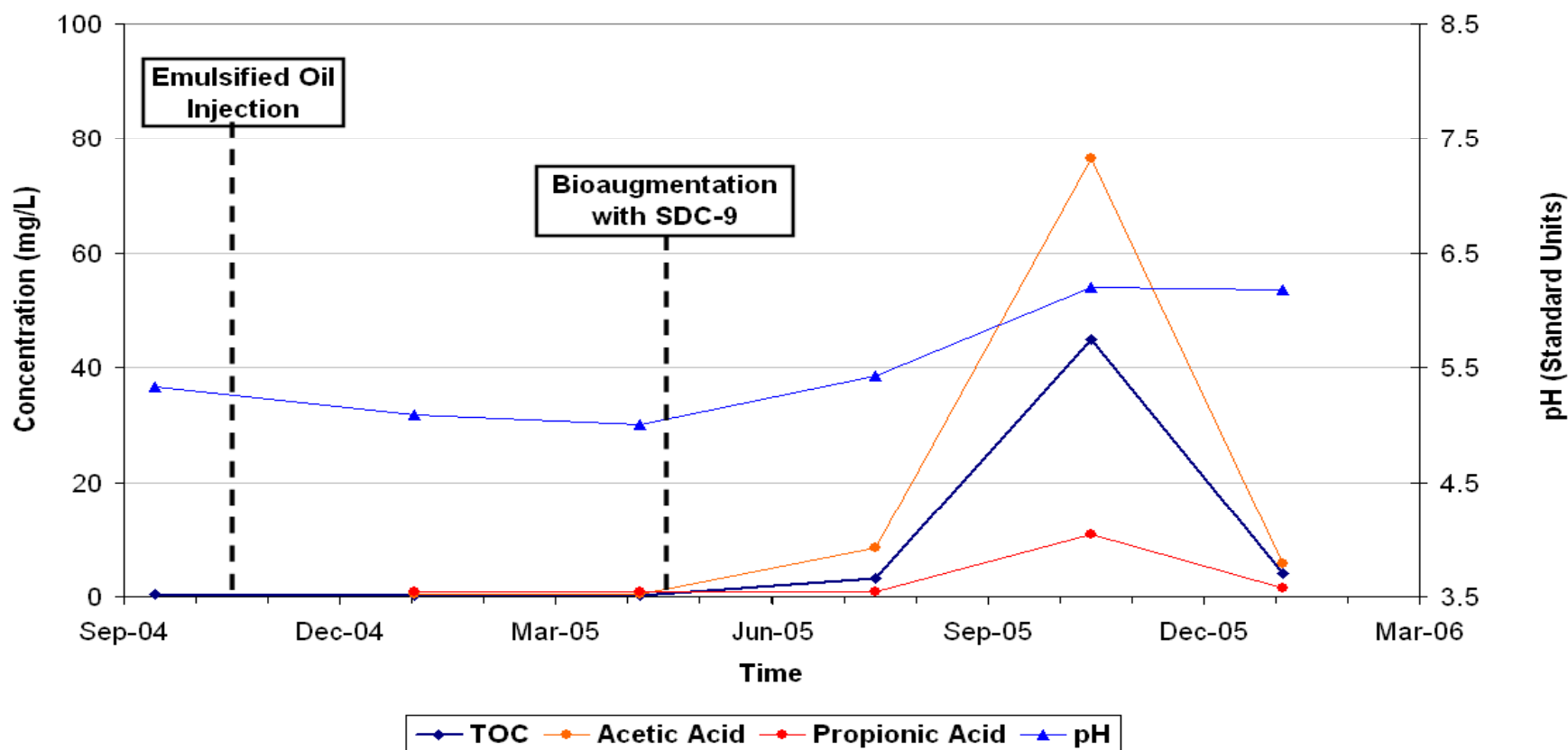
Flightline Storm Drain Outfall & Mission Lake (SD-16) Performance Monitoring Results

Geochemical Trends over Time Monitoring Well SD16-MW111



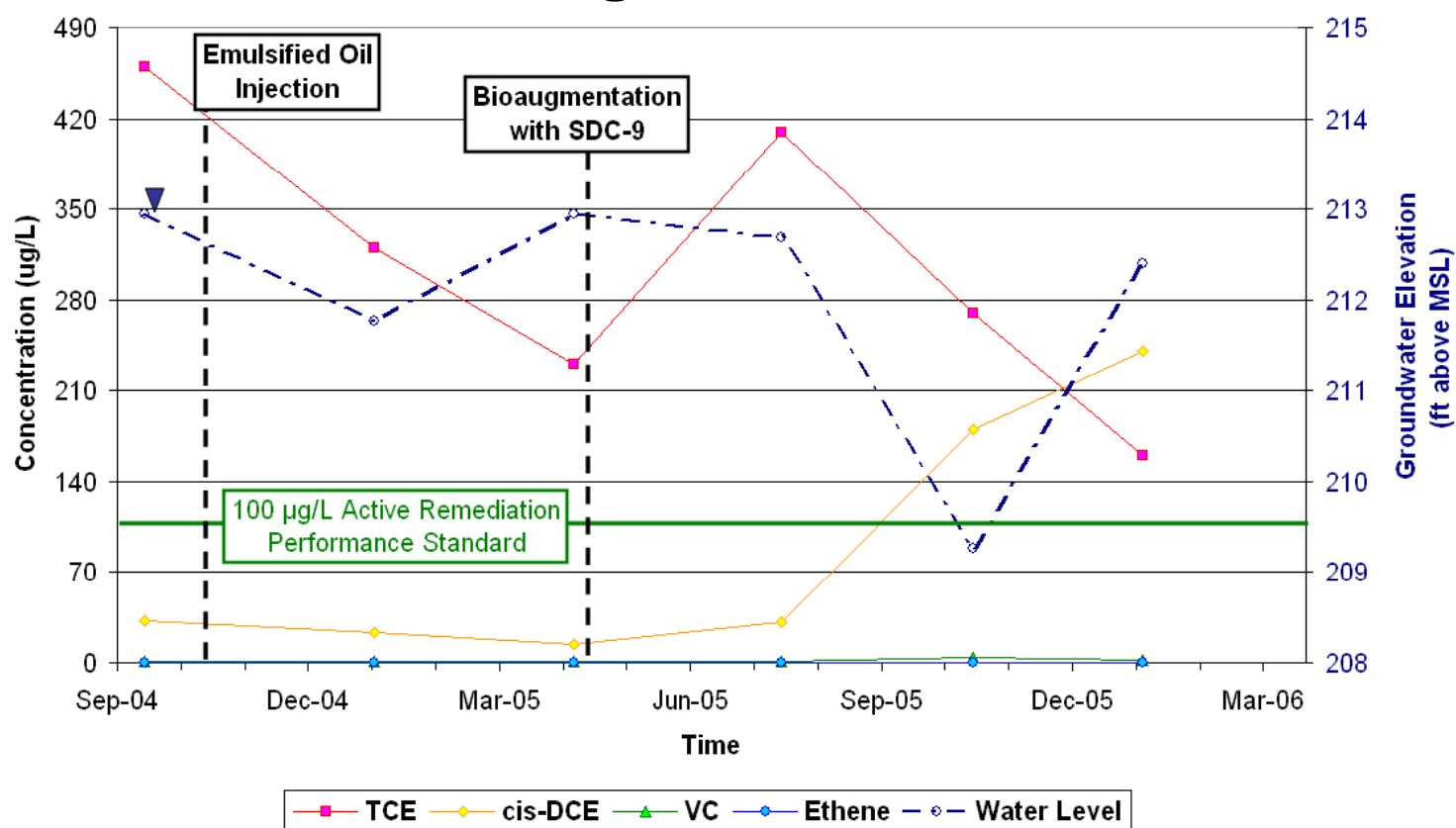
Flightline Storm Drain Outfall & Mission Lake (SD-16) Performance Monitoring Results

Carbon, Metabolic Acids, and pH Trends over Time Monitoring Well SD16-MW111



Flightline Storm Drain Outfall & Mission Lake (SD-16) Performance Monitoring Results

VOC Concentration Trends over Time Monitoring Well SD16-MW111



Flight Line Full Scale

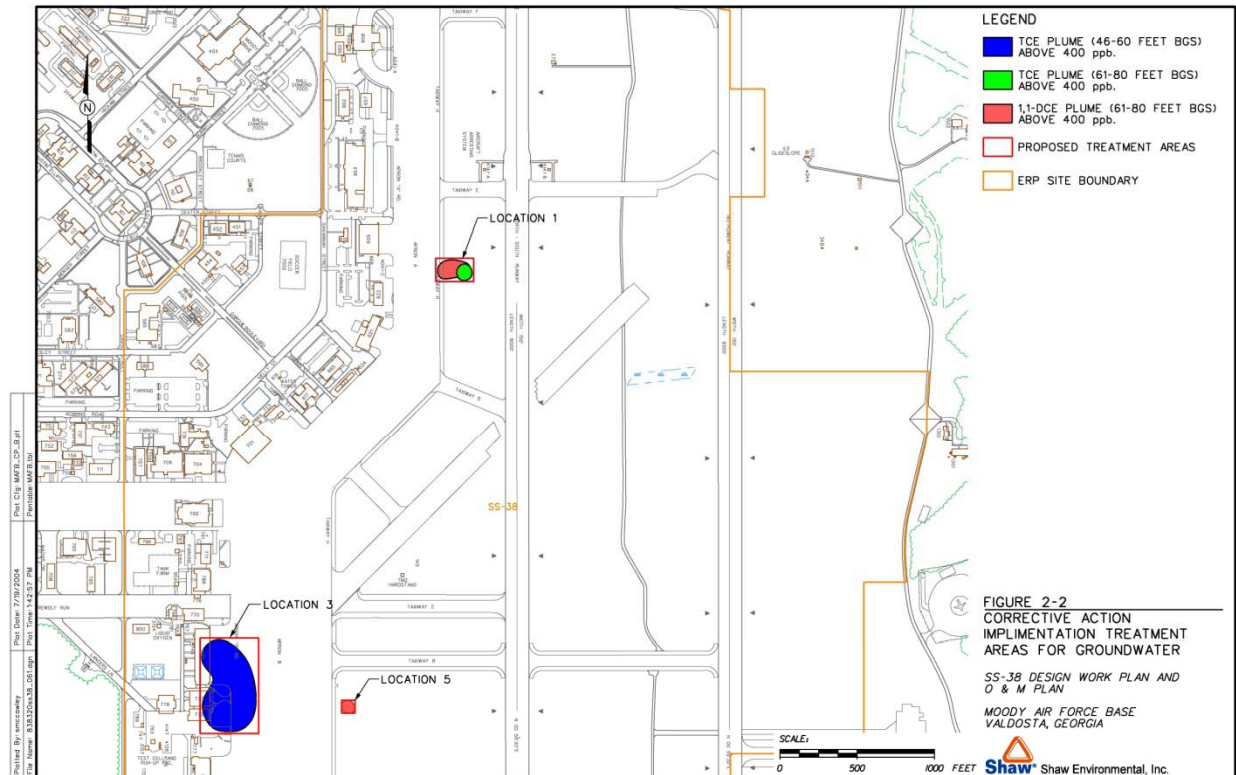
- **Recirculation – Horizontal Wells**
- **Lactate**
- **Bioaugmentation with SDC-9**

Flightline Area (SS-38)

Full-Scale Anaerobic Bioremediation

Treatment Areas

- TCE and 1,1-DCE > 400 µg/L
- Locations 1 and 5
Passive Distribution
- Location 3 Ground-
water Recirculation
- Lactate
- Bioaug. with SDC-9

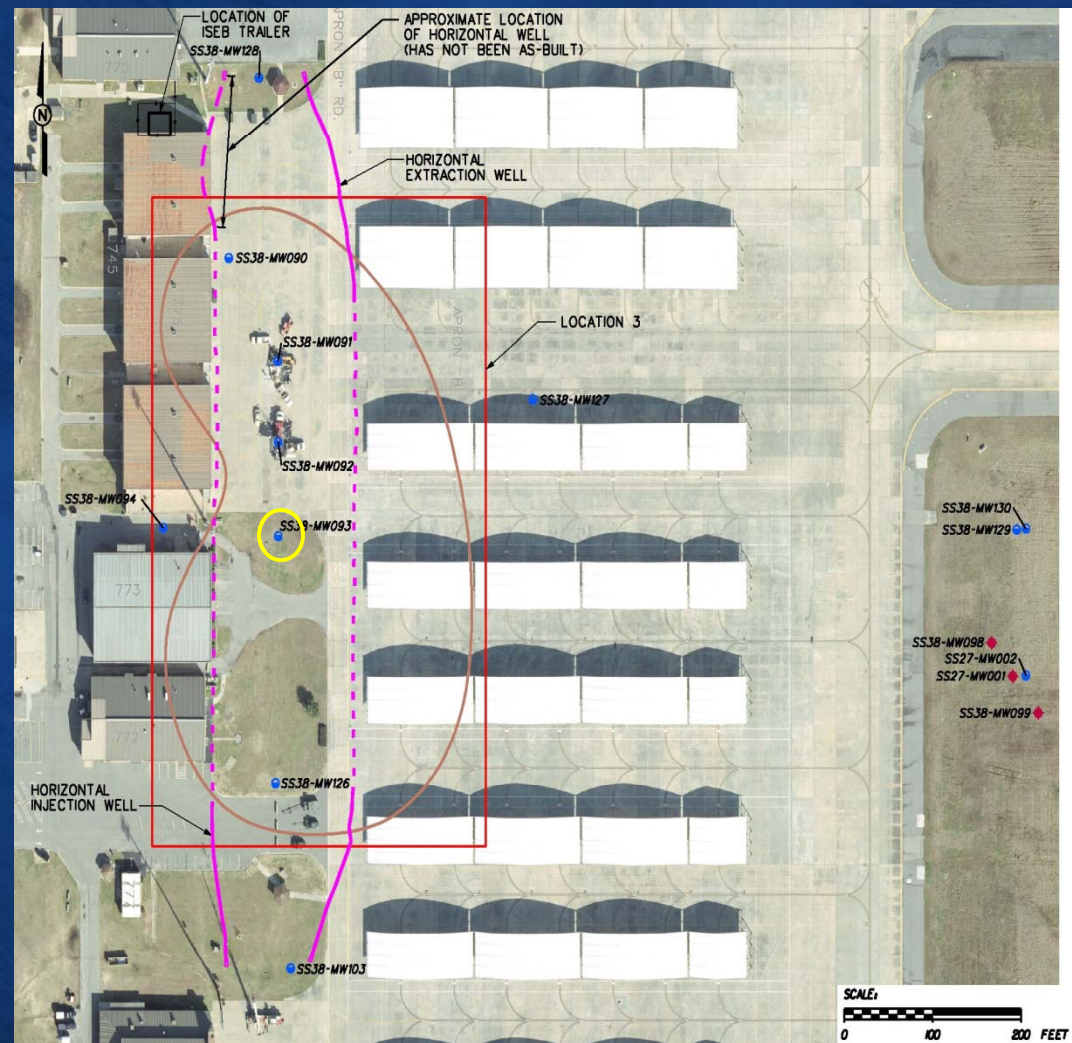


Flightline Area (SS-38)

Anaerobic Bioremediation w/ Groundwater Recirculation

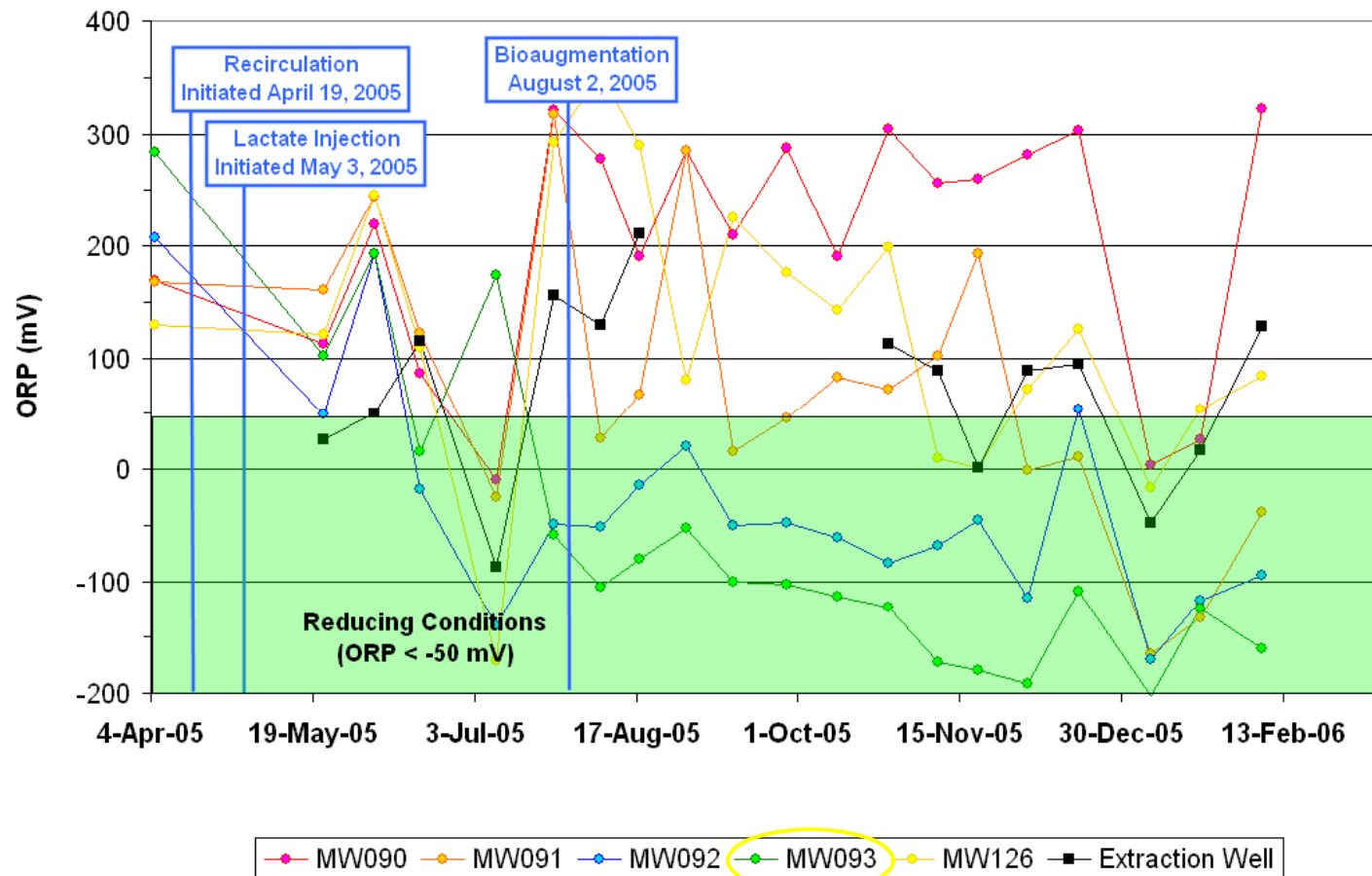
Location 3 Treatment Area

- 1 Horizontal Injection Well
- 1 Horizontal Extraction Well
 - 700 ft, 500 ft. screened
- Carbon Source – Lactate
- Bioaugmentation – SDC-9
 - 925 L



Flightline Area (SS-38) Performance Monitoring Results

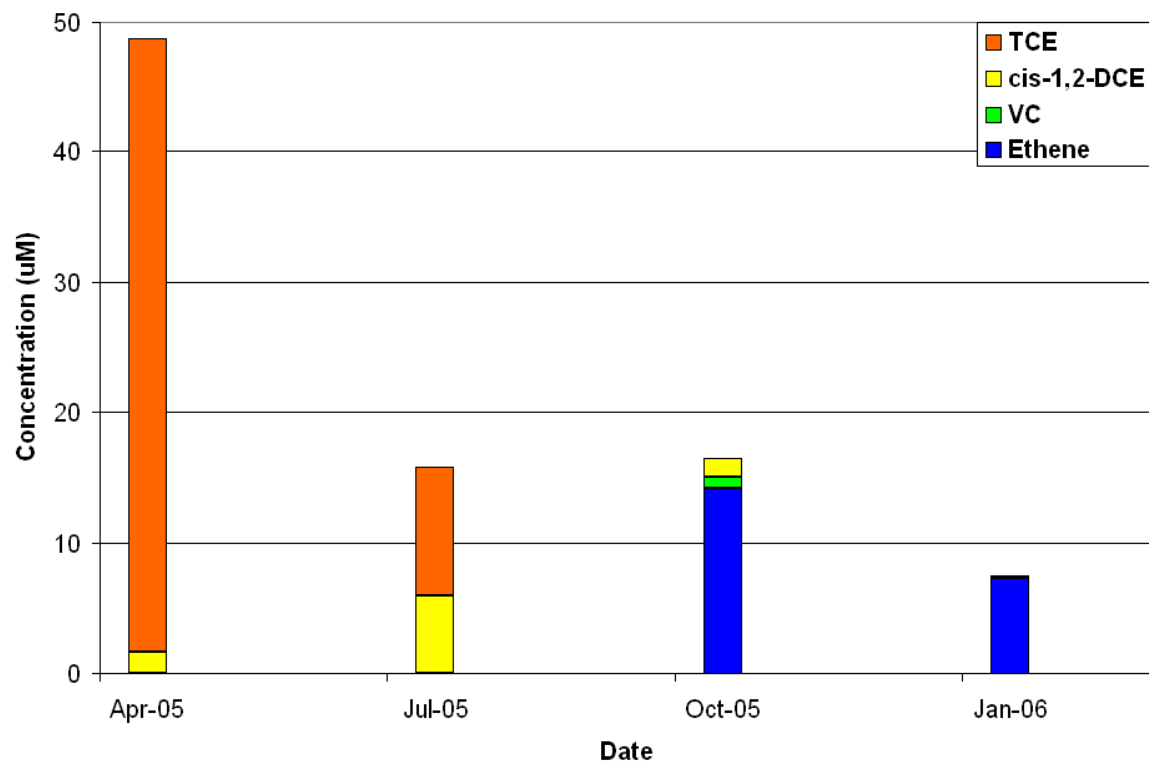
ORP Readings in Treatment Area Monitoring Wells



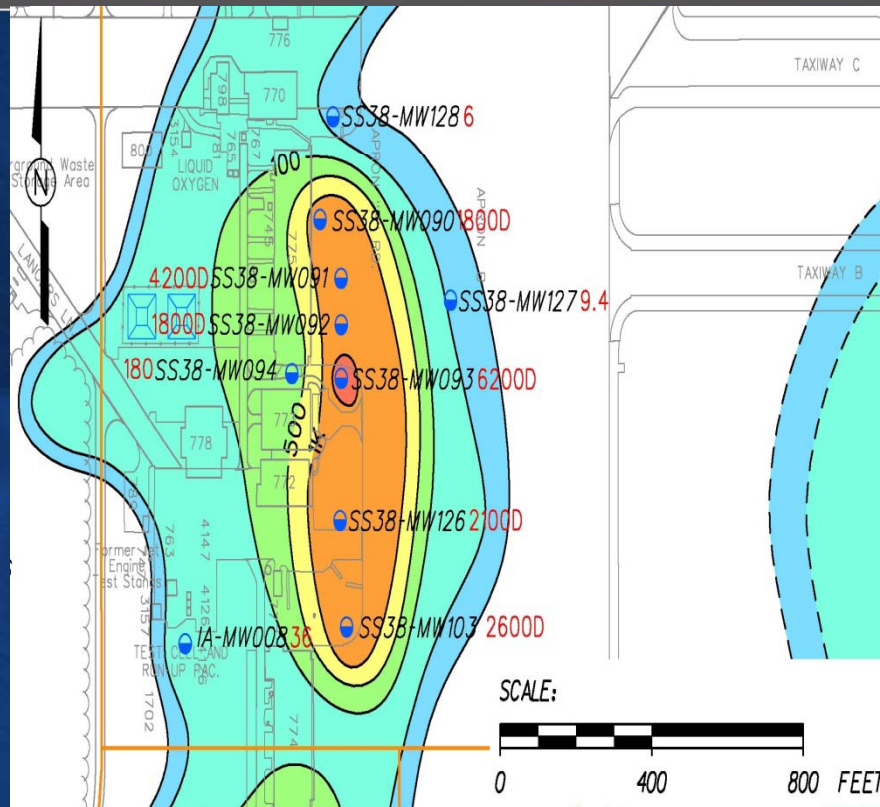
Flightline Area (SS-38)

Monitoring Well SS38-MW093 Results

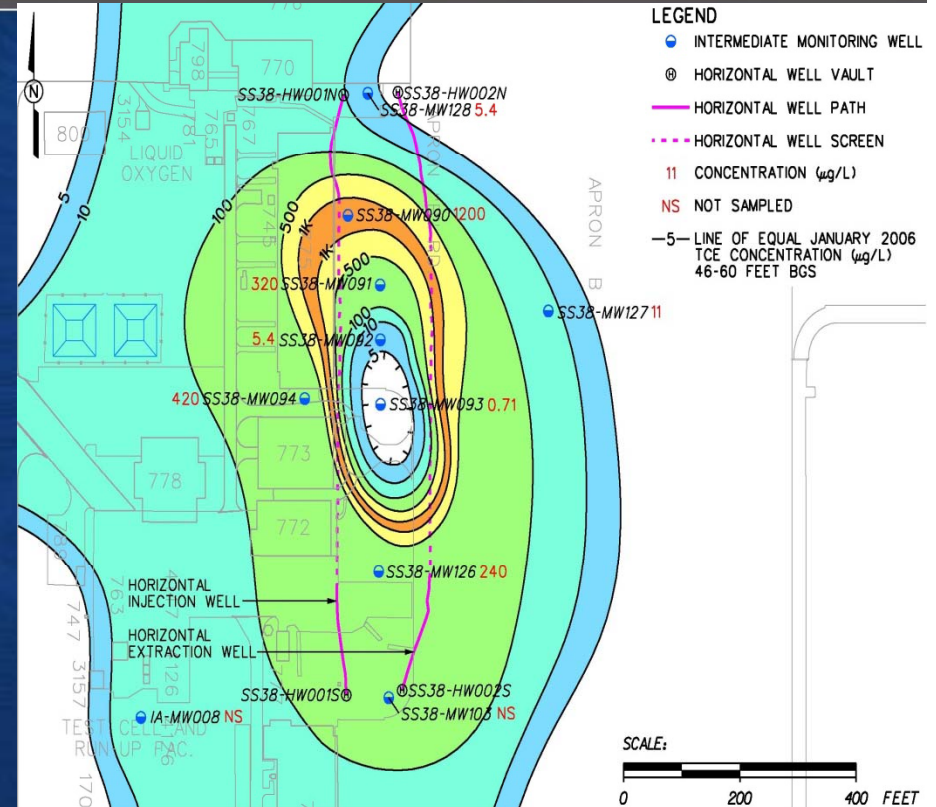
Monitoring Well SS38-MW093
Chlorinated Ethene Mass
Flightline Area (SS-38)
Moody Air Force Base, Valdosta, Georgia



Flightline Area (SS-38) Performance TCE Monitoring Results



**Base line
April 2005**



**January 2006
9-months post system
start-up**

System currently shut down – No further action required

Active versus Passive Distribution

| Site | Anaerobic Conditions | Daughter Products | Adequate Distribution |
|-----------------------|---|----------------------|-----------------------|
| SS-39 (active) | < 3 months | < 3 months | Yes |
| FT-07 (passive) | NA | 3-6+ months | OK |
| SD-16 (passive) | 3-6 months | 9-12 months | Yes |
| SS-38 (active) | < 3 months | < 3 months | Yes |
| SS-38 (passive) | 3-6 months | 9-12 months | Yes |
| LF-04 (passive) | Performance Monitoring Data Not Yet Available | | |