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December 28, 2021

Mr. Glenn May, PG New York State Department of Environmental Conservation, Region 9 270 Michigan Avenue Buffalo, NY 14203-2999

Subject: Bioaugmentation Injection Program Summary Report Former Scott Aviation Facility – West of Plant 2 Lancaster, New York NYSDEC Site Code No. 9-15-149

Dear Mr. May:

On behalf of Tyco International and its successor Scott Figgie LLC, AECOM Technical Services, Inc. (AECOM) is providing this letter report detailing a summary of the September 2021 bioaugmentation injection program and associated comparison between the pre- and post-bioaugmentation injection groundwater data at the Former Scott Aviation Facility – West of Plant 2 site (the "Site") in Lancaster, New York (refer to **Figure 1** for the Site location), New York State Department of Environmental Conservation (NYSDEC) Site No. 915149. The objective of this bioaugmentation injection program was to further enhance remediation of impacted Site groundwater.

This work was performed in accordance with the NYSDEC-approved Bioaugmentation Injection Work Plan (Work Plan) date August 30, 2021 (Attachment 1).

This summary report provides the following information:

- Introduction and summary of the scope of work.
- A summary of Site remedial action objectives (RAOs).
- Summary of bioaugmentation injection program.
- Pre- and post-bioaugmentation injection groundwater data collection.
- Conclusions and recommendations.

INTRODUCTION

The bioaugmentation injection program was performed by AECOM's subcontractor Matrix Environmental Technologies, LLC (METI), with oversight provided by AECOM. The injectate used was a combination of KB-1[®] Plus (a bioengineered microbial culture by SiREM containing *Dehalococcoides* (Dhc), which is designed to promote the complete dechlorination of chlorinated ethenes to non-toxic ethene and other non-chlorinated end products) and KB-1[®] Primer (a liquid used to prepare anaerobic water to disperse electron donors and protect KB-1[®] Plus during injection into the targeted aquifer). The total injection area was approximately 50-foot by 50-foot. The targeted injections directly addressed monitoring wells MW-8R and MW-16S and dual-phase extraction (DPE) wells DPE-4, DPE-7, and DPE-8; these are the locations with the highest current total volatile organic compound (VOC) concentrations detected in Site groundwater. These injections also indirectly addressed locations with elevated VOCs at MW-4 and DPE-3, which are located farther away from the injection locations but are still expected to be impacted.



The scope of work for the bioaugmentation injection program consisted of three tasks: Task 1 – Project Management / Premobilization Activities; Task 2 – Bioaugmentation Injection Program; and Task 3 – Bioaugmentation Injection Program Summary Report. These tasks are summarized below:

Task 1 - Project management activities included updating the health and safety plan, preparing the Bioaugmentation Injection Program Work Plan (**Attachment 1**) and performing other organizational activities to prepare for the implementation of the bioaugmentation injection program. As part of the pre-mobilization activities, AECOM marked out the injection locations for DigSafely New York to complete the utility mark outs.

Task 2 – The bioaugmentation injection program included pre- and post-injection groundwater sampling and the bioaugmentation injections, as detailed in the Work Plan (**Attachment 1**) and summarized below.

Task 3 – The Bioaugmentation Injection Program Summary Report included the writing of this letter report, summarizing the bioaugmentation injection activities, and presenting the pre- and post-bioaugmentation injection groundwater data.

REMEDIAL ACTION OBJECTIVES

Cleanup criteria for Site soil and groundwater are based on the RAOs established in the Record of Decision. The table below presents the Site-specific cleanup criteria.

	Remedial Ac	tion Objectives
Volatile Organic Compounds	Soil	Groundwater
	(mg/kg)	(µg/L)
Chloroethane	1	5
1,1-Dichloroethane	1	5
1,2-Dichloroethene	1	5
1,1,1-Trichloroethane	1	5
Trichloroethene	1	5
Vinyl chloride	1	5
Ethylbenzene	1	5
Toluene	1	5
Xylenes	1	5
Total VOCs	10	Not Applicable

The RAOs for the combined soil and groundwater remediation system include:

- 1. Maintain hydraulic control of shallow groundwater and eliminate potential off-Site migration of VOCs along the western property boundary.
- 2. Lower the groundwater table within the impacted source area to expose the aquifer matrix and subsequently extract soil vapors containing VOCs using enhanced vacuum extraction. By lowering the water table surface, the DPE system induces groundwater flow toward the system extraction wells, thereby allowing the applied vacuum to more effectively remove VOCs in the exposed aquifer matrix.
- 3. Reduce the mass of VOCs in the subsurface and remediate Site soil and groundwater toward meeting RAOs.
- 4. Obtain No Further Action status for the Site.



SUMMARY OF BIOAUGMENTATION ACTIVITIES

Utility Survey

Prior to beginning any intrusive activities, AECOM marked out the bioaugmentation injection locations with white spray paint and flagging. METI contacted the Underground Facilities Protection Organization (i.e., DigSafely New York) to mark out utilities in the proposed injection areas. There were no utilities identified by DigSafely New York in the injection area. To confirm there were no facility-related utilities in the injection area, AECOM met with facility maintenance to review injection locations and historic subsurface geophysical; no issues were identified. In addition, METI performed real-time ground-penetrating radar surveys around each injection point to obtain information on subsurface conditions and features, including utilities, remedial system conveyance piping, and any other obstructions. Per the data collected and reviewed, injection locations did not need to be adjusted.

Bioaugmentation Injections

METI mobilized equipment to the Site on September 14, 2021. Prior to the bioaugmentation injections, AECOM and subcontractor METI took the DPE remedial system and the groundwater collection trench (GWCT) temporarily off-line to accommodate the bioaugmentation injection program (refer to **Figure 2** for the location of the DPE and GWCT remedial systems. Bioaugmentation injections were performed September 15, 2021 and September 16, 2021. The bioaugmentation injection program consisted of injecting KB-1[®] Plus and KB-1[®] Primer at nine locations within an approximately 50-foot by 50-foot area as shown in **Figure 3**. The total injection area was designed to address suspected VOC impacted groundwater around monitoring wells MW-4, MW-8R and MW-16S and DPE wells DPE-3, DPE-4, DPE-7, and DPE-8; these are the locations with the highest total VOC concentrations detected in Site groundwater. As shown in **Figure 3**, three injection points were located around two targeted monitoring wells (MW-8R and MW-16S), with injection points biased to the upgradient groundwater side of each of the wells, and one injection point was located on the upgradient side of each DPE-4, DPE-7, and DPE-8 (note DPE-3 is located in the center of the previously mentioned injection points and well MW-4 is located immediately downgradient of the injection points near locations DPE-7 and DPE-8). Photographs taken during the injection activities are presented in **Attachment 2**.

The microbial culture KB-1[®] Plus and the KB-1[®] Primer was supplied by SiREM. The KB-1[®] Plus and the KB-1[®] Primer were mixed and injected according to the specification sheets with no deviations (refer to **Attachment 1** for SiREM's detailed specifications sheets and detailed mixing and injection procedures).

The bioaugmentation solution was injected in to the subsurface via direct push technology injections, targeting either 3 or 4 discrete intervals ranging between 5 and 20 feet below ground surface (ft bgs) depending on the location. Injection points were advanced using a Geoprobe[®] 6620DT drill rig, using 1.5-inch diameter drill rods. Each injection point around locations MW-8R, DPE-4, and DPE-8 received approximately 200 gallons of KB-1[®] Plus/Primer (i.e., injectate) which was distributed at 5-foot depth intervals (5, 10, 15, and 20 ft bgs), targeting either the shallow or shallow and deep overburden groundwater zones. Each injection point around locations MW-16S and DPE-7 received approximately 150 gallons of injectate and was distributed at three depth intervals (8, 13, and 18 ft bgs), targeting the shallow overburden groundwater zone (refer to **Table 1** for a summary of the nine injection locations, injection intervals, and KB-1[®] Primer solution and KB-1[®] Plus bioaugmentation amounts injected).

The KB-1[®] Primer came in pouches suitable for mixing with approximately 250 gallons of potable water. An appropriate amount of the KB-1[®] Primer was weighed with a scale provided by SiREM and mixed with the amount of water required for each injection location (i.e. 60% of a KB-1[®] Primer pouch for 150 gallons or 80% of a pouch for 200 gallons). The KB-1[®] Primer water mix was ready to inject when fully dissolved and upon pH and oxygen reduction potential (ORP) readings meeting the specifications designated by SiREM (i.e., 6 to 8.3 standard units for pH, and < -75 milliVolts for ORP).

Injection flow rates for the injections ranged from approximately 3 to 12.5 gallons per minute. The target volume of injections for each discrete interval regardless of location was 50 gallons (to minimize short circuiting or breakthrough). This is the minimum amount of KB-1[®] Primer water recommended by SiREM to support the KB-1[®] Plus. At each interval, approximately half the injection amount of KB-1[®] Primer water (25 gallons) was injected. A target amount of KB-1[®] Plus



(approximately 0.6 liters) was then injected using nitrogen gas to push the anaerobic microbial injectate into the targeted interval. The remaining half of the primer water was subsequently injected. Injections were conducted using a bottom-up approach, starting at the lowest designated interval, and raising the rods up the next interval following completion of the lower interval injection.

After each injection location was completed, injection boreholes were backfilled with a mixture of bentonite chips and granules and hydrated to minimize the potential for short circuiting of injection fluids from adjacent injection points to the ground surface.

The target volume for each interval was successfully delivered at each location (refer to **Table 1**). There were no deviations for location or amounts injected when compared to the Work Plan (**Attachment 1**). During injections, breakthrough was observed at locations MW-16S-B, MW-8R-B, MW-8R-C and DPE-4-A. In all cases, breakthrough was very minor with minimal injection loss except for DPE-4-A (15 ft bgs interval) where injection fluid daylighted via an old borehole from a previous drilling event. METI was successful at repacking the old borehole with a bentonite chips/granule mixture, which stopped the daylighting and allowed the remaining intervals to be injected without further injectate loss.

On October 4, 2021, two weeks prior to the fourth quarter 2021 (October 2021) groundwater sampling event, the GWCT was brought back on-line. On November 23, 2021, approximately 40 days following the bioaugmentation injection event, AECOM and METI performed operation and maintenance activities on the DPE system (including winterization activities) and brought DPE-1, DPE-2, and DPE-5 back on-line. Note DPE-1, DPE-2, and DPE-5 are located up/side-gradient of the regional groundwater flow and outside the bioaugmentation injection area. Contradictory to the Work Plan (Attachment 1), during the winter months, DPE-1, DPE-2, and DPE-5 will not be cycled, but rather will remain on-line to keep the DPE remedial system components from freezing if the system were to be kept totally off-line. Per Periodic Review Report (PRR) #16, the combined DPE system only removed about 3 pounds of VOCs during the year ending in April 2021, so the effect of having a portion of that system off-line (i.e., DPE-3, DPE-4, DPE-7, and DPE-8) is expected to be minimal compared to the benefit of the bioaugmentation injections. The GWCT will remain on-line as there were no deep injection locations near the GWCT (i.e., GWCT is upgradient [regional groundwater flow] of the bioaugmentation injection points).

PRE- AND POST- BIOAUGMENTATION INJECTION GROUNDWATER ANALYTICAL DATA COLLECTION

Groundwater analytical data obtained following the bioaugmentation injections has been compared to the prebioaugmentation injection data and evaluated to initially assess the performance of the bioaugmentation injection program. The data have been interpreted to evaluate the effectiveness of the bioaugmentation injections in terms of contaminant reduction and bacteria population.

Pre-Injection Groundwater Data Collection

Total Organic Carbon (TOC) and VOC groundwater samples were collected July 14 and 15, 2021, as part of the third quarter 2021 groundwater sampling event, from monitoring wells MW-4, MW-8R, MW-16S, and MW-16D, and from DPE wells DPE-3, DPE-4, DPE-7, and DPE-8 and submitted to Eurofins TestAmerica for analysis. This data was used to establish a pre-injection baseline for comparison to the post-injection TOC and VOC groundwater data (refer to **Table 2** and **Table 3** for monitoring well and DPE well data respectively).

On August 26, 2021, AECOM collected pre-bioaugmentation injection groundwater samples from monitoring wells MW-16S and MW-8R for volatile fatty acids (VFA) analysis and a groundwater sample from monitoring well MW-16S for Gene-Trac[®] analysis. The pre-injection VFA data was used to establish a baseline and monitor the quality and form of fermentation byproducts of electron donors previously injected at the site. The pre-injection Gene-Trac[®] data was used as a baseline for confirming the post-injection distribution of the primary microorganisms (Dhc and *Dehalobacter* [Dhb]) in the KB-1[®] Plus culture. The VFA and Gene-Trac[®] samples were submitted to SiREM for analysis. Sample collection procedures are detailed in the Work Plan (**Attachment 1**). Pre-injection VFA and Gene-Trac[®] analytical data are summarized in **Table 4** and **Table 5** respectively).



Post-Injection Groundwater Data Collection

During the week of October 18, 2021, approximately 35 days following the bioaugmentation injection, AECOM performed the fourth quarter 2021 groundwater sampling event. Post-injection TOC and VOC groundwater data from monitoring wells MW-4, MW-8R, MW-16S, and MW-16D, and from DPE wells DPE-3, DPE-4, DPE-7, and DPE-8 were used for comparison against the previously collected pre-injection groundwater data (refer to **Table 1** and **Table 2** for monitoring well and DPE well data respectively). Below is a summary illustrating the percent reduction of VOC concentrations of RAOs between pre- and post-bioaugmentation injection samples collected from monitoring wells and DPE extraction wells.

Remedial Action Objectives	MW-4	MW-8R	MW-16S	MW-16D	DPE-3	DPE-4	DPE-7	DPE-8
1,1,1-Trichloroethane	ND	ND	ND	ND	ND	ND	ND	ND
1,1-Dichloroethane	54%	ND	16%	55%	69%	increase	ND	14%
Chloroethane	ND	ND	12%	21%	ND	ND	ND	ND
cis-1,2-Dichloroethene	ND	93%	84%	92%	88%	increase	ND	81%
Toluene	0%	increase	ND	ND	ND	ND	ND	ND
Trichloroethene	ND	ND	ND	ND	85%	37%	ND	ND
Vinyl chloride	25%	50%	40%	91%	59%	increase	ND	increase
Xylenes	ND	ND	ND	ND	ND	ND	ND	ND

On December 9, 2021, 85 days following the bioaugmentation injection, AECOM collected groundwater samples from monitoring wells MW-16S and MW-8R for VFA analysis and one groundwater sample from monitoring well MW-16S for Gene-Trac[®] analysis. A summary of the post-injection VFA and Gene-Trac[®] analytical data reports are presented in **Table 4** and **Table 5** respectively; laboratory data reports are included in **Attachment 3**.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions for the September 2021 bioaugmentation injection event and the associated pre- and post-injection analytical results are presented in the following subsections.

Volatile Organic Compounds and Total Organic Carbon

A comparison of the pre- and post-injection VOCs in groundwater monitoring wells and DPE wells targeted by the bioaugmentation injection program is presented in **Table 2** and **Table 3**, respectively. Although the post-injection groundwater data was collected only approximately 35 days following the bioaugmentation injection event, post-injection results show a decrease in most VOC concentrations compared to the pre-injection groundwater VOC concentration data. At monitoring well MW-8R, toluene increased from 12 micrograms per liter (μ g/L) to 21 μ g/L and at monitoring well MW-16D, methylcyclohexane increased from <1.0 μ g/L to an estimated concentration of 0.66 μ g/L. Bioaugmentation with KB-1[®] Plus would not impact the concentration of these two compounds. At DPE-4, 1,1-dichloroethane increased from an estimated concentration of 1.6 μ g/L to an estimated concentration of 2.1 μ g/L, cis-1,2-dichloroethene (cis-1,2-DCE) increased from 180 μ g/L to 240 μ g/L, and vinyl chloride (VC) increased from 200 μ g/L to 300 μ g/L. At DPE-8, VC increased from 1,400 μ g/L to 5,000 μ g/L.

A TOC concentration of 20 milligrams per liter (mg/L) is commonly considered the minimum concentration of carbon necessary for effective reductive dechlorination to occur. During the October 2021 monitoring event, locations MW-4 (22.7 mg/L), MW-8R (56.8 mg/L), MW-16S (300 mg/L), DPE-3 (40.5 mg/L), and DPE-8 (26.2 mg/L) all had TOC concentrations above 20 mg/L. During this same event, locations MW-16D (3.8 mg/L), DPE-4 (9.3 mg/L), and DPE-7 (11.5 mg/L) all had TOC concentrations below 20 mg/L. TOC concentrations decreased at monitoring well MW-16S from 400 mg/L to 300 mg/L, at DPE-4 from 11.6 mg/L to 9.3 mg/L, and at DPE-8 from 37.7 mg/L to 26.2 mg/L.



The bioaugmentation event in September 2021 would not be expected to increase the concentration of TOC in the area targeted by the injections. The TOC detected is the result of natural organic carbon present in site groundwater and also from previous injections of an organic carbon substrate. The most recent organic carbon injection event conducted at the site was performed during the week of November 26, 2018. At that time, Anaerobic BioChem-Ole' (ABC-Ole') with zero valent iron was injected within a 4,500 square foot area that surrounded the aforementioned monitoring wells and DPE wells.

Volatile Fatty Acids

In addition to a TOC concentration greater than 20 mg/L, the quantification of VFAs is useful to assess the form of TOC present and its availability to promote the reductive dechlorination process. VFAs are fermented by a variety of pathways to produce the hydrogen necessary for complete reductive dechlorination to occur. In general, VFAs should be in excess of 10 to 20 mg/L. Pre- and post-injection VFA data is summarized in **Table 4**; the associated laboratory data reports are included in **Attachment 3**.

Six VFAs were analyzed for by SiREM. Lactate is a component of the ABC-Ole' that was previously injected at the Site. Lactate ferments to the VFAs acetate and propionate. Lactate can be used as a measure of the remaining unused reducing potential of the previously injected ABC-Ole'. For monitoring well MW-8, lactate reduced from a low detected concentration of 1.2 mg/L in August 2021 down to the detection limit (<0.39 mg/L) in December 2021. This indicates the depletion of this VFA at this well. For monitoring well MW-16S, lactate increased from <0.39 mg/L to <7.8 mg/L between August and December 2021; however, the sample dilution factor increased from 50 to 1,000 so there likely is little lactate left in the vicinity of this well.

Acetate is fermented from lactate, ABC-Ole', and sugars. Dhb can use acetate as a low energy source while Dhc cannot. Dhb is implicated in the biodegradation of chlorinated ethenes such as tetrachloroethene (PCE) and trichloroethene (TCE) to cis-1,2-dichloroethene and in the biodegradation of the chlorinated ethane 1,1,1-trichloroethane to 1,1-dichloroethane and subsequently to chloroethane. As a result, the presence of acetate indicates that partial reductive dechlorination can occur. However, complete reductive dechlorination to ethene and ethane will not occur without the presence of other VFAs and Dhc. Acetate decreased in monitoring well MW-8R (70 mg/L to 28 mg/L) and increased in monitoring well MW-16S (495 mg/L to 921 mg/L).

Propionate is fermented from lactate, ABC-Ole', and alcohols. Propionate subsequently ferments to produce hydrogen and formate. Hydrogen is the preferred electron acceptor for reductive dechlorination because of the high energy yield. Dhc can only use hydrogen as an energy source. Slow fermentation of propionate results in efficient reductive dechlorination (less methanogenesis) and optimal Dhc growth. Propionate was not detected in MW-8R in August or December 2021. Propionate was detected in monitoring well MW-16S in August 2021 (12 mg/L) and also in December 2021 (14 mg/L).

Formate is created from the fermentation of propionate. Formate is fermented to produce hydrogen and bicarbonate. Formate was not detected in monitoring wells MW-8R or MW-16S in August or December 2021.

Pyruvate is created from the fermentation of sugars. Pyruvate is subsequently fermented to propionate and acetate with some hydrogen production. Pyruvate was not detected in monitoring well MW-8R during either sampling event. A low concentration of pyruvate was detected (0.71 mg/L) in monitoring well MW-16S in August 2021, and it was not detected (<13.8 mg/L) in December 2021.

Butyrate is created from the fermentation of ABC-Ole' and alcohols. Butyrate ferments to produce hydrogen and acetate. Slow fermentation of butyrate results in efficient reductive dechlorination (less methanogenesis) and optimal Dhc growth. Butyrate was not detected in monitoring well MW-8R in August or December 2021. Butyrate was detected at MW-16S in August 2021 (81 mg/L) and also in December 2021 (98 mg/L).

Overall, the December 2021 VFA results for monitoring well MW-8R indicate that the remaining TOC in the vicinity of this well is insufficient to promote complete reductive dechlorination. While reduction to cis-1,2-DCE by Dhb may still be possible due to the presence of acetate, this process will be slow since hydrogen is the preferred energy source. No



other VFAs were detected in this well. For monitoring well MW-16S, there was an increase in the concentration of three VFAs (acetate, propionate, and butyrate) between August and December 2021. Both propionate and butyrate produce hydrogen when they are fermented, which is essential for complete reductive dechlorination to occur. These results indicate that completed reductive dechlorination can occur in the vicinity of this well if Dhc is present is sufficient quantity. A discussion of Dhc, Dhb, and reductase results is provided in the next subsection.

Gene-Trac[®]

Gene-Trac[®] Dhc is used to detect Dhc in a groundwater sample. The detection of Dhc is significant as Dhc contain the greatest number of reductive dehalogenase genes of any microbial group. Dhc is capable of the reductive dechlorination of PCE, TCE, cis-1,2-DCE, 1,1-dichloroethene, trans-1,2-dichloroethene, and VC. Pre- and post-injection Gene-Trac[®] data is summarized in **Table 5**; laboratory data reports are included in **Attachment 3**.

Both the pre- and post-injection Gene-Trac[®] Dhc results indicate 1×10^9 Dhc gene copies per liter. Per the technical notes from SiREM regarding interpretation of data (refer to **Attachment 4**), when the density of Dhc gene copies per liter is 1×10^9 or higher, this concentration is generally associated with very high rates of dechlorination.

Gene-Trac[®] *vcrA*, *bvcA*, and vinyl chloride reductase *tceA* quantifies genes that code for reductase enzymes that dechlorinate chlorinated ethenes and other compounds. The *vcrA*, *bvcA*, and *tceA* genes play specific roles in reductive dechlorination. Specifically, the Gene-Trac[®] *vcrA* and *bvcA* test quantifies VC-reductase genes that produce enzymes that convert VC to ethene. The *vcrA* reductase gene is reported to be the most commonly identified VC reductase gene in the environment, whereas *bvcA* is generally less common but can predominate in more oxidizing groundwater and possibly where DCE is dominant. The Gene-Trac[®] *tceA* test quantifies the TCE reductase gene that produces an enzyme that primarily converts TCE to cis-1,2-DCE and VC.

The *vcrA* reductase gene was detected in monitoring well MW-16S at 1×10^9 gene copies per liter in both the pre-and post-injection samples collected. The *bvcA* reductase gene was detected in monitoring well MW-16S at 1×10^8 gene copies per liter in August 2021 and at 6×10^7 gene copies per liter in December 2021. The *tceA* reductase gene was detected in monitoring well MW-16S at 1×10^9 gene copies per liter in August 2021 and at 3×10^8 gene copies per liter in December 2021. The *tceA* reductase gene was detected in monitoring well MW-16S at 1×10^9 gene copies per liter in August 2021 and at 3×10^8 gene copies per liter in December 2021. Per the technical notes from SiREM, the potential for complete dechlorination is very high when Dhc, *vcrA*, *bvcA*, and *tceA* are present at greater than or equal to 1×10^7 . Additionally, VC stall is unlikely when *vcrA* greater than 1×10^7 gene copies per liter, and ethene is detectable. At monitoring well MW-16S, ethene was detected at 33,000 µg/l and 51,000 µg/l in April 2021 and October 2021, respectively.

Gene-Trac[®] Dhb is used to detect Dhb in a groundwater sample. Dhb are implicated in the biodegradation of PCE and TCE to cis-1,2-DCE. The detection of Dhb indicates that dechlorination activities attributed to Dhb may be active. Increasing concentrations of Dhb indicative of increased potential for degradation. Dhb was detected at 5 x 10^7 gene copies per liter in August 2021 and at 2 x 10^7 gene copies per liter in December 2021.

In summary, Dhc, *vcrA*, *bvcA*, and *tceA* are present at monitoring well MW-16S at concentrations that indicate a very high potential for complete dechlorination to occur. Additional time is needed to evaluate the overall impact of the bioaugmentation event in the vicinity of this well.

Monitored natural attenuation data from the second quarter 2021 and fourth quarter 2021 groundwater sampling events are summarized in **Table 6**. Per **Table 6**, three of the four wells sampled for MNA parameters within the targeted bioaugmentation area show strong evidence for anerobic biodegradation of chlorinated organics to occur (i.e., monitoring wells MW-4, MW-16S, and MW-16D); the remaining well (MW-8R) shows adequate evidence for anerobic biodegradation of chlorinated organics as well as a slight increase in the total screening score from 19 points to 20 points (note > 20 points indicate there is a strong evidence for anaerobic biodegradation of chlorinated organics. Ethene, which is the ultimate product of reductive dechlorination for chlorinated ethenes, increased in both monitoring wells MW-8R and MW-16S and remained relatively the same in monitoring well MW-4 when comparing the pre- and postbioaugmentation results.



Recommendations

Based on the information presented above, more time is needed to evaluate the impact of the bioaugmentation injection program. In January 2022, the first quarter 2022 groundwater monitoring event will be performed; groundwater samples from select monitoring wells will be analyzed for VOCs and TOC during this event. In April 2022, the 2022 comprehensive annual groundwater monitoring event will be performed; groundwater samples from the entire monitoring well network will be analyzed for VOCs, TOC, and select monitoring wells will be sampled for MNA parameters. Additionally, in April 2022, VFA samples will be collected at monitoring wells MW-8R and MW-16S, and a Gene-Trac[®] sample will be collected at monitoring well MW-16S; these data will be used to track the performance of the bioaugmentation injection program (refer to attached **Table 7** for a summary of the groundwater sampling schedule). Groundwater data from the two beforementioned sampling events will be summarized in the 2022 PRR as related to the performance of the 2021 bioaugmentation injection program.

If you have any questions regarding this submission, please do not hesitate to contact me at (716) 923-1125 or via email at <u>dino.zack@aecom.com</u>.

Yours sincerely,

Dino J. Gack

Dino L. Zack, PG, STS Project Manager dino.zack@aecom.com

\Enclosures

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Figures







BIOAUGMENTATION INJECTION LOCATION MW-13S/D -Ò-NESTED PIEZOMETER LOCATION MONITORING WELL LOCATION MW-9 🕀 DUAL-PHASE EXTRACTION WELL LOCATION (OFF-LINE) DPE-6 Ә DUAL-PHASE EXTRACTION DPE-1 WELL LOCATION (ACTIVELY EXTRACTING PRIOR TO INJECTION) [13.4] TOTAL VOC CONCENTRATION (µg/L) TOTAL VOC CONTOUR - 10 SHALLOW PIEZOMETER (S) DEEP PIEZOMETER (D)

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GROUNDWATER COLLECTION TRENCH (GWCT) APPROXIMATE PROPERTY BOUNDARY

NOTES

- 1. GROUNDWATER DATA IS FROM APRIL 2021.
- 2. TOTAL VOC FROM THE SHALLOW PIEZOMETER PAIR LOCATIONS (i.e. MW-13S, MW-14S, MW-15S, MW-16S) WERE USED TO CREATE THE TOTAL VOC CONTOURS.
- 3. VFA SAMPLES COLLECTED AT MW-8R AND MW-16S.
- 4. GENE-TRAC SAMPLE COLLECTED AT MW-16S.
- SHALLOW/DEEP OVERBURDEN GROUNDWATER 5. FLOW IS TO THE NORTHWEST.

0 15 30 60 SCALE IN FEET	
FIGURE 3 BIOAUGMENTATION INJECTION POINTS	;
FORMER SCOTT AVIATION FACILITY LANCASTER, NEW YORK	

Bioaugmentation Injection Intervals and Injectate Volumes Former Scott Aviation Facility - West of Plant 2 NYSDEC Site Code No. 9-15-149 Lancaster, New York

<u>MW-16S</u>

Injection point MW-16S-A-18' - 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer Injection point MW-16S-A-13' - 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer Injection point MW-16S-A-08' - 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer

Injection point MW-16S -B-18' – 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer Injection point MW-16S -B-13' – 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer Injection point MW-16S -B-08' – 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer

Injection point MW-16S -C-18' – 0.16 gallons KB-1^{\circ} Plus / 50 gallons KB-1^{\circ} Primer Injection point MW-16S -C-13' – 0.16 gallons KB-1^{\circ} Plus / 50 gallons KB-1^{\circ} Primer Injection point MW-16S -C-08' – 0.16 gallons KB-1^{\circ} Plus / 50 gallons KB-1^{\circ} Primer

MW-8R

Injection point MW-8R-A-20' – 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer Injection point MW-8R-A-15' – 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer Injection point MW-8R-A-10' – 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer Injection point MW-8R-A-05' – 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer

Injection point MW-8R-B-20' – 0.16 gallons KB-1° Plus / 50 gallons KB-1° Primer Injection point MW-8R-B-15' – 0.16 gallons KB-1° Plus / 50 gallons KB-1° Primer Injection point MW-8R-B-10' – 0.16 gallons KB-1° Plus / 50 gallons KB-1° Primer Injection point MW-8R-B-05' – 0.16 gallons KB-1° Plus / 50 gallons KB-1° Primer

Injection point MW-8R-C-20' – 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer Injection point MW-8R-C-15' – 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer Injection point MW-8R-C-10' – 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer Injection point MW-8R-C-05' – 0.16 gallons KB-1[°] Plus / 50 gallons KB-1[°] Primer

DPE-4

Injection point DPE-4-A-20' – 0.16 gallons KB-1° Plus / 50 gallons KB-1° Primer Injection point DPE-4-A-15' – 0.16 gallons KB-1° Plus / 50 gallons KB-1° Primer Injection point DPE-4-A-10' – 0.16 gallons KB-1° Plus / 50 gallons KB-1° Primer Injection point DPE-4-A-05' – 0.16 gallons KB-1° Plus / 50 gallons KB-1° Primer

DPE-7

Injection point DPE-7-A-15' – 0.16 gallons KB-1^{\circ} Plus / 50 gallons KB-1^{\circ} Primer Injection point DPE-7-A-10' – 0.16 gallons KB-1^{\circ} Plus / 50 gallons KB-1^{\circ} Primer Injection point DPE-7-A-05' – 0.16 gallons KB-1^{\circ} Plus / 50 gallons KB-1^{\circ} Primer

<u>DPE-8</u>

Injection point DPE-8-A-20' – 0.16 gallons KB-1° Plus / 50 gallons KB-1° Primer Injection point DPE-8-A-15' – 0.16 gallons KB-1° Plus / 50 gallons KB-1° Primer Injection point DPE-8-A-10' – 0.16 gallons KB-1° Plus / 50 gallons KB-1° Primer Injection point DPE-8-A-05' – 0.16 gallons KB-1° Plus / 50 gallons KB-1° Primer



Pre- and Post-Bioaugmentation Injection Monitoring Well Analytical Data Comparison Former Scott Aviation Facility - West of Plant 2 NYSDEC Site Code No. 9-15-149 Lancaster, New York

Sample ID	Groundwater		MW-4	4		MW-4	4		MW-8F	2		MW-8R			MW-16S			MW-16S		Ν	/W-16	D	I	W-16C)
Date Collected	RAO/TOGS 1.1.1		07/15/2	21	· ·	10/19/2	21	(07/14/2	1		10/19/21			07/14/21			10/20/21		C)7/14/2	1		10/20/21	1
Lab Sample ID	Objective	48	0-1872	292-2	480)-1910	95-1	480	0-18729	92-3	4	80-19109	5-1	48	30-187292	2-8	48	80-191185-	·1	480	-18729	92-9	480)-18729:	2-9
Volatile Organic Compounds (µg/L)											_														
1,1-Dichloroethane*	5*	<	4.0	U	<	4.0	U		7.1	J	<	25	U		510	J		430	J		2.0	J		0.91	J
Acetone	50	<	40	U	<	40	U	<	80	U	<	250	U	<	10,000	U	<	10,000	U		4.7	J	<	10	U
Chloroethane*	5*		91			42			26		<	25	U		1,700			1,500			73			58	
Chloroform	5	<	4.0	U	<	4.0	U	<	8.0	U	<	8.0	U	<	1,000	U	<	1,000	U		0.42	J		0.42	J
cis-1,2-Dichloroethene*	5*	<	4.0	U	<	4.0	U		1,700			120			34,000			5,600			12			0.91	J
Methylcyclohexane	5	<	4.0	U	<	4.0	U	<	10	U	<	25	U	<	1,000	U	<	1,000	U	<	1.0	U		0.66	J
Toluene*	5*		3.6	J		3.6	J		12			21			560	J	<	1,000	U	<	1.0	U	<	1.0	U
Trichloroethene*	5*	<	4.0	U	<	4.0	U	<	8.0	U	<	25	U	<	1,000	U	<	1,000	U		1.5		<	1.0	U
Vinyl chloride*	5*		12.0			9.0			2,000			1,000			58,000			35,000			16			1.5	
Total Volatile Organic Compounds	NL		107			55			3,745			1,145			94,770			42,530			110			73	
Total Organic Carbon (mg/L)	NL		15.7			22.7			28.0			56.8			400			300			1.6			3.8	

Notes:

Bold font indicates the analyte was detected.

Bold font and bold outline indicates the screening criteria was exceeded.

VOC - Volatile Orgainic Compound

TOC - Total Organic Carbon

Volatile Organic Compounds - Green font indicates decrease in post-injection VOC concentrations and red font indicates increase in post-injection VOC concentrations.

Total Organic Carbon - Green font indicates increase in post-injection TOC concentrations and red font indicates decrease in post-injection TOC concentrations.

RAO - Remedial Action Objectives

TOGS 1.1.1 - NYSDEC Technical and Operational Guidance Series 1.1.1

* Site-specific RAO per Record of Decision (November 1994).

Site-specific RAO's 1,1,1-Trichloroethane, Ethylbenzene, and Xylenes were not detected above the reporting limit.

Volatile Organic Compounds by Environmental Protection Agency Method 8260D (SW-846).

Total Organic Carbon by Environmental Protection Agency Method 9060A (SW-846).

µg/L - microgram per liter.

mg/L - milligrams per liter.

J - Result is less than the reporting limit but greater than or equal to the method detection limit and the concentration is an approximate value.

U - Not detected at or above reporting limit.

NL - Not listed.

Pre- and Post-Bioaugmentation Injection Dual Phase Extraction Well Analytical Data Comparison Former Scott Aviation Facility - West of Plant 2 NYSDEC Site Code No. 9-15-149 Lancaster, New York

Sample ID	Groundwater	DPE-3		DPE-3	}	DPE-4	ļ	DPE-4	ŀ	DPE-7	,	DPE-7	7	DPE-8		DPE-8	
Date Collected	RAO/TOGS 1.1.1	07/15/21	1	10/19/2	1	07/15/2	!1	10/19/2	1	07/15/2	1	10/19/2	21	07/15/21		10/19/21	1
Lab Sample ID	Objective	480-187292	2-12	480-19109	5-13	480-18729	2-13	480-19109	95-5	480-18729	2-16	480-19109	95-8	480-187292	2-17	480-19109	5-9
Volatile Organic Compounds (µg/l	_)																
1,1,1-Trichloroethane*	5*	20	U	4.0	U	4.0	U	4.0	U	2.0	U	2.0	U	54		100	U
1,1-Dichloroethane*	5*	12	J	3.7	J	1.6	J	2.1	J	2.3		2.0	U	140		120	
Acetone	50	200	U	40	U	40	U	40	U	9.2	J	20	U	400	U	1,000	U
Chloroethane*	5*	20	U	4.0	U	4.0	U	4.0	U	80		2.0	U	66		100	U
cis-1,2-Dichloroethene*	5*	940		110		180		240		2.4		2.0	U	21,000		4,100	
Toluene*	5*	20	U	4.0	U	4.0	U	4.0	U	2.0	U	2.0	U	22	J	100	U
Trichloroethene*	5*	120		18		19		12		2.0	U	2.0	U	24	J	100	U
Vinyl chloride*	5*	170		70		200		300		35		2.0	U	1,400		5,000	
Xylenes, Total*	5	40	U	8.0	U	8.0	U	8.0	U	4.0	U	4.0	U	80	U	100	U
Total Volatile Organic Compounds	s NL	1,242		202		401		554		128.9		28.0		22,706		9,220	
Total Organic Carbon (mg/L)	NL	4.9		40.5		11.6		9.3		5.9		11.5		37.7		26.2	

Notes:

Bold font indicates the analyte was detected.

Bold font and bold outline indicates the screening criteria was exceeded.

VOC - Volatile Orgainic Compound

TOC - Total Organic Carbon

Volatile Organic Compounds - Green font indicates decrease in post-injection VOC concentrations and red font indicates increase in post-injection VOC concentrations.

Total Organic Carbon - Green font indicates increase in post-injection TOC concentrations and red font indicates decrease in post-injection TOC concentrations.

RAO - Remedial Action Objectives

TOGS 1.1.1 - NYSDEC Technical and Operational Guidance Series 1.1.1

* Site-specific RAO per Record of Decision (November 1994).

Site-specific RAO's 1,1,1-Trichloroethane, Ethylbenzene, and Xylenes were not detected above the reporting limit.

Volatile Organic Compounds by Environmental Protection Agency Method 8260D (SW-846).

Total Organic Carbon by Environmental Protection Agency Method 9060A (SW-846).

µg/L - microgram per liter.

mg/L - milligrams per liter.

J - Result is less than the reporting limit but greater than or equal to the method detection limit and the concentration is an approximate value.

NL - Not listed.

Pre- and Post-Bioaugmentation Injection VFA Data Comparison Former Scott Aviation Facility - West of Plant 2 NYSDEC Site Code No. 9-15-149 Lancaster, New York

Sample ID	Sample Date	Sample Dilution	Lactate	Acetate	Propionate	Formate	Butyrate	Pyruvate
		Factor	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
MW-8R	8/26/2021	50	1.2	70	<0.31	<0.22	<0.41	<0.69
MW-8R	12/9/2021	50	<0.39	28	<0.31	<0.22	<0.41	<0.69
MW-16S	8/26/2021	50	<0.39	495	12	<0.22	81	0.71
MW-16S	12/9/2021	1000	<7.8	921	14	<4.4	98	<13.8

Notes:

VFA - Volatile fatty acid

mg/L - milligram per liter

Pre- and Post-Bioaugmentation Injection Gene-Trac Data Comparison Former Scott Aviation Facility - West of Plant 2 NYSDEC Site Code No. 9-15-149 Lancaster, New York

Sampla ID	Sample Date	Deha	alococcoides (Dhc)	Del	halobacter (Dhb)	VC R	eductase vcrA)	BAV1 V(C Reductase ovcA)	TCE Reductase (tcaA)		
Sample ID	Sample Date	Percent Dhc	Enumeration/Liter	Percent Dhb	Gene Copies/Liter	Percent vcrA	Gene Copies/Liter	Percent bvcA	Gene Copies/Liter	Percent tceA	Gene Copies/Liter	
MW-16S	8/26/2021	8 - 23 %	1 x 10 ⁹	0.3 - 1 %	5 x 10 ⁷	8 - 22 %	1 x 10 ⁹	1 - 3 %	1 x 10 ⁸	7 - 18 %	1 x 10 ⁹	
MW-16S	12/9/2021	6 - 17 %	1 x 10 ⁹	0.08 - 0.2 %	2 x 10 ⁷	5 - 15 %	1 x 10 ⁹	0.3 - 1 %	6 x 10 ⁷	2 - 5 %	3 x 10 ⁸	

Pre- and Post-Bioaugmentation Injection Bioattenuation Screening Summary Former Scott Aviation Facility - West of Plant 2 NYSDEC Site Code No. 9-15-149 Lancaster, New York

Table 6

											Monitorin	g Well Ide	entification							
Parameter	Units	Criteria		Score	MW	-4	MV	V-4	MW	-8R	MW	-8R	MW-	16S	MW	-16S	MW-	-16D	MW-	16D
				Value	Plume	Well	Plume	e Well	Plume	e Well	Plume	Well	Plume	Well	Plume	Well	Plume	e Well	Plume	Well
					4/8/21	Score	10/19/21	Score	4/7/21	Score	10/19/21	Score	4/9/21	Score	10/20/21	Score	4/7/21	Score	10/20/21	Score
Dissolved Oxygen	mg/L	< 0.5 mg/L	Tolerated, suppresses the reductive pathway at higher concentrations	3	0.81	0	3.0	0	0.63	0	1.8	0	2.24	0	0.34	0	0.92	0	1.10	0
		> 5 mg/L	Not tolerated; however, VC may be oxidized aerobically	-3																
Nitrate	mg/L	< 1 mg/L	At higher concentrations may compete with reductive pathway	2	0.032	2	0.035	2	< 0.050	2	0.022	2	< 0.050	2	< 0.050	2	< 0.050	2	0.031	2
Ferrous Iron	µg/L	> 1 mg/L	Reductive pathway possible	3	0.63	0	0.11	0	0.33	0	0.69	0	2.7	3	0.77	0	<0.10	0	2.90	0
Sulfate	mg/L	< 20 mg/L	At higher concentrations may compete with reductive pathway	2	8.2	2	4.1	2	8.5	2	6.0	2	17.9	2	<20	2	<20	2	<20	2
Sulfide	mg/L	> 1 mg/L	Reductive pathway possible	3	1.6	3	1.2	3	<1.0	0	0.8	0	<1	0	<1.0	0	<1.0	0	<1.0	0
Methane	µg/L	< 500 µg/L	VC oxidizes	0																1
		> 500 µg/L	Ultimate reductive daughter product, VC accumulates	3	14,000	3	25,000	3	21,000	3	12,000	3	13,000	3	10,000	3	17,000	3	21,000	3
Ethene	µg/L	> 10 µg/L	Daughter product of VC	2	600	2	580	2	460	2	620	2	33,000	2	51,000	2	320	2	<770	2
Ethane	µg/L	> 100 µg/L	Daugher product of Ethene	3	140	3	500	3	<1,700	0	360	3	710	3	510	3	330	3	630	3
ORP	mV	< 50 mV	Reductive pathway possible	1											-94.7	1				1
		< -100 mV	Reductive pathway likely	2	-170.4	2	-185.9	2	-185.8	2	-161.8	2	-101.0	2			-158.5	2	-164.3	2
pН	s.u.	5 < pH < 9	Optimal range for reductive pathway	0	7.60	0	7.71	0	7.34	0	7.56	0	6.84	0	6.65	0	7.47	0	7.52	0
		5 > pH > 9	Outside optimal range for reductive pathway	-2																1
Temperature	°C	> 20°C	At temperature > 20°C, biochemical process is accelerated	1	11.64	0	15.20	0	14.63	0	14.60	0	10.48	0	14.80	0	12.10	0	13.50	0
TOC	mg/L	> 20 mg/L	Carbon and energy source, drives dechlorination (natural or anthropogenic)	2	146	2	22.7	2	16.8	0	56.8	2	204	2	300	2	2.8	0	3.8	0
Carbon Dioxide	µg/L	> 2x background	Ultimate oxidative product	1	60,000	0	25,000	0	22,000	0	30,000	0	89,000	0	64,000	0	16,000	0	28,000	0
Alkalinity	mg/L	> 2x background	Results from interaction of between carbon dioxide and aquifer minerals	1	1040	0	756	0	281	0	458	0	472	0	470	0	314	0	302	0
Chloride	mg/L	> 2x background		2	494	0	NS	0	207	0	NS	0	866	0	NS	0	204	0	NS	0
PCE ¹	µg/L		N/A	0	<4	0	<4	0	<10	0	<25	0	<1,000	0	<1,000	0	<1	0	<1	0
TCE ²	µg/L		Material Released	0	<4	0	<4	0	<10	0	<25	0	<1,000	0	<1,000	0	<1	0	<1	0
DCE ³	µg/L		Daugher product of TCE (score if cis-1,2-DCE is 80% of total DCE)	2	4.4	2	<4	0	300	2	120	2	57,000	2	5,600	2	2.5	2	0.91	2
VC ⁴	µg/L		Daugher product of DCE	2	14	2	9	2	400	2	1,000	2	71,000	2	35,000	2	2.7	2	1.5	2
1,1,1-TCA ⁵	µg/L		Material Released	0	<4	0	<4	0	<10	0	<25	0	<1000	0	<1,000	0	<1	0	<1	0
1,1-DCA ⁶	µg/L		Daugher product of 1,1,1-TCA under reducing conditions	2	<4	0	<4	0	4.1	2	<25	0	440	2	430	2	0.84	2	0.91	2
CA ⁷	µg/L		Daughter product of 1,1-DCA or VC under reducing conditions	2	93	2	42	2	20	2	<25	0	1,300	2	15,000	2	50	2	58	2
			TOTAL SCOP	RE		25		23		19		20		27		23		22		22
Notes:					* MNA param	neters not o	collected so	cannot ade	quately eval	uate and s	core									

DCE = dichloroethene °C = degrees Celsius

µg/L = micrograms per liter

mg/L = milligrams per liter

mV = millivolts

ORP = oxidation-reduction potential

s.u. = standard unit

PCE = tetrachloroethene

TCE = trichloroethene

TOC = total organic carbon

1,1,1-TCA = 1,1,1-trichloroethane

1,1-DCA = 1,1-dichloroethane

VC = vinyl chloride

CA = chloroethane

0 to 5 points: There is inadequate evidence for anaerobic biodegradation of chlorinated organics.

6 to 14 points: There is limited evidence for anaerobic biodegradation of chlorinated organics.

15 to 20 points: There is adequate evidence for anaerobic biodegradation of chlorinated organics.

>20 points: There is strong evidence for anaerobic biodegradation of chlorinated organics.

1 = Material Released

² = Daugher product of PCE

³ = Daugher product of TCE (score if cis-1,2-DCE is 80% of total DCE)

⁴ = Daugher product of DCE

5 = Material Released

⁶ = Daugher product of 1,1,1-TCA under reducing conditions

⁷ = Daughter product of 1,1-DCA or VC under reducing conditions

Post-Bioaugmentation Injection Groundwater Monitoring Schedule Former Scott Aviation Facility - West of Plant 2 NYSDEC Site Code No. 9-15-149 Lancaster, New York

Event Date	Number of Locations Scheduled for Sampling		Locations Sch VOC and TOC	eduled for Sampling	
Quarterly Groun	dwater Monitoring				
		MW-2	MW-3	MW-4	MW-8R
		MW-11	MW-13S	MW-13D	MW-16S
January 2022	18	MW-16D	DPE-1	DPE-2	DPE-3
		DPE-4	DPE-5	DPE-6	DPE-7
		DPE-8	GWCT		
Comprehensive	Annual Groundwater Monito	oring			
		MW-2	MW-3	MW-4*	MW-8R* ⁺
		MW-9	MW-11*	MW-13S*	MW-13D
April 2022	22	MW-14S	MW-14D	MW-15S	MW-15D
April 2022	23	MW-16S*^*	MW-16D	DPE-1	DPE-2
		DPE-3	DPE-4	DPE-5	DPE-6
		DPE-7	DPE-8	GWCT	

Notes:

MW-## - Monitoring Well

MW-##S - Shallow Piezometer

MW-##D - Deep Piezometer

DPE-## - Dual Phase Extraction Well

GWCT - Groundwater Collection Trench

VOC - Volatile organic compound

TOC - Total organic carbon

* - Locations to be included for MNA sampling

^ - Location tentatively to be included for dechlorinating bacteria sampling

+ - Locations tentatively to be included for volatile fatty acid sampling

Attachment 1



AECOM 1 John James Audubon Parkway Suite210 Amherst, NY 14228 aecom.com

August 30, 2021

Mr. Glenn May, PG New York State Department of Environmental Conservation, Region 9 270 Michigan Avenue Buffalo, NY 14203-2999

Subject: Bioaugmentation Injection Work Plan Former Scott Aviation Facility – West of Plant 2 Lancaster, New York NYSDEC Site Code No. 9-15-149

Dear Mr. May:

On behalf of Tyco International and its successor Scott Figgie LLC, AECOM Technical Services, Inc. (AECOM) is pleased to provide for your review and approval this letter work plan for completing a bioaugmentation injection event at the Former Scott Aviation Facility – West of Plant 2 site (the Site) in Lancaster, New York (refer to **Figure 1** for the Site location). The objective of this injection event is to further remediate impacted Site groundwater. This injection work will be performed by subcontractor Matrix Environmental Technologies, LLC. (Matrix), with oversight by AECOM, using KB-1[®] Plus and KB-1[®] Primer; a bioengineered microbial culture by SiREM that contains Dehalococcoides (Dhc), to promote the complete dechlorination of chlorinated ethenes to non-toxic ethene and other non-chlorinated end products. The total injection area is approximately 50-foot by 50-foot and encompasses monitoring wells MW-8R and MW-16S and dual-phase extraction (DPE) wells DPE-3, DPE-4, DPE-7, and DPE-8; these are the locations with the highest total volatile organic compound (VOC) concentrations detected in Site groundwater.

This letter work plan provides the following information:

- A brief summary of the Site background, including Site history, Site geology/hydrogeology, previous investigation and remediation activities, and Site remedial action objectives (RAOs),
- A summary of the groundwater analytical data including monitored natural attenuation occurring at the Site,
- A detailed scope of work for the proposed bioaugmentation injection of KB-1® Plus and KB-1® Primer; and
- A schedule.

SITE BACKGROUND

The following discussion presents a brief summary of Site history, Site geology/hydrogeology, previous investigation and remediation activities, and Site RAOs.

Site History

A 3,000-gallon underground storage tank (UST) was previously located at the Site, immediately adjacent to the southwest corner of Scott Aviation Plant 2 (refer to attached **Figure 2** for Site features). The UST was used to store waste cutting oil and spent chlorinated organic solvents generated during manufacturing operations conducted in Plant 2.

During April 1991, the former Site owner, Figgie International, removed the aforementioned UST. Based on impacts discovered during the removal of the UST, Figgie entered into a remedial investigation/feasibility study (RI/FS) Order



on Consent with the New York State Department of Environmental Conservation (NYSDEC) on July 9, 1992, and an RI was initiated by Versar, Inc. on behalf of Figgie in the immediate area surrounding the former UST. The final RI report, approved by the NYSDEC on December 13, 1993, indicated the presence of VOCs in excess of NYSDEC soil and groundwater guidance values to the west of Plant 2. A subsequent FS report was prepared by Figgie and approved by the NYSDEC on August 29, 1994.

Based on the results of the RI/FS, the NYSDEC prepared a Record of Decision (ROD), dated November 7, 1994, which required remedial actions to be initiated to address contaminated soils and groundwater at the Site. The ROD specified that soil remediation would be accomplished by excavating all soils with VOCs above Site-specific RAOs and subsequently treating the soil on-Site using an ex-situ soil vapor extraction system.

The ROD also specified that groundwater remediation would be performed by installing a groundwater collection trench (GWCT) west of Plant 2 to induce hydraulic capture of groundwater impacted with VOCs and by constructing an associated groundwater treatment system.

Site Geology/Hydrogeology

The native soils underlying the Site generally consist of interbedded silts and clays with discontinuous sporadic fine sand lenses (shallow overburden). A thin coarse-grained layer is located above the bedrock (deep overburden). Based on the deep overburden wells installed at the Site, the average thickness of the overburden is approximately 21 feet below ground surface (bgs); ranging from 20 feet in the south to 26 feet in the north.

Groundwater is first encountered at the Site in the shallow overburden and then again just above the bedrock. The natural flow of groundwater at the Site in both the shallow overburden and deep overburden is to the northwest.

Previous Investigation and Remediation Activities

Source Area Soil Excavation and Treatment

Following approval of the Remedial Design by the NYSDEC in September 1995, soil remediation actions were initiated. Soils to the west of Plant 2 in the vicinity of the former UST were excavated and treated on-Site (refer to attached **Figure 2**). Approximately 5,600 cubic yards of soil were excavated from depths ranging between 2 feet and 21 feet bgs (bedrock contact) and treated. Based on analytical results for the treated soil (each individual VOC <1 milligram per kilogram (mg/kg) and total VOCs <10 mg/kg), the NYSDEC approved backfilling the excavation with the originally excavated soil treated on-Site. Backfilling of the excavation was completed on December 19, 1995.

Groundwater Collection Trench

In accordance with the ROD, a 200-foot long GWCT was constructed approximately 90 feet west of Plant 2 during February 1996 (refer to attached **Figure 2**). The purpose of the trench was to maintain hydraulic control of VOC-impacted groundwater. The bottom of the trench was excavated down to bedrock (approximately 25 feet bgs). The bottom five feet of the trench consists of rounded pea gravel and the top 20 feet of the trench was backfilled with remediated soils. A 6-inch diameter, slotted high density polyethylene pipe located at the bottom of the trench conveys water to a wet well located at the north end of the trench. The water is transferred from the wet well using a submersible pump through a 1-inch diameter Schedule 80 polyvinyl chloride pipe to a treatment system located in the Groundwater Treatment Building (GWTB) immediately west of Plant 2.

The groundwater treatment system consists of a low-profile shallow tray air stripper (AS) unit. Treated water from the AS unit is discharged under a City of Buffalo Pollutant Discharge Elimination System permit via a 2-inch diameter force main to the local sanitary sewer located south of the GWTB at Erie Street. Start-up of the groundwater treatment system occurred on March 1, 1996. Attached **Figure 2** shows the location of the GWCT and GWTB.



Additional Investigation Activities

Annual groundwater monitoring completed in April 1998 indicated an increasing trend in VOC concentrations in MW-4, located to the west of the GWCT at the western property boundary of the Site. Additionally, light non-aqueous phase liquid (LNAPL) was observed at MW-4 on the water level probe during a quarterly monitoring event conducted in November 1998. In April 1999, four new monitoring wells (designated MW-7, MW-8, MW-9, and MW-10) were installed to evaluate the extent and potential source of VOCs and LNAPL observed in MW-4. Based on repeated detections of VOCs and LNAPL in the groundwater to the west of the GWCT, a comprehensive Site investigation was conducted in February 2003 to further assess the vertical and horizontal extent of VOCs and LNAPL.

During the 2003 investigation, LNAPL was observed in MW-8 only (note MW-8 was retrofitted from a 4-inch diameter casing to a 2-inch diameter casing and finer sand pack in February of 2004 and renamed MW-8R). A total of 21 direct push technology borings were advanced to the east and west of the GWCT to further assess the extent of impacted soils west of Plant 2. Results were summarized in the June 2003 Site Investigation Completion Report (SICR), and the data indicated the continued presence of VOCs above the RAOs in the saturated soil and groundwater, primarily to the west of the GWCT.

Remedial Alternatives Analysis

Based upon the results of the 2003 investigation, a remedial alternatives analysis was completed, and the results were reported in the SICR. DPE was recommended to be implemented to supplement the existing groundwater remediation system and to further remediate VOCs in soil and groundwater at the Site.

At the request of the NYSDEC, a Remedial Design Work Plan was prepared that provided a detailed description of the proposed DPE system recommended in the SICR. A discussion of DPE system construction, startup, and operation and maintenance activities during approximately the first year of operation (May 14, 2004 through July 19, 2005) is provided in the first Remedial Action Engineering Report.

Previous Groundwater Injections

Beginning on July 28, 2010 and concluding on October 29, 2010, O&M, Inc., on behalf of Scott and with NYSDEC approval, initiated an in situ chemical oxidation (ISCO) pilot test. The test consisted of injection of sodium persulfate with chelated iron activation at 10 injection points located within the area of the >100 micrograms per liter (μ g/L) trichloroethene (TCE) plume as defined in 2010. A second series of ISCO injections was performed between June and October 2011; refer to attached **Figure 3** for the previous injection locations. A review of groundwater data at the source wells following the pilot test indicated a spike in TCE concentrations, possibly due to mobilization of product from the vadose zone and/or back diffusion from the treated aquifer matrix.

On November 6, 2014, AECOM submitted an Injection Pilot Test Work Plan to NYSDEC outlining a pilot test injection program to be conducted with the injectate Anaerobic BioChem and zero valent iron (ABC+[®]). Following NYSDEC approval, the pilot test was performed in November 2014 in a 1,200 square foot area centered between source area wells MW-4, MW-8R, and MW-16S; refer to attached **Figure 3** for previous ABC+[®] injection points. A total of eight injection points were completed with approximately 480 gallons of ABC+[®] injected at each location. Following the November 2014 injection of ABC+[®], two rounds of groundwater samples were collected and analyzed for VOCs. The groundwater VOC data collected in January 2015 and April 2015 showed significant decreases in TCE concentrations in the area of the injections, with corresponding increases in cis-1,2-dichlorethene, chloroethane, and vinyl chloride.

On April 28, 2015, AECOM submitted an addendum to the Injection Pilot Test Work Plan to NYSDEC outlining a second phase of injections to be conducted with the injectate ABC+[®]. Following NYSDEC approval, the injection program was performed between April and May 2015 in an approximate 3,600 square foot area centered between monitoring wells MW-4, MW-8R, MW-13S/D, and MW-16S/D, and DPE wells DPE-3, DPE-4, DPE-5, DPE-7, and DPE-8; refer to attached **Figure 3** for previous injection points. A total of 21 injection points were completed with approximately 410 gallons of ABC+[®] injected at each location. Note that this area was expanded vertically and horizontally from the first phase of injections in 2014 as well as overlapping (offset from) the first phase of injections.



During the week of November 26, 2018, AECOM completed a five-day supplemental injection program per the 2018 Injection Work Plan submitted to NYSDEC on October 31, 2018. ABC-Ole[®] with ZVI, a mixture of Anaerobic Biochem, ZVI, and emulsified fatty acids, was selected to remediate impacted groundwater in an approximate 4,500 square foot area within the 100 µg/L total VOC plume, which was based on October 2018 groundwater sample data. This area encompassed monitoring wells MW-4, MW-8R, MW-16S/D and MW-13S/D and dual phase extraction wells DPE-3, DPE-4, DPE-5, DPE-7, and DPE-8. The injectate ABC-Ole[®] with ZVI, mixed as an approximately 15 percent by weight solution, was injected at 20 locations (**Figure 4**). Sixteen injection points received approximately 400 gallons of solution each, with the four locations adjacent to monitoring well cluster MW-16 receiving approximately 500 gallons of injectate each. The injectate was distributed at depth intervals of 11, 14, 17, and 20 ft bgs and targeted the shallow water bearing unit.

REMEDIAL ACTION OBJECTIVES

Cleanup criteria for Site soil and groundwater are based on the RAOs established in the ROD. The table below presents the Site-specific cleanup criteria.

	Remedial Ac	tion Objectives
VOC	Soil	Groundwater
VOC	(mg/kg)	(µg/L)
Chloroethane	1	5
1,1-Dichloroethane	1	5
1,2-Dichloroethene	1	5
1,1,1-Trichloroethane	1	5
Trichloroethene	1	5
Vinyl chloride	1	5
Ethylbenzene	1	5
Toluene	1	5
Xylenes	1	5
Total VOCs	10	Not Applicable

The RAOs for the combined soil and groundwater remediation system include:

- 1. Maintain hydraulic control of shallow groundwater and eliminate potential off-Site migration of VOCs along the western property boundary.
- 2. Lower the groundwater table within the impacted source area to expose the aquifer matrix and subsequently extract soil vapors containing VOCs using enhanced vacuum extraction. By lowering the water table surface, the DPE system induces groundwater flow toward the system extraction wells, thereby allowing the applied vacuum to more effectively remove VOCs in the exposed aquifer matrix.
- 3. Reduce the mass of VOCs in the subsurface and remediate Site soil and groundwater toward meeting RAOs.
- 4. Obtain No Further Action status for the Site.

GROUNDWATER ANALYTICAL DATA

During the most recent groundwater sampling event (July 2021), nine VOCs were detected in groundwater from monitoring wells and piezometers sampled above their associated detection limits during the monitoring period. Six of the nine VOCs detected exceeded either the Site-specific RAOs for groundwater or the NYCRR criteria. The occurrences of constituents of potential concern were detected primarily in the vicinity of monitoring wells MW-16S and



MW-8R, and DPE wells DPE-3, DPE-4, DPE-7, and DPE-8. Attached **Tables 1** and **2** summarize VOC data for groundwater samples collected in July 2021 from the monitoring wells and DPE wells. VOC concentrations decrease significantly in the vicinity of the perimeter monitoring wells.

The presence and distribution of TCE degradation products cis-1,2-dichlorethene (cis-1,2-DCE) and vinyl chloride (VC), and of 1,1,1-trichloroethane (1,1,1-TCA) degradation products 1,1-dichlorethane (1,1-DCA) and chloroethane, provides supportive evidence that the attenuation of TCE and 1,1,1-TCA continues to occur on the Site via reductive dechlorination. The occurrence of these degradation products appears to be directly related to the historic distribution of TCE and 1,1,1-TCA in the subsurface. In addition, the virtual elimination of TCE and 1,1,1-TCA concentrations between the Third Quarter of 2015 and the Third Quarter of 2021 can be attributed to the injection pilot test performed in November 2014 using ABC+[®], the injection treatment in April/May 2015 using ABC+[®], and the most recent injection treatment in November 2018 using ABC-Ole+[®].

Monitored Natural Attenuation

To monitor the effectiveness of the November 2018 supplemental injections over time, monitored natural attenuation (MNA) parameters were collected from five monitoring wells (MW-4, MW-8R, MW-13S, MW-16S, and MW-16D) prior to the November 2018 injection event. MNA samples were also collected from the same five wells during the April 2019, July 2019, October 2019, April 2020, and April 2021 sampling events.

Results of the April 2021 MNA samples are summarized in attached **Table 3** (note MNA data were not collected in July 2021). Per **Table 3**, four of the five wells sampled for MNA parameters (not including background monitoring well MW-11) show strong evidence for anerobic biodegradation of chlorinated organics to occur (i.e., MW-4, MW-13S, MW-16S, and MW-16D); the remaining well (MW-8R) shows adequate evidence for anerobic biodegradation of chlorinated organics.

The initial concentrations of known TCA degradation products (1,1-DCA and chloroethane), as well as of TCE degradation products (1,2-DCE isomers and VC), suggest that reductive dechlorination of the chlorinated solvents present in Site groundwater as a result of the November 2018 ABC+[®] injection event is occurring. The induction of reducing conditions by the injection of ABC+[®] can accelerate the reductive dechlorination of parent chlorinated VOCs and increase the relative accumulation of degradation intermediates such as cis-1,2-DCE and VC before complete mineralization. As the naturally more aerobic aquifer conditions return after treatment using ERD, VC oxidizing bacteria should increase and complete the dechlorination process to ethene followed by complete mineralization.

Dechlorinating Bacteria Analysis

The use of the enhanced reductive dechlorination (ERD) amendments ABC+[®] and ABC-Ole[®] with ZVI were designed to provide needed nutrients, such as a soluble lactic acid carbon source, a phosphate buffer to control pH for optimum microbial growth, and ZVI which accelerates abiotic dechlorination of chlorinated ethenes and ethanes. The microbial analyses from "Bio-traps" placed on-Site indicates that the necessary concentrations for bacteria such as DHC species producing the enzymes tceA Reductase and VC reductase, remain present in the subsurface. Stimulation of the native bacteria by the injection of ABC+[®] and extra nutrients in the presence of chlorinated solvents in Site groundwater have dramatically reduced the concentrations of the original parent chlorinated VOCs, TCE and 1,1,1-TCA, over time.

AECOM deployed "Bio-traps" at MW-4 and MW-16S during three events, to determine the concentration (i.e., cells/bead) of dechlorinating bacteria. On January 4, 2016, April 9, 2020, and April 6, 2021, after approximately 30 days following deployment of the "Bio-Traps", AECOM extracted the "Bio-traps" and submitted them to Microbial Insights, Inc., in Knoxville, Tennessee for analysis. Per the table below summarizing the dechlorinating bacteria data from January 4, 2016, April 9, 2020, and April 6, 2021, the dechlorinating bacteria and degradative enzymes concentrations have decreased over time.



Sample ID	MW-16S	MW-16S	MW-16S	MW-4	MW-4	MW-4
Sample Date	1/4/16	4/9/20	4/6/21	1/4/16	4/9/20	4/6/21
Units	Cells/bead	Cells/bead	Cells/bead	Cells/bead	Cells/bead	Cells/bead
Dechlorinating Bacteria	and Degradativ	e Enzymes				
Dehalococcoides	2.28x10 ⁶	2.30x10 ⁵	1.26x10 ⁴	5.36x10 ⁵	6.21x10 ³	5.52x10 ³
tceA Reductase	1.02x10 ⁵	1.14x10 ⁴	1.63x10 ³	1.63x10 ⁴	4.59x10 ²	6.32x10 ²
BAV1 VC Reductase	6.80x10 ⁴	1.36x10 ³	2.84x10 ²	2.80x10 ⁴	4.27x10 ¹	9.49x10 ¹
VC Reductase	2.07x10 ⁴	1.96x10 ⁴	1.99x10 ²	4.81x10 ⁴	2.27x10 ²	7.40x10 ¹

Dechlorinating Chemical Analysis

In addition to the dechlorinating bacteria and degradative enzyme results, the presence and distribution of TCE degradation products (cis-1,2-DCE and VC) and 1,1,1-TCA degradation products (1,1-DCA and chloroethane) provide supportive evidence that the attenuation of TCE and 1,1,1-TCA and their degradation products via reductive dechlorination continues to occur in-situ at the Site. The occurrence and concentrations of these degradation products are directly related to the historic distribution of TCE and 1,1,1-TCA in the subsurface. Following the July 2021 quarterly groundwater sampling event, degradation products of TCE and 1,1,1-TCA were detected at their highest concentrations within the suspected source area near monitoring wells MW-8R, and MW-16S, and DPE wells DPE-4, DPE-7, and DPE-8. A limited number of other VOCs were sporadically detected in groundwater at the Site, with the majority of these detections located in groundwater at MW-15S (Note, MW-15S/D is located in the lime-stabilized excavation fill area and were not targeted during previous injection events because the pH of the groundwater in this area is too high to promote biological activity). The *Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Ground Water* indicates that a pH value greater than 9 is outside the range for reductive dechlorination to occur.

Total Organic Carbon

Samples were collected in July 2021 for total organic carbon (TOC) analysis to monitor the concentration of organic carbon sources available for optimum microbial growth. Although TOC concentrations have decreased over time in the areas targeted during the last injection event in 2018, the locations with the highest historical concentrations of contaminants of concern (MW-8R and MW-16S) still have TOC concentrations above 20 μ g/L, which is the minimum TOC concentration required to maintain effective ERD. Refer to **Table 1** for TOC data.

SCOPE OF WORK

The scope of work for the proposed bioaugmentation injections consists of three tasks: Task 1 – Project Management / Premobilization Activities; Task 2 – Bioaugmentation Injection; and Task 3 – Bioaugmentation Injection Summary Report. These tasks are described below.

Task 1 – Premobilization Activities

Under Task 1, AECOM will provide project management and coordination, premobilization activities, and communication with GSF, current Site owner AVOX Systems Inc., and NYSDEC.

The premobilization activities are summarized below:

- Amend the health and safety plan to address the bioaugmentation injection scope of work and physical and chemical elements of concern.
- Prepare a work plan describing the scope of work for the bioaugmentation injection program.
- Issue purchase orders to the injection subcontractor (Matrix) and the microbial culture manufacturer (SiREM).
- Prepare forms for use by AECOM personnel to document daily health and safety meetings, injection tracking, and/or other daily general notes.
- Mark out injection locations for Dig Safely 811 utility mark outs. Note a previous geophysical survey completed for utility locations will be referenced during mark out of the injection points.



Task 2 – Bioaugmentation Injection

AECOM will collect groundwater grab samples from MW-16S and MW-8R for volatile fatty acids (VFA) analysis, both prior to completing the bioaugmentation injections and 90 days following the injections. The data will be used to establish a baseline and monitor the quality and form of fermentation byproducts of electron donors to manage potential reapplication requirements. In addition, AECOM will collect groundwater grab sample from MW-16S both prior to completing the bioaugmentation injections and 90 days following the injections for Gen-Trac analysis to confirm the successful introduction and distribution of the organisms in the KB-1 Plus culture. The VFA and Gen-Trac samples will be submitted to SiREM for analysis. Refer to **Attachment 1** for the sample collection procedures.

Prior to the bioaugmentation injections, AECOM and subcontractor Matrix will take the DPE system off-line. A couple days prior to the fourth quarter groundwater sampling event (currently scheduled for the week of October 25, 2021), DPE-1, -2, and -5 would be brought back on-line for approximately one week to prevent any potential issues with the DPE system being off-line for an extended period of time. Note DPE-1, -2, and -5 are located up/side-gradient of the regional groundwater flow and outside the area of the bioaugmentation injection. DPE-1 and -5 have elevated VOCs that are not targeted by the injections due to their locations to the previously "remediated" soil on the east side of the GWCT. The DPE system would be cycled on-line for approximately one week and off-line for approximately three weeks until March 2023; i.e., the month of annual operations and maintenance, and just prior to the 2023 annual groundwater sampling event. The GWCT would remain on line as there are no deep injections near the GWCT (i.e., GWCT is upgradient [regional groundwater flow] of the proposed injection points). Per PRR #16, the combined DPE system only removed about 3 pounds of VOCs during the year ending in April 2021, so the effect of having a portion of that system offline is expected to be minimal compared to the benefit of the additional injections.

The microbial culture KB-1® Plus and the KB-1® Primer (used to prepare anaerobic water to disperse electron donors and protect anaerobic bioaugmentation cultures during injection into the subsurface) will be supplied by SiREM; refer to **Attachment 2** for specifications (material safety sheets are included in the Site specific health and safety plan (HASP)). The KB-1® Plus and the KB-1® Primer will be mixed and injected by Matrix at nine locations using a direct push technology drill rig (refer to **Attachment 3** for the SiREM's detailed mixing and injection procedures). As shown in **Figure 5**, three injection points will be located around two targeted monitoring wells (MW-8R and MW-16S) with injection points biased to the upgradient groundwater side of each of the wells, and one injection point will be located on the upgradient side of DPE-4, DPE-7, and DPE-8 (note DPE-3 is located in the center of the previously mentioned injection points).

Each injection point around MW-8R, DPE-4, and DPE-8 will receive approximately 200 gallons of KB-1® Plus/Primer (i.e., injectate) and will be distributed at four depth intervals (5, 10, 15 and 20 feet bgs), targeting both the shallow and deep overburden groundwater zone. Each injection point around MW-16S and DPE-7 will receive approximately 150 gallons of injectate and will be distributed at three depth intervals (8, 13, and 18 feet below ground surface), targeting the shallow overburden groundwater zone (refer to **Table 4** for a summary of injection depths and injectate volumes). The injections are expected to take three 10-hour days to complete. Following the injection, Matrix will complete Site restoration activities (i.e., plug injection boreholes). AECOM will oversee and track the injection program and restoration activities with support from the AECOM project engineer as needed.

Task 3 – Bioaugmentation Injection Summary Report

Following the completion of the bioaugmentation injection program (i.e., receipt of the post-injection 90-day sample data from SiREM), AECOM will draft a brief letter report for submittal to NYSDEC. The report will describe the activities performed and summarize the pre- and post-injection VFA and Gen-Trac data. In addition, the summary report will compare the July 2021 VOC groundwater data (pre-bioaugmentation injection) against the October 2021 VOC groundwater data (post-bioaugmentation injection) to demonstrate the ongoing dechlorination process. The final bioaugmentation injection summary report is expected to be finalized and submitted to NYSDEC prior to December 31, 2021.



HEALTH AND SAFETY PLAN

The Site-specific HASP was updated and approved by AECOM's District Safety, Health and Environment Manager on July 26, 2021 and was updated to include safety precautions regarding the bioaugmentation injections. A copy of the HASP is currently available on Site.

SCHEDULE

Following NYSDEC approval of this work plan, the bioaugmentation injection program will be initiated. AECOM is tentatively scheduled to begin the injections in September 2021; however, the pre-injection groundwater samples will be collected in August 2021.

If you have any questions regarding this submission, please do not hesitate to contact me at (716) 923-1125 or via e-mail at <u>dino.zack@aecom.com</u>.

Yours sincerely,

Dino J. Gack

Dino L. Zack, PG, STS Project Manager dino.zack@aecom.com

\Enclosures

cc: Mr. Stuart Rixman, GSF Management Company, LLC (electronic copy) Mr. Troy Chute, GSF Management Company, LLC (electronic copy) Mr. Raymond DeCarlo, AVOX Systems Inc. (electronic copy) Mr. Allen Thomalla, AVOX Systems Inc. (electronic copy) Mr. Hunter Bogdan, AVOX Systems Inc. (electronic copy) Project File 60538931

Figures







	MW-9 -	MONITORING WELL LOCATION
MW	-13S/D -ф-	NESTED PIEZOMETER LOCATION
	DPE-1 💿	DUAL-PHASE EXTRACTION WELL LOCATION (ACTIVELY EXTRACTING)
	DPE-6 🖨	DUAL-PHASE EXTRACTION WELL LOCATION (OFF-LINE)
		NOVEMBER 2014 INJECTION POUNTS (ABC+)
		MAY 2015 INJECTION POUNTS (ABC+)
	۲	OCT2010/OCT2011 INJECTION POINTS (PERSULFATE)
	[85]	TRICHLOROETHENE CONCENTRATION (μg/L) (APRIL 2018)
—	10 —	TRICHLOROETHENE ISOCONCENTRATION CONTOUR (µg/L) (APRIL 2018)
	- 5	REMEDIAL ACTION OBJECTIVE FOR TRICHLOROETHENE (μg/L) (APRIL 2018)
	[160]	TRICHLOROETHENE CONCENTRATION (μg/L) (APRIL 2014)
_	10 —	TRICHLOROETHENE ISOCONCENTRATION CONTOUR (μg/L) (APRIL 2014)
	- 5	REMEDIAL ACTION OBJECTIVE FOR TRICHLOROETHENE (µg/L) (APRIL 2014)
	<	BELOW REPORTING LIMIT
	(S)	SHALLOW PIEZOMETER
	(D)	DEEP PIEZOMETER
		GROUNDWATER COLLECTION TRENCH (GWCT)
-		APPROXIMATE PROPERTY BOUNDARY
	J	RESULT IS LESS THAN THE RL BUT GREATER THAN OR EQUAL TO THE MDL AND THE CONCENTRATION IS AN APPROXIMATE VALUE
	D	COMPOUND ANALYZED AT A DILUTION
	NM	NOT MEASURED
	NOTE	
	1. THE HIG	- HEST CONCENTRATION OF TCE WAS
	USED A GENERA	T PIEZOMETER PAIR LOCATIONS TO ATE ISOCONCENTRATION CONTOURS.
		0 15 30 60
	LOCA	FIGURE 3 TION OF PREVIOUS INJECTION POINTS
		FORMER SOOTT AMATION FACILITY
		LANCASTER, NEW YORK



IP-1 🔷	INJECTION LOCATION
MW-13S/D -�-	NESTED PIEZOMETER LOCATION
MW-9 🔶	MONITORING WELL LOCATION
DPE-6 🖨	DUAL-PHASE EXTRACTION WELL LOCATION (OFF-LINE)
[69]	TOTAL VOC CONCENTRATION (µg/L) (April 2018)
10	TOTAL VOC CONTOUR (April 2018) (DASHED WHERE INFERRED)
(S)	SHALLOW PIEZOMETER
(D)	DEEP PIEZOMETER
]	GROUNDWATER COLLECTION TRENCH (GWCT)
	APPROXIMATE PROPERTY BOUNDARY
NM	NOT MEASURED

N

NOTE

1. TOTAL VOC FROM THE SHALLOW PIEZOMETER PAIR LOCATIONS (i.e. MW-13S, MW-15S, MW-16S) WERE USED TO CREATE THE TOTAL VOC CONTOURS.





PROPOSED BIOAUGMENTATION INJECTION LOCATION

Ν

- MW-13S/D -Ò-NESTED PIEZOMETER LOCATION
 - MONITORING WELL LOCATION MW-9 🕀
 - DUAL-PHASE EXTRACTION WELL LOCATION (OFF-LINE) DPE-6 🝚
 - DUAL-PHASE EXTRACTION DPE-1 WELL LOCATION (ACTIVELY EXTRACTING)
 - [13.4] TOTAL VOC CONCENTRATION (µg/L)
- TOTAL VOC CONTOUR • 10
 - SHALLOW PIEZOMETER (S)
 - DEEP PIEZOMETER (D)
 - GROUNDWATER COLLECTION TRENCH (GWCT)
 - APPROXIMATE PROPERTY BOUNDARY

NOTES

- 1. GROUNDWATER DATA IS FROM APRIL 2021.
- 2. TOTAL VOC FROM THE SHALLOW PIEZOMETER PAIR LOCATIONS (i.e. MW-13S, MW-15S, MW-16S) WERE USED TO CREATE THE TOTAL VOC CONTOURS.
- 3. PROPOSED VFA SAMPLES TO BE COLLECTED AT MW-8R AND MW-16S.
- 4. PROPOSED GENE-TRAC SAMPLE TO BE COLLECTED AT MW-16S.
- 5. SHALLOW/DEEP OVERBURDEN GROUNDWATER FLOW IS TO THE NORTHWEST.

0	1	5 3	0	60	C							
SCALE IN FEET												

FIGURE 5 PROPOSED BIOAUGMENTATION INJECTION POINTS

FORMER SCOTT AVIATION FACILITY LANCASTER, NEW YORK

Summary of Monitoring Well Analytical Data - July 2021 Former Scott Aviation Facility NYSDEC Site Code No. 9-15-149 Lancaster, New York

Sample ID	Groundwater	MW-2		MW-3		MW-4		MW-8R			MW-11			Duplicate [^]					
Date Collected	RAO/TOGS 1.1.1	07/13/21		07/13/21		07/15/21		1	07/14/21			07/13/21			07/13/21				
Lab Sample ID	Objective	480-187292-1		480-187292-4		480-187292-2		92-2	480-187292-3			480-187292-5			480-187292-19				
Volatile Organic Compounds by Method 8260 (µg/L)				-				_		-				_					
1,1-Dichloroethane	5*	<	2.0	U		11		<	4.0	U		7.1	J		0.61	J		0.64	J
Acetone	50	<	20	U	<	10	U	<	40	U	<	80	U	<	10	U	<	10	U
Chloroethane	5*	<	2.0	U	<	1.0	U		91			26		<	1.0	U	<	1.0	U
Chloroform	5	<	2.0	U	<	1.0	U	<	4.0	U	<	8.0	U	<	1.0	U	<	1.0	U
cis-1,2-Dichloroethene	5*	<	2.0	U		3.0		<	4.0	U		1,700			1.6			1.5	
Toluene	5*	<	2.0	U	<	1.0	U		3.6	J		12		<	1.0	U	<	1.0	U
Trichloroethene	5*	<	2.0	U	<	1.0	U	<	4.0	U	<	8.0	U	<	1.0	U	<	1.0	U
trans-1,2-Dichloroethene	5	<	2.0	U	۷	1.0	U	<	4.0	U		8.4		<	1.0	U	<	1.0	U
Vinyl chloride	5*	<	2.0	U		9.6			12			2,000			2.2			2.2	
Total Volatile Organic Compounds	NL		0.0			24			107			3,754			5.4			4.3	
Total Organic Carbon	NL		25.0			2.9			15.7			28.0			2.5			NS	
Table 1

Summary of Monitoring Well Analytical Data - July 2021 Former Scott Aviation Facility NYSDEC Site Code No. 9-15-149 Lancaster, New York

Sample ID	Groundwater	Ν	/W-135	S		MW-13D			MW-16S			MW-16D	
Date Collected	RAO/TOGS 1.1.1	0	7/14/2 ⁻	1		07/14/21			07/14/21			07/14/21	
Lab Sample ID	Objective	480	-18729	2-6	48	0-187292	2-7	48	30-187292-	-8	480	0-187292	2-9
Volatile Organic Compounds by Met	hod 8260 (µg/L)						-						
1,1-Dichloroethane	5*	<	2.0	U	<	1.0	U		510	J		2.0	J
Acetone	50	<	20	U	<	10	U	<	10,000	U		4.7	J
Chloroethane	5*		2.7			2.2			1,700			73	
Chloroform	5	<	2.0	U	<	1.0	U	<	1,000	U		0.42	J
cis-1,2-Dichloroethene	5*	<	2.0	U	<	1.0	U		34,000			12	
Toluene	5*	<	2.0	U	<	1.0	U		560	J	<	1.0	U
Trichloroethene	5*	<	2.0	U	<	1.0	U	<	1,000	U		1.5	
trans-1,2-Dichloroethene	5	<	2.0	U	<	1.0	U	<	1,000	U	<	1.0	U
Vinyl chloride	5*		3.5		<	1.0	U		58,000			16	
Total Volatile Organic Compounds	NL		6.2			2.2			94,770			110	
Total Organic Carbon	NL		4.9			2.7			400			1.6	

Notes:

Bold font indicates the analyte was detected.

Bold font and bold outline indicates the screening criteria was exceeded.

^ - Duplicate collected at MW-11.

* Site-specific RAO per ROD (November 1994).

Site-specific RAO's 1,1,1-Trichloroethane, Ethylbenze, and Xylenes were not detected above the reporting limit.

J - Result is less than the reporting limit but greater than or equal to the method detection limit and the concentration is an approximate value.

U - Not detected at or above reporting limit.

NL - Not listed.

Table 2

Summary of Dual Phase Extraction Well Groundwater Analytical Data Former Scott Aviation Facility - West of Plant 2 NYSDEC Site Code No. 9-15-149 Lancaster, New York

Sample ID	Groundwater	DPE-1		DPE-2)	DPE-3		DPE-4	1	DPE-5	5	DPE-6	3	DPE-	7	DPE-8	
Date Collected	RAO/TOGS 1.1.1	07/15/2	1	07/15/2	1	07/15/2	1	07/15/2	21	07/15/2	1	07/15/2	21	07/15/2	21	07/15/2	1
Lab Sample ID	Objective	480-18729	2-10	480-18729	2-11	480-18729	2-12	480-18729	92-13	480-18729	2-14	480-18729	92-15	480-18729	92-16	480-18729	2-17
Volatile Organic Compounds by M	lethod 8260 (µg/L)																
1,1,1-Trichloroethane	5*	4.0	U	1.0	U	20	U	4.0	U	2.0	U	1.0	U	2.0	U	54	
1,1-Dichloroethane	5*	72		1.0	U	12	J	1.6	J	0.80	J	6.0		2.3		140	
2-Butanone (MEK)	50	62		10	U	200	U	40	U	20	U	10	U	20	U	400	U
Acetone	50	180		10	U	200	U	40	U	6.4	J	10	U	9.2	J	400	U
Chloroethane	5*	8.4		1.0	U	20	U	4.0	U	14		1.0	U	80		66	
cis-1,2-Dichloroethene	5*	96		1.0	U	940		180		1.7	J	6.9		2.4		21,000	
Ethylbenzene	5	4.2		1.0	U	20	U	4.0	U	2.0	U	1.0	U	2.0	U	40	U
Toluene	5*	11		1.0	U	20	U	4.0	U	2.0	U	1.0	U	2.0	U	22	J
Trichloroethene	5*	8.0		1.0	U	120		19		2.0	U	1.4		2.0	U	24	J
Vinyl chloride	5*	23		1.0	U	170		200		2.0	U	2.8		35		1,400	
Xylenes, Total	5	2.8	J	2.0	U	40	U	8.0	U	4.0	U	2.0	U	4.0	U	80	U
Total Volatile Organic Compounds	NL	467		0		1,242		401		23		17.1		128.9		22,706	
Total Organic Carbon	NL	142		7.4		4.9		11.6		27.5		4.5		5.9		37.7	

Notes:

Bold font indicates the analyte was detected.

Bold font and bold outline indicates the screening criteria was exceeded.

* Site-specific RAO per ROD (November 1994).

J - Result is less than the reporting limit but greater than or equal to the method detection limit and the concentration is an approximate value.

U - Not detected at or above reporting limit.

NS - Not sampled.

NL - Not listed.

Table 3 Bioattenuation Screening Summary Scott Figgie Area 2 Site Lancaster, New York

								Monito	oring Well	Identificatio	on				
Parameter	Units	Criteria	Score	MW-	4	MW	-8R	MW	-11	MW-	13S	MW-	16S	MW-	16D
			Value	Plume V	Vell	Plume	Well	Backgrou	und well	Plume	Well	Plume	Well	Plume	Well
				4/8/21	Score	4/7/21	Score	4/6/21	Score	4/7/21	Score	4/9/21	Score	4/7/21	Score
Dissolved Oxygen	mg/L	< 0.5 mg/L	3	0.81	0	0.63	0	1.21	0	1.07	0	2.24	0	0.92	0
		> 5 mg/L	-3												
Nitrate	mg/L	< 1 mg/L	2	0.032	2	<0.050	2	<0.050	2	<0.050	2	<0.050	2	<0.050	2
Ferrous Iron	µg/L	> 1 mg/L	3	0.63	0	0.33	0	0.33	0	0.088	0	2.7	3	<0.10	0
Sulfate	mg/L	< 20 mg/L	2	8.2	2	8.5	2	18.7	2	7.5	2	17.9	2	<20	2
Sulfide	mg/L	> 1 mg/L	3	1.6	3	<1.0	0	<1.0	0	<1	0	<1	0	<1.0	0
Methane	µg/L	< 500 µg/L	0												
		> 500 µg/L	3	14,000	3	21,000	3	2,100	3	20,000	3	13,000	3	17,000	3
Ethene	µg/L	> 10 µg/L	2	600	2	460	2	<150	2	<770	2	33,000	2	320	2
Ethane	µg/L	> 100 µg/L	3	140	3	<1,700	0	<170	0	710	3	710	3	330	3
ORP	mV	< 50 mV	1					-17.3	1	-97.3	1				
		< -100 mV	2	-170.4	2	-185.8	2					-101.0	2	-158.5	2
pН	s.u.	5 < pH < 9	0	7.60	0	7.34	0	6.64	0	6.88	0	6.84	0	7.47	0
		5 > pH > 9	-2												
Temperature	°C	> 20°C	1	11.64	0	14.63	0	12.70	0	11.41	0	10.48	0	12.10	0
тос	mg/L	> 20 mg/L	2	146	2	16.8	0	4.5	0	6.8	0	204	2	2.8	0
Carbon Dioxide	µg/L	> 2x background	1	60,000	0	22,000	0	140,000	0	120,000	0	89,000	0	16,000	0
Alkalinity	mg/L	> 2x background	1	1040	0	281	0	428	0	539	0	472	0	314	0
Chloride	mg/L	> 2x background	2	494	0	207	0	1,310	0	163	0	866	0	204	0
PCE ¹	µg/L		0	<4	0	<10	0	<1	0	<1	0	<1000	0	<1	0
TCE ²	µg/L		0	<4	0	<10	0	<1	0	0.77	0	<1000	0	<1	0
DCE ³	µg/L		2	4.4	2	300	2	1.8	2	1.8	2	57,000	2	2.5	2
VC ⁴	µg/L		2	14	2	400	2	2.1	2	7.1	2	71,000	2	2.7	2
1,1,1-TCA⁵	µg/L		0	<4	0	<10	0	<1	0	<1	0	<1000	0	1	0
1,1-DCA ⁶	µg/L		2	<4	0	4.1	2	0.66	2	0.40	2	440	2	0.84	2
CA7	µg/L		2	93	2	20	2	<1	2	3.3	2	1,300	2	50	2
					25		19		18		21		27		22

Notes:

DCE = dichloroethene

°C = degrees Celsius

µg/L = micrograms per liter

mg/L = milligrams per liter

mV = millivolts

ORP = oxidation-reduction potential

s.u. = standard unit

- PCE = tetrachloroethene
- TCE = trichloroethene

* MNA parameters not collected so cannot adequately evaluate and score

0 to 5 points: There is inadequate evidence for anaerobic biodegradation of chlorinated organics.

6 to 14 points: There is limited evidence for anaerobic biodegradation of chlorinated organics.

15 to 20 points: There is <u>adequate</u> evidence for anaerobic biodegradation of chlorinated organics.

>20 points: There is strong evidence for anaerobic biodegradation of chlorinated organics.

¹ = Material Released

- ² = Daugher product of PCE
- 3 = Daugher product of TCE (score if cis-1,2-DCE is 80% of total DCE)
- ⁴ = Daugher product of DCE
- ⁵ = Material Released
- ⁶ = Daugher product of 1,1,1-TCA under reducing conditions

⁷ = Daughter product of 1,1-DCA or VC under reducing conditions

Table 4

Bioaugmentation Injection Intervals and Injectate Volumes

<u>MW-16S</u>

Injection point MW-16S-A-18' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-16S-A-13' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-16S-A-08' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer

Injection point MW-16S -B-18' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-16S -B-13' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-16S -B-08' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer

Injection point MW-16S -C-18' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-16S -C-13' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-16S -C-08' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer

MW-8R

Injection point MW-8R-A-20' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-8R-A-15' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-8R-A-10' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-8R-A-05' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer

Injection point MW-8R-B-20' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-8R-B-15' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-8R-B-10' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-8R-B-05' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer

Injection point MW-8R-C-20' - 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-8R-C-15' - 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-8R-C-10' - 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point MW-8R-C-05' - 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer

DPE-4

Injection point DPE-4-A-20' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point DPE-4-A-15' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point DPE-4-A-10' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point DPE-4-A-05' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer

<u>DPE-7</u>

Injection point DPE-7-A-15' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point DPE-7-A-10' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point DPE-7-A-05' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer

<u>DPE-8</u>

Injection point DPE-8-A-20' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point DPE-8-A-15' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point DPE-8-A-10' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer Injection point DPE-8-A-05' – 0.6 liters KB-1 Plus / 50 gallons KB-1 Primer

Note:

Injection volumes are based on 6 packets of KB-1[°] Plus mixed with 20 liters of KB-1[°] Primer.



Attachment 1



GROUNDWATER COLLECTION AND SHIPPING PROTOCOL FOR VOLATILE FATTY ACIDS & DISSOLVED HYDROCARBON GASES ANALYSIS

This document provides procedures for collecting and shipping volatile fatty acids (VFA) samples and dissolved hydrocarbon gases (DHG) samples.

Sampling Supplies: SiREM provides sampling supplies (VOA vials, blue ice, coolers, shipping documents) free of charge upon request, please provide 3 days advance notice for this service. Customers are responsible for return shipping charges for the samples.

For shipping inquiries and sampling supplies please use our online form:

http://siremlab.com/sampling-supply-form/

Or contact:

- Ximena Druar: 519-515-0838/xdruar@siremlab.com
- Jennifer Wilkinson: 519-822-2265/jwilkinson@siremlab.com

Sample Collection: Prior to sample collection, sampling points should be purged using industry-accepted groundwater purging protocols to obtain representative groundwater. Duplicate samples are collected in 40 mL VOA vials that are unpreserved for VFA analysis, or preserved with hydrochloric (HCI) acid for DHG analysis.

- 1) Vials should be completely filled with no headspace (to the extent possible). Fill the VOA vial so there is a convex meniscus above the rim of the vial making sure not to overflow, to ensure that the preservative (HCI) is not washed out in the case of DHG analysis.
- 2) Cap each vial tightly and invert to confirm the absence of air bubbles. If air bubbles are present, uncap the vial and add a few more drops of sample and re-check for bubbles.
- 3) Fill two 40 mL VOA vials for each sample location.
- 4) Samples should be stored at 4°C and shipped on blue ice or double bagged wet ice in a plastic or Styrofoam cooler.

Sample Labeling and Handling: Samples should be clearly labeled using permanent marker with sample ID and sampling date and individually sealed in bubble wrap and then placed in a cooler with blue ice packs (preferred). If wet ice is used it should be double bagged. Sample hold time is 14 days at 4°C.

Chain-of-Custody: See Attachment 1 for a sample chain-of-custody, printable chain of custody forms are available online at <u>http://siremlab.com/forms/</u> the completed chain-of-custody should be placed in a ziplock bag inside the cooler. If applicable, purchase order number and quotation number should be entered in the chain of custody. Please indicate which analysis is requested.

siremlab.com



Shipping: Ship samples by priority overnight courier to SiREM Knoxville, TN (address below). When using FedEx, if a shipment value exceeding \$100 is declared additional charges may apply. Please see terms and conditions on reverse of waybill or contact FedEx directly for more information.

Shipping Documentation: A US domestic waybill is required. See Attachment 2 for sample FedEx waybill.

Section 1: Fill in date, complete shipping address and include your FedEx account number Section 2: Your internal reference number/project number (if required) Section 3: To address is: (already completed)

SiREM Knoxville 180A Market Place Boulevard Knoxville, TN 37922

Section 4a: Express package Service – mark FedEx priority overnight Section 5: Other packaging Section 7: Payment and by Sender Section 8: Signature

Place completed waybill in plastic sleeve on exterior of cooler.

Technical Inquiries: Should you require technical assistance with sampling or if you have questions regarding the analysis, data interpretation etc. please contact:

- Jeff Roberts 519-515-0840/jroberts@siremlab.com
- Phil Dennis 519-515-0836/pdennis@siremlab.com

Attachments: (1) Sample Chain-of-Custody (2) Sample FedEx Domestic US Air Waybill



Attachment 1: Sample Chain-of-Custody

	1		Lustody Form										180A Market Place Blvd. Knoxville, TN 37922 Phone: 865.330.0037					
Project Name		*F	roject #				Analysis											
Project Manager		*(ompany															Preservative Kev
Email Address													8		\neg			0. None
ddress (Street)							1					/ Acids	bon ga					1. HCL 2. Other
ity	State/Province		Co	ountry			Ŷ	-	뿌	Q.	ABO		drocarl	tudy			3. Other	3. Other
Phone #							frac D	frac V(frac D	Irac D	Trac to	e Fath	red hy	bility 9				4. Other
Sampler's Signature	*	'Sampler's Pri	nted				Gene-	Gene-	Gene-	Gene-	Gene	Volatil	Dissol	Treata				6. Other
Client Sample ID			Sam Date	npling Time	Matrix	∉ of Containers									+	+	╈	Other Information
					-				\vdash						+	+	+	
					<u> </u>										\rightarrow	\rightarrow	+	
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																+	+	
O. # Billing Inf	ormation		Turnard	ound Time R	equested	Cooler C	ondition	C.	For	Lab Use	Only							
Bill To:			No	ormal 🗌		Cooler Te	emperat	ture:							-			
			R	ish 🗌		Custodu	Scolor		~ □						-			
						Guaday	Joara.	1	00	,						-		
Relinquished By: gnature	Re Signature	ceived By:		Relinquished By: Signature				gnature	Rec	eived B	y:		Signati	Re	linquisho	ed By:		Received By: Signature
inted me	Printed Name		Printed Name				Prin Na	nted me					Printed Name					Printed Name
m	Firm		Firm				Firr	m					Firm					Firm
te/Time	Date/Time		Date/Time				Date/Time Date/Time							Date/Time				



Attachment 2: Sample Domestic Waybill

	ost locations. Packages up to 150 lbs.
Service order has changed. Please select ca	For packages over 150 lbs., use the new FedEx Express Freight US Airbill.
t Business Day	2 or 3 Business Days
x First Overnight st next business morning delivery to select yns. Friday shipments will be delivered on ay unless SATURDAY Delivery is selected.	FedEx 2Day A.M. Second business morning* Saturday Delivery NOT available.
Ex Priority Overnight usiness morning.* Friday shipments will be red on Monday unless SATURDAY Delivery cted.	FedEx 2Day Second business afternoon.* Thursday shipments will be delivered on Monday unless SATURIDAY Delivery is selected.
Ex Standard Overnight usiness afternoon.* Jay Delivery NOT available.	Third business day.* Saturday Delivery NOT available.
kaging * Declared value limit \$500.	and and and a set of the set of
ix Envelope* 📃 FedEx Pak*	* FedEx FedEx Tube
cial Handling and Delivery S	ignature Options
URDAY Delivery vailable for FedEx Standard Overnight, FedEx 2Day	A.M., or FedEx Express Saver.
Signature Required Direct Someo signature for delivery.	ot Signature Indirect Signature In a trecipient's address gn for delivery. Foe applies.
this shipment contain dangerous gor	ods?
Yes As per attached Yes Shipper's D	Declaration Dry Ice
shipper's Declarabon. — not required sods (including dry ice) cannot be shipped in FedEx pa FedEx Express Drop Box.	ickaging Cargo Aircraft Only
ment Bill to:	and the second of the second of the second of the
der Enter FedEx Acct.	No. or Credit Card No. below.
lo in Section Recipient	Third Party Credit Card Cash/Check
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GROUNDWATER SAMPLE COLLECTION AND SHIPPING FOR GENE-TRAC® ANALYSIS

This document provides sampling and shipping instructions for Gene-Trac[®] quantitative polymerase chain reaction (qPCR) (e.g., Gene-Trac[®] Dhc or FGA analysis) and Gene-Trac[®] next generation sequencing (NGS) tests performed on groundwater.

Sample Collection Methods: There are two groundwater sampling methods for Gene-Trac®:

- Method A: conventional groundwater sample collection; and
- Method B: field filtration (i.e., groundwater solids collected on a filter).

Both methods yield equivalent results; however, shipping charges for the field filters will be lower due to reduced size and weight of the samples retuned to the lab.



Ordering Sampling Supplies: SiREM is pleased to provide sampling supplies (containers or filters, coolers, ice packs upon request) free of charge. Note: Please provide 7 days advance notice for this service, otherwise a \$50 shipping surcharge may apply. Please contact Ximena Druar 519-515-0838 / <u>xdruar@siremlab.com</u> or use our **online sample kit order portal** <u>http://www.siremlab.com/sampling-supply-form</u> to order sampling supplies.

Figure 1: Gene-Trac samples can be provided as either 1 L Wide mouth (HDPE) bottles (left) or Sterivex® filters (right). Each filter is provided in an outer storage tube that contains the filter, a screw cap and a sample label

Table 1: Sample Requirements for Gene-Trac[®] qPCR Testing and NGS Analysis

	Method A: Groundwater Sample	Method B: Field Filtration	Hold Time
Gene-Trac [®] qPCR Tests (e.g., Dhc/FGA)	One-1L Wide mouth Nalgene	One -Sterivex [®] filter with up to 1 L water passed through	7 days at 4°C
Gene-Trac [®] NGS	Two-1L Wide mouth Nalgene	Two-Sterivex [®] filters with up to 1 L water passed through	7 days at 4°C





Collecting Samples

For all Gene-Trac[®] (qPCR) tests, only one bottle or filter is required per sample. Please note that for next generation sequencing (NGS) analysis duplicate samples are required to provide sufficient biomass for analysis (SeeTable 1)

Groundwater Purging: Prior to groundwater sample collection, sampling points should be purged using industry-accepted well purging protocols to obtain representative groundwater. Note: turbidity in groundwater samples is not a concern.

Method A: Conventional Groundwater Samples in 1 Liter Bottles

Following purging, 1-liter (L) groundwater samples are collected in large mouth 1L high-density polyethylene (HDPE) bottles (e.g., Nalgene or equivalent) with minimal headspace. No preservatives are required; samples should be stored and shipped at 4°C on blue or double bagged wet ice. The hold time is 7 days.

Method B: Field Filtration

- Following ground water purging, remove Sterivex filter from storage container and insert luer-lock adapter (white barbed fitting) into pump effluent tubing (1/4"-5/16" inside diameter) and securely fasten using a hose clamp if required (Figure 1B).
- Remove the white rubber nipple cover from the effluent end of the filter (do not discard cap-this will be used to seal after sampling).
- 3) Turn on pump and direct filter discharge into a graduated container (Figure 1B). Pass up to 1L of water through the filter. Note that the filter often clogs before a full 1L of sample is filtered. If this occurs, record the measured volume of water passed through the filter (in milliliters [mL]) on the label provided (Figure 1A) and the provided chain of custody. Shut off the pump.
- 4) Cap the effluent end of the filter (while full of water) with the small white nipple cap provided; decouple the tubing/luer-lock fitting from the influent end of the filter and seal the filter unit with the white screw-cap (Figure 1C). Place the sealed filter in the storage tube, label with the sample location, date and total volume of groundwater passed through the filter. The filter should be stored and shipped at 4°C in the provided cooler (Figure 1D).
- 5) Remove the luer-lock fitting in the pump tubing and discard. Dispose of effluent groundwater in accordance with applicable site procedures.



Figure 2: Use and Shipping of Field Filters



Labelling, Storage and Shipping

Sample Labelling and Handling: Samples should be clearly labeled (including sample ID and date) and individually sealed in re-sealable freezer bags provided and placed in a cooler with cool packs. If wet ice is used it must be double bagged. Sample hold time for 1L groundwater and filter samples is 7 days at 4°C.

Chain-of-Custody: Include the total volume passed through the filter for each sample (Method B only), the applicable purchase order number and quotation number where applicable. Please indicate which analysis is requested by noting the test method (refer to Attachment 1 for a list of Gene-Trac[®] analyses provided by SiREM). The completed chain-of-custody (Attachment 2) should be placed in a zip-lock bag inside the cooler with the samples.

Shipping: For samples originating in the USA ship samples by priority overnight courier to SiREM Knoxville, TN (address below). Samples should be given a nominal value of no more than \$10.

Please note that SiREM is not open on Saturdays

The following shipping document is required:

Domestic Waybill (e.g., FedEx) see sample FedEx waybill (Attachment 3). Complete shipper specific information, other information should be completed as indicated.

Section 1: Fill in date, complete shipping address and include your FedEx account number Section 2: Your internal reference number/project number (if required) Section 3: To address is: (already completed)

SiREM Knoxville 180A Market Place Boulevard Knoxville, TN 37922

Section 4a: Express package Service – mark FedEx priority overnight Section 5: Other packaging Section 7: Payment and by Sender Section 8: Signature

Technical Inquiries:

- Ximena Druar 519-515-0838 xdruar@siremlab.com
- Phil Dennis 519-515-0836 pdennis@siremlab.com

Attachments: (1) Available Gene-Trac® Tests

- (2) SiREM Chain-of-Custody
- (3) Example FedEx Waybill





Attachment 1: Available Gene-Trac® Tests

Contaminant Class	Redox	Gene-Trac® Test Name	Target	Relevance					
		Dhc	Dehalococcoides	Dechlorinates PCE, TCE, all DCE isomers, VC					
		Dhb	Dehalobacter	Dechlorination of PCE &TCE to cDCE					
		Dsm	Desulfuromonas	Dechlorination of PCE & TCE to cDCE					
		Dsb	Desulfitobacterium	Partial dechlorination of PCE and TCE to cDCE					
	Anaerobic	Geo	Geobacter	Dechlorinates PCE to cDCE/biogeochemical degradation					
Chlorinated Ethenes		Dhg	Dehalogenimonas	Dechlorination of tDCE to VC and VC to ethene					
		Oblessethese	Vinyl Chloride Reductase (vcrA)	Dechlorination of cDCE & VC to ethene					
		FGA	BAV1 Reductase (bvcA)	Dechlorination of cDCE and VC to ethene					
			Trichloroethene Reductase (tceA)	Dechlorination of PCE and TCE to cDCE and VC					
	Aerobic	Polaromonas	Polaromonas	Aerobic dechlorination of cDCE					
	Логовіо	etn	etnE	Aerobic degradation of VC					
		Dhb	Dehalobacter	Dechlorinates 1,1,1-TCA/1,2-DCA /1,1,2-TCA/ 1,1,2,2-TeCA					
		Dhg	Dehalogenimonas	Dechlorinates 1,2- DCA, 1,1,2,2-TeCA, 1,1,2-TCA					
	Anaerobic	Dhc	Dehalococcoides	Dechlorinates 1,2-DCA to ethene					
Chlorinated Ethanes		Dsb	Desulfitobacterium	Dechlorinates 1,1,2-TCA &1,2-DCA					
		cfrA/dcrA	Dichloroethane Dehalogenase (dcrA)	Dechlorinates 1, 1, 1-TCA & 1, 1-DCA					
		sMMO	Soluble Methane Monooxygenase	Co-metabolism of 1,1,1-TCA & 1,1-DCA by methanotrophs					
	Aerobic	PMO	Propane Monooxygenase	Co-metabolism of chlorinated ethanes by propanotrophs					
		dhIA	Haloalkane Dehalogenase (dhlA)	Aerobic dechlorination of 1,2-DCA					
	Anaerobic	Dhb	Dehalobacter	Dechlorination of chloroform to DCM; DCM to acetate					
Chlorinated Methanes	Anderobie	cfrA/dcrA	Chloroform Reductase (cfrA)	Converts chloroform to dichloromethane					
	Aerobic	sMMO	Soluble Methane Monooxygenase	Co-metabolism of chloroform & dichloromethane					
		Dhg	Dehalogenimonas	Converts TCP to allyl chloride; DCP to propene					
Chlorinated Propanes	Anaerobio	Dhc	Dehalococcoides	Converts DCP to propene					
cinomateu Propanes	Anadiobic	Dhb	Dehalobacter	Converts DCP to propene					
		Dsb	Desulfitobacteriu m	Dechlorination of TCP & DCP					
Chlorinated Benzenes	Anaerobic	Dhc	Dehalococcoides	Partial dechlorination of HCB/PCB					
omornitated Belizenes	Anderobie	Dhb	Dehalobacter	Reductive dechlorination of DCB, MCB					
Chlorinated Phenols	Anaerobic	Dhc	Dehalococcoides	Dechlorination of 2,3-dichlorophenol, TCP and PCP					
		Dhc	Dehalococcoides	Dechlorinates select Arochlor 1260 congeners					
PCBs	Anaerobic	Dhb	Dehalobacter	Dechlorinates 2,3,4-trichorobiphenyl; 2,3,4,5-tetrachlorobiphenyl					
		Dhg	Dehalogenimonas	Dechlorinates select Arochlor 1260 congeners					
		SRB	Sulfate reducing bacteria (dsrA)	Partners to ORM-2 in anaerobic benzene degradation					
BTEX	Anaerobic	ORM-2	Deltaproteobacterium ORM-2	Anaerobic benzene degrader (SO ₄ /CH ₄ reducing conditions)					
D TEN	T ind of obio	Pepto-ben	Benzene degrading Peptococcaceae	Anaerobic benzene degrader under NO3 reducing conditions					
		abcA	Benzene Carboxylase (abcA)	Involved in benzene ring deavage					
			Methylibium petroleiphilum PM1	MTBE/TBE degrading microorganism					
Fuel Oxygenates	Aerobic	MTBE/TBA	tert-butyl alcohol hydroxylase (mdpJ)	Active on TBA in aerobic MTBE degradation pathway					
			HIBA mutase (<i>hcmA</i>)	Active on 2-HIBA in aerobic MTBE degradation pathway					
	Aerobic	1,4-dioxane	Dioxane monooxygenase (<i>dxmb</i>)	Energy yielding 1,4-dioxane degradation					
	metabolism	1,4-dioxane	Aldehyde Dehydrogenase	Energy yielding 1,4-dioxane degradation					
1,4-Dioxane	Assobis	рММО	Particulate Methane Monooxygenase	Co-oxidation of 1,4-dioxane in presence of methane					
	Cometabolism	sMMO	Soluble Methane Monooxygenase	Co-oxidation of 1,4-dioxane					
		PMO	Propane Monooxygenase	Co-oxidation of 1,4-dioxane in presence of propane					
Nitrogen	Anaerobic	Anammox	Major anammox genera	Anaerobic co-removal of ammonium and nitrite					
		Universal	Bacteria	Quantifies Bacteria-measure of total biomass					
Prokaryotic Groups	Variable	Arch	Archaea	Quantifies Archaea biomass					
r ontary out on oup o	i anabio	SRB	Sulfate reducing bacteria (dsrA)	Anaerobic hydrocarbon oxidation/biogeochemical reduction/MIC					
		NGS	Bacteria/Archaea	Comprehensive characterization of microbial communities					



Attachment 2:

SiREM Chain-of-Custody



SiREM

*Project Name

*Project Manager

*Email Address

Address (Street)

City

*Phone #

*Sampler's Signature

P.O. #

*Bill To:

Signature

Printed Name Firm Date/Time

Chain-of-Custody Form

siremlab.com

Analysis

*Project #

*Company

Preservative Key

0. None 1. HCL

Lab #

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Distribution: White - return to * Mandatory Fields

SiREM

Attachment 3:

Example FedEx Waybill

Charles and and a second s
FedEx First Overnight Extension and the solution of t
FedEx Priority Overnight Next business morning* Fridary shipments will be delivered on Monday unless SATURDAY Delivery is selected. FedEx 2Day Delivery is selected. Second business afternoon* Thursday shipments will be delivered on Monday unless SATURDAY Delivery is selected.
FedEx Standard Overnight Next business afterroon.* Saturday Delvery NOT available.
5 Packaging * Declared value limit \$500.
FedEx Envelope* FedEx Pak* FedEx PedEx Tube V Other
Special Handling and Delivery Signature Options SATURDAY Delivery MOT conside for FedEx Standard Overnight, FedEx 20ey A.M., or FedEx Express Sever.
No Signature Required Direct Signature Indirect Signature Indirect Signature Indirect Signature Indirect Signature for fellowry. Me applies without got technic a signature for fellowry.
Does this shipment contain dangerous goods?
V No Yes Shipper's Declaration. Shipper's Declaration. Dry Ice Dry Ice, 8, UN 1845 x kg Dangerous goods (including dry ice) cannot be objeged in FedEx packaging or pleced in effects between too pack Disperious goods (including dry ice) cannot be objeged in FedEx packaging Cargo Aircraft Only
7 Payment Bill to:
Sender Enter FedEx Acct. No. or Credit Card No. below.



Attachment 2



KB-1^{plus}

Use KB-1[®] Plus for Bioaugmentation at Mixed Chlorinated Solvent Sites



toll free: 1-866-251-1747 phone: (519) 822-2265

Contact SiREM for a quotation or more information on our line of leading bioaugmentation products.

Bioaugmentation Cultures

KB-1[®] Plus bioaugmentation cultures are custom-blended microbial formulations capable of biodegradation of chlorinated solvents including complex contaminant mixtures. These cultures have been developed by SiREM in collaboration with the University of Toronto^{1,2} and the United States Geological Survey³.

KB-1[®] Plus Cultures are used for the Remediation of:

- Chlorinated ethanes (1,1,1-trichloroethane and 1,1-dichloroethane to chloroethane) (1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane and 1,2-dichloroethane to ethene);
- Chlorinated methanes (carbon tetrachloride, chloroform and dichloromethane to nonchlorinated end products);
- Chlorinated propanes (1,2,3-trichloropropane and 1,2-dichloropropane to allyl alcohol and propene);
- Chlorofluorocarbons (1,1,2-trichloro-1,2,2-trifluoroethane);
- · Explosives (RDX); and
- Chlorinated ethenes (tetrachloroethene, trichloroethene, dichloroethene isomers and vinyl chloride to ethene)

A key benefit of KB-1[®] Plus cultures is their effectiveness on contaminant mixtures. First, specialized microbes degrade inhibitory compounds (e.g., 1,1,1-TCA /chloroform/CFCs), followed by the complete degradation of the remaining chlorinated compounds (e.g., chlorinated ethenes).

SiREM's bioremediation culture capabilities are always growing. If you have a site with compounds not on this list and are interested in advanced bioremediation approaches, please contact SiREM to enquire about our expanding culture capabilities and latest innovations in groundwater remediation.

Benefits of KB-1® Plus include:

- First rate technical support ensures a successful bioaugmentation application
- · Custom blended formulations optimize biodegradation for chlorinated VOC mixtures
- Only a single application required
- · Works with all commonly used electron donors
- Natural microbial culture (not genetically modified)
- Pathogen free
- · Rigorous quality control ensures each shipment is effective, stable and safe
- Shipped in specially designed vessels that prevent exposure to air and are safe and easy to handle

All KB-1[®] Plus purchases include:

- KB-1[®] Plus Guarantee*
- Complimentary Gene-Trac[®] Dehalococcoides, Dehalobacter and Dehalogenimonas tests to verify the successful delivery, growth and persistence of KB-1[®] Plus microbes in site groundwater

References

¹Grostern, A. and E. A. Edwards. 2006. Growth of *Dehalobacter* and *Dehalococcoides* spp. during Degradation of Chlorinated Ethanes. Appl. Environ. Microbiol. 72: 428–436.

²Grostern, A., M. Duhamel, S. Dworatzek and E. A. Edwards. 2010. Chloroform respiration to dichloromethane by a *Dehalobacter* population. *Environmental Microbiology*.

³Jones E. J. P., M. A. Voytek, M.M. Lorah, J. D. Kirshtein. 2006. Characterization of a Microbial Consortium Capable of Rapid and Simultaneous Dechlorination of 1,1,2,2-Tetrachloroethane and Chlorinated Ethane and Ethene Intermediates. *Bioremediation Journal*, Volume 10: 153-168.

siremlab.com

*some conditions apply

KB-1[®] Primer – Instruction Sheet



KB-1[®] Primer is used to prepare anaerobic water to disperse electron donors and protect anaerobic bioaugmentation cultures during injection into aquifers. KB-1[®] Primer is provided:

- In pre-weighed pouches that are designed to treat 250 gallons (or ~ 1,000 Liters) of water;
- In pre-weighed pouches that are designed to treat 1,000 gallons (or ~3,800 Liters) of water; or
- In 50-pound buckets that can be weighed in the field for custom sizes.

The recommended dosing is 0.8 g/L of KB-1[®] Primer in water. KB-1[®] Primer at this dosing rate is designed to reduce water to <-75 mV ORP within two hours well keeping the pH in the 6.0 to 8.5 range. The ORP will continue to decrease with time, the more time the KB-1[®] Primer has to react the better its performance will be.

PREPARING TOTES/TANKS OF ANAEROBIC WATER

- Start by filling the tote/tank with water (ground or municipal water source) up to approximately 25% of the volume
- While the tote/tank is filling with water, prepare the KB-1[®] Primer slurry;
 - Add contents of the KB-1[®] Primer pouch to an empty pail and fill partially with water. If using KB-1[®] Primer from a bucket, weigh the amount of KB-1[®] Primer required for the tote/tank, add into the empty pail and fill partially with water.
 - Mix the pail thoroughly in most cases the action of the water filling the bucket will provide enough mixing to make the slurry. If required, paint mixers are an effective method for mixing the KB-1[®] Primer slurry.
- Pour the slurry into the tote/tank, rinsing out any undissolved solids
- Finish filling the tote/tank. Fill the tote/tank as full as possible to limit headspace. Cover the tote/tank with a vented lid.

KB-1[®] PRIMER WATER STORAGE

- Keep a minimal headspace within the tote/tank to reduce possible oxygen exposure
- When a minimal headspace is not possible, purge the headspace with nitrogen/argon gas

TIPS FOR OPTIMAL KB-1® PRIMER PERFORMANCE

- Avoid mixing KB-1[®] Primer continuously for long periods of time as it may reintroduce oxygen into the solution.
- Avoid adding KB-1® Primer powder to a full tote/tank without first making the slurry as it may not dissolve fully, resulting in reduced product performance.
- If electron donor solution is being made anaerobic with KB-1[®] Primer; it is recommended to prepare the KB-1[®] Primer water in the tote/tank first. After the water has achieved reducing conditions it can be used to prepare the electron donor solution.

For additional information refer to the KB-1[®] Primer safety data sheet (SDS) Contact SiREM for Customer Support Toll free: 1-866-251-1747 Bioaugmentation Coordinator, Corey Scales: (519) 515-0848

Attachment 3

Leading Science - Lasting Solutions



Anaerobic Injection Water Preparation

Rapidly Prepare Anaerobic Injection Water for Remediation Applications



Field technician preparing anaerobic injection water with KB-1[®] Primer slurry

toll free: 1-866-251-1747 phone: (519) 822-2265 KB-1[®] Primer is used to prepare anaerobic water to disperse electron donors and protect anaerobic bioaugmentation cultures during injection into aquifers. In the past, production of anaerobic water was time consuming, and often produced water with solids that required filtration and that had pH impacts. SiREM has developed KB-1[®] Primer as an easy to use product to facilitate anaerobic conditions during remediation injections.

KB-1[®] and KB-1[®] Plus cultures contain microorganisms that promote dechlorination of chlorinated solvents. These cultures are strictly anaerobic, which can present challenges during injection into non-reducing aquifers and when electron donor and bioaugmentation cultures are applied simultaneously. KB-1[®] Primer does not adversely impact bioaugmentation culture activity or viability.

Use KB-1[®] Primer to:

- · Rapidly prepare anaerobic water from municipal water supplies
- Inject anaerobic bioaugmentation cultures and electron donor simultaneously
- · Save money on lengthy tank rentals/incubation periods

KB-1[®] Primer: Safe and Simple to Use

- Conveniently packaged in foil pouches
- · Easily dissolved; no need to filter water
- · Works within hours of application in most water types
- Prepare anaerobic water even at low temperatures

Anaerobic injection water prepared with KB-1[®] Primer meets the following criteria:

- ORP less than -75 mV
- pH between 6 and 8
- provides the conditions to maintain healthy dechlorinating populations



KB-1[®] Primer powder is shipped in vacuum sealed pouches

Contact SiREM for more information on KB-1[®] Primer and our other leading remediation products and testing services.

KB-1[®] Injection Summary



TOOL KIT CONTENTS

- 1. Toolkit Case
- 2. Quick Connect Fittings
- 3. Scale
- 4. Tubing
- 5. Regulator
- 6. Tools
- 7. KB-1[®] Vessel in Overpack Case

*Please note that the nitrogen/argon gas cylinder is not included with the culture shipment. Gas can be obtained from a local gas supplier.



VESSEL PORT FUNCTIONS

- 1. Inoculation Port (YELLOW) To allow KB-1[®] to flow out of the vessel.
- 2. Purge Port (GREEN) To purge tubing with inert gas.
- **3.** Pressurization Port (RED) To pressurize KB-1[®] vessel.

KB-1[®] Injection Summary

SETUP TO PURGE INJECTION TUBING



1. Gas In: The inert gas tubing remains in the pressurization port (**RED**) for the duration of the injection.

2. Gas Out: Initially the tubing used to inject the KB-1[®] will be connected to the purge port (GREEN).

SETUP TO INJECT KB-1®



1. Gas In: The pressurization port (**RED**) remains in the open position for the duration of the injection.

2. KB-1[®] **Out:** The KB-1[®] injection tubing is moved from the purge port (**GREEN**) to the KB-1[®] inoculation port (**YELLOW**).



Turn scale on by pressing the lbs/kg button and ON buttons simultaneously



Change the units to kg by pressing Ibs/kg button



Press Zero/Hold to tare scale

USING THE SCALE



Place KB-1[®] vessel on scale and record the weight



Weight will decrease with each injection of $\mathsf{KB-1}^{\circledast}$

KB-1[®] Injection Summary



ANAEROBIC WATER DRIVEN KB-1[®] INJECTION SETUP

- **1.** Gas Tubing
- 2. KB-1[®] Injection Tubing
- **3.** Female Quick Connect (1/4" Male NPT)
- 4. Ball Valve with ¼" Female NPT Fitting*
- 5. T-Fitting*
- 6. Ball Valve*
- 7. Anaerobic water line*

*not included with shipment

KB-1[®] Injection Summary

KB-1[®] INJECTION DISPENSER OPERATION

- 1. Gas Line
- 2. Female Quick Connect (item #3 as shown in anaerobic water driven KB-1 injection set-up)



Step 1: Cut the length of tubing that will span from the gas cylinder to the culture vessel (5-10' should be sufficient). Attach one end to the hosebarb on the regulator and the other to the hosebarb on a quick connect. Connect the quick connect to the top port of the injection dispenser.

Step 2: Cut the length of tubing that will span from the injection dispenser to the injection location (5-10' should be sufficient). Attach one end to the hosebarb on the injection dispenser and the other to the hosebarb on a quick connect. Open the valve on the gas cylinder, followed by the regulator, the top of the injection dispenser and finally the bottom of the injection dispenser. Push on the bottom of the quick connect to allow gas to flow through the injection equipment.

Step 3: Close the bottom port on the injection dispenser and allow pressure to build to 5 psi in the dispenser. Close the top port of the injection dispenser.

Step 4: Connect the bottom quick connect into the inoculation port **(YELLOW)**. Move the gas line from the top of the injection dispenser to the pressurization port **(RED)** on the culture vessel. Connect a quick connect into the top port of the injection dispenser.

Step 5: Open the inoculation port **(YELLOW)** and allow KB-1[®] to flow into the injection dispenser to the desired volume.

Step 6: Pressure will increase as the injection dispenser fills. Release the pressure by opening the top port. Close the top port before the target volume is reached, this will ensure that there is always pressure in the dispenser.

Step 7: Once the target volume is reached close the bottom port and remove the quick connect from the top port.

Step 8: Move the injection dispenser from the inoculation port **(YELLOW)** to the port on the anaerobic water line set up. Connect the gas line to the top of the injection dispenser. Open the top port followed by the bottom port of the injection dispenser. Once the culture has been injected, close the bottom port followed by the top port to keep pressure in the injection dispenser.

Step 9: Repeat steps 4-8 until all injections are complete.

Step 10: Once the injections are complete, pack the vessel(s) in the white over pack(s) & place all tools into the tool kit. Contact Corey Scales at 519-515-0848 for return shipping instructions and paperwork.

For additional information refer to the Culture safety data sheet (SDS) Contact SiREM for Customer Support Toll free: 1-866-251-1747 Bioaugmentation Coordinator, Corey Scales: (519) 515-0848

Attachment 2











Attachment 3



Technical Note 1.5: Interpretation of Gene-Trac[®] Dhc, *vcrA*, *bvcA and tceA* Assays

This note provides technical background and guidelines for interpretation of the following Gene-Trac[®] assays:

- (1) Gene-Trac[®] Dhc
- (2) Gene-Trac[®] vcrA
- (3) Gene-Trac[®] bvcA
- (4) Gene-Trac[®] tceA

Gene-Trac[®] Dhc-Total *Dehalococcoides* Test

Background

Gene-Trac[®] Dhc is a quantitative polymerase chain reaction (qPCR) test for the microbial species *Dehalococcoides mccartyi* (i.e., *Dehalococcoides* [Dhc]). The Gene-Trac[®] Dhc test targets sequences of the 16S ribosomal ribonucleic acid (16S rRNA) gene unique to Dhc. Note the 16S rRNA gene does not directly participate in dechlorination, but is used as a molecular fingerprint in the identification and quantification of a wide variety of microbial groups. The detection of Dhc in environmental samples is significant as Dhc contain the greatest number of reductive dehalogenase genes of any microbial group (Tas et al., 2010). Dhc are capable of reductive dechlorination of a wide variety compounds/compound classes including:

- Chlorinated ethenes (tetrachloroethene [PCE], trichloroethene [TCE], cis-1,2-dichloroethene [cDCE], 1,1-dichloroethene [1,1-DCE], trans-1,2-dichloroethene [tDCE, vinyl chloride [VC]) (Duhamel et al., 2002);
- 1,2-dichloroethane (1,2-DCA) to ethene (Grostern and Edwards, 2006);
- Selected polychlorinated biphenyl [PCB] congeners (Bedard et al., 2007);
- Selected chlorinated benzene compounds (Adrian et al., 2000; Fennell et al., 2004);
- Chlorophenols and polychlorinated dibenzo-*p*-dioxins (Fennell et al., 2004) and;
- 1,2-dibromoethane (Magnusson et al., 2000).



In addition to screening for diverse dechlorinating activities, Gene-Trac[®] Dhc can also be used to assess the *in situ* growth of Dhc containing bioaugmentation cultures such as KB-1[®] (Major et al., 2002).

Gene-Trac[®] Dhc Results Interpretation

Negative (Non-detect [ND]) Gene-Trac[®] Dhc Test Results

The absence of Dhc is associated with a lack of dechlorination or only partial reductive dechlorination of chlorinated ethenes. Where Dhc are absent the accumulation of cDCE is commonly observed, particularly after electron donor addition, often due to the presence of partial dechlorinators (e.g., *Dehalobacter, Geobacter*). Bioaugmentation with Dhc containing cultures (e.g., KB-1[®]) often improves bioremediation performance at sites lacking indigenous Dhc.

Positive (Detect) Gene-Trac[®] Dhc Test Results

The detection of Dhc is correlated with the complete biological dechlorination of chlorinated ethenes to non-toxic ethene at contaminated sites (Hendrickson et al., 2002). A positive Gene-Trac[®] Dhc test indicates that Dhc DNA was detected and is correlated with the occurrence of reductive dechlorination. Note, not all Dhc can convert vinyl chloride to ethene; this capability can be determined by quantifying the functional genes (vcrA, bvcA, tceA) (see following section). In most cases Dhc must be present at sufficient concentrations in order for significant dechlorination to be observed, guidelines for expected impacts on chlorinated ethenes at various Dhc concentrations in groundwater are indicated below.

- **10⁴ Dhc gene copies per liter (or lower):** indicates low concentrations of Dhc which may indicate site conditions that are sub-optimal for high rates of dechlorination. Increases in Dhc concentrations at the site may be possible if conditions are optimized (e.g., electron donor addition/pH adjustment).
- 10⁵-10⁶ Dhc gene copies per liter: indicates the sample contains moderate concentrations of Dhc which may, or may not, be associated with observable dechlorination activity.
- **1 x 10⁷ Dhc gene copies per liter (or above):** indicates that the sample contains high concentrations of Dhc often associated with significant dechlorination rates (Lu et al., 2006).
- **10⁹-10¹⁰ Dhc gene copies per liter:** are generally the highest observed for groundwater samples and are associated with very high rates of dechlorination



Interpretation of Functional Gene Assays for vcrA, bvcA and tceA

Background

Gene-Trac[®] *vcrA*, *bvcA* and *tceA* tests are provided combined as a functional gene assay package. These tests quantify genes that code for enzymes that dechlorinate chlorinated ethenes and other compounds. The *vcrA*, *bvcA* and *tceA* genes play specific roles in reductive dechlorination, specifically *tceA* converts TCE and cDCE to VC and *vcrA* and *bvcA* convert cDCE and VC to non-toxic ethene (Figure 1).



Figure 1: Major (energy yielding) activities against chlorinated ethene of enzymes coded for by the *tceA*, *vcrA* and *bvcA* genes.

Results Interpretation

Table 1 provides interpretation guidelines for different scenarios for Gene-Trac[®] Dhc, *vcrA*, *bvcA* and *tceA* tests. In general, accumulation of VC is more likely where Gene-Trac[®] *vcrA/bvcA* results are ND, or significantly lower than Gene-Trac[®] Dhc/*tceA*. Where abundance of *vcrA/bvcA* is similar to total Dhc the chances of VC accumulation are reduced.



Table 1: Interpretation of Gene-Trac[®] Dhc, *vcrA*, bvcA, *tceA* test results

	Gene C	opies/L				
Dhc	vcrA	bvcA	tceA	Summary	Interpretation	Remediation Implications
ND	ND	ND	ND	ND for Dhc and functional genes	Site lacks Dhc	Complete dechlorination unlikely, may observe cis-DCE accumulation Site may require bioaugmentation
<u>≥</u> 1 x 10 ⁷	<u>≥</u> 1 x 10 ⁷	<u>≥</u> 1 x 10 ⁷	<u>≥</u> 1 x 10 ⁷	Dhc and <i>vcrA/bvcA/tceA</i> are the same	Entire Dhc population has <i>tceA, vcrA</i> and <i>bvcA</i> gene	Potential for complete dechlorination very high. VC stall unlikely-sites with <i>vcr</i> A above 1 x 10 ⁷ /L typically have detectable ethene
<u>></u> 1 x 10 ⁷	ND	<u>></u> 1 x 10 ⁷	ND	Total Dhc and <i>bvcA/</i> are the same <i>vcrA/tceA</i> ND	Dhc at high concentrations entire Dhc population has <i>bvcA</i> gene	Potential for complete dechlorination high. VC stall unlikely
<u>≥</u> 1 x 10 ⁷	<u>></u> 1 x 10 ⁷	ND	ND	Total Dhc and <i>vcrA/</i> are the same <i>bvcA/tceA</i> ND	Dhc at high concentrations entire Dhc population has <i>vcrA</i> gene	Potential for complete dechlorination high. VC stall unlikely-sites with <i>vcr</i> A above 1 x 10 ⁷ /L often have detectable ethene
<u>≥</u> 1 x 10 ⁷	ND	ND	<u>≥</u> 1 x 10 ⁷	Total Dhc high; <i>vcrA</i> and <i>bvcA</i> non-detect <i>tceA</i> same as Dhc	High concentration of Dhc, entire Dhc population has <i>tceA</i> but lacks the <i>vcrA/bvcA</i> genes	Likelihood for VC accumulation high as <i>vcrA</i> and <i>bvcA</i> both ND
1 x 10 ⁷	1 x 10⁵	1 x 10 ⁶	1 x 10 ⁷	Total Dhc and <i>tceA</i> is significantly higher 10-100 fold) than <i>vcrA/bvcA</i>	Dhc population consists of different types, some with the vcrA/gene (10%) some with bvcA gene (1%) all contain tceA gene	VC-accumulation possible; Dhc: <i>vcrA:bvcA:tceA</i> ratios may evolve over the course of remediation
1 x 10 ⁷	1 x 10 ⁷	1 x 10 ⁶	ND	Total Dhc is high <i>vcrA/bvcA</i> high <i>tceA</i> ND	<i>tceA</i> negative population	cDCE to ethene dechlorination likely PCE and TCE dechlorination possible via <i>pceA</i> commonly found in other dechlorinators such as <i>Dehalobacter</i>
a = f	avorable 1	for comple	te dechlorir	nation, B = some	potential for VC stall	= complete dechlorination unlike



Gene-Trac[®] vcrA/bvcA

Gene-Trac[®] *vcrA* and *bvcA* tests quantify VC-reductase genes that produce enzymes that convert VC to non-toxic ethene; a critical step in reductive dechlorination. The VC reductase genes (*vcrA*, *bvcA*) (Müller et al., 2004; Krajmalnik-Brown et al., 2004) produce enzymes found in many (but not all) Dhc. The *vcrA* gene is reported to be the most commonly identified VC reductase gene in the environment, whereas *bvcA* is generally less common but can predominate especially in more oxidizing groundwater (van der Zaan et al., 2010) and possibly where DCE is dominant. The *vcrA* gene can be used for tracking bioaugmentation cultures including KB-1[®] and is typically present at a 1:1 ratio with total Dhc whereas the *bvcA* gene is not predominant in the KB-1[®] culture and is present at less than a 1:1 ratio with total Dhc, therefore *bvcA* is not generally used for tracking KB-1[®] bioaugmentation and may be negative even after bioaugmentation with KB-1[®].

Positive Gene-Trac[®] vcrA, bvcA Tests

Positive Gene-Trac[®] *vcrA* or *bvcA* tests indicate that the Dhc population has the *vcrA* and/or the *bvcA* gene and complete dechlorination to ethene is likely. As a minimal requirement, *vcrA* and/or *bvcA* copies exceeding 10^5 /L combined with observed increases over time (i.e., cell growth) are required for robust VC dechlorination (van der Zaan et al., 2010). In one study, more than 90% of samples where *vcrA* enumeration exceeded 1 x 10^7 gene copies/L of groundwater had detectable ethene (Dennis, 2009). The enzyme produced by the *bvcA* genes has also been shown to degrade 1,2-DCA directly to ethene (Grostern and Edwards 2009) and the *bvcA* is used for tracking the KB-1[®] 1,2-DCA culture.

Non-Detect in Gene-Trac[®] vcrA/bvcA Test

A ND in the Gene-Trac[®] *vcrA* and *bvcA* test indicates that *vcrA/bvcA* gene sequences in the sample were below the detection limit of the assay. In cases where *vcrA/bvcA* are ND the chances of VC accumulation are increased compared to samples with detectable *vcrA/bvcA*. In such cases, *tceA* may promote limited and slow cometabolic degradation of VC to ethene (Lee et al., 2008) that may account for (generally low) detections of ethene where *vcrA* and *bvcA* are ND.

Gene-Trac[®] tceA

Gene-Trac[®] *tceA* test targets the trichloroethene reductase gene that produces an enzyme that primarily converts TCE to *c*DCE and VC. Studies have shown that this gene is commonly expressed under more oxidized conditions compared to *vcrA* (van der Zaan et al., 2010). Note the *tceA* gene is not predominant in the KB-1[®] culture and therefore *tceA* is not used for tracking KB-1[®] bioaugmentation.



Positive *tceA* test

A positive *tceA* test indicates that the Dhc population has the potential to dechlorinate TCE to cDCE and VC and VC to ethene cometabolically at relatively slow rates (Lee et al. 2008). Detection of *tceA* in the absence of *vcrA/bvcA* also indicates an increased likelihood for VC accumulation. The enzyme produced by *tceA* is also reported to dehalogenate 1,2-DCA and 1,2 dibromoethane (Magnussen et al., 2000).

Negative *tceA* test

A ND *tceA* test indicates that the Dhc population may lack the ability to convert TCE to cDCE and VC, nevertheless, conversion of PCE to cDCE is relatively common amongst other dechlorinators that harbor the *pceA* gene (Maillard et al., 2003; Wagner et al., 2012). Therefore *tceA is* not essential for complete dechlorination of TCE provided that *pceA* harboring microorganisms are present. Gene-Trac[®] Dhb (*Dehalobacter*) and Gene-Trac[®] Geo (*Geobacter*) can be used to quantify these common *pceA* containing microorganisms.

Sites with mixed Dhc populations

At some sites the Dhc population is homogenous while other sites have Dhc populations that are mixtures of different Dhc types. These scenarios can lead to differing proportions for Gene-Trac[®] Dhc *vcrA bvcA* and *tceA* test results. If the numerical results of Gene-Trac[®] *vcrA*, *bvcA or tceA* tests are identical to those obtained in the Gene-Trac[®] Dhc test it suggests that the entire Dhc population contains that gene. In other cases, Gene-Trac[®] *vcrA*, *bvcA*, *tceA* results may differ significantly (i.e., more than an order of magnitude) from total Dhc. For example, the *vcrA* gene may be 100-fold lower than the total Dhc. This scenario would suggest that only 1% of the Dhc population harbors the *vcrA* gene and the remaining 99% of the Dhc population does not contain the *vcrA* gene. In such cases the proportions of the functional genes may change over time (e.g., the proportion of *vcrA* may increase as the VC concentration increases favoring Dhc that contain *vcrA*).


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SiREM Technical Note 1.6:

Interpretation of Gene-Trac[®]-*Dhb* and Gene-Trac[®]-*cfrA* Assays

Background

This technical note provides background information and guidelines for interpretation of the following Gene-Trac[®] tests:

- (1) Gene-Trac[®]-Dhb (Dehalobacter), and
- (2) Gene-Trac[®]-*cfrA* functional gene.

These tests are used to assess: (1) the activities of indigenous microorganisms, and (2) the impact of bioaugmentation with the KB-1[®] Plus cultures that contain high concentrations of *Dhb*. SiREM Technical Note 1.4 - *Quantitative Gene-Trac[®] Assay Test Procedure and Reporting Overview* provides detailed information on general aspects Gene-Trac[®] test procedures and reporting including data qualifiers and commonly used notes.

Gene-Trac[®]-Dhb and cfrA Biodegradation Pathways

Dehalobacter (*Dhb*) and its functional genes *cfrA/dcrA* are implicated in the biodegradation of chlorinated ethenes, ethanes and methanes. Gene-Trac[®]-*Dhb* is a quantitative polymerase chain reaction (qPCR) test targeting 16S rRNA gene sequences unique to *Dhb*. Gene-Trac[®]-*cfrA* targets two key *Dhb* functional genes (*cfrA* and *dcrA*) that produce enzymes that participate in degradation pathways for chloroform and 1,1,1-trichlorethane (1,1,1-TCA).

Dhb are implicated in the biodegradation of tetrachlorethene (PCE) and trichloroethene (TCE) to cis-1,2-dichloroethene (cDCE) (Figure 1), 1,1,1-TCA to 1,1-dichloroethane (1,1-DCA) to chloroethane (Figure 2), 1,2-dichloroethane (1,2-DCA) to ethene (Figure 3), chloroform (CF) to dichloromethane (DCM) and fermentation to acetate (Figure 4), 1,1,2,2-tetrachloroethane (TeCA) degradation to trans-1,2-dichloroethene (tDCE), and 1,1,2-trichlorethane (1,1,2-TCA) and 1,1,2-TCA to vinyl chloride (VC) (Figure 5).



Figure 1: *Dhb* can dechlorinate PCE and TCE to cDCE.





Figure 2: Pathway for the biodegradation of chlorinated ethanes. The *Dhb cfrA* gene mediates dechlorination of 1,1,1-TCA to 1,1-DCA, 1,1,-DCA to chloroethane is mediated by the *dcrA* gene. The conversion of chloroethane to ethane is reported but is not widely observed and is considered unconfirmed.



Figure 3: *Dhb* converts 1,2-DCA to ethene by dihaloelimination, this reaction is also performed by *Dehalococcoides* (Gene-Trac[®]-*Dhc*) and *Dehalogenimonas* (Gene-Trac[®]-*Dhg*)



Figure 4: Dechlorination of chlorinated methanes. CTC is converted to CF abiotically. CF can be degraded to DCM by reductive dechlorination by *Dhb* species containing the *cfrA* functional gene. DCM is fermented to acetate by *Dhb*.

Attachment 4



Analytical Results

Client: AECOM Client Project Number: 60538931-1 Date Samples Received: August 27, 2021 Date Samples Analyzed: September 13, 2021 SiREM File Reference: S-8336

Client Sample ID	SiREM Reference ID	Client Sample	Sample Dilution	Lactate	Acetate	Propionate	Formate	Butyrate	Pyruvate
		Date	Factor	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
MW-16S	21-6044	26-Aug-21	50	<0.39	495	12	<0.22	81	0.71
MW-8R	21-6045	26-Aug-21	50	1.2	70	<0.31	<0.22	<0.41	<0.69
		QL	50	0.39	0.54	0.31	0.22	0.41	0.69

Comments:

QL = Quantitation limit

< = compound analysed for but not detected, associated value is QL. Sample QL is corrected for dilution.

Analyst:

Kela Ashworth, B.Sc. Senior Laboratory Technician

Results approved:

14-Sep-21

Date:

Michael Healey, B.Sc. Treatability and SP3™ Services Coordinator



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Analytical Results

Client: AECOM Client Project Number: 60538931 Date Samples Received: December 10, 2021 Date Samples Analyzed: December 20, 2021 SiREM File Reference: S-8744

Client Sample ID	SiREM Reference ID	Client Sample Dilution		Lactate	Acetate	Propionate	Formate	Butyrate	Pyruvate
		Date	Factor	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
MW-16S	21-8384	09-Dec-21	1,000	<7.8	921	14	<4.4	98	<13.8
MW-8R	21-8385	09-Dec-21	50	<0.39	28	<0.31	<0.22	<0.41	<0.69
		QL	50	0.39	0.54	0.31	0.22	0.41	0.69
		QL	1,000	7.8	10.8	6.2	4.4	8.2	13.8

Comments:

QL = Quantitation limit

< = compound analysed for but not detected, associated value is QL. Sample QL is corrected for dilution.</p>

Analyst:

Kahl Malter

Rachel Hallman, B.Sc. Laboratory Technician

Results approved:

Date:

22-Dec-21

Michael Healey, B.Sc. Treatability and SP3™ Services Coordinator



Certificate of Analysis: Gene-Trac® Dehalococcoides Assay

Customer: Dino Zack, AECOM Project: Scott Figgie West Plant 2 Customer Reference: 60538931-1 SiREM Reference: S-8336 Report Date: 13-Sep-21 Data Files: QS3A-DHCT-TM-QPCR-1914 QS3A-DB-DHC-TM-QPCR-1229

Table 1a: Test Results

Sample ID	Dehalococcoides (Dhc)							
	Percent Dhc ⁽¹⁾	Enumeration/Liter ⁽²⁾						
MW-16S	8 - 23 %	1 x 10 ⁹						

See final page for notes.

Taylor A

Analyst:

Taylor Aris, B.Sc. Laboratory Technician II

Jumena Druar Approved:

Ximena Druar, B.Sc. Genetic Testing Supervisor



Certificate of Analysis: Gene-Trac® Functional Gene Assay

Customer: Dino Zack, AECOM Project: Scott Figgie West Plant 2 Customer Reference: 60538931-1 SiREM Reference: S-8336 Report Date: 13-Sep-21 Data Files: QS3A-FGA-QPCR-1266 QS3A-DB-FGA-QPCR-0957

Table 1b: Test Results

Sample ID	VC R (۱	eductase /crA)	BAV1 VC (<i>k</i>	CReductase	TCE Reductase (<i>tceA</i>)			
	Percent vcrA ⁽³⁾	Gene Copies/Liter	Percent bvcA ⁽³⁾	Gene Copies/Liter	Percent tceA ⁽³⁾	Gene Copies/Liter		
MW-16S	8 - 22 %	1 x 10 ⁹	1 - 3 %	1 x 10 ⁸	7 - 18 %	1 x 10 ⁹		

See final page for notes.

Taylor A

Analyst:

Taylor Aris, B.Sc. Laboratory Technician II

Jumena Druar

Approved: _____ Ximena Druar, B.Sc.

Almena Druar, B.Sc. Genetic Testing Coordinator



Certificate of Analysis: Gene-Trac® Dehalobacter Assay

Customer: Dino Zack, AECOM Project: Scott Figgie West Plant 2 Customer Reference: 60538931-1 SiREM Reference: S-8336 Report Date: 13-Sep-21 Data Files: iQ5B-DHB-QPCR-0562 iQ5B-DB-DHB-QPCR-0369

Table 1c: Test Results

Sample ID	Dehalobacter (Dhb)							
	Percent Dhb ⁽¹⁾	Gene Copies/Liter						
MW-16S	0.3 - 1 %	5 x 10 ⁷						

See final page for notes.

Taylor A

Analyst:

Taylor Aris, B.Sc. Laboratory Technician II

Jumena Druar Approved:

Ximena Druar, B.Sc. Genetic Testing Coordinator

Table 2: Detailed Test Parameters, Test Reference S-8336

Customer Sample ID	MW-16S
SiREM Dhc Test ID	DHC-21783
SIREM FGA Test ID	FGA-10754
SiREM Dhb Test ID	DHB-2658
Date Sampled ⁽⁴⁾	26-Aug-21
Matrix	Groundwater
Date Received ⁽⁴⁾	27-Aug-21
Sample Temperature	4.0 °C
Filtration Date ⁽⁴⁾	30-Aug-21
Volume Used for DNA Extraction	100 mL
DNA Extraction Date	7-Sep-21
DNA Concentration in Sample (extractable)	30075 ng/L
PCR Amplifiable DNA	Detected
Dhc qPCR Date Analyzed	7-Sep-21
FGA qPCR Date Analyzed	9-Sep-21
Dhb qPCR Date Analyzed	8-Sep-21
Laboratory Controls (see Tables 3, 4 & 5)	Passed
Comments	

			Dhc 16	S rRNA	
Laboratory Control	tory Control Analysis Control Description		Spiked Gene Copies per Liter	Recovered Gene Copies per Liter	Comments
Positive Control Low Concentration	7-Sep-21	Genomic DNA (CSLD-1552)	1.4 x 10 ⁶	1.1 x 10 ⁶	Passed
Positive Control High Concentration	7-Sep-21	Genomic DNA (CSHD-1552)	1.8 x 10 ⁸	2.3 x 10 ⁸	Passed
Extraction Control	7-Sep-21	Extraction Control (KB-0831)	1.0 x 10 ¹¹	1.6 x 10 ¹¹	Passed
DNA Extraction Blank	7-Sep-21	Sterile Water (FB-3881)	0	2.6 x 10 ³ U	Passed
Negative Control	7-Sep-21	Reagent Blank (TBD-1511)	0	2.6 x 10 ³ U	Passed

			VC	rA	bv	сA	tc	eA	
Laboratory Control	Analysis Date	Control Description	Spiked Gene Copies per Liter	Recovered Gene Copies per liter	Spiked Gene Copies per liter	Recovered Gene Copies per liter	Spiked Gene Copies per liter	Recovered Gene Copies per liter	Comments
Positive Control Low Concentration	9-Sep-21	Genomic DNA (CSLF-1134)	2.5 x 10 ⁶	2.9 x 10 ⁶	5.4 x 10 ⁵	8.2 x 10 ^{5 (5)}	6.5 x 10 ⁵	1.5 x 10 ^{6 (5)}	See Note 5
Positive Control High Concentration	9-Sep-21	Genomic DNA (CSHF-1134)	4.7 x 10 ⁸	5.1 x 10 ⁸	1.3 x 10 ⁸	1.4 x 10 ⁸	1.7 x 10 ⁸	2.0 x 10 ⁸	Passed
DNA Extraction Blank	9-Sep-21	Sterile Water (FB-3881)	0	2.6 x 10 ³ U	0	2.6 x 10 ³ U	0	2.6 x 10 ³ U	Passed
Negative Control	9-Sep-21	Reagent Blank (TBF-1105)	0	2.6 x 10 ³ U	0	2.6 x 10 ³ U	0	2.6 x 10 ³ U	Passed

			Dhb 16	Dhb 16S rRNA								
Laboratory Control	Analysis Date	Control Description	Spiked Gene Copies per Liter	Recovered Gene Copies per Liter	Comments							
Positive Control Low Concentration	8-Sep-21	Genomic DNA (CSLDB-0521)	2.9 x 10 ⁷	3.7 x 10 ⁷	Passed							
Positive Control High Concentration	8-Sep-21	Genomic DNA (CSHDB-0521)	5.1 x 10 ⁹	4.7 x 10 ⁹	Passed							
DNA Extraction Blank	8-Sep-21	Sterile Water (FB-3881)	0	2.6 x 10 ³ U	Passed							
Negative Control	8-Sep-21	Test Reagent Blank (TBDB-0521)	0	2.6 x 10 ³ U	Passed							

Notes:

Dhc = *Dehalococcoides* vcrA = VC reductase bvcA = BAV1 VC reductasetceA = TCE reductase FGA = functional gene assay Dhb = Dehalobacter J The associated value is an estimated quantity between the method detection limit and quantitation limit. U Not detected, associated value is the quantitation limit. B Analyte was detected in the method blank within an order of magnitude of the test sample. E Extracted genomic DNA was not detected in the sample. I Sample inhibited the test reaction based on inability to PCR amplify extracted DNA with universal primers. ng/L = nanograms per liter mL = milliliter NA = not applicableND = not detected DNA = deoxyribonucleic acid 16S rRNA = 16S ribosomal ribonucleic acid PCR = polymerase chain reaction qPCR = quantitative PCR °C = degrees Celsius

¹ Percent *Dehalococcoides* (Dhc) or *Dehalobacter* (Dhb) in microbial population. This value is calculated by dividing the number of Dhc or Dhb 16S ribosomal ribonucleic acid (rRNA) gene copies by the total number of bacteria as estimated by the mass of DNA extracted from the sample. Range represents normal variation in Dhc enumeration.

² Based on quantification of Dhc or Dhb 16S rRNA gene copies. Dhc or Dhb are generally reported to contain one 16S rRNA gene copy per cell; therefore, this number is often interpreted to represent the number of Dhc or Dhb cells present in the sample.

³ Percent of functional gene in microbial population. This value is calculated by dividing the functional gene copies quantified by the total number of estimated prokaryotes in the sample (based on the total quantity of DNA extracted from the sample). A value of 100% would suggest that all microbes in the sample contain the gene.

⁴ Samples are stabilized by freezing at -80 °C upon sample reception (field filters) or in-lab filtration (groundwater). Hold time not exceeded if sampling date is within 14 days of date received or filtration date.

⁵ Control was outside recovery limit guidelines (+/- 50%), however, test results are deemed acceptable if one of two positive controls fall within the recovery limit guidelines.



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Certificate of Analysis: Gene-Trac® Dehalococcoides Assay

Customer: Dino Zack, AECOM Project: Scott Figgie West Plant 2 Customer Reference: 60538931 SiREM Reference: S-8744 Report Date: 21-Dec-21 Data Files: QS3A-DHCT-TM-QPCR-1963 QS3A-DB-DHC-TM-QPCR-1279

Table 1a: Test Results

Sample ID	De	halococcoides (Dhc)
	Percent Dhc ⁽¹⁾	Enumeration/Liter ⁽²⁾
MW-16S	6 - 17 %	1 x 10 ⁹

See final page for notes.

Taylor A

Analyst:

Taylor Aris, B.Sc. Laboratory Technician II

Jumena Druar Approved:

Ximena Druar, B.Sc. Genetic Testing Supervisor



Certificate of Analysis: Gene-Trac® Functional Gene Assay

Customer: Dino Zack, AECOM Project: Scott Figgie West Plant 2 Customer Reference: 60538931 SiREM Reference: S-8744 Report Date: 21-Dec-21 Data Files: QS3A-FGA-QPCR-1295 QS3A-DB-FGA-QPCR-0986

Table 1b: Test Results

Sample ID	VC R (1	eductase /crA)	BAV1 VC (<i>k</i>	CReductase	TCE Reductase (tceA)			
	Percent vcrA ⁽³⁾	Gene Copies/Liter	Percent bvcA ⁽³⁾	Gene Copies/Liter	Percent tceA ⁽³⁾	Gene Copies/Liter		
MW-16S	5 - 15 %	1 x 10 ⁹	0.3 - 1 %	6 x 10 ⁷	2 - 5 %	3 x 10 ⁸		

Taylor A

Analyst: _____ Taylor Aris

Taylor Aris, B.Sc. Laboratory Technician II

Jumena Druar

Approved: _

Ximena Druar, B.Sc. Genetic Testing Supervisor



Certificate of Analysis: Gene-Trac® Dehalobacter Assay

Customer: Dino Zack, AECOM Project: Scott Figgie West Plant 2 Customer Reference: 60538931 SiREM Reference: S-8744 Report Date: 21-Dec-21 Data Files: iQ5B-DHB-QPCR-0577 iQ5B-DB-DHB-QPCR-0384

Table 1c: Test Results

Sample ID	Dehalobacter (Dhb)					
	Percent Dhb ⁽¹⁾	Gene Copies/Liter				
MW-16S	0.08 - 0.2 %	2 x 10 ⁷				

See final page for notes.

Taylor A

Analyst:

Taylor Aris, B.Sc. Laboratory Technician II

Jemena Druar Approved:

Ximena Druar, B.Sc. Genetic Testing Supervisor

Table 2: Detailed Test Parameters, Test Reference S-8744

Customer Sample ID	MW-16S
SIREM Dhc Test ID	DHC-22680
SIREM FGA Test ID	FGA-11257
SiREM Dhb Test ID	DHB-2777
Date Sampled ⁽⁴⁾	9-Dec-21
Matrix	Groundwater
Date Received ⁽⁴⁾	10-Dec-21
Sample Temperature	5.6 °C
Filtration Date ⁽⁴⁾	10-Dec-21
Volume Used for DNA Extraction	100 mL
DNA Extraction Date	20-Dec-21
DNA Concentration in Sample (extractable)	37200 ng/L
PCR Amplifiable DNA	Detected
Dhc qPCR Date Analyzed	21-Dec-21
FGA qPCR Date Analyzed	20-Dec-21
Dhb qPCR Date Analyzed	21-Dec-21
Laboratory Controls (see Tables 3, 4 & 5)	Passed
Comments	

			Dhc 16	S rRNA		
Laboratory Control	Analysis Date	Control Description	Spiked Gene Copies per Liter	Recovered Gene Copies per Liter	Comments	
Positive Control Low Concentration	21-Dec-21	Genomic DNA (CSLD-1601)	3.9 x 10 ⁶	2.0 x 10 ⁶	Passed	
Positive Control High Concentration	21-Dec-21	Genomic DNA (CSHD-1601)	5.2 x 10 ⁸	3.6 x 10 ⁸	Passed	
Extraction Control	20-Dec-21	Extraction Control (KB-0846)	7.0 x 10 ¹⁰	8.8 x 10 ¹⁰	Passed	
DNA Extraction Blank	21-Dec-21	Sterile Water (FB-3967)	0	1.0 x 10 ³ U	Passed	
Negative Control	21-Dec-21	Reagent Blank (TBD-1560)	0	1.0 x 10 ³ U	Passed	

			VC	rA	bv	сA	tc		
Laboratory Control	Analysis Date	Control Description	Spiked Gene Copies per Liter	Recovered Gene Copies per liter	Spiked Gene Copies per liter	Recovered Gene Copies per liter	Spiked Gene Copies per liter	Recovered Gene Copies per liter	Comments
Positive Control Low Concentration	20-Dec-21	Genomic DNA (CSLF-1163)	6.0 x 10 ⁶	3.9 x 10 ⁶	5.7 x 10 ⁵	2.7 x 10 ^{5 (5)}	3.6 x 10 ⁵	4.0 x 10 ⁵	See Note 5
Positive Control High Concentration	20-Dec-21	Genomic DNA (CSHF-1163)	5.9 x 10 ⁸	6.6 x 10 ⁸	5.7 x 10 ⁷	7.8 x 10 ⁷	4.7 x 10 ⁷	4.8 x 10 ⁷	Passed
DNA Extraction Blank	20-Dec-21	Sterile Water (FB-3967)	0	1.0 x 10 ³ U	0	1.0 x 10 ³ U	0	1.0 x 10 ³ U	Passed
Negative Control	20-Dec-21	Reagent Blank (TBF-1134)	0	1.0 x 10 ³ U	0	1.0 x 10 ³ U	0	1.0 x 10 ³ U	Passed

			Dhb 16			
Laboratory Control	Analysis Date	Control Description	Spiked Gene Copies per Liter	Recovered Gene Copies per Liter	Comments	
Positive Control Low Concentration	21-Dec-21	Genomic DNA (CSLDB-0536)	1.7 x 10 ⁶	2.0 x 10 ⁶	Passed	
Positive Control High Concentration	21-Dec-21	Genomic DNA (CSHDB-0536)	2.4 x 10 ⁸	2.7 x 10 ⁸	Passed	
DNA Extraction Blank	21-Dec-21	Sterile Water (FB-3967)	0	1.0 x 10 ³ U	Passed	
Negative Control	21-Dec-21	Test Reagent Blank (TBDB-0536)	0	1.0 x 10 ³ U	Passed	

Notes:

Dhc = *Dehalococcoides* vcrA = VC reductase bvcA = BAV1 VC reductasetceA = TCE reductase FGA = functional gene assay Dhb = Dehalobacter J The associated value is an estimated quantity between the detection limit and quantitation limit. U Not detected, associated value is the detection limit. B Analyte was detected in the method blank within an order of magnitude of the test sample. E Extracted genomic DNA was not detected in the sample. I Sample inhibited the test reaction based on inability to PCR amplify extracted DNA with universal primers. ng/L = nanograms per liter mL = milliliter NA = not applicableND = not detected DNA = deoxyribonucleic acid 16S rRNA = 16S ribosomal ribonucleic acid PCR = polymerase chain reaction qPCR = quantitative PCR °C = degrees Celsius

¹ Percent *Dehalococcoides* (Dhc) or *Dehalobacter* (Dhb) in microbial population. This value is calculated by dividing the number of Dhc or Dhb 16S ribosomal ribonucleic acid (rRNA) gene copies by the total number of bacteria as estimated by the mass of DNA extracted from the sample. Range represents normal variation in Dhc enumeration.

² Based on quantification of Dhc or Dhb 16S rRNA gene copies. Dhc or Dhb are generally reported to contain one 16S rRNA gene copy per cell; therefore, this number is often interpreted to represent the number of Dhc or Dhb cells present in the sample.

³ Percent of functional gene in microbial population. This value is calculated by dividing the functional gene copies quantified by the total number of estimated prokaryotes in the sample (based on the total quantity of DNA extracted from the sample). A value of 100% would suggest that all microbes in the sample contain the gene.

⁴ Samples are stabilized by freezing at -80 °C upon sample reception (field filters) or in-lab filtration (groundwater). Hold time not exceeded if sampling date is within 14 days of date received or filtration date.

⁵ Control was outside recovery limit guidelines (+/- 50%), however, test results are deemed acceptable if one of two positive controls fall within the recovery limit guidelines.



Chain-of-Custody Form

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Preservative Key

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Dhc. NCrA. DVCA. TCEADAb

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1. HCL

Analysis

gases



one tonn tames and upon parkway June 210 Dissolved hydrocarbon State/Province Volatile Fatty Acids City Country Amhuist Gene-Trac DHGM Treatability Study 2U Gene-Trac DHC Gene-Trac DHB Gene-Trac SRB Gene-Trac FGA *Phone # 716.8106.8222 *Sampler's Signature *Sampler's Printed Name Kuthund Mutho vaa Sampling **Client Sample ID** # of Matrix Date Time Containers MW-16S 12/10/21 ivals 1500 × 3 × × X MW-812 vials 1100 12/13/21 X 7 **Billing Information Turnaround Time Requested** P.O. # For Lab Use Only Cooler Condition good Normal *Bill To: -21.5°C Cooler Temperature Rush \square No TY Yes Custody Seals: **Relinquished By: Received By: Relinquished By: Received By:** Signature Signature Signature Signature ndoracchiola Printed Punted Printed ansman Name Name Name Firm Firm Firm Date/Time Date/Time 21321 Date/Time

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Figure 5: Role of *Dhb* in dechlorination of 1,1,2,2-tetrachloroethane (TeCA). *Dhb* dechlorinates TeCA to 1,1,2-TCA which it then converts to vinyl chloride. TeCA is also dechlorinated to trans-1,2-DCE by *Dhb* (Figure courtesy of Manchester et al., 2012).

Interpretation of Gene-Trac[®]-Dhb/cfrA Results

Positive Gene-Trac[®]-Dhb Test Results (Detects)

A positive Gene-Trac[®]-*Dhb* test indicates that a member of the *Dehalobacter* genus was detected in the sample. The detection of *Dhb* indicates that dechlorination activities attributed to *Dhb* may be active. Increasing concentrations of *Dhb* are indicative of increased potential for degradation of some or all of these compounds.

Dechlorination Pathways which may be active with Dhb present include:

- PCE and TCE to cDCE (Holliger et al., 1998);
- 1,1,1-TCA to CA, and 1,2-DCA to ethene (Grostern and Edwards, 2006/2008);
- CF to DCM (Tang et al., 2014);
- DCM to acetate (Lee et al., 2011); and





• 1,1,2,2-TeCA to 1,1,2-TCA, and 1,1,2-TCA to VC, and 1,1,2,2-TeCA to tDCE (Manchester et al., 2012)

Considerations

- Gene-Trac[®]-*Dhb* will not differentiate the type of *Dhb*; therefore, observations of the specific biodegradation pathways and end products based on chemical analyses in conjunction with Gene-Trac[®]-*Dh*b and the functional gene test Gene-Trac[®]-*cfrA* will increase the interpretability of Gene-Trac[®]-*Dhb* results.
- Dhb have been reported to contain multiple copies (up to 4 per cell) of the 16S rRNA gene (Grostern and Edwards, 2008). This means that, unlike Dhc, there is not typically a 1:1 ratio between the 16S rRNA gene copy and the number of Dhb cells in a sample. Calculating the number of Dhb cells requires dividing the Gene-Trac[®]-Dhb test result by the 16S rRNA gene copy number (often 3-4 copies/cell).

Positive Gene-Trac[®]-cfrA Test Results (Detects)

A positive Gene-Trac[®]-*cfrA* test indicates that some or all of the *Dhb* present contain the functional genes *cfrA* and /or *dcrA* implicated in the dechlorination of:

- 1,1,1-TCA to CA (Grostern and Edwards, 2006);
- CF to DCM (Tang and Edwards, 2013); and
- DCM to acetate (Lee et al. 2011).

Increasing concentrations of these functional genes indicate increased likelihood and increased rates for the above pathways (Figures 2 & 4).

Negative Gene-Trac®-Dhb and cfrA Results (Non-Detects)

In cases where Gene-Trac[®]-*Dhb* and *cfrA* results are non-detect this indicates that *Dhb* species and key functional genes were not identified in the sample and that anaerobic reductive dechlorination of 1,1,1-TCA, 1,1,2-TCA, chloroform/DCM and TeCA may not be observed. It is noted that several other dechlorinating genera are implicated in degradation pathways for chlorinated ethenes and 1,2-DCA, and dechlorination of these compounds is more likely in the absence of *Dhb*.



Overview of Results Interpretation

Table 1 provides an overview of biodegradation under various scenarios for Gene-Trac[®]-*Dhb* Gene-Trac[®]-*cfrA* results.

Table 1: Interpretation of	Gene-Trac [®] -Dhb and	Gene-Trac [®] -cfrA result	S

Gene-Trac [®]	Test Result	Compound of Potential Concern							
Dhb	cfrA/dcrA	PCE/TCE	CF/DCM	1,1,1-TCA/ 1,1-DCA	1,1,2,2- TeCA	1,2-DCA			
Negative	Negative								
Positive	Negative								
Positive	Positive								

= No evidence for pathway due to absence of targeted microorganism or functional gene, note other organisms or pathways may be active

= Test results provide evidence for incomplete dechlorination of compound

=Test results provide evidence for complete dechlorination of compound



References

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Holliger, C., D. Hahn, H. Harmsen, W. Ludwig, W. Schumacher, B. Tindall, F. Vazquez, N. Weiss, and A.J.B. Zehnder, 1998. Dehalobacter restrictus gen. nov. and sp. nov., a strictly anaerobic bacterium that reductively dechlorinates tetra and trichloroethene in an anaerobic respiration. *Arch Microbiol* (1998) 169 : 313–321.

Lee, M., A. Low, O. Zemb, J. Koenig, A. Michaelsen, M. Manefield, 2011. Complete chloroform dechlorination by organochlorine respiration and fermentation. *Society for Applied Microbiology and Blackwell Publishing Ltd. Environmental Microbiology* 1-11.

Manchester, M, J., L.A. Hug, M. Zarek, A. Zila, E. A, Edwards, 2012. Discovery of a trans-Dichloroethene dehalogenating Dehalogenimonas Species in the 1,1,2,2-Tetrachloroethane-Dechlorinating WBC-2 Consortium. *Appl. Environ. Microbiol.* 78: 5280-5287.

Tang, S. and E.A. Edwards, 2013. Identification of *Dehalobacter* reductive dehalogenases that catalyse dechlorination of chloroform, 1,1,1-trichloroethane and 1,1-dichloroethane. *Phil. Trans. R. Soc. B* 2013 368, 20120318.