Site Investigation Work Plan

Bio-Trap Study Field Work

December 2020
Revision 0

Wauleco
Wausau, Wisconsin

BRRTS #02-37-000006

Prepared For:

Wauleco, Inc.

Prepared By:

TRC
708 Heartland Trail, Suite 3000
Madison, Wisconsin 53717

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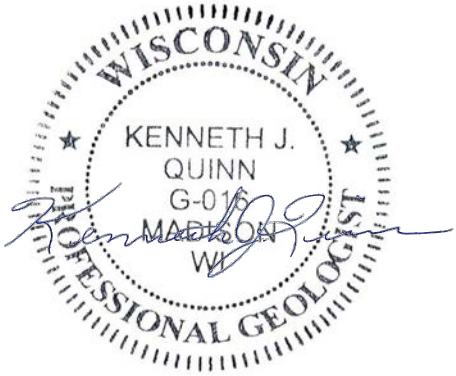
APPENDICES

1.0 Professional Certification

"I, Kenneth J. Quinn, hereby certify that I am a hydrogeologist as that term is defined in s. NR 712.03 (1), Wis. Adm. Code, am registered in accordance with the requirements of ch. GHSS 2, Wis. Adm. Code, or licensed in accordance with the requirements of ch. GHSS 3, Wis. Adm. Code, and that, to the best of my knowledge, all of the information contained in this document is correct and the document was prepared in compliance with all applicable requirements in chs.NR 700 to 726, Wis. Adm. Code."



Senior Project Hydrogeologist / G-016

P.G. Stamp

2.0 Project Management Plan

Consistent with NR 716.09(2)(a), (b), and (c) Wis. Adm. Code, the following information is provided:

1. **Site Address and Location:**

   Wauleco, Inc.
   125 Rosecrans Street
   Wausau, WI 54402
   Marathon County
   N½ of SE¼ of Section 35, Township 29 North, Range 7 East

2. **Responsible Party:**

   Wauleco, Inc.
   1800 North Point Drive
   Stevens Point, WI 54481

   Contact: Mr. Evan Schreiner
   (715) 346-8530

3. **Name of the consultant involved with the project:**

   TRC Environmental Corporation
   708 Heartland Trail, Suite 3000
   Madison, WI 53717

   Attention: Mr. Bruce Iverson
   Senior Project Manager
   (608) 826-3644
   e-mail: biverson@trcsolutions.com

4. **Site Location Map:** See Figure 1

3.0 Introduction

Consistent with NR 716.09(2)(d) Wis. Adm. Code, the following applicable information per NR 716.07 (Site Investigation Scoping) Wis. Adm. Code is provided:

3.1 Site History and Background

The Wauleco, Inc. (Wauleco) facility is located at 125 Rosecrans Street, Wausau, Wisconsin (Figure 1). The property is located in an area of mixed industrial and residential land use. The property is the location of a former window and patio door manufacturer.

As was common in the wood window manufacturing industry, surface coating on the exterior portions of wood windows manufactured at the site was performed using a wood preservative solution of pentachlorophenol (PCP) and mineral spirits. Over time, releases of the solution occurred, and residual-phase light non-aqueous phase liquid (LNAPL) is present in the smear zone (i.e., the interface of the unsaturated zone and the water table).

Natural attenuation of PCP via biological degradation has been documented in the TRC document titled Technical Memorandum Lines of Evidence of PCP Degradation dated August 2020 (PCP Degrades TM). In particular, biodegradation is occurring in the northeast and southeast portions of the plume as shown by the Northern, Northeast, Southeast, and Stagnation Zone profiles shown on Figures 9, 10 and 11 of the PCP Degrades TM. The PCP Degrades TM included findings and conclusions (refer to Sections 7.1 and 7.2 respectively, of the PCP Degrades TM). Conclusions included:

- "That PCP at Wauleco is degrading via biological degradation methods that are well accepted and described in literature…"
- "That the decreasing PCP concentration observed in groundwater in monitoring wells within or consistently downgradient of the residual phase LNAPL are due to biodegradation, and not other natural attenuation mechanisms, like dispersion, or dilution or due to changes in groundwater flow direction."

The Wisconsin Department of Natural Resources (WDNR's) technical review letter dated October 30, 2020 concerning the PCP Degrades TM stated: "The Department conceptually agrees with the findings and conclusions of this technical memorandum, however, additional field studies are necessary to support the conclusion that biodegradation is the primary natural attenuation mechanism for the central portion of the groundwater plume."

This Site Investigation Work Plan, Bio-Trap Study Field Work (Bio-Trap Work Plan) is designed to address the WDNR's request for additional site-specific field studies.

The additional field studies and results of this Bio-Study Work Plan will be used to evaluate if biological degradation of PCP is occurring through the central portion of the groundwater plume.

Additional information on site history and background is presented in documents on the WDNR's Bureau of Remediation and Redevelopment Tracking System (BRRTS) site.

3.2 Purpose and Approach

The purposes of this Bio-Trap Study Work Plan are to:

- Respond to the WDNR's October 30, 2020 request for "additional field studies… necessary to support the conclusion that biodegradation is the primary natural attenuation mechanism for the central portion of the groundwater plume." See Section 3.1.
- Determine whether bacteria capable of degrading PCP are present at the Wauleco site.
- Gather additional data to demonstrate that biodegradation of PCP is occurring within the central portion of the PCP groundwater plume. This data will be used to demonstrate whether PCP degradation is occurring throughout the PCP groundwater plume as well as beneath of the area where residual phase LNAPL is present.
- Fulfill Wauleco's commitment in the PCP Degrades TM (refer to Section 1) to perform a study to assess the presence of necessary bacteria.

The proposed approach is to conduct field sampling to demonstrate whether the bacteria understood to degrade PCP are present and to demonstrate whether PCP is degrading in the groundwater. Bio-Trap samplers are passive sampling tools that collect microbes over time for the purpose of better understanding biological activity occurring in the groundwater. The approach to this field investigation is to deploy Bio-Trap Samplers that contain beads that will be spiked with PCP, so that bacteria present within the groundwater that can degrade PCP will grow on and within the beads, providing an effective medium to collect the bacteria for analysis. In addition, stable isotope probing (SIP) will be used to demonstrate that PCP is being degraded. SIP starts with the PCP spiked in the beads containing PCP labeled with ^{13}C, a stable isotope. The analysis of ^{13}C in the PCP remaining in the beads, and in potential PCP breakdown products (i.e., in the bacteria present in the beads, in carbon dioxide, and within dissolved inorganic carbon) will provide information to determine the fate of the ^{13}C in the spiked PCP. These techniques will provide further evidence to evaluate whether PCP is degrading even in the area beneath the residual phase LNAPL.

3.3 Previous Investigations and Reports

Several investigations have been conducted at the site. Documents associated with these investigations are on the WDNR's BRRTS site. Among these documents, documents closely aligned with this additional investigation, include the following:

- Characterization of the LNAPL residual phase has been completed and reported in TRC's 2015 report titled Extent of Residual Phase Product/2015 LIF Survey Memo.
- The PCP Degrades TM (TRC, 2020b) demonstrates that PCP is degrading within the groundwater plume.

4.0 Site Description

Consistent with NR 716.09((2)(e) Wis. Adm. Code, this section provides information on the site setting.

4.1 Site Location and Features

According to the U.S. Geological Survey 7.5-Minute Quadrangle (USGS, see Figure 1), Wauleco is located in the N½ of SE¼ of Section 35, Township 29 North, Range 7 East, at an approximate elevation of 820 feet above mean sea level (amsl). The site is located within the limits of the City of Wausau, in a mixed industrial, commercial and residential area, approximately 500 feet to 1,000 feet west of the Wisconsin River.

Marathon County has a temperate climate with cold winters and warm summers. Total annual precipitation is approximately 32 inches.

4.2 Geology and Hydrogeology

The Wauleco site is located within the Wisconsin River bedrock valley and south of the southern extent of glacial advance. In general, the geology consists of a valley in the PreCambrian bedrock created by pre-glacial erosion with subsequent deposition in the valley of glacial aged outwash and lake deposits. The depth to the top of bedrock at the Wauleco site ranges from 58 feet on the west side of the site at well W-1B to greater than 60 feet near the Wisconsin River at well W-10B. The bedrock valley fill consists of sand, and sand and gravel glacial outwash from the surface to the top of bedrock on the western portion of the site (i.e., at 58 feet at well W-1B). A continuous silty clay to clayey silt deposit is present on top of bedrock, below the sand and gravel outwash, extending from the center of the site, near well PW-12, to the east, past well W-10B and under the Wisconsin River.

The groundwater in the vicinity of Wauleco occurs within the sand and gravel outwash within the Wisconsin River bedrock valley. Depth to groundwater ranges from approximately 33 feet (at well W-8) upgradient, west of Wauleco, to approximately 19 feet (at well W-10A) near the Wisconsin River shoreline.

4.3 LNAPL Distribution

The distribution of the residual phase LNAPL and the PCP concentration within the residual phase LNAPL was thoroughly characterized in the Technical Memorandum Residual Phase LNAPL Investigation dated December 2019 (Residual Phase LNAPL TM; i.e., cryogenic coring) (TRC, 2019). The horizontal extent of the residual phase LNAPL is shown in Figure 2. As shown in the Residual Phase LNAPL TM, the PCP concentration within the LNAPL has decreased significantly from the original 5%. However, there is still sufficient PCP present within the residual phase LNAPL to act as a source of PCP to groundwater. Hence, within the footprint of the residual phase LNAPL, if there is biodegradation of PCP in groundwater, the PCP may be replaced with dissolution of PCP from the residual phase LNAPL, slowing the decline in groundwater PCP concentration with distance from the site. The decline in PCP concentration shown in the Northern, Northeastern, Stagnation Zone, and Southeastern profiles can be used to estimate the decay rate because there is no other source of PCP except in the upgradient end of the profiles. However, the Centerline Profile, through the area with residual phase LNAPL acting as a source of PCP to groundwater, cannot be used to estimate a decay rate.

4.4 PCP Plume in Groundwater

The extent and isoconcentration contours of PCP in groundwater is shown routinely in the Annual Groundwater Monitoring Reports (e.g., TRC, 2020a). The 2019 PCP isoconcentration map is shown in Figure 2, with the extent of residual phase LNAPL also shown.

5.0 Sampling and Analysis Strategy

Consistent with NR 716.09(2)(f) and (g) Wis. Adm. Code, this section provides information on the proposed sampling and analysis strategy.

5.1 Scope of Work

To achieve the purposes discussed in Section 3.2, the proposed Scope of Work includes:

- Deploying eight (8) Bio-Traps that have been spiked with ^{13}C labeled PCP. The Bio-Traps will be provided by Microbial Insights, Inc.
- Leaving the Bio-Traps in place for 90 days, allowing for time for bacterial growth and PCP degradation within the Bio-Traps.
- Retrieving the Bio-Traps with analysis described in Section 5.2.
- The 8 Bio-Traps are proposed to be installed in monitoring wells:
  - Within the residual phase LNAPL at the head of the Northeast Profile, at well W02.
  - Within the central portion of the residual phase LNAPL, at wells W06R, W44, W03A, W22, and W10A
  - Within the Southeast Profile line at wells W41 and W11.

5.2 Analytical Strategy

Analysis of the Bio-Trap beads will be conducted for:

- Phospholipid Fatty Acids (PLFA)
- Stable Isotope Probing (i.e., ^{13}C analysis) of:
  - Remaining PCP in the beads
  - Within bacterial mass
  - Dissolved inorganic carbon
- CENSUS-DNA of the bacteria within the beads, including:
  - PCP-4-Monooxygenase--pcpB
  - Maleylacetate Reductase--pcpE
  - PCP Regulator Gene--pcpR
  - Dehalococcoides--DHC
  - Desulfitobacterium spp.--DSB)

5.3 Sample Preservation and Shipping

Bio-Traps will be handled as described in Microbial Insights protocols contained in Appendix A. In general, samples are packed in a cooler with ice or blue ice for next day delivery.

5.4 Decontamination of Equipment

Equipment will be provided by Microbial Insights and will be specific to each well. Therefore, no equipment decontamination is required.

5.5 Investigation Derived Waste (IDW)

Equipment used in this study will be returned to Microbial Insights, so no IDW will be generated on site.

5.6 Analytical Methods

Analyses will be conducted by Microbial Insights using their protocols for the analyses listed in Section 5.2.

5.7 Quality Assurance /Quality Control Protocols

QA/QC on the laboratory analyses will include the protocols described by Microbial Insights in their QA/QC protocol presented in Appendix A. The tables summarizing each analytical method includes the limit of detection (LOD) and limit of quantitation (LOQ) along with the quality control methods, acceptance criteria, and corrective actions when needed.

5.8 Bio-Trap Results Technical Memorandum

A Bio-Trap Study Technical Memorandum will be prepared and submitted to summarize the activities discussed in this Bio-Trap Work Plan. It will be provided to the WDNR within 60 days of receipt of all laboratory data described in this Bio-Trap Work Plan. Results from the Bio-Trap Study will inform the revised Groundwater Remedial Action Options Report developed after completion of the Bio-Trap Study.

6.0 Schedule

Consistent with NR 716.09(2)(h) Wis. Adm. Code, based on the approach described in this Bio-Trap Work Plan, the targeted schedule, after WDNR review and approval of this Bio-Trap Work Plan, is as follows:

Task	Completion Time
Lead time to obtain labeled PCP	18 weeks
Bio-Traps in wells	13 weeks
Laboratory analysis	9 weeks
Preparation of Technical Memorandum	8 weeks
Total	**48 weeks**

7.0 Technical Review Request

Wauleco is submitting a technical review fee for this Bio-Trap Work Plan per NR 749 Wisc. Adm. Code, for the following topics associated with this document:

- The sampling and analysis strategy discussed in Section 5.
- The anticipated schedule discussed in Section 6.

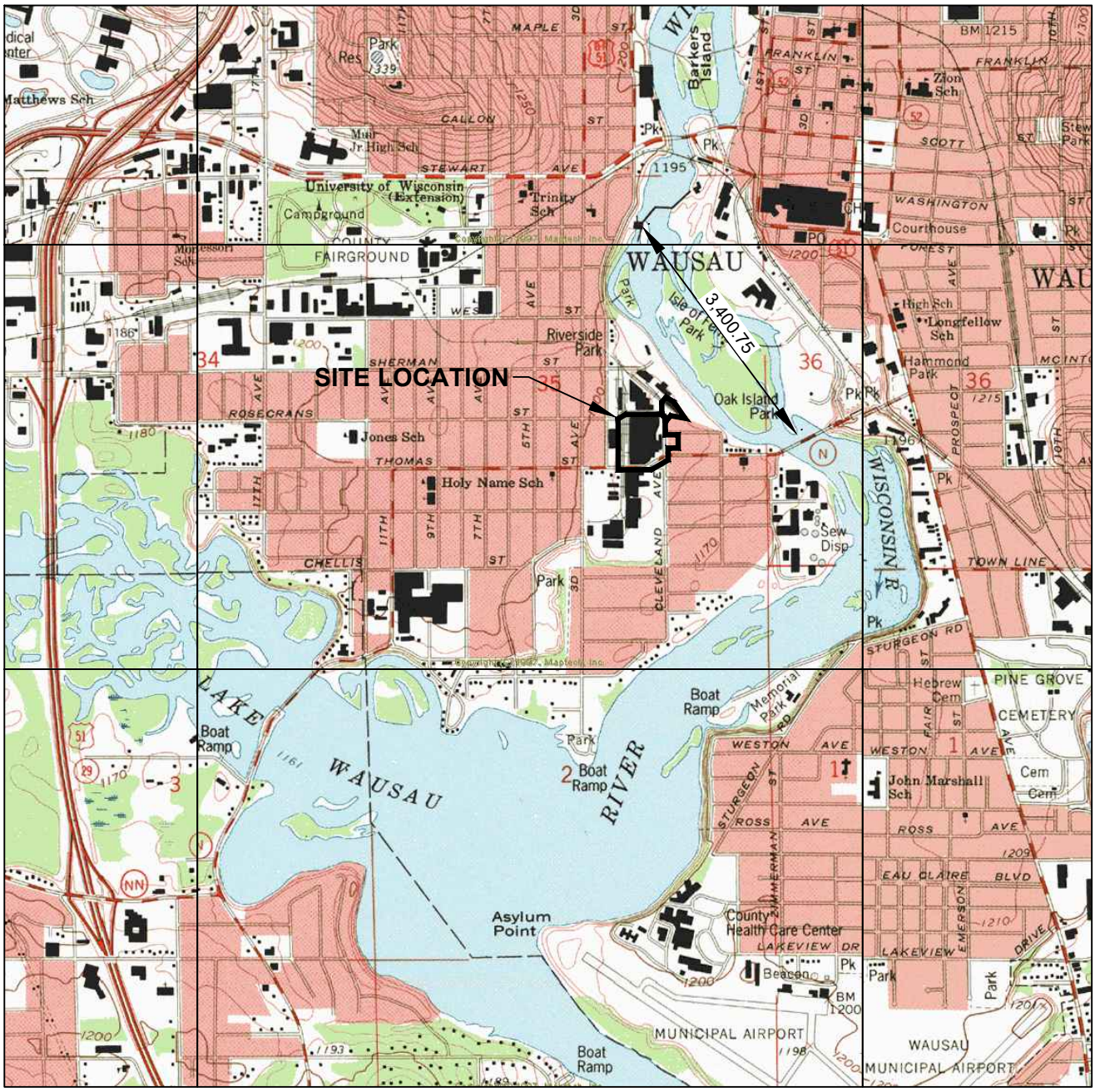
8.0 References

TRC. 2019. Technical Memorandum Residual Phase LNAPL Investigation. December 2019.

TRC. 2020a. 2019 Annual Groundwater Monitoring Report. April 2020.

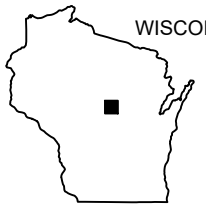
TRC. 2020b. Technical Memorandum Lines of Evidence of PCP Degradation. August 2020.

WDNR. 2020. Department Review of Technical Memorandum Lines of Evidence of PCP Degradation. Wauleco, Inc. October 30, 2020.



NOTE

BASE MAP DEVELOPED FROM THE WAUSAU WEST AND WAUSAU EAST, WISCONSIN 7.5 MINUTE U.S.G.S. TOPOGRAPHIC QUADRANGLE MAPS, DATED 1993. PART OF SECTION 35, T29N, R8E



QUADRANGLE LOCATION

TRC
708 Heartland Trail
Suite 3000
Madison, WI 53717
Phone: 608.826.3600

PROJECT:

WAULECO, INC.

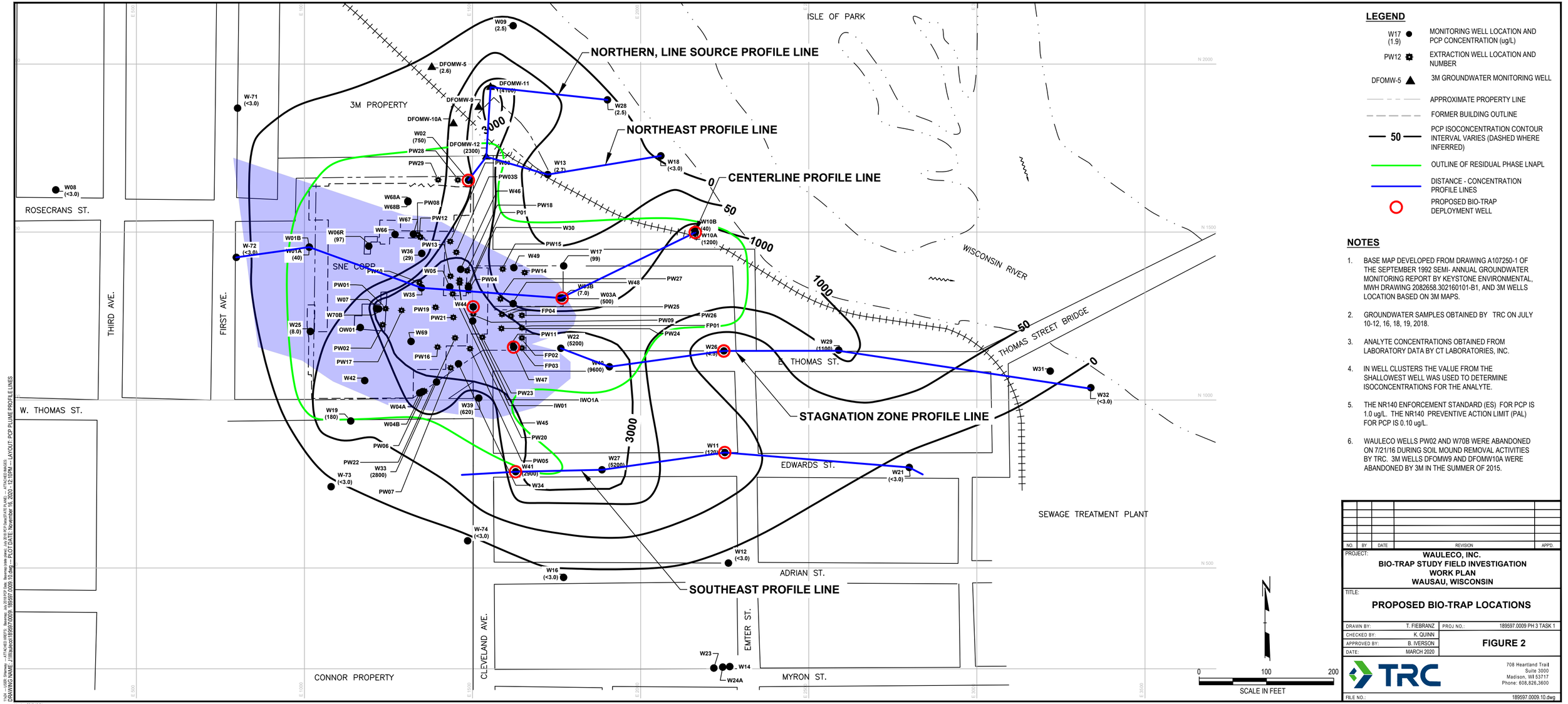
WAUSAU, WISCONSIN

TITLE:

SITE LOCATION MAP

DRAWN BY:	T. FIEBRANZ
CHECKED BY:	K. QUINN
APPROVED BY:	B. IVERSON
DATE:	MARCH 2020
PROJ. NO.:	189597.0009 PH 3 TASK 1
FILE:	189597.0009.03.dwg

FIGURE 1



LEGEND

- W17 (1.9) — MONITORING WELL LOCATION AND PCP CONCENTRATION (ug/L)
- PW12 — EXTRACTION WELL LOCATION AND NUMBER
- DFOMW-5 — 3M GROUNDWATER MONITORING WELL
- APPROXIMATE PROPERTY LINE
- FORMER BUILDING OUTLINE
- 50 — PCP ISOCONCENTRATION CONTOUR INTERVAL VARIES (DASHED WHERE INFERRED)
- OUTLINE OF RESIDUAL PHASE LNAPL
- DISTANCE - CONCENTRATION PROFILE LINES
- PROPOSED BIO-TRAP DEPLOYMENT WELL

NOTES

1. BASE MAP DEVELOPED FROM DRAWING A107250-1 OF THE SEPTEMBER 1992 SEMI- ANNUAL GROUNDWATER MONITORING REPORT BY KEYSTONE ENVIRONMENTAL, MWH DRAWING 2082658.302160101-B1, AND 3M WELLS LOCATION BASED ON 3M MAPS.
2. GROUNDWATER SAMPLES OBTAINED BY TRC ON JULY 10-12, 16, 18, 19, 2018.
3. ANALYTE CONCENTRATIONS OBTAINED FROM LABORATORY DATA BY CT LABORATORIES, INC.
4. IN WELL CLUSTERS THE VALUE FROM THE SHALLOWEST WELL WAS USED TO DETERMINE ISOCONCENTRATIONS FOR THE ANALYTE.
5. THE NR140 ENFORCEMENT STANDARD (ES) FOR PCP IS 1.0 ug/L. THE NR140 PREVENTIVE ACTION LIMIT (PAL) FOR PCP IS 0.10 ug/L.
6. WAULECO WELLS PW02 AND W70B WERE ABANDONED ON 7/21/16 DURING SOIL MOUND REMOVAL ACTIVITIES BY TRC. 3M WELLS DFOMW9 AND DFOMW10A WERE ABANDONED BY 3M IN THE SUMMER OF 2015.

NO.	BY	DATE	REVISION	APPD.

PROJECT:
WAULECO, INC.
BIO-TRAP STUDY FIELD INVESTIGATION
WORK PLAN
WAUSAU, WISCONSIN

TITLE:
PROPOSED BIO-TRAP LOCATIONS

DRAWN BY:	T. FIEBRANZ	PROJ NO.:	189597.0009 PH 3 TASK 1
CHECKED BY:	K. QUINN		
APPROVED BY:	B. IVERSON		**FIGURE 2**
DATE:	MARCH 2020		

TRC
708 Heartland Trail
Suite 3000
Madison, WI 53717
Phone: 608.826.3600

FILE NO.: 189597.0009.10.dwg

Appendix A: Microbial Insights Protocols

Bio-Trap – PLFA Sampling Protocol

Sampling Instructions

Storage:

It is important to minimize the amount of time that the Bio-Trap Samplers are stored prior to being installed in the field. The physical properties of the Bio-Trap Samplers that make them an ideal medium for collecting microbes also increase the chances of microbial or chemical contamination. The Bio-Trap Samplers need to remain sealed and refrigerated (not frozen) until they can be installed in the field.

Note: Clean latex gloves (or similar) should be used at all times when handling the Bio-Trap Samplers.

Installation:

- Prior to installing the Bio-Trap Sampler, the monitoring well may need to be purged if it has not been sampled in a while. If purging is necessary, MI recommends that three well volumes be removed to ensure contact with formation water and reduce well bore effect.
- Attach the Bio-Trap Sampler's nylon loop (provided) to a nylon line (not provided) and suspend the Bio-Trap Sampler at a depth where significant contaminant concentrations exist. If no data is available on the vertical distribution of contaminants, then suspend the Bio-Trap Sampler in the middle of the saturated screened interval.
- If large fluctuations in the water level are anticipated during the period of incubation, the Bio-Trap Sampler should be suspended from a float (contact MI for further details). Be sure not to suspend the Bio-Trap in the NAPL zone.
- Once installed, incubation times can vary depending upon the scope of the project (routine monitoring and stable isotope probing (SIP) - 30 days and "baited" - 60 days).

Retrieval:

- Open the monitoring well and pull up the Bio-Trap Sampler. Cut and remove the braided nylon line used to suspend the Bio-Trap Sampler.
- Transfer the recovered Bio-Trap Sampler to labeled (well number and date) zippered bags, seal and then double bag in a larger (one-gallon) zippered bag, immediately place on blue ice in a cooler.
- Repeat the above for all Bio-Trap Samplers from the site. Individual zippered bags containing the Bio-Trap Samplers can be placed in the same one-gallon zippered bag (if there is enough space).
- A chain of custody (COC) form must be included with each shipment of samples.

Hold time for this analysis is 24-48 hours.

Shipping Instructions

Packaging Samples:

1. Samples should be shipped in a cooler with ice or blue ice for next day delivery. If regular ice is used, the ice should be double bagged.
2. A chain of custody form must be included with each shipment of samples. Access our chain of custody at www.microbe.com.

Shipment for Weekday Delivery:

Samples for weekday delivery should be shipped to:

Sample Custodian
Microbial Insights, Inc.
10515 Research Drive
Knoxville, TN 37932
(865) 573-8188

Shipment for Saturday Delivery:

Coolers to be delivered on Saturday must be sent to our FedEx Drop Location. To ensure proper handling the following steps must be taken:

1. FedEx shipping label should be marked under (6) Special Handling, check Hold Saturday.
2. The cooler must be taped with FedEx SATURDAY tape.
3. The shipping label must be filled out with the Drop Location address below. Our laboratory name must be on the address label.
4. You MUST ***notify by email*** customerservice@microbe.com with the tracking number of the package on Friday (prior to 4pm Eastern Time) to arrange for Saturday pickup. Please make sure you write "Saturday Delivery" in the subject line of the message. Without proper labeling and the tracking number, there is no guarantee that the samples will be collected.

Samples for Saturday delivery should be shipped to:

Microbial Insights, Inc.
FedEx Drop Location
10601 Murdock Drive
Knoxville, TN 37932
(865) 300-8053

Bio-Trap – DNA Sampling Protocol

Sampling Instructions

Storage:

It is important to minimize the amount of time that Bio-Trap Samplers are stored prior to being installed in the field. The physical properties of the Bio-Trap Samplers that make them an ideal medium for collecting microbes also increase the chances of microbial or chemical contamination. Bio-Trap Samplers need to remain sealed and refrigerated (not frozen) until they can be installed in the field.
Note: Clean latex gloves (or similar) should be used at all times when handling Bio-Trap Samplers.

Installation:

- Prior to installing the Bio-Trap Sampler, the monitoring well may need to be purged if it has not been sampled in a while. If purging is necessary, MI recommends that three well volumes be removed to ensure contact with formation water and reduce well bore effect.
- Attach the Bio-Trap Sampler's nylon loop (provided) to a nylon line (not provided) and suspend the Bio-Trap Sampler at a depth where significant contaminant concentrations exist. If no data is available on the vertical distribution of contaminants, then suspend the Bio-Trap Sampler in the middle of the saturated screened interval.
- If large fluctuations in the water level are anticipated during the period of incubation, the Bio-Trap Sampler should be suspended from a float (contact MI for further details). Be sure not to suspend the Bio-Trap in the NAPL zone.
- Once installed, incubation times can vary depending upon the scope of the project (routine monitoring and stable isotope probing (SIP) - 30 days and "baited" - 60 days).

Retrieval:

- Open the monitoring well and pull up the Bio-Trap Sampler. Cut and remove the braided nylon line used to suspend the Bio-Trap Sampler.
- Transfer the recovered Bio-Trap Sampler to labeled (well number and date) zippered bags, seal and then double bag in a larger (one-gallon) zippered bag, immediately place on blue ice in a cooler.
- Repeat the above for all Bio-Trap Samplers from the site. Individual zippered bags containing the Bio-Trap Samplers can be placed in the same one-gallon zippered bag (if there is enough space).
- A chain of custody (COC) form must be included with each shipment of samples.

Hold time for this analysis is 24-48 hours.

Shipping Instructions

Packaging Samples:

1. Samples should be shipped in a cooler with ice or blue ice for next day delivery. If regular ice is used, the ice should be double bagged.
2. A chain of custody form must be included with each shipment of samples. Access our chain of custody at www.microbe.com.

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2. The cooler must be taped with FedEx SATURDAY tape.
3. The shipping label must be filled out with the Drop Location address below. Our laboratory name must be on the address label.
4. You MUST ***notify by email*** customerservice@microbe.com with the tracking number of the package on Friday (prior to 4pm Eastern Time) to arrange for Saturday pickup. Please make sure you write "Saturday Delivery" in the subject line of the message. Without proper labeling and the tracking number, there is no guarantee that the samples will be collected.

Samples for Saturday delivery should be shipped to:

Microbial Insights, Inc.
FedEx Drop Location
10601 Murdock Drive
Knoxville, TN 37932
(865) 300-8053

Bio-Trap – Stable Isotope Probing Protocol

Sampling Instructions

Handling:

- Bio-Trap Samplers used for Stable Isotope Probing (SIP) are baited with ^{13}C-labeled contaminant of interest (e.g. benzene, MTBE, chlorobenzene) adsorbed onto the powder activated carbon (PAC). Controlled laboratory conditions show only minimal loss of contaminant due to volatilization. However, special considerations must be taken into account when handling SIP Bio-Trap Samplers in order to reduce the risk of volatilization.
- SIP Bio-Trap Samplers are shipped out chilled, on blue ice, and it is essential that they should be kept cool (not frozen) until deployment.
- When retrieving the Bio-Trap Samplers that have been deployed in the field, they should immediately be placed on ice and shipped on ice for next day delivery. These steps will ensure the most accurate results.
- Although the contaminant is absorbed onto the beads, caution should be used in handling these Bio-Trap Samplers because the contaminant compounds are associated with possible health and safety risks.

Note: Clean latex gloves (or similar) should be used at all times when handling the Bio-Trap Samplers.

Storage:

It is important to minimize the amount of time that Bio-Trap Samplers are stored prior to being installed in the field. The physical properties of the Bio-Trap Samplers that make them an ideal medium for collecting microbes also increase the chances of microbial or chemical contamination. Bio-Trap Samplers need to remain sealed and refrigerated (not frozen) until they can be installed in the field.

Installation:

- Prior to installing Bio-Trap Sampler, the monitoring well may need to be purged if it has not been sampled in a while. If purging is necessary, MI recommends that three well volumes be removed to ensure contact with formation water and reduce well bore effect.
- Attach the Bio-Trap Sampler's nylon loop (provided) to a nylon line (not provided) and suspend Bio-Trap Sampler at a depth where significant contaminant concentrations exist. If no data are available on the vertical distribution of contaminants, then suspend the Bio-Trap Sampler in the middle of the saturated screened interval.
- If large fluctuations in the water level are anticipated during the period of incubation, the Bio-Trap Sampler should be suspended from a float (contact MI for further details). Be sure not to suspend the bio-trap in the NAPL zone.
- Once installed, incubation times can vary depending upon the scope of the project. A typical Stable Isotope Probing (SIP) study incubation period is 30 days but is project dependant. Please contact us if you have questions regarding the optimum deployment period for your samples.

Retrieval:

- Open the monitoring well and pull up the Bio-Trap Sampler. Cut and remove the braided nylon line used to suspend the Bio-Trap Sampler.
- Transfer the recovered Bio-Trap Sampler to labeled (well number and date) zippered bags, seal and then double bag in a larger (one-gallon) zippered bag, immediately place on blue ice in a cooler.
- Repeat above for all the Bio-Trap Samplers from the site.
- A chain of custody (COC) form must be included with each shipment of samples.
- In order to minimize the potential effect of these samplers on the monitoring well, MI recommends purging three well volumes from the test well following the retrieval of the SIP Bio-Trap Samplers.

Hold time for this analysis is 24-48 hours.

Shipping Instructions

Packaging Samples:

1. Samples should be shipped in a cooler with ice or blue ice for next day delivery. If regular ice is used, the ice should be double bagged.
2. A chain of custody form must be included with each shipment of samples. Access our chain of custody at www.microbe.com.

Shipment for Weekday Delivery:

Samples for weekday delivery should be shipped to:

Sample Custodian
Microbial Insights, Inc.
10515 Research Drive
Knoxville, TN 37932
(865) 573-8188

Shipment for Saturday Delivery:

Note: Microbial Insights, Inc is **closed** on Sunday, however we can receive samples on Saturday. Please contact us prior to shipping if the delivery of the samples is going to be on a Saturday.

Samples for Saturday delivery should be shipped to:

Microbial Insights, Inc.
FedEx Drop Location
10601 Murdock Drive
Knoxville, TN 37932
(865) 300-8053

Notes:

- **Stable Isotope Probing (SIP) may preclude subsequent Compound Specific Isotope Analysis (CSIA) in the study well for a period of time. CSIA can be performed prior to SIP or at another location.**

Microbial Insights
Analytical Methods and QA/QC

Parameter	Method	Holding Time/Volume/	LOD/LOQ	Reporting Units	Quality Control	Minimum Frequency	Acceptance Criteria	Corrective Action
13C compound	GC/MS	14 days holding time/1 x 40 mL Volatile Organic Acid (VOA) Vial or 6 bio-sep beads/No preservative/On Ice at 4c	0.03 / 0.1	mg/bd	Initial Calibration (ICAL)	Once per assay	r2 ≥ 0.995	Re-Anayze / check reagents.
					Continuing Calibration Verification (CCV)	Bracketed by CCV at a 10% frequency and at the	All analytes ±20% of expected value from ICAL	Correct problem, repeat CCV and reanalyze all samples since last successful calibration verification
					Laboratory Method Blank (LMB)	Once per analytical batch	No analytes detected > RL. DoD: No analytes detected > 1/2 RL.	Correct problem, reanalyze LMB and all samples processed with contaminated blank.
					Laboratory Control Sample (LCS)	Once per event batch	Percent recovery between 80% and 120%	Reprep and reanalyze LCS, if fails again, correct problem and renalyze all samples in the associated batch, if sufficient material is available
					Replicates	All samples analyzed in Triplicate	+/- 20% of expected value	If criterion fails, but all other instrument run criteria pass, then simply noted in folder. Othewsie reanalyze batch

Microbial Insights
Analytical Methods and QA/QC

Parameter	Method	Holding Time/Volume/ Preservation/Storage	LOD/LOQ	Reporting Units	Quality Control	Minimum Frequency	Acceptance Criteria	Corrective Action
Phospholipid Fatty Acid Analysis (PLFA)	Modified Bligh & Dyer lipid extraction	48 hrs/1-2L for water samples, 50 grams for soil samples, 1 Bio-Trap/No preservative/On Ice at 4c	150 picomoles of PLFA/500 picomoles of PLFA	Picomoles & % of total	Initial Calibration (ICAL)	Once per assay	r2 ≥ 0.95	Re-Anayze / check reagents.
					Continuing Calibration Verification (CCV)	Bracketed by CCV at a 10% frequency and at the end of the sample batch	selected analyte ±20% of expected value from ICAL	Correct problem, repeat CCV and reanalyze all samples since last successful calibration verification
					Laboratory Method Blank (LMB)	Once per event batch	No biomarkers detected > RL. DoD: No biomarkers detected > 1/2 RL.	Field samples below our LMB are reported below our practical quantification limit. Biomarkers found in higher levels in blank then field samples are removed from profile
					Laboratory Control Sample (LCS)	Once per event batch	Serves as a postive control to evaluate peak seperation and community composition, Percent recovery between 60% and 140%	If criterion fails, but all other instrument run criteria pass, then simply noted in folder.

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Parameter	Method	Holding Time/Volume/ Preservation/Storage	LOD/LOQ	Reporting Units	Quality Control Check	Minimum Frequency	Acceptance Criteria	Corrective Action
DIC	Trace Gase System+IRMS	days hold time/2 x 40mL VOA vials with bio-sep beads/No preservative/on ice at 4c	250 uM C in the bulk sample	Del	Laboratory Method Blank (LMB)	Once per event batch	Any analytes detected in these blanks must not be present in concentrations greater than in the lowest standard	If this criterion is not met, inspect all glassware, etc. and then prepare and analyze another blank. Blanks should be run until this criterion is met.
					Laboratory Reference Gas	every sample	+ / - 0.5 del	If this criterion is not met, samples with a reference gas outside the acceptance criteria are flagged.

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Parameter	Method	Holding Time/Volume/ Preservation/Storage	LOD/LOQ	Reporting Units	Quality Control Check	Minimum Frequency	Acceptance Criteria	Corrective Action
13C-FAME	GC-IRMS	Not Applicable/2 mL GC Vial/No preservative/stored frozen or dried down	0.2 Vs peak area	Del	Laboratory Method Blank (LMB)	Once per event batch	No biomarkers detected > RL. DoD: No biomarkers detected > 1/2 RL.	Field samples below our LMB are reported below our practical quantification limit. Biomarkers found in higher levels in blank then field samples are removed from profile
					Laboratory Reference Gas	every sample	+ / - 0.5 del	If this criterion is not met, samples with a reference gas outside the acceptance criteria are flagged

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Method	Quality Control Check	Minimum Frequency	Acceptance Criteria	Corrective Action
DNA (qPCR)	Assay Calibration (Standard Curve)	Primary – initial	Standard curve R2 >0.95	Rerun assay / check reagents.
DNA (qPCR)		Secondary – every plate (assay)	CT value within +/- 20% of known value	
DNA (qPCR)	Assay Negative Control (Blank)	1 per analytical assay plate in duplicate	values for positive samples are set above any fluorescence for the negative control	Rerun assay; may have to reoptimize assay
DNA (qPCR)	DNA extraction negative control	1 per analytical batch	CT < or = Assay Negative Control	Rerun assay or reextract samples if problem persists
DNA (qPCR)	Positive Control	1 per analytical assay plate in duplicate	CT value within +/- 20% of known value	Rerun assay / check reagents