

Bioinjection Performance Review for the Building 100 Area and 4.5 Acre Site at the Pinellas County, Florida, Site

May 2016



**U.S. DEPARTMENT OF
ENERGY**

Legacy
Management

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1.0 Purpose

This document provides a summary of a review of the performance of bioinjection activities at the Building 100 Area and the 4.5 Acre Site at the Pinellas County, Florida, Site since 2010, determines how best to optimize future injection events, and identifies the approach for the bioinjection event in 2016 at (1) the 4.5 Acre Site and (2) the Essentra property at the Building 100 Area.

Because this document describes the next bioinjection event at the 4.5 Acre Site, it also serves as an addendum to the *Interim Remedial Action Plan for Emulsified Edible Oil Injection at the 4.5 Acre Site* (DOE 2013). In addition, this document revises the injection layout and injection intervals for the Essentra property that were originally described in the *Interim Corrective Measure Work Plan for Source and Plume Treatment at the Building 100 Area* (DOE 2014), and is a de facto update of that document.

2.0 Introduction

Bioinjection consists of injection of emulsified vegetable oil (EVO) and the microorganism *Dehalococcoides mccartyi* (DHM; formerly known as *Dehalococcoides ethenogenes*) into the subsurface to enhance biodegradation of trichloroethene (TCE), dichloroethene (DCE), and vinyl chloride (VC). VC is the only contaminant that exceeds its maximum contaminant level (10 micrograms per liter onsite and 1 microgram per liter onsite) on the 4.5 Acre Site and the Essentra property.

Bioinjection was conducted at the 4.5 Acre Site in 2010 and 2013. Approximately 49,900 gallons of EVO and DHM were injected at 95 injection points in February 2010, and approximately 22,900 gallons of EVO and DHM were injected at 46 injection points in July 2013. The injection locations are shown on Figure 1. The goal of bioinjection at the 4.5 Acre Site is to decrease contaminant concentrations to maximum contaminant levels along the west and southwest property boundaries (to meet risk-based corrective action requirements) and to minimize the extent of the contaminant plume in the interior of the site.

Bioinjection was conducted at the Building 100 Area in 3 phases from October 2014 through November 2015 (Figures 2–4). Approximately 32,850 gallons of EVO and DHM were injected using 62 temporary injection points in the onsite plumes in October and November 2014, 16,500 gallons were injected using 33 temporary injection points in the offsite plumes in February 2015, and 51,000 gallons were injected beneath the building using 8 horizontal wells in November 2015. The goal of bioinjection at the Building 100 Area is to enhance contaminant biodegradation to stabilize or shrink the contaminant plumes.

3.0 Performance Review

Terra Systems, Incorporated was the vendor of all the EVO and DHM that was injected previously at the two sites. DOE collaborated with Terra Systems to conduct a detailed review of the performance of the injections, and this document summarizes that review. As part of the

review, a special sampling event was conducted in January 2016 during which a subset of 19 wells was sampled for a suite of biogeochemical parameters to provide current geochemical data. These data are included as Tables 1 and 2, and Table 2 also includes methane, ethane, and ethene data from five wells beneath Building 100 from March 2016.

The review of current and historical data involved the following components.

- Contaminant concentration trending.
- An evaluation of field parameters, such as dissolved oxygen (DO), oxidation-reduction potential (ORP), and specific conductance (SC).
- An evaluation of historical and current geochemical data, such as the electron acceptors nitrate, iron, and sulfate.
- An evaluation of microbial parameters, consisting of DHM concentrations and the presence (in the microorganisms) of three different functional genes (two responsible for VC biodegradation and one for TCE biodegradation).

The results of the review of the 4.5 Acre Site data demonstrated that EVO and DHM injection had resulted in significant concentration decreases at most wells (Figures 5 and 6), but two main factors have limited the effectiveness at reaching the desired contaminant concentration goals. The first factor is elevated sulfate concentrations. Sulfate reduction must occur to reach the optimum conditions for contaminant biodegradation, and reduction of sulfate uses some of the electrons generated by EVO injection. Sulfate concentrations up to 690 milligrams per liter were observed in January 2016 (Table 2), indicating that previous EVO injections have not completely reduced sulfate at all areas. This suggests that insufficient electrons were available for sulfate reduction, resulting in limited contaminant biodegradation. The second factor limiting contaminant biodegradation is lack of contact of the injected EVO and DHM with the contaminants. This most likely is a result of preferential flow through the surficial aquifer during the injections and the limitations imposed on the maximum volume of injectate by the aquifer characteristics.

At the Building 100 Area, some wells have shown significant contaminant concentration decreases and other wells have shown little to no change since the injections (Figures 7 and 8). The results of the data review indicate that a lack of contact of the injectate with the contaminants is the main factor limiting biodegradation, similar to the situation at the 4.5 Acre Site. Sulfate concentrations are relatively low at the Building 100 Area and thus are not a factor influencing biodegradation.

The DHM concentration goal after injection is 1×10^7 cells per liter, or higher. The DHM concentrations measured during the special sampling event in January 2016 were lower than this concentration in the 5 wells sampled at the 4.5 Acre Site (average of 8×10^5 cells per liter), while 5 wells at the Building 100 Area exceeded the goal and the remainder were less than the goal (average of 5×10^5 cells per liter for the remainder) (Table 1). Results from the functional gene analysis also suggest that the concentrations of DHM containing the necessary functional genes were lower than optimal at almost all locations.

Contaminant and geochemical parameter concentration trends suggest that the longevity of the injectate (the duration for which it remains significantly effective) is generally about 2 to 3 years.

4.0 Optimization of Future Bioinjection Events

As a result of the performance review, actions to optimize future bioinjection events at the 4.5 Acre Site and the Building 100 Area were identified.

The approach to increase contact of the injectate with the contaminants at both sites is to focus the injection intervals and to inject in more locations. Previously, injection generally was conducted over a 20-foot vertical interval spanning both the shallow and deep portions of the surficial aquifer with about a 15-foot horizontal spacing between injection points. In the future, injection will be implemented using a shorter vertical interval (focused on the portion of the aquifer with the highest contaminant concentrations) and with a generally tighter spacing between injection points. As a general example, 5 injection points with a 12-foot vertical interval and 10-foot horizontal spacing would be used instead of 3 injection points with a 20-foot vertical interval and 15-foot horizontal spacing.

Identification of the depths at which to focus injection will be based on a detailed review of data from existing monitoring wells and an evaluation of previous bioinjection performance. In addition for the Building 100 Area, this will include a detailed review of the drive-point sampler data collected at the Building 100 Area in 2010–2012. During the drive-point sampling, groundwater samples were collected about every 4 feet vertically at locations that were about 10–15 feet apart, resulting in a very detailed delineation of the depth and lateral extent of the plumes at the sampling locations.

To address the lower-than-optimal DHM and functional gene concentrations, the concentration of DHM in the injectate will be increased by approximately a factor of three.

The solution to the high sulfate concentrations at the 4.5 Acre Site is to inject enough EVO to reduce the concentrations to desirable levels. This will be accomplished by focusing the injection intervals and increasing the number of injection points as described above. There is a possibility that multiple injection events will be necessary to decrease the sulfate concentrations sufficiently to reach the remediation goals.

The performance review identified several areas at the 4.5 Acre Site where EVO and DHM were injected within about 10 feet of a monitoring well but no effect was seen in the well. This most likely is due to preferential flow during the injection. To counter this lack of effect and to improve the understanding of the significance of the preferential flow pathways, injection points generally will be clustered closely together near the monitoring wells.

The performance review found that injected EVO and DHM remained effective for 2–3 years. Given the sulfate limitation at the 4.5 Acre Site and the limited-contact issue at both sites, bioinjection events may be needed about every 2–3 years if monitoring indicates that the remediation goals have not been met.

5.0 Bioinjection at the Essentra Property and the 4.5 Acre Site

Based on the performance review, additional bioinjection is required to meet remediation goals at the 4.5 Acre Site. Implementation of the optimization actions described in Section 4 resulted in the injection intervals listed in Table 3 and the injection point layout shown in Figure 9. Well M068 is the only shallow well with VC above the maximum contaminant level, so injection points N01–N03 have a 20-foot vertical injection interval that covers both the deep and shallow portions of the surficial aquifer at this location. The 12-foot vertical injection interval for the remaining 29 injection points is focused on the deeper part of the aquifer containing the highest contaminant concentrations, as defined by the screened intervals of the monitoring wells.

DOE had planned bioinjection for all four offsite properties at the Building 100 Area in February 2015, but access to the Essentra property was not obtained in time for the event. However, now that access has been granted, DOE will implement the optimization actions described in Section 4 to treat the plume on this property. The injection intervals are listed in Table 3 and the injection point layout is shown in Figure 10. The injection intervals are defined by the contaminant concentrations measured recently in monitoring wells and in water samples collected during the drive-point sampler work. The drive-point sampler results are described in detail in the *Building 100 Area Site Assessment Report* (DOE 2012). The contaminant plume is located only in the deep portion of the surficial aquifer in the north part of the Essentra property, but appears to spread into the shallow surficial aquifer by the time it reaches well 12-0574, adjacent to Belcher Road. Thus, injection points K01–K09 have a 12-foot vertical interval in the deep surficial aquifer and injection points L01–L10 have a 20-foot vertical interval covering both the deep and shallow surficial aquifers.

Zebra Technical Services of Tampa, Florida, has conducted all the previous bioinjection events at both the 4.5 Acre Site and the Building 100 Area and will conduct the upcoming bioinjection events as well. Concentrated EVO will be diluted 9:1 with municipal water and DHM will be added prior to injection. Each 4-foot injection interval will receive 100 gallons of EVO and DHM mixture. Table 3 lists the injectate volumes and injection intervals. The detailed injection procedure is included as Attachment A.

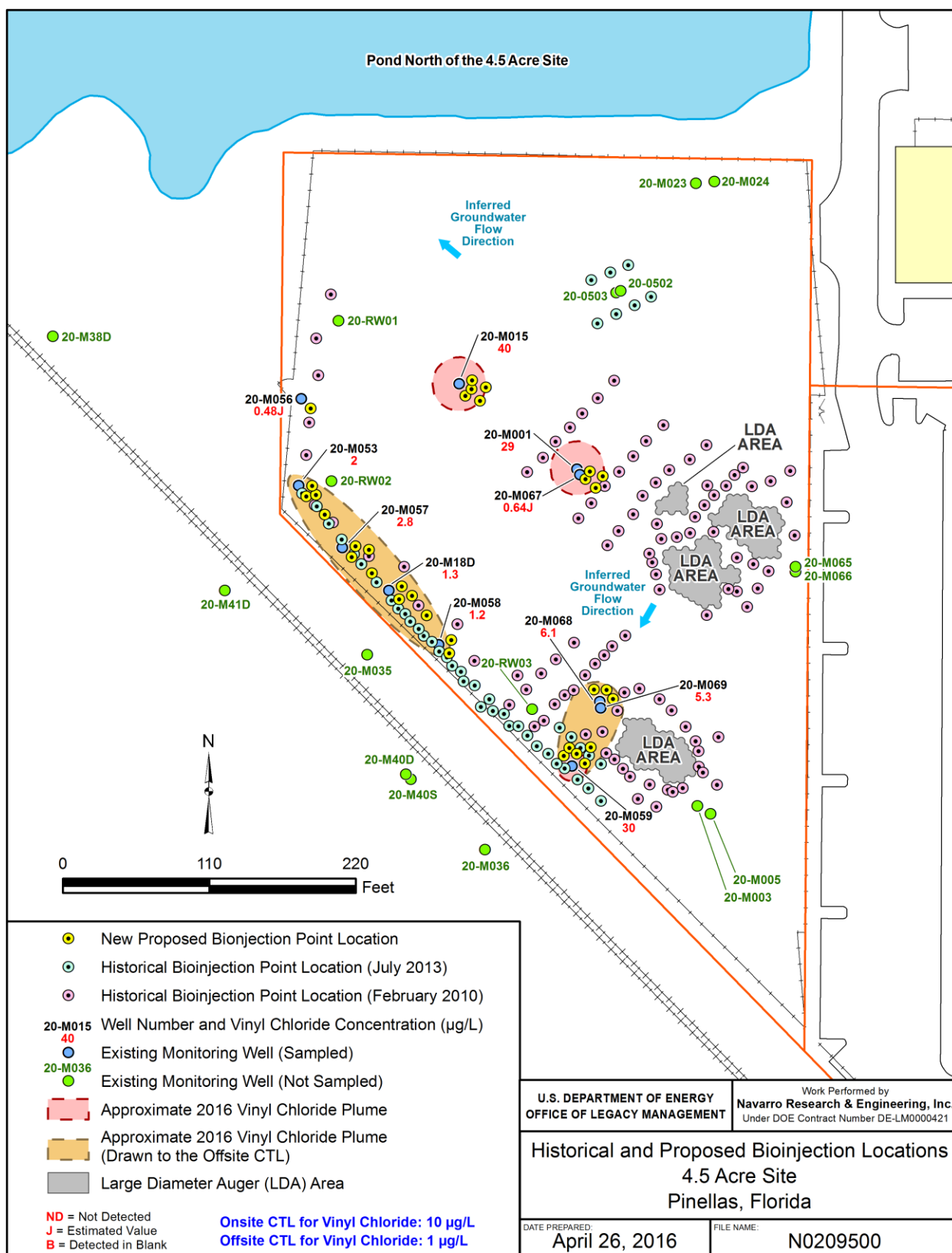
These two bioinjection projects will be conducted sequentially and the work is planned for August 2016. Currently, DOE has access to conduct the injection event at the Essentra property, but is in the process of gaining access for remediation at the 4.5 Acre Site. If access to the 4.5 Acre Site cannot be obtained in time for the work to be performed in fiscal year 2016, then injection at the 4.5 Acre Site may be postponed to a later date.

6.0 References

DOE (U.S. Department of Energy), 2012. *Building 100 Area Site Assessment Report*, LMS/PIN/N01747, Office of Legacy Management, August.

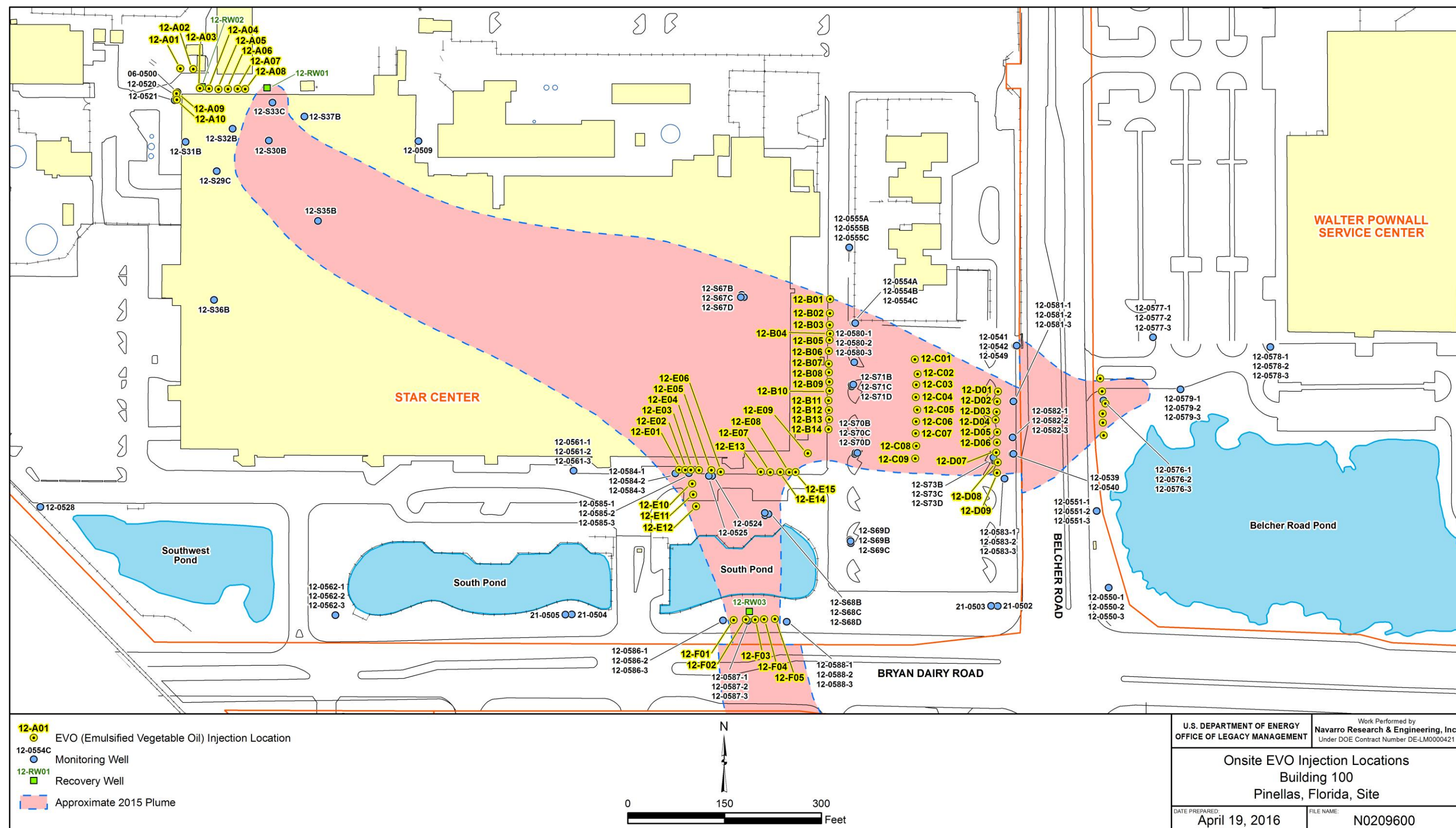
DOE (U.S. Department of Energy), 2013. *Interim Remedial Action Plan for Emulsified Edible Oil Injection at the 4.5 Acre Site*, LMS/PIN/N01776, Office of Legacy Management, April.

DOE (U.S. Department of Energy), 2014. *Interim Corrective Measure Work Plan for Source and Plume Treatment at the Building 100 Area*, LMS/PIN/N01868, Office of Legacy Management, October.



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Figure 1. 4.5 Acre Site Historical Bioinjection Locations



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Figure 2. Building 100 Onsite Historical Bioinjection Locations

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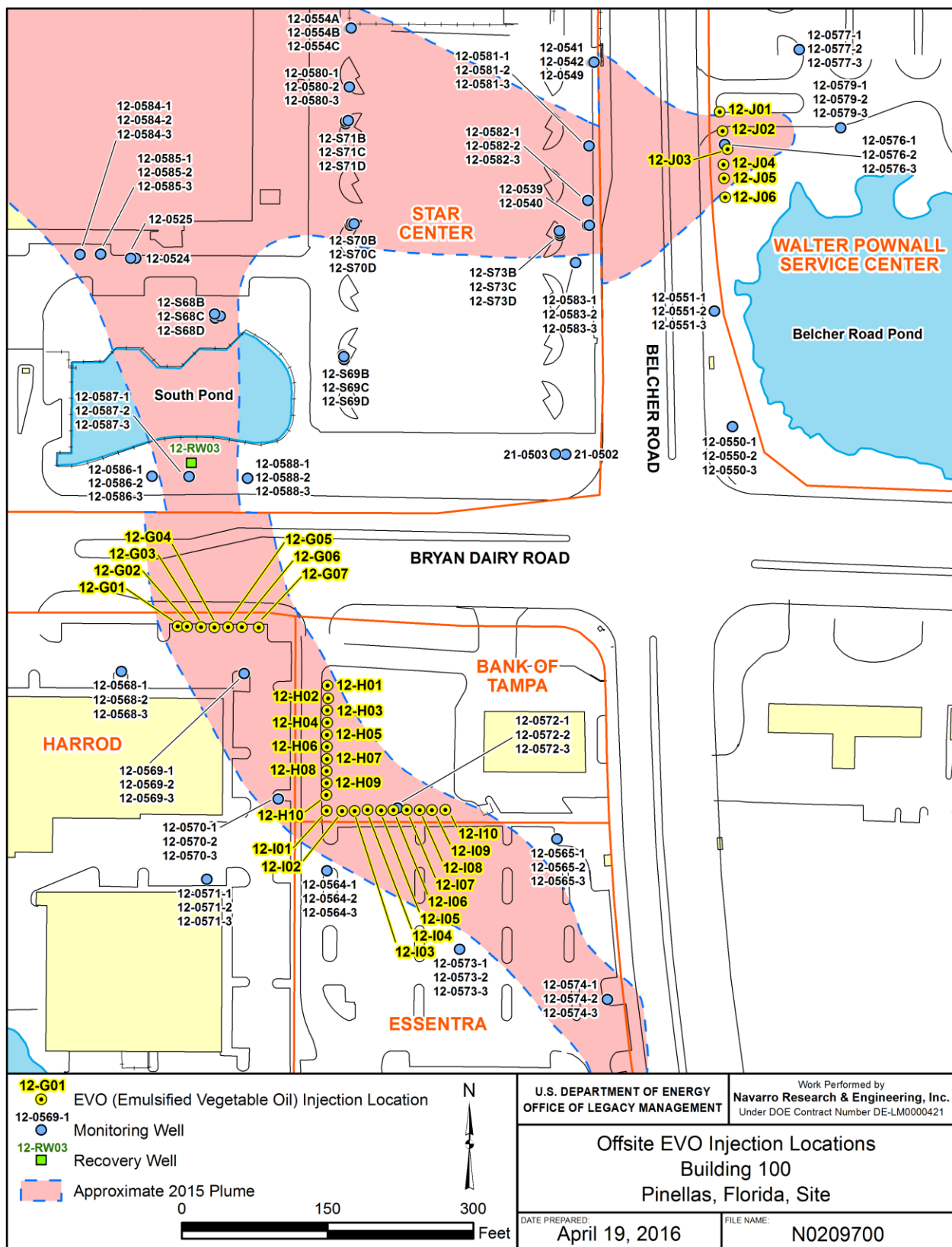


Figure 3. Building 100 Offsite Historical Bioinjection Locations

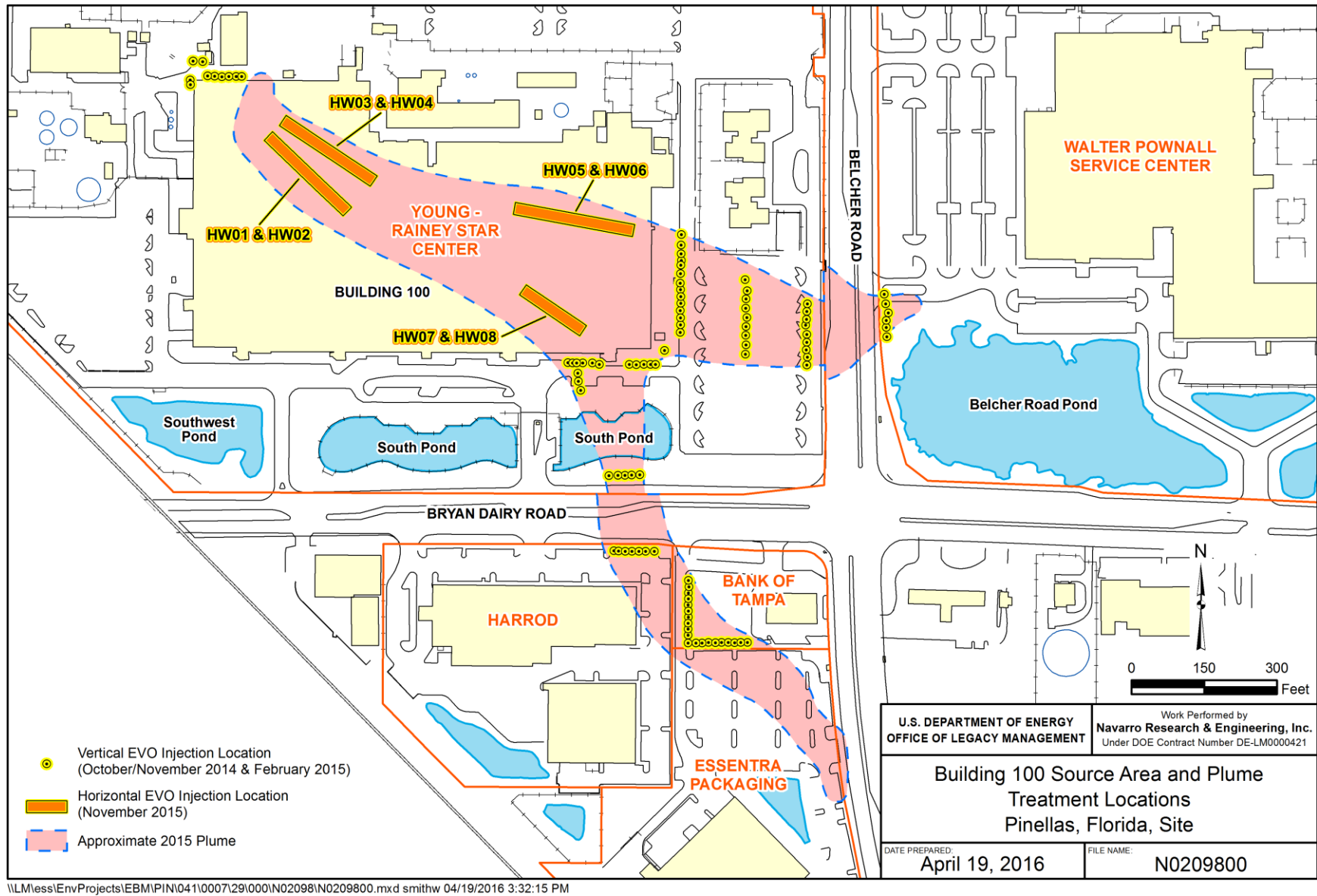


Figure 4. Summary of Building 100 Historical Bioinjection Locations

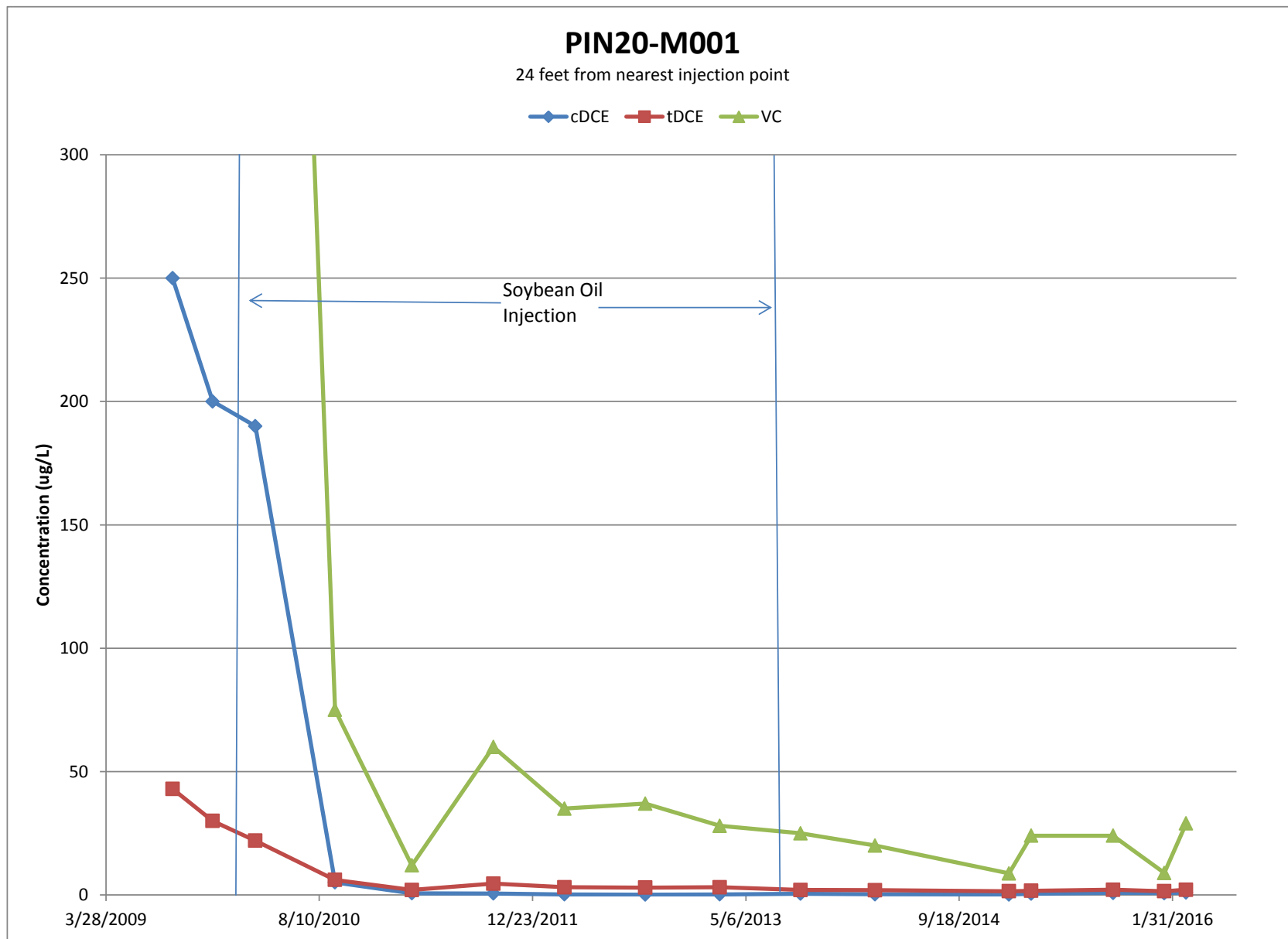


Figure 5. Contaminant Concentration Trends in Well PIN20-M001

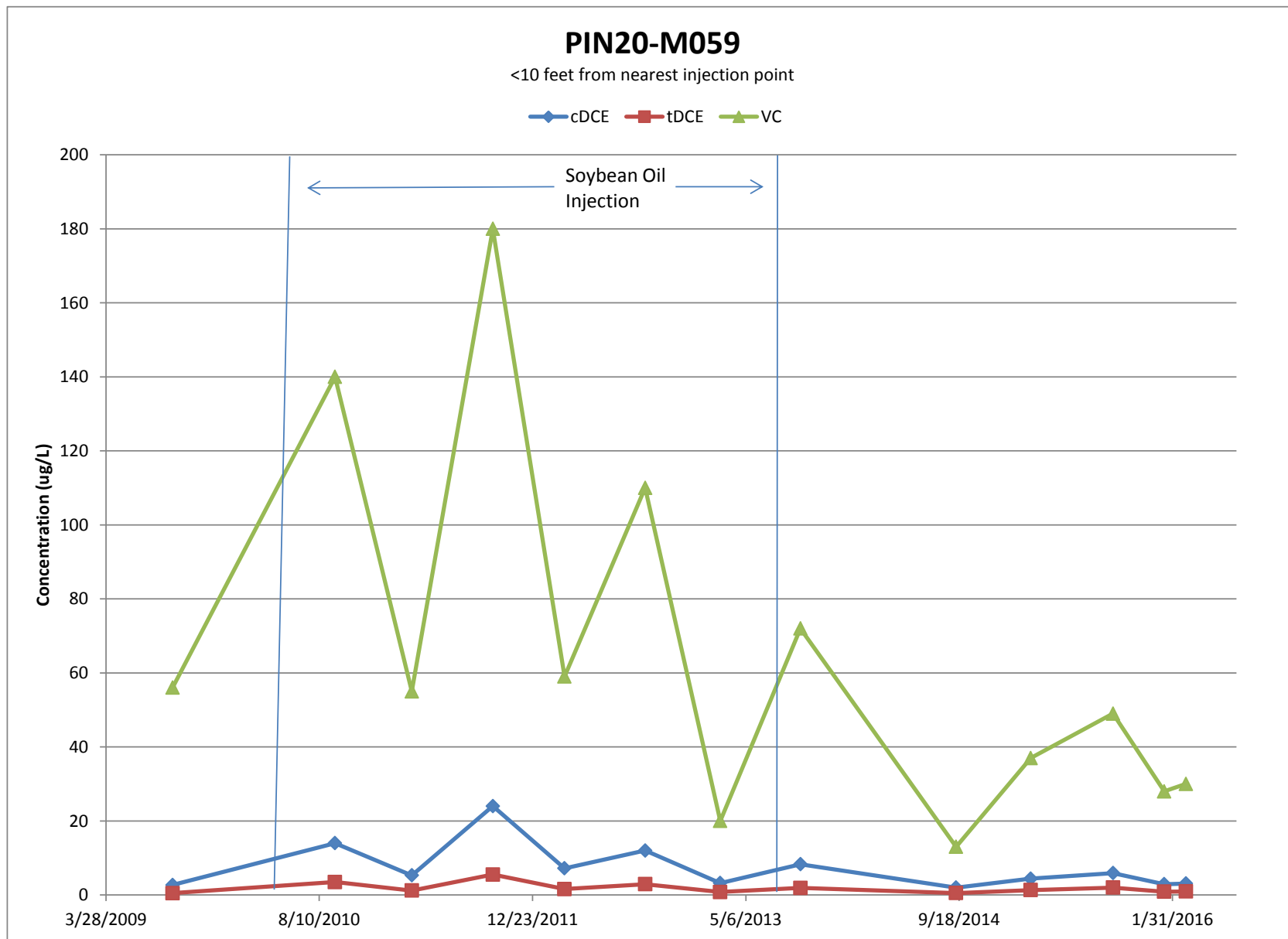


Figure 6. Contaminant Concentration Trends in Well PIN20-M059

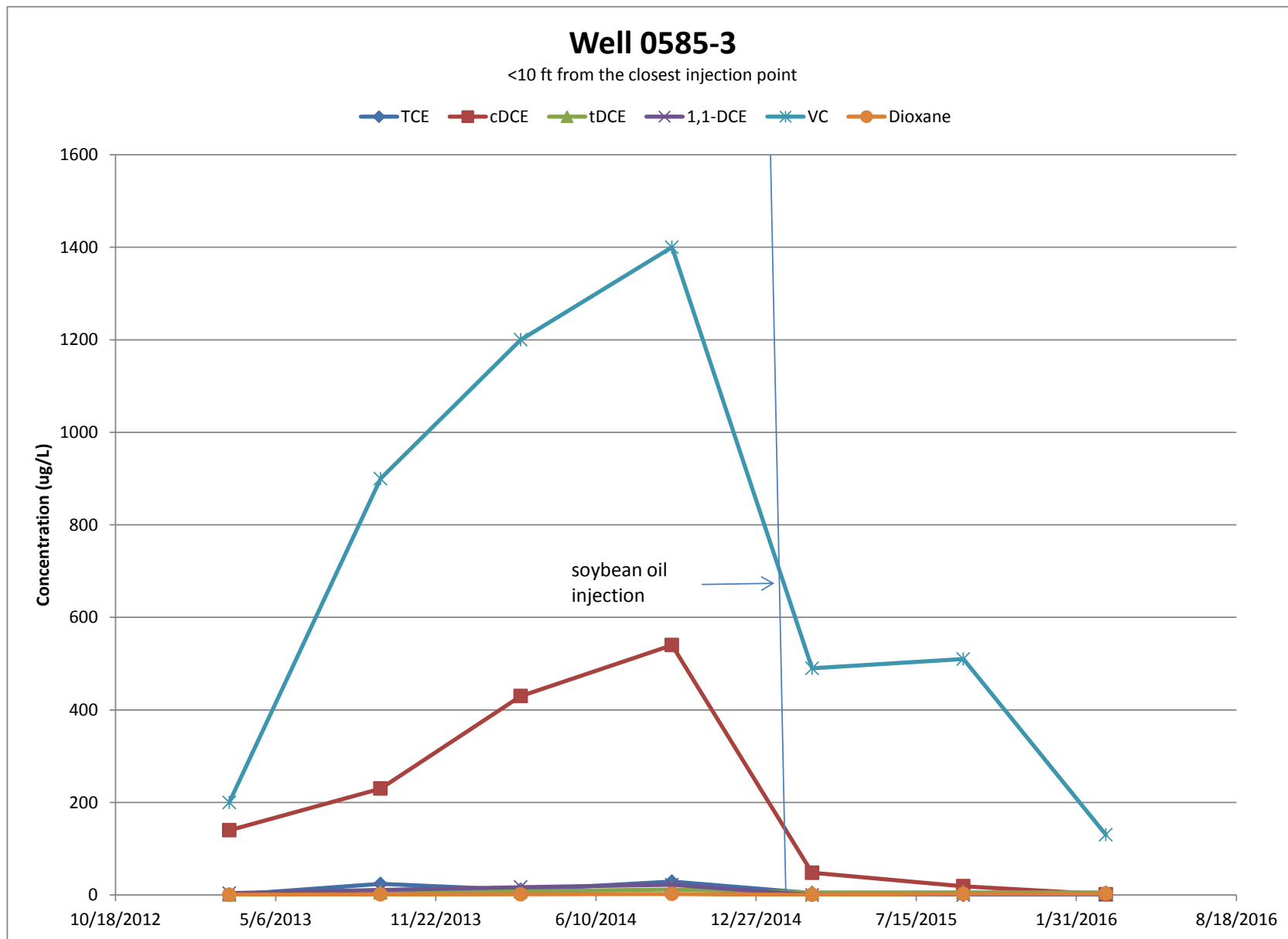


Figure 7. Contaminant Concentration Trends in Well PIN12-0585-3

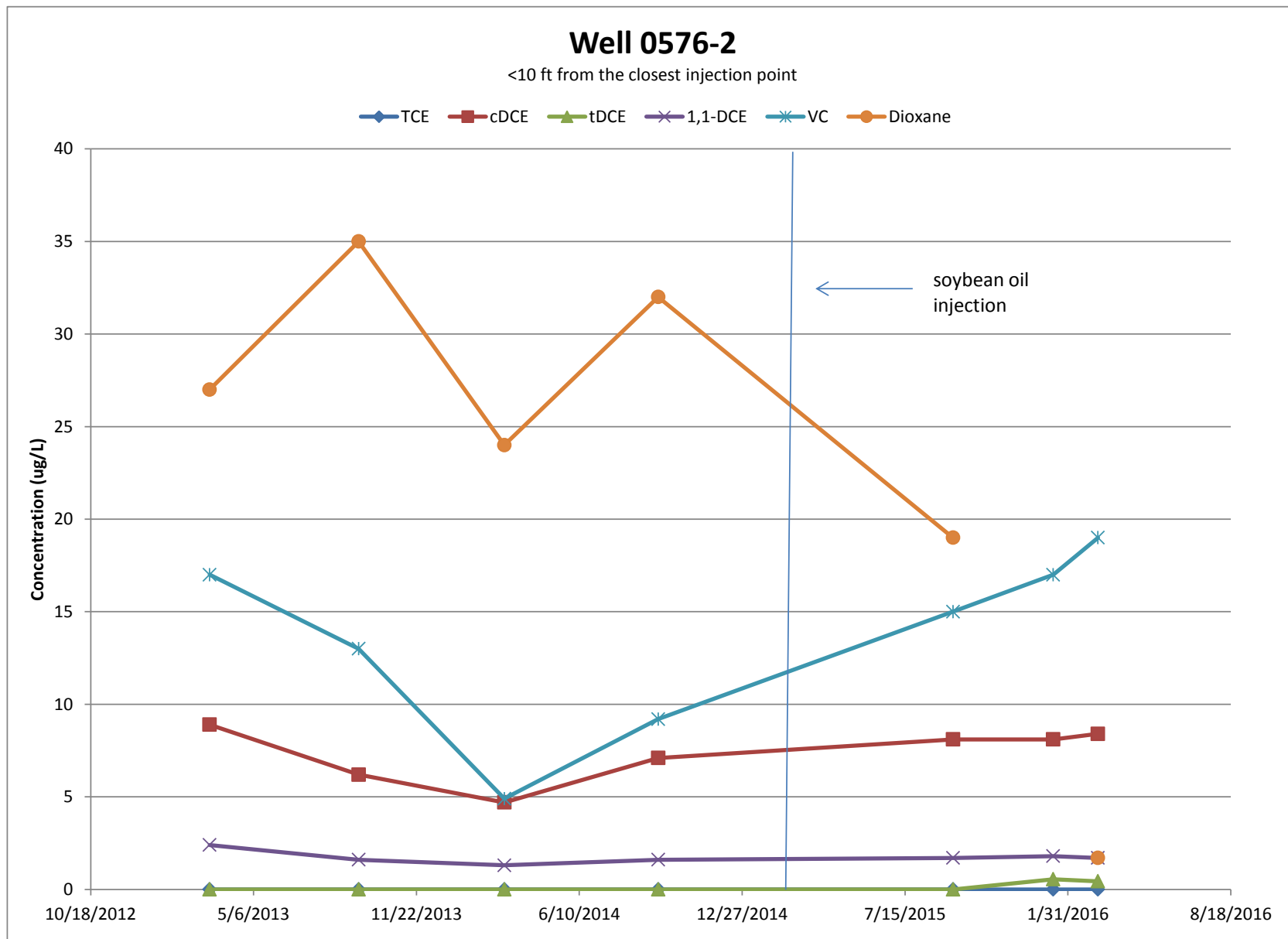
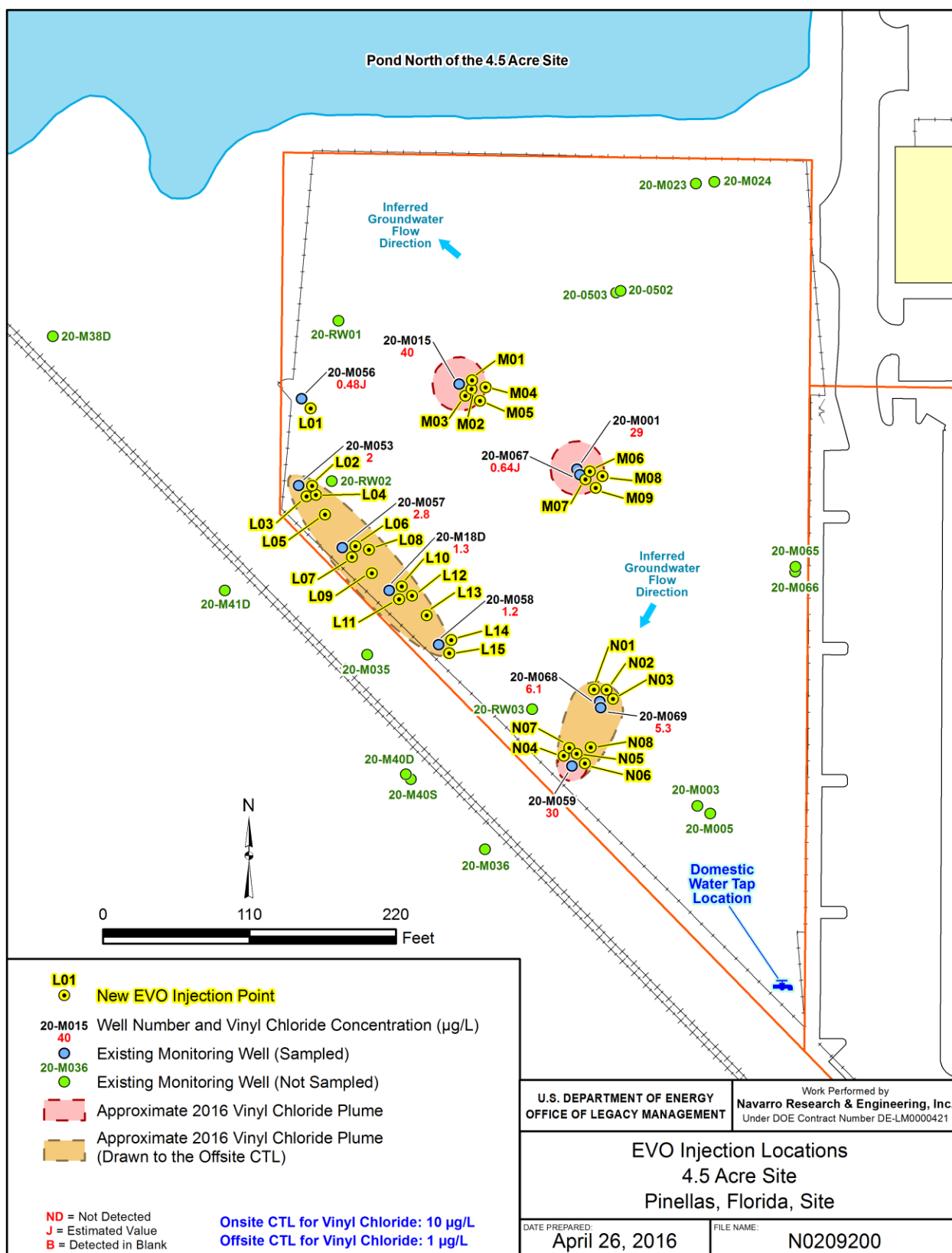


Figure 8. Contaminant Concentration Trends in Well PIN12-0576-2



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Figure 9. Bioinjection Layout at the 4.5 Acre Site

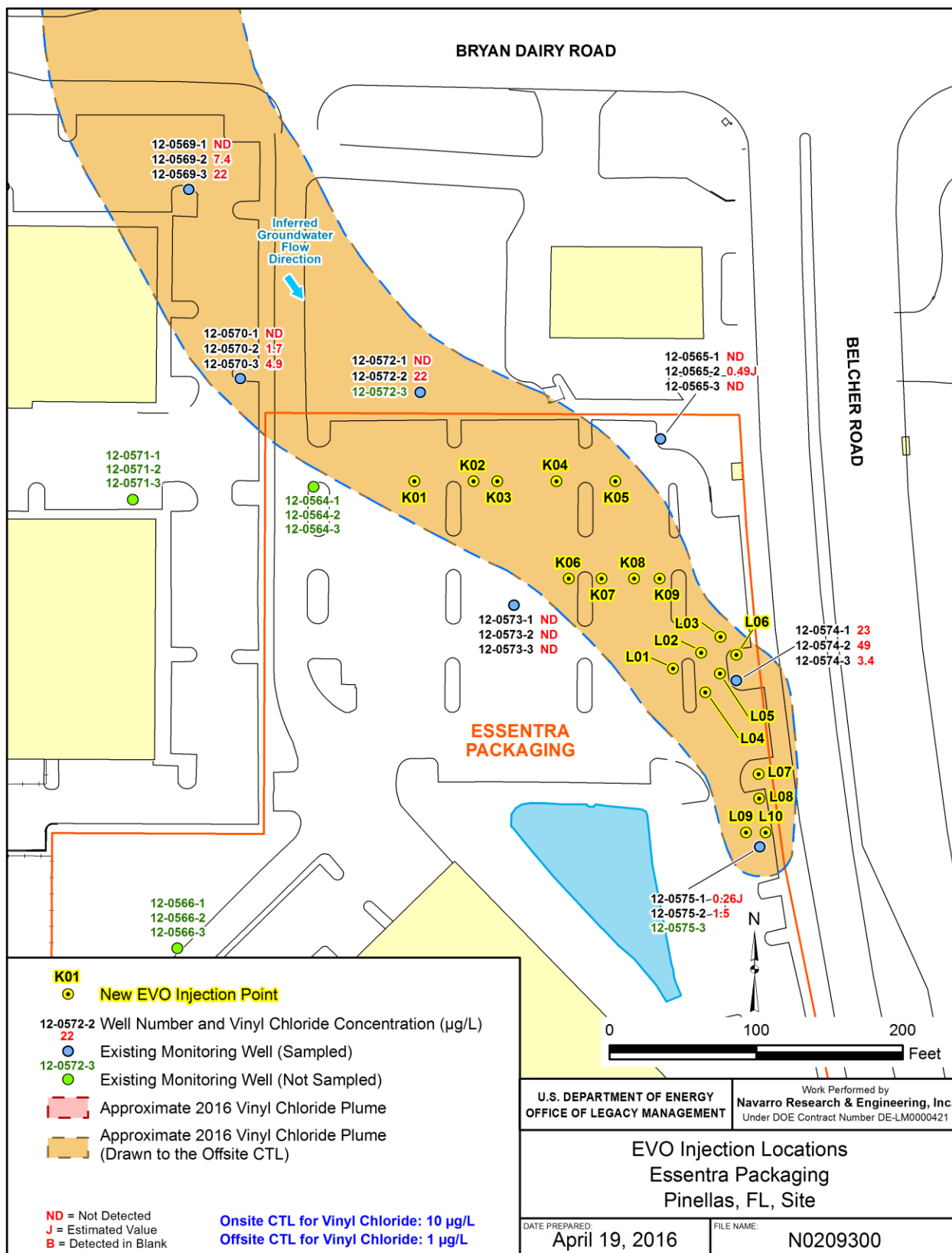


Figure 10. Bioinjection Layout at the Essentra Property

Table 1. January 2016 Microbial Parameters (cells per liter)

Location	Screen Depth (ft bls)	<i>Dehalococcoides mccartyi</i>	BAV1 Vinyl Chloride Reductase	Vinyl Chloride Reductase	tceA Reductase
4.5 Acre Site (PIN20)					
M001	20–25	1.11E+06	5.00E+02	7.66E+04	4.42E+04
M015	20.8–25.8	7.36E+05	5.00E+02	4.12E+04	3.97E+04
M057	20–30	2.70E+05	5.00E+02	1.26E+04	1.75E+04
M059	19–29	6.96E+05	3.10E+03	3.08E+04	8.40E+03
M068	20–30	1.24E+06	1.09E+04	8.68E+04	4.33E+04
Building 100 Area (PIN12)					
0569-2	20–29	9.88E+05	3.31E+05	5.00E+02	5.00E+02
0572-2	20–29	1.18E+07	5.99E+06	4.79E+04	8.37E+04
0574-1	9–18	4.12E+05	1.28E+05	5.00E+02	5.00E+02
0574-2	20–29	6.89E+05	1.12E+05	5.00E+02	1.00E+02
0574-3	31–40	1.06E+07	3.89E+06	5.00E+02	5.00E+02
0575-1	9–18	2.32E+04	5.00E+02	5.00E+02	5.00E+02
0575-2	20–29	5.59E+04	5.00E+02	5.00E+02	5.00E+02
0576-2	15–24	2.12E+05	3.00E+03	1.30E+03	4.10E+03
0580-2	20–29	4.37E+05	1.30E+03	5.00E+02	7.00E+02
0582-2	20–29	1.04E+07	5.00E+02	1.21E+06	5.91E+05
0585-2	20–29	1.70E+09	2.07E+08	4.70E+08	4.38E+07
0586-2	19–28	8.96E+05	1.88E+05	5.00E+02	4.00E+02
0587-2	20–29	1.21E+08	5.23E+07	5.27E+06	2.72E+06
S35B	5–15	6.91E+05	6.48E+04	5.00E+02	2.00E+02

ft bls = feet below land surface

Table 2. January and March 2016 Geochemical Parameters

Location	Screen Depth (ft bls)	Date Sampled	Methane (µg/L)	Ethane (µg/L)	Ethene (µg/L)	Nitrate (mg/L)	Ferrous Iron (Field) (mg/L)	Total Iron (Field) (mg/L)	Sulfate (mg/L)	Manganese (mg/L)	TOC (mg/L)	Alkalinity (mg/L)
4.5 Acre Site (PIN20)												
M001	20–25	1/12/2016	26,000	<1.7	<1.2	<0.019	0.23	0.24	44	0.029	54	590B
M015	20.8–25.8	1/12/2016	400	12	<0.4	<0.019	4.6	7.4	690	0.031	53	300B
M057	20–30	1/12/2016	21,000	<1.1	<0.8	<0.019	0	0	480	0.0022J	32	790B
M059	19–29	1/12/2016	5,500	2.6J	<0.4	<0.019	2.6	3.4	510	0.019	38	370B
M068	20–30	1/12/2016	17,000	61	<0.8	<0.019	0.19	0.29	8.7	0.0092	56	430B
Building 100 Area (PIN12)												
0569-2	20–29	1/14/2016	9,400	<0.57J	<0.4	<0.019	2.4	2.5	3.7J	0.012	43	380B
0572-2	20–29	1/14/2016	14,000	<0.57	11	<0.019	2.45	2.52	2.6J	0.027	65	420B
0574-1	9–18	1/13/2016	1,200	<0.57	1.1J	<0.019	2.7	3.5	18	0.018	51	390B
0574-2	20–29	1/13/2016	2,400	<0.57	1.3J	<0.019	0.6	1.1	12	0.014	61	420B
0574-3	31–40	1/13/2016	4,000	<0.57	<0.4	<0.019	1.07	1.1	<.23	0.12	70	470B
0575-1	9–18	1/14/2016	2,700	<0.57	<0.4	<0.019	3.6	3.8	1.1J	0.023	66	420B
0575-2	20–29	1/14/2016	3,400	<0.57	<0.4	<0.019	2.6	3.8	<.23	0.0095	70	480B
0576-2	15–24	1/13/2016	6,300	<0.57	<0.4	<0.019	2.61	2.71	50	0.0056	44	340B
0580-2	20–29	1/19/2016	1,100	2.2J	1.1J	<0.019	2.3	5	71	0.0093	53	360B
0582-2	20–29	1/14/2016	6,500	5.9J	41	<0.019	4.5	6.1	260	0.013	48	340B
0585-2	20–29	1/19/2016	13,000	<10J	2,000	<0.019	1.7	3.8	3.1J	0.014	70	400B
0586-2	19–28	1/19/2016	1,500	<0.57J	<0.4	<0.019	0.1	3.1	4.9J	0.013	34	310B
0587-2	20–29	1/19/2016	17,000	<2.3J	1,400	<0.019	0.9	1.6	<0.51J	0.012	210	260B
S30B	5–15	3/4/2016	260J	3.6J	0.64J	-	-	-	-	-	-	-
S35B	5–15	1/13/2016	2,500	9.5	320	0.16	1.81	2.04	160	0.038	48	380B
S35B	5–15	3/4/2016	2,000	13	240	-	-	-	-	-	-	-
S67B	10–19.8	3/4/2016	1,200	49	1.4J	-	-	-	-	-	-	-
S67C	20–29.8	3/4/2016	620	9.7	0.85J	-	-	-	-	-	-	-
S67D	30–39.8	3/4/2016	380	<0.57	<0.4	-	-	-	-	-	-	-

µg/L = micrograms per liter - = not measured B = Result is between the instrument detection limit and the contract-required detection limit
 J = estimated value ft bls = feet below land surface

Table 3. Injection Intervals for the 4.5 Acre Site and the Essentra Property

Location	Injection Interval (ft bls)	Number of Injection Points	Number of 4-foot Intervals Per Point	Total Number of 4-foot Intervals	Concentrated DHM Volume (liters)	Concentrated EVO Volume (gallons)	Dilution Water Volume (gallons)	EVO Plus Water Volume (gallons)	Volume of Injectate at Each Injection Point (gallons)
4.5 Acre Site									
N01–N03 Points	10–30	3	5	15	3.0	150	1,350	1,500	500
Remainder of Points	18–30	29	3	87	17.4	870	7,830	8,700	300
4.5 Acre Site Totals:	–	32	-	102	20.4	1,020	9,180	10,200	–
Essentra									
K Points	20–32	9	3	27	5.4	270	2,430	2,700	300
L Points	10–30	10	5	50	10.0	500	4,500	5,000	500
Essentra Totals:	–	19	-	77	15.4	770	6,930	7,700	–
Totals for Both Sites:	–	51	-	179	35.8	1,790	16,110	17,900	–

ft bls = feet below land surface

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Attachment A

EVO and DHM Injection Procedure

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A1.0 EVO and DHM Dilution Procedure

The emulsified vegetable oil (EVO) will be shipped to the site in concentrated form (approximately 60% EVO). Prior to injection, the EVO will be diluted with water at a ratio of 9:1. Various amendments will be added to the mixture to remove chlorine and dissolved oxygen (DO). The concentrated microbial culture will be mixed with diluted EVO prior to injection to facilitate distribution in the subsurface. The process is shown on Figure A-1.

- [1] The U.S. Department of Energy (DOE) will provide access to a domestic water line. The water will be treated and stored in a large tank ranging in volume from a few thousand gallons to 21,000 gallons. Chlorine content is estimated to range from 1 to 5 milligrams per liter (mg/L) based on information from the water provider. The domestic water will be amended with sodium thiosulfate at a concentration of 3 mg/L to remove chlorine. The chlorine concentration needs to be less than 0.1 mg/L in the amended water to ensure fitness of the *Dehalococcoides mccartyi* (DHM) culture. Sodium sulfite will be added at a concentration of 90 mg/L to remove DO.

Goals for tap water treatment are as follows:

- a. Chlorine concentration of less than 0.1 mg/L
- b. DO concentration of less than 1 mg/L

Oxidation–reduction potential (ORP) does **not** need to be measured at this stage.

- [2] About 1,800 gallons of amended water will be transferred from the amended domestic water tank to the 2,000-gallon mixing tank, and 200 gallons of EVO will then be added to the 2,000-gallon mixing tank (Table A-1). Batches smaller than 2,000 gallons may be mixed, but the ratio of water to EVO will remain at 9:1. The solution will be mixed and recirculated using a centrifugal pump. The DO, ORP, and pH of the EVO mixture will be measured in the 2,000-gallon tank.

The mixture must meet these criteria:

- a. DO concentration of less than 1 mg/L.
- b. pH between 6.0 and 9.0.
- c. Ideally the ORP value will be less than –200 millivolts. However, meeting an ORP value of –100 millivolts will be acceptable in most circumstances, as determined during the 2013 bioinjection event. Higher (less negative) ORP values are allowed if only the ORP measurement is holding up injection. The mixture likely meets the requirements, but the ORP meter can take a very long time to produce a stable reading, so the meter should not be allowed to hold up the injection.

If the EVO mixture does not meet the DO or pH criteria, more time should be allowed for the mixture to react. A second 2,000-gallon mixing tank should be used to improve efficiency.

- [3] Once the mixing is completed inside the 2,000-gallon mixing tank, the microbial culture will be added at a ratio of 1 liter of concentrated microbial culture for every 500 gallons of mixture (4 liters of culture for a 2,000 gallon batch; Table A-1). The concentrated microbial culture will be added to the mixing tank directly from the pressurized, 20-liter culture keg using the sight glass attached to the culture keg to measure the culture

volume. Details on the operation, delivery, and storage of the culture keg will be provided by the vendor.

- [4] The mixture will be dispensed into 500-gallon tanks before injection. Digital inline flow meters will be used to calculate volumes.



The microbial culture must not be exposed to oxygen.

Warning 1



The culture keg must be kept chilled at all times.

Warning 2

Table A-1. Mixing Volumes for a 2,000-Gallon Batch

Volume of Concentrated EVO	Volume of Concentrated Microbial Culture	Volume of Amended Water	Total Volume
200 gallons	4 liters	1,800 gallons	2,001 gallons

A2.0 Direct-Push Injection Procedure

- [1] DOE will verify that no underground utilities or structures are in the work areas.
- [2] The direct-push unit will be set up over each specific installation point. Two individual direct-push units may be used to inject simultaneously at different injection points.
- [3] The drive rods will be advanced into the subsurface, and the drive rod assembly will be pushed to the maximum planned depth as listed in Table A-2. The injection intervals are also specified in Table A-2.
- [4] A diaphragm pump will be used to transfer the EVO mixture from the 500-gallon tanks into the drive rod assembly. Digital inline flow meters will be used to calculate injected volumes. The application of pressure exposes the injection holes of the drive rod. 100 gallons of mixture will be injected at each 4-foot interval. Lithology permitting, an average injection flow rate of 4–10 gallons per minute is anticipated.
- [5] Step 4 is repeated at different depth intervals at each injection point until treatment of the entire vertical zone is achieved. The injection intervals at each site are listed in Table A-2.
- [6] After each injection is completed, the boreholes will be abandoned by sealing them with bentonite chips from above the EVO material to the land surface.

Surfacing/daylighting of product: Bentonite chips will be placed around the injection rods during injection to minimize the potential of surfacing/daylighting of the injectate. If surfacing/daylighting should occur, the injection pumps (adjustable rate) will be throttled down.

If these mechanical controls are insufficient, the injection will be stopped at the current location and moved to another injection point.

Table A-2. Specified Injection Depths and Intervals

Injection Locations	Injection Depth Range (feet bls)	Number of 4-foot Injection Intervals per Injection Point	Injection Interval (feet bls)
4.5 Acre Site			
N01–N03	10–30	5	1. 26–30 2. 22–26 3. 18–22 4. 14–18 5. 10–14
Remainder of Points	18–30	3	1. 26–30 2. 22–26 3. 18–22
Essentra			
K01–K09	20–32	3	1. 28–32 2. 24–28 3. 20–24
L01–L10	10–30	5	1. 26–30 2. 22–26 3. 18–22 4. 14–18 5. 10–14

bls = below land surface

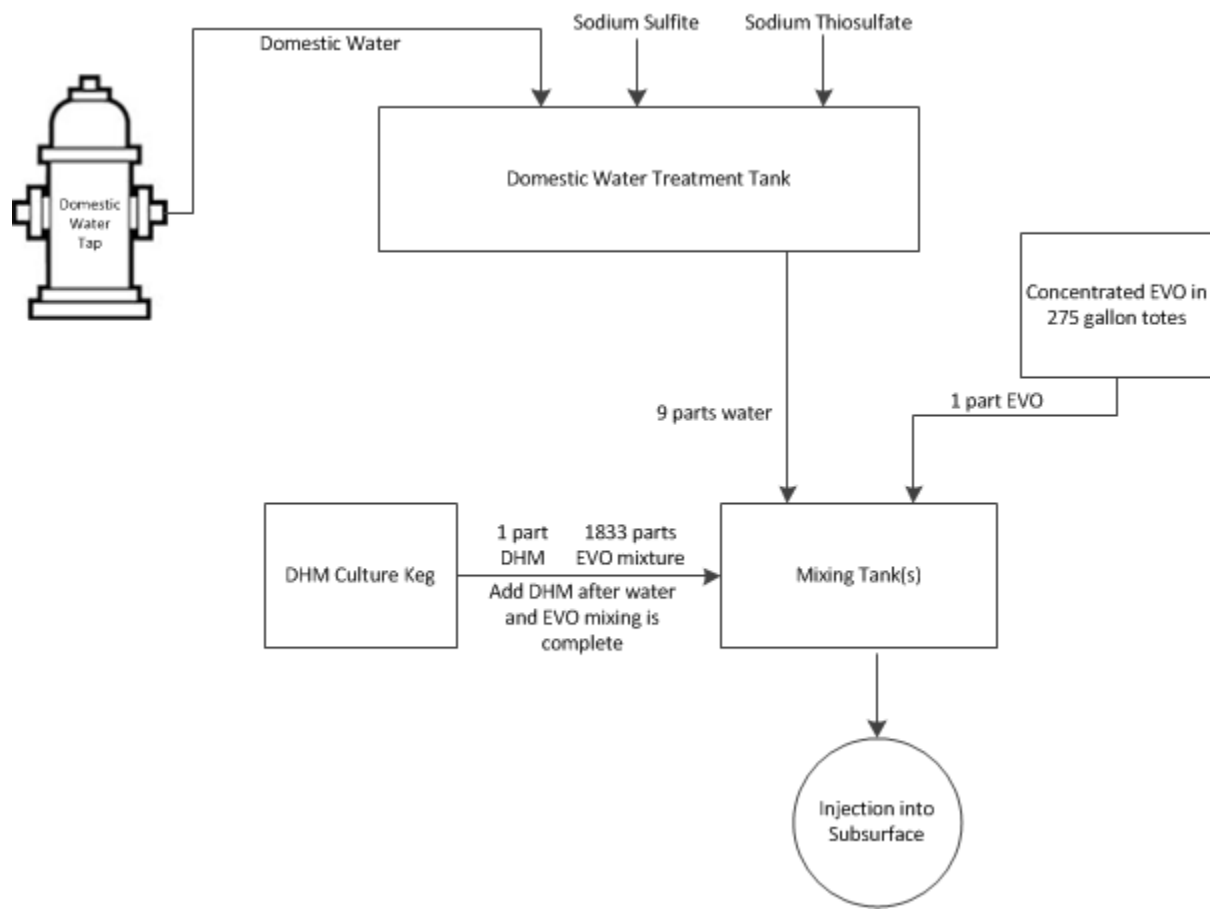


Figure A-1. EVO and DHM Injection Procedure Flow Chart

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