State of Wisconsin
Department of Natural Resources
PO Box 7921, Madison WI 53707-7921
dnr.wi.gov

Technical Assistance, Environmental Liability Clarification or Post-Closure Modification Request

Form 4400-237 (R 12/18)

Notice: Use this form to request **a written response (on agency letterhead)** from the Department of Natural Resources (DNR) regarding technical assistance, a post-closure change to a site, a specialized agreement or liability clarification for Property with known or suspected environmental contamination. A fee will be required as is authorized by s. 292.55, Wis. Stats., and NR 749, Wis. Adm. Code., unless noted in the instructions below. Personal information collected will be used for administrative purposes and may be provided to requesters to the extent required by Wisconsin's Open Records law [ss. 19.31 - 19.39, Wis. Stats.].

Definitions

"Property" refers to the subject Property that is perceived to have been or has been impacted by the discharge of hazardous substances.

"Liability Clarification" refers to a written determination by the Department provided in response to a request made on this form. The response clarifies whether a person is or may become liable for the environmental contamination of a Property, as provided in s. 292.55, Wis. Stats.

"Technical Assistance" refers to the Department's assistance or comments on the planning and implementation of an environmental investigation or environmental cleanup on a Property in response to a request made on this form as provided in s. 292.55, Wis. Stats.

"Post-closure modification" refers to changes to Property boundaries and/or continuing obligations for Properties or sites that received closure letters for which continuing obligations have been applied or where contamination remains. Many, but not all, of these sites are included on the GIS Registry layer of RR Sites Map to provide public notice of residual contamination and continuing obligations.

Select the Correct Form

This from should be used to request the following from the DNR:

- Technical Assistance
- Liability Clarification
- Post-Closure Modifications
- Specialized Agreements (tax cancellation, negotiated agreements, etc.)

Do not use this form if one of the following applies:

- Request for an **off-site liability exemption or clarification** for Property that has been or is perceived to be contaminated by one or more hazardous substances that originated on another Property containing the source of the contamination. Use DNR's Off-Site Liability Exemption and Liability Clarification Application Form 4400-201.
- Submittal of an Environmental Assessment for the **Lender Liability Exemption**, s 292.21, Wis. Stats., **if no response or review by DNR is requested**. Use the Lender Liability Exemption Environmental Assessment Tracking Form 4400-196.
- Request for an **exemption to develop on a historic fill site** or licensed landfill. Use DNR's Form 4400-226 or 4400-226A.
- **Request for closure** for Property where the investigation and cleanup actions are completed. Use DNR's Case Closure - GIS Registry Form 4400-202.

All forms, publications and additional information are available on the internet at: dnr.wi.gov/topic/Brownfields/Pubs.html.

Instructions

1. Complete sections 1, 2, 6 and 7 for all requests. Be sure to provide adequate and complete information.
2. Select the type of assistance requested: Section 3 for technical assistance or post-closure modifications, Section 4 for a written determination or clarification of environmental liabilities; or Section 5 for a specialized agreement.
3. Include the fee payment that is listed in Section 3, 4, or 5, unless you are a "Voluntary Party" enrolled in the Voluntary Party Liability Exemption Program **and** the questions in Section 2 direct otherwise. Information on to whom and where to send the fee is found in Section 8 of this form.
4. Send the completed request, supporting materials and the fee to the appropriate DNR regional office where the Property is located. See the map on the last page of this form. A paper copy of the signed form and all reports and supporting materials shall be sent with an electronic copy of the form and supporting materials on a compact disk. For electronic document submittal requirements see: http://dnr.wi.gov/files/PDF/pubs/rr/RR690.pdf"

The time required for DNR's determination varies depending on the complexity of the site, and the clarity and completeness of the request and supporting documentation.

Section 1. Contact and Recipient Information

Requester Information

This is the person requesting technical assistance or a post-closure modification review, that his or her liability be clarified or a specialized agreement and is identified as the requester in Section 7. DNR will address its response letter to this person.

Last Name	First	MI	Organization/ Business Name		
Schreiner	Evan		Wauleco, Inc.		
Mailing Address			**City**	**State**	**ZIP Code**
18 North Point Drive			Stevens Point	WI	54481
Phone # (include area code)	**Fax # (include area code)**		**Email**		
(715) 346-8530	(715) 346-7842		Evan.Schreiner@Sentry.com		

The requester listed above: (select all that apply)

- ☒ Is currently the owner
- ☐ Is considering selling the Property
- ☐ Is renting or leasing the Property
- ☐ Is considering acquiring the Property
- ☐ Is a lender with a mortgagee interest in the Property
- ☐ Other. Explain the status of the Property with respect to the applicant:

Contact Information (to be contacted with questions about this request) ☒ Select if same as requester

Contact Last Name	First	MI	Organization/ Business Name		
Schreiner	Evan		Wauleco, Inc.		
Mailing Address			**City**	**State**	**ZIP Code**
18 North Point Drive			Stevens Point	WI	54481
Phone # (include area code)	**Fax # (include area code)**		**Email**		
(715) 346-8530	(715) 346-7842		Evan.Schreiner@Sentry.com		

Environmental Consultant (if applicable)

Contact Last Name	First	MI	Organization/ Business Name		
Iverson	Bruce	A	TRC		
Mailing Address			**City**	**State**	**ZIP Code**
708 Heartland Trail, Suite 3000			Madison	WI	53717
Phone # (include area code)	**Fax # (include area code)**		**Email**		
(608) 826-3644	(608) 826-3941		biverson@trccompanies.com		

Section 2. Property Information

Property Name			FID No. (if known)		
Wauleco, Inc.			737079310		
BRRTS No. (if known)			**Parcel Identification Number**		
02-37-000006			291-2907-354-0972		
Street Address			**City**	**State**	**ZIP Code**
125 Rosecrans Street			Wausau	WI	54402
County	**Municipality where the Property is located**		**Property is composed of:**		**Property Size Acres**
Marathon	◉ City ○ Town ○ Village of Wausau		◉ Single tax parcel ○ Multiple tax parcels		8

1. Is a response needed by a specific date? (e.g., Property closing date) Note: Most requests are completed within 60 days. Please plan accordingly.

◉ No ○ Yes

Date requested by: _____

Reason:

2. Is the "Requester" enrolled as a Voluntary Party in the Voluntary Party Liability Exemption (VPLE) program?

◉ No. **Include the fee that is required for your request in Section 3, 4 or 5.**

○ Yes. **Do not include a separate fee.** This request will be billed separately through the VPLE Program.

Fill out the information in Section 3, 4 or 5 which corresponds with the type of request:
Section 3. Technical Assistance or Post-Closure Modifications;
Section 4. Liability Clarification; or Section 5. Specialized Agreement.

Section 3. Request for Technical Assistance or Post-Closure Modification

Select the type of technical assistance requested: **[Numbers in brackets are for WI DNR Use]**

☐ No Further Action Letter (NFA) (Immediate Actions) - NR 708.09, [183] - **Include a fee of $350.** Use for a written response to an immediate action after a discharge of a hazardous substance occurs. Generally, these are for a one-time spill event.

☐ Review of Site Investigation Work Plan - NR 716.09, [135] - **Include a fee of $700.**

☐ Review of Site Investigation Report - NR 716.15, [137] - **Include a fee of $1050.**

☐ Approval of a Site-Specific Soil Cleanup Standard - NR 720.10 or 12, [67] - **Include a fee of $1050.**

☐ Review of a Remedial Action Options Report - NR 722.13, [143] - **Include a fee of $1050.**

☐ Review of a Remedial Action Design Report - NR 724.09, [148] - **Include a fee of $1050.**

☐ Review of a Remedial Action Documentation Report - NR 724.15, [152] - **Include a fee of $350**

☐ Review of a Long-term Monitoring Plan - NR 724.17, [25] - **Include a fee of $425.**

☐ Review of an Operation and Maintenance Plan - NR 724.13, [192] - **Include a fee of $425.**

Other Technical Assistance - s. 292.55, Wis. Stats. [97] (For request to build on an abandoned landfill use Form 4400-226)

☐ Schedule a Technical Assistance Meeting - **Include a fee of $700.**

☐ Hazardous Waste Determination - **Include a fee of $700.**

☒ Other Technical Assistance - **Include a fee of $700.** Explain your request in an attachment.

Post-Closure Modifications - NR 727, [181]

☐ Post-Closure Modifications: Modification to Property boundaries and/or continuing obligations of a closed site or Property; sites may be on the GIS Registry. This also includes removal of a site or Property from the GIS Registry. **Include a fee of $1050, and:**

☐ Include a fee of $300 for sites with residual soil contamination; and

☐ Include a fee of $350 for sites with residual groundwater contamination, monitoring wells or for vapor intrusion continuing obligations.

Attach a description of the changes you are proposing, and documentation as to why the changes are needed (if the change to a Property, site or continuing obligation will result in revised maps, maintenance plans or photographs, those documents may be submitted later in the approval process, on a case-by-case basis).

Skip Sections 4 and 5 if the technical assistance you are requesting is listed above and complete Sections 6 and 7 of this form.

Section 5. Request for a Specialized Agreement

Select the type of agreement needed. Include the appropriate draft agreements and supporting materials. Complete Sections 6 and 7 of this form. More information and model draft agreements are available at: dnr.wi.gov/topic/Brownfields/lgu.html#tabx4.

☐ Tax cancellation agreement - s. 75.105(2)(d), Wis. Stats. [654]

- ❖ **Include a fee of $700, and the information listed below:**

(1) Phase I and II Environmental Site Assessment Reports,
(2) a copy of the Property deed with the correct legal description.

☐ Agreement for assignment of tax foreclosure judgement - s.75.106, Wis. Stats. [666]

- ❖ **Include a fee of $700, and the information listed below:**

(1) Phase I and II Environmental Site Assessment Reports,
(2) a copy of the Property deed with the correct legal description.

☐ Negotiated agreement - Enforceable contract for non-emergency remediation - s. 292.11(7)(d) and (e), Wis. Stats. [630]

- ❖ **Include a fee of $1400, and the information listed below:**

(1) a draft schedule for remediation; and,
(2) the name, mailing address, phone and email for each party to the agreement.

Section 6. Other Information Submitted

Identify all materials that are included with this request.

Send both a paper copy of the signed form and all reports and supporting materials, and an electronic copy of the form and all reports, including Environmental Site Assessment Reports, and supporting materials on a compact disk.

Include one copy of any document from any state agency files that you want the Department to review as part of this request. The person submitting this request is responsible for contacting other state agencies to obtain appropriate reports or information.

☐ Phase I Environmental Site Assessment Report - Date: _____
☐ Phase II Environmental Site Assessment Report - Date: _____
☐ Legal Description of Property (required for all liability requests and specialized agreements)
☐ Map of the Property (required for all liability requests and specialized agreements)
Analytical results of the following sampled media: Select all that apply and include date of collection.
☐ Groundwater ☐ Soil ☐ Sediment ☐ Other medium - Describe: _____
Date of Collection: _____
☐ A copy of the closure letter and submittal materials
☐ Draft tax cancellation agreement
☐ Draft agreement for assignment of tax foreclosure judgment
☒ Other report(s) or information - Describe: Technical Memorandum Lines of Evidence of PCP Degradation

For Property with newly identified discharges of hazardous substances only: Has a notification of a discharge of a hazardous substance been sent to the DNR as required by s. NR 706.05(1)(b), Wis. Adm. Code?

◉ Yes - Date (if known): _____
○ No

Note: The Notification for Hazardous Substance Discharge (non-emergency) form is available at: dnr.wi.gov/files/PDF/forms/4400/4400-225.pdf.

Section 7. Certification by the Person who completed this form

☒ I am the person submitting this request (requester)

☐ I prepared this request for: _____
Requester Name

I certify that I am familiar with the information submitted on this request, and that the information on and included with this request is true, accurate and complete to the best of my knowledge. I also certify I have the legal authority and the applicant's permission to make this request.

Signature

8/21/20
Date Signed

Treasurer
Title

(715) 346-8530
Telephone Number (include area code)

Section 8. DNR Contacts and Addresses for Request Submittals

Send or deliver one paper copy and one electronic copy on a compact disk of the completed request, supporting materials, and fee to the region where the property is located to the address below. Contact a DNR regional brownfields specialist with any questions about this form or a specific situation involving a contaminated property. For electronic document submittal requirements see: http://dnr.wi.gov/files/PDF/pubs/rr/RR690.pdf.

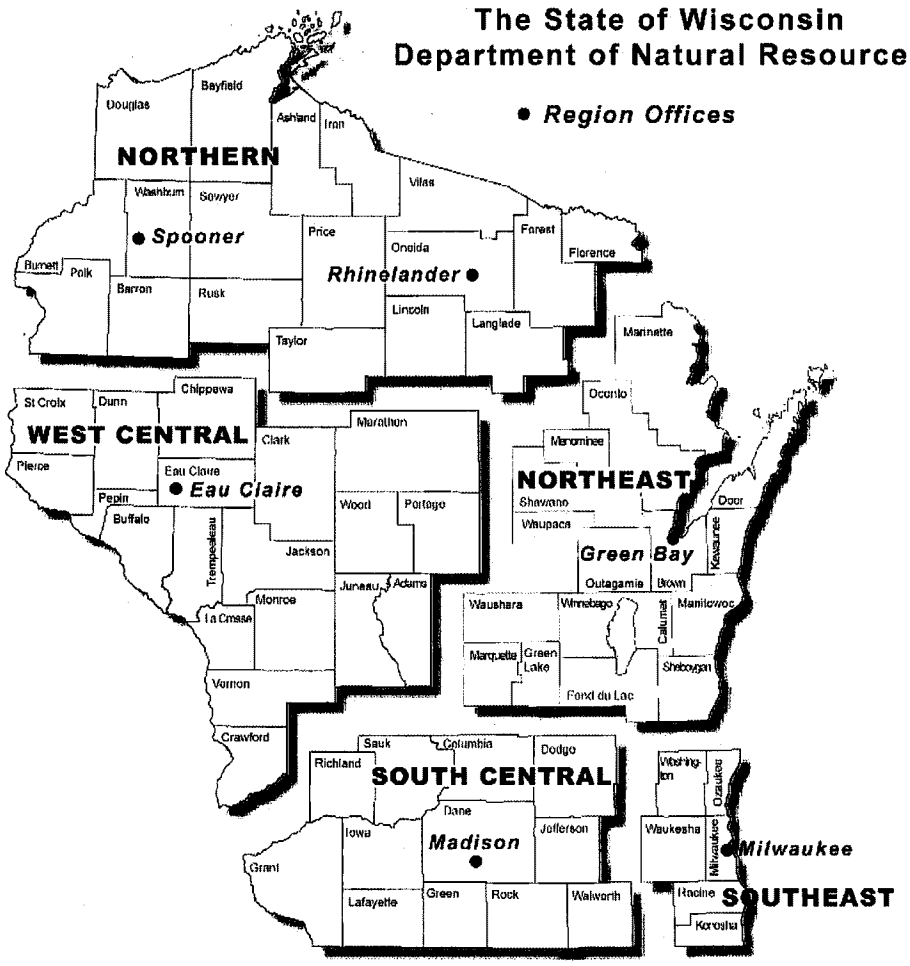
DNR NORTHERN REGION
Attn: RR Program Assistant
Department of Natural Resources
223 E Steinfest Rd Antigo, WI 54409

DNR NORTHEAST REGION
Attn: RR Program Assistant
Department of Natural Resources
2984 Shawano Avenue
Green Bay WI 54313

DNR SOUTH CENTRAL REGION
Attn: RR Program Assistant
Department of Natural Resources
3911 Fish Hatchery Road
Fitchburg WI 53711

DNR SOUTHEAST REGION
Attn: RR Program Assistant
Department of Natural Resources
2300 North Martin Luther King Drive
Milwaukee WI 53212

DNR WEST CENTRAL REGION
Attn: RR Program Assistant
Department of Natural Resources
1300 Clairemont Ave.
Eau Claire WI 54702



Note: These are the Remediation and Redevelopment Program's designated regions. Other DNR program regional boundaries may be different.

DNR Use Only			
Date Received	Date Assigned	BRRTS Activity Code	BRRTS No. (if used)
DNR Reviewer	Comments		
Fee Enclosed? ◯ Yes ◯ No	Fee Amount $	Date Additional Information Requested	Date Requested for DNR Response Letter
Date Approved	Final Determination		

Technical Memorandum
Lines of Evidence of PCP Degradation

BRRTS No. 02-37-000008

Wauleco, Inc.
Wausau, Wisconsin

August 2020

Prepared For
Wauleco, Inc.

Prepared By
TRC Environmental Corporation
708 Heartland Trail, Suite 3000
Madison, Wisconsin

Table of Contents

List of Tables

List of Figures

List of Appendices

Section 1
Executive Summary

This Technical Memorandum compiles, evaluates and presents the multiple lines of evidence that natural attenuation/contaminant degradation is occurring at the Wauleco Site, consistent with both the EPA and WDNR guidance documents. This Technical Memorandum also responds to questions raised by the WDNR concerning Wauleco site conditions relative to the occurrence of natural attenuation/contaminant degradation. The below table presents those lines of evidence in summary fashion; each topic is addressed more fully in the body of this Technical Memorandum.

NO.	LINE OF EVIDENCE	ASSESSMENT SUMMARY	REFERENCE
1	**Literature Review of PCP Biodegradation Mechanisms:** A review of relevant literature demonstrates that PCP can and does naturally degrade in soils and groundwater, under appropriate conditions. This line of evidence is consistent with the WDNR Guidance line of evidence #1 and EPA Guidance line of evidence #2, described below.	Numerous publications, summarized in Bosso (2014) demonstrate that natural biodegradation of PCP occurs through several biological processes both aerobically and anaerobically. WDNR's Site, Penta Wood Products, demonstrated naturally occurring PCP biodegradation both aerobically and anaerobically.	See Section 3, with backup summary journal article in Appendix A, and Penta Wood Products Site's support for biodegradation in Appendix B.
2	**CSM Update:** The updated Wauleco site CSM was analyzed to evaluate the presence of EPA and WDNR lines of evidence for natural attenuation (NA) of PCP. This update specifically identifies and evaluates data outside the influence of the groundwater extraction system to respond to WDNR's request.	The LNAPL CSM demonstrated substantial reduction in the LNAPL volume and PCP concentration in the LNAPL in the Residual Phase LNAPL Investigation. The Northeast (NE) Profile and Southeast (SE) Profile lines (described below) include wells along groundwater migration pathways outside the influence of the extraction system. Biodegradation demonstrated along these profiles is unaffected by pumping. Seasonal trend evaluations of the PCP in groundwater at wells with multiple samples collected per year shows that either the declines in PCP through multi-year trends dwarf any seasonal changes or that the peaks and valleys in PCP concentration are not specific to a particular season. Therefore, there is no indication that rainfall recharge results in significant dilution during the spring, summer, or fall seasons.	See Section 4.1 for the LNAPL CSM. See Section 4.2.3 for description and Figure 4 for the NE and SE Profile lines on the water table map. See Section 5.3.

NO.	LINE OF EVIDENCE	ASSESSMENT SUMMARY	REFERENCE
3	**Concentration and Mass Trends:** Concentration trend lines support the conclusion that natural attenuation is occurring at the Wauleco Site. This is consistent with EPA's Guidance Primary line of evidence EPA #1 and WDNR lines of evidence #2, #3, and #4, described below.	The NE and SE Profile lines' distance-concentration trends and the analysis of biodegradation rates also includes the effect of contaminated water mixing with clean groundwater in the aquifer (i.e., dispersion). Dispersion, which causes dilution in the groundwater, and biodegradation are the only two natural attenuation mechanisms for PCP in groundwater. EPA (Newell, 2002) describes use of distance-concentration trends as a method to determine whether biodegradation is occurring. Distance-concentration trends at several wells support that PCP is biodegrading at the Wauleco Site.	See Section 5.4 for the distance-concentration trend evaluation.
		The biodegradation rate along the NE profile (11 day half-life) is faster than the SE profile (86 day half-life) because of the aerobic conditions in the NE area and anaerobic conditions in the SE area. Because these profile lines are unaffected by pumping, these decay rates will continue even after shutdown of pumping.	Table 2 presents the analysis of decay rate considering dispersion as well.
		Site-specific testing that necessary bacteria are present at the Wauleco site has not been performed. However, consistent with EPA Guidance, this is not a requirement to demonstrate whether natural attenuation (NA) is occurring. The EPA Guidance states that historical data (EPA line of evidence #1) and data characterizing the nature and rates of natural attenuation processes (EPA line of evidence #2) should be presented for all sites. Where data from lines of evidence 1 and 2 need additional support, then data from microcosm studies (EPA line of evidence #3) may also be necessary. For Wauleco, lines of evidence 1 and 2 do not need additional support. Nevertheless, Wauleco will perform a study to assess the presence of necessary bacteria.	Section 5.3 describes the PCP time-concentration trends and other factors (e.g., a water main leak) that affected the PCP trend at some wells. Section 2.2 describes that the EPA Guidance specifies the use of three lines of evidence to demonstrate whether NA is occurring. Section 5 describes the lines of evidence assessment for Wauleco.

NO.	LINE OF EVIDENCE	ASSESSMENT SUMMARY	REFERENCE
4	**Geochemistry:** Geochemical conditions at the Wauleco Site are appropriate for degradation of PCP. This is consistent with EPA Guidance line of evidence #2 and WDNR Guidance line of evidence #1, described below.	The geochemical conditions at Wauleco illustrate anaerobic conditions in most areas of the site, with primarily aerobic conditions in the NE profile area. The consumption of a large quantity of electron acceptors indicate prolific biological activity throughout the PCP plume. Biodegradation rates at Wauleco are consistent with the literature and conclusions reached for the Penta Wood Products Site: biodegradation is faster in the aerobic conditions along the NE Profile and slower along the primarily anaerobic SE Profile. However, redox in the SE Profile becomes progressively more aerobic in the downgradient extent of the migration pathway.	See Section 6, and Appendix D.

As noted above, in addition to the above referenced general lines of evidence, this Technical Memorandum addresses WDNR comments and questions about the degradation of PCP at the Wauleco Site:

NO.	WDNR REQUESTS REGARDING PCP BIODEGRADATION EVALUATION	ASSESSMENT SUMMARY	SECTION TO REFER TO
1	Provide evidence that biodegradation is occurring, rather than other natural attenuation mechanisms	Biodegradation and dispersion are the only two natural attenuation processes acting on PCP at the Wauleco Site. See Section 3.4. Typical dispersion values are used in EPA's distance-concentration analysis that supports the conclusion that biodegradation is occurring at the Wauleco site and is the predominant reason that PCP concentrations are declining in both aerobic and anaerobic environments.	See Section 3.4 regarding NA processes. See Section 5.4 for the NE and SE Profile lines analysis of dispersion and biodegradation.
		Other factors potentially affecting PCP time-concentration trends, such as a City water main leak or pumping rate changes, are addressed in the context of the analysis of each well's trend. While these factors affect PCP concentrations in limited time frames, they do not affect the overall trend of declines.	See Section 5.3 for a description of other factors affecting PCP trends.

NO.	WDNR REQUESTS REGARDING PCP BIODEGRADATION EVALUATION	ASSESSMENT SUMMARY	SECTION TO REFER TO
2	Address whether inflow from the Wisconsin River results in dilution causing the decreasing PCP concentrations in some groundwater monitoring wells	Inflow from the River occurred during most of the time while pumping at 42 gpm with PCP concentrations steady at well W10A during much of this time. However, since 2010 the documented dominant groundwater flow has been toward the River, especially near well W10A, with declining PCP concentrations and anaerobic conditions reflective of flow from the LNAPL area and not affected by aerobic River water.	See Section 5.3.5 on Groundwater Gradients and General Water Chemistry, and Appendix C.
3	Address the decay rate to account for dispersion	The biological decay rate outside the cone of depression and accounting for dispersion is an 11 day half-life along the NE Profile and 86 day half-life for the SE Profile. These different decay rates are due to the different geochemistries in these areas (i.e., aerobic in the NE and anaerobic in the SE).	See Section 5.4
4	Describe the biodegradation mechanisms for PCP	There are several PCP biodegradation mechanisms described in the literature. The evidence for PCP biodegradation is similar to that for benzene; to wit, loss of the contaminant in the documented presence of biological activity (either aerobic or anaerobic) with no observable decay or “daughter” compounds. As with benzene, biodegradation of PCP is faster in aerobic environments and somewhat slower in anaerobic environments.	See Section 3
5	Address whether on the north side of the Wauleco Site, there are other processes that could be affecting the decreasing concentrations of PCP in groundwater not associated with biodegradation.	Assessment of the NE Profile line demonstrates that no other processes currently affect the decline in PCP concentration, including pumping (as the NE Profile is outside the capture zone) or inflow from the River (flow is towards the River). In addition, the only other property affecting transport of PCP in groundwater, dispersion, is accounted for in the distance-concentration trend analysis which demonstrates that dispersion is not a significant factor.	See Sections 5.3.3 for time-concentration trends and 5.4.1 for analysis of the NE Profile trends.

NO.	WDNR REQUESTS REGARDING PCP BIODEGRADATION EVALUATION	ASSESSMENT SUMMARY	SECTION TO REFER TO
6	Address whether at groundwater monitoring well MW10A the decline in PCP concentrations in groundwater is because this well is no longer being impacted by the source area due to the capture zone of the groundwater pump and treatment system	As illustrated by the current water table map and CSM, well W10A is located outside the capture zone of the current extraction system, but is within and downgradient of the residual phase LNAPL and has been since 2011 (extraction rate reduced). Despite that, PCP concentration decline at well W10A has been continuous since 2010.	See Section 3, and 5.3.5 for a detailed evaluation of W10A, including its historical PCP trends, groundwater gradients and geochemistry.
7	Address the correlation between fluctuating groundwater concentration results at groundwater monitoring wells W29 and W10A (i.e., if W29 goes up, then W10A goes down) and how this relates to evidence of biodegradation.	PCP concentrations in groundwater at W10A have been declining since 2010 while W29 has had two spikes in PCP concentration. The source of these spikes (i.e., a water main leak and small changes in flow direction) are unrelated to W10A's reduction. In addition, both wells have exhibited overall declining trends in PCP concentrations since 2016.	See Section 5.3 for a comparison of the trends at W10A and W29, and Figure 6 for a time-concentration graph of PCP detected at W10A and W29.

Conclusions

The conclusions from this analysis are:

- That PCP at Wauleco is degrading via biological degradation methods that are well accepted and described in the literature. The PCP degradation at Wauleco is faster in an aerobic area (the NE Profile Line) than an anaerobic area (SE Profile Line), which is also consistent with conditions documented at other sites in the literature and at the Penta Woods Site in Wisconsin. The biodegradation at Wauleco will very reliably continue to occur under the current pumping conditions and will also continue to occur under non-pumping conditions.
- That the decreasing PCP concentrations in groundwater are due to biodegradation, and not other natural attenuation mechanisms, like dispersion or dilution or due to changes in groundwater flow directions.
- Some monitoring well locations, especially W26 and W29, that are located adjacent to the LNAPL footprint, are subject to small changes in groundwater flow directions that result in differences in flow distances from the LNAPL and, hence, fluctuations in PCP concentrations at these wells. However, as shown on the Stagnation Zone Profile concentration-distance graph (Figure 11), these intermittent PCP concentrations are degraded before reaching the next downgradient well.

Section 2
Background

2.1 Introduction

The Wauleco, Inc. (Wauleco) facility is located at 125 Rosecrans Street, Wausau, Wisconsin (Site; see 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 from the early 1900s to the early 1990s. Manufacturing operations ceased in March 1991 and nearly all site buildings were demolished by 1993.

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 trade named Woodtox Preprime, manufactured by Kopper Chemical and Coating Company. Woodtox Preprime, commonly referred to as Penta, was a 5% solution of pentachlorophenol (PCP) dissolved in 85% mineral spirits, and 10% inerts. Penta was used at the site from approximately 1944 until 1986.

2.2 Purpose and Background

As requested by the Wisconsin Department of Natural Resources (WDNR), this Technical Memorandum presents the data and interpretation of site conditions to assess natural attenuation of PCP that is occurring at the Wauleco Site. The purpose of this Technical Memorandum is to present the lines of evidence addressing the question of whether PCP is naturally degrading in the groundwater at and downgradient of the Wauleco Site. This Technical Memorandum also responds to questions raised by the WDNR regarding the occurrence of natural attenuation/contaminant degradation at the Wauleco Site.

This assessment follows two guidance documents to assess natural attenuation of chlorinated compounds:

- EPA (1998) Technical Protocol for Evaluation Natural Attenuation of Chlorinated Solvents in Ground Water. EPA/600/R-98/128 ("EPA Guidance"). This guidance document was developed to provide appropriate and adequate methods for assessment of natural attenuation and to provide remediation managers the ability to incorporate natural attenuation into an integrated remediation approach. While PCP is not a chlorinated solvent, this EPA Guidance is useful for assessment of natural attenuation of PCP in that the Guidance details methods to evaluate multiple lines of evidence for natural attenuation of any contaminant. For example, two central evaluation methods addressed in this

Guidance-- time-concentration and distance-concentration trends-- are used for assessing natural attenuation of both petroleum and chlorinated hydrocarbons.

- WDNR (2014), Understanding Chlorinated Hydrocarbon Behavior in Groundwater: Guidance on the Investigation, Assessment and Limitations of Monitored Natural Attenuation. RR-699 October 2014 ("WDNR Guidance"). This WDNR Guidance "is intended to provide guidance on characterizing and monitoring sites where monitored natural attenuation of chlorinated hydrocarbons is being considered as part of a cleanup remedy." It was developed for use by "responsible parties, consultants or other interested parties, and WDNR staff."

Both of these documents focus on chlorinated solvents, like tetrachloroethene (PCE), although the WDNR Guidance is more inclusive in referring to chlorinated hydrocarbons. PCP is a relatively uncommon contaminant, so there are no EPA or WDNR guidance documents specifically addressing the natural attenuation of PCP. Therefore, these guidance documents for chlorinated compounds are used to guide the evaluation approaches presented in this Technical Memorandum. Although PCP is not a chlorinated VOC, the Guidances' recommended evaluation tools can still be utilized to evaluate natural biodegradation of PCP.

The EPA Guidance recommends the use of three lines of evidence to demonstrate whether natural attenuation (NA) is occurring. These are:

1. Historical ground water and/or soil chemistry data that demonstrate a clear and meaningful trend of decreasing contaminant mass and/or concentration over time at appropriate monitoring or sampling points.
2. Hydrogeologic and geochemical data that can be used to demonstrate indirectly the type(s) of natural attenuation processes active at the site, and the rate at which such processes will reduce contaminant concentrations to required levels.
3. Data from field or microcosm studies to directly demonstrate a particular NA process is occurring.

The EPA Guidance states that historical data (EPA line of evidence #1) and data characterizing the nature and rates of natural attenuation processes (EPA line of evidence #2) should be presented for all sites. Where data from lines of evidence 1 and 2 need additional support, then data from microcosm studies (EPA line of evidence #3) may also be necessary. Therefore, EPA lines of evidence #1 and #2 are required and microcosm studies (EPA line of evidence #3) are optional (EPA, 1998, page 6, last paragraph). This is the process used for demonstration of natural attenuation of PCP at the WDNR's Penta Wood Products Site, where the pump and treatment system was shutdown, the PCP plume was shown to be stable, and field and microcosm studies were completed to show PCP degradation in both the aerobic and anaerobic conditions found on the Site (GHD, 2017).

The WDNR's Guidance (WDNR's RR-699) relies on development and continual refinement of a Conceptual Site Model (CSM) that includes recommendations for field investigations to characterize the site hydrogeologic conditions and include water quality parameters to characterize groundwater quality. The multiple lines of evidence for NA of chlorinated hydrocarbons in the WDNR Guidance are not as explicit as in the EPA Guidance, but are generally described by WDNR (2014, page 7) to include:

1. Assessing NA mechanisms and demonstrating whether they can be relied on for plume control and protection of the environment and human health.
2. Demonstration that the NA mechanisms can achieve plume control typically requires the use of predictive tools (e.g., statistical trends or fate and transport models), which can be used to develop specific performance measures.
3. Predicting the capacity for NA in the aquifer.
4. Long term monitoring to confirm adequacy of the CSM and that remedial goals are met.

An important component of the WDNR Guidance and, as requested specifically by WDNR for the Wauleco Site, is to demonstrate that there are natural biodegradation mechanisms for PCP and that those mechanisms are naturally occurring at the Wauleco Site.

This Technical Memorandum evaluates and presents multiple lines of evidence which indicate natural attenuation is occurring at the Wauleco Site:

1. **Literature Review of PCP Biodegradation Mechanisms:** A presentation of literature evidence that PCP can and does naturally degrade in soils and groundwater, given the appropriate conditions. This line of evidence is provided in response to WDNR's specific request for Wauleco and consistent with the WDNR Guidance line of evidence #1 and EPA Guidance line of evidence #2.
2. **CSM Update:** An update to the Wauleco CSM is presented to describe site conditions, the nature of the source of PCP to groundwater, and to select appropriate wells and profiles for the assessment of concentration and mass trends.
3. **Concentration and Mass Trends:** These lines of evidence present trends in concentration and mass estimates to assess whether natural attenuation is occurring at the Wauleco Site. This is consistent with EPA's Guidance Primary line of evidence lines of evidence EPA #1 and WDNR Guidance lines of evidence #2, #3, and #4.
4. **Geochemistry:** A review of site-specific data from the Wauleco Site demonstrating the presence of appropriate geochemical conditions for degradation of PCP. This is consistent with EPA Guidance line of evidence #2 and WDNR Guidance line of evidence #1.

2.3 Report Organization

Based on the lines of evidence discussed above in Section 2.2, the remainder of this Technical Memorandum is organized as follows:

- **Literature Review of PCP Biodegradation Mechanism** – Section 3
- **Current Conceptual Site Models** – Section 4
- **Lines of Evidence for PCP Degradation at Wauleco** – Section 5
- **Geochemical Data** – Section 6
- **Findings and Conclusions** – Section 7
- **References** – Section 8

The residual phase LNAPL is referred to in this Technical Memorandum as simply LNAPL as no mobile phase LNAPL remains. If a reference to mobile phase is needed, it will be specifically referred to it as mobile phase LNAPL.

Section 3
Literature Review of PCP Degradation Mechanisms

3.1 Literature Review

This section provides a brief summary of the literature describing when, where, and how PCP is naturally degraded. The technical literature contains numerous examples of PCP degradation by bacteria, describing the types of bacteria and conditions that are effective in biodegrading PCP. An excellent summary is presented in a recent publication (Bosso, 2014) that contains a table describing numerous (more than 60) bacteria capable of degrading PCP in natural conditions, in waste materials and in inoculated cultures. This article is included in Appendix A.

The Bosso (2014) article provides in Table 1 (see Bosso, 2014) a summary of numerous articles demonstrating bacterial degradation of PCP in numerous settings. In this table, there are more than 60 microbial species shown to degrade PCP. Several (i.e., 11) of these bacteria are shown to naturally be present in contaminated soils. Their final conclusion includes the statement:

> "Analyzing all the works of PCP bioremediation, we have found that aerobic microorganisms have been shown to be highly efficient at degrading and mineralizing [PCP] at higher PCP concentrations, more than the anaerobic microorganisms. Furthermore, bacteria showed the greatest efficiency, in regards to, degradation of PCP, whereas the fungi demonstrated lower capability and efficiency. In addition, all the bacteria and fungi, in a pure or mixed culture, act much better when used with an amendment (wheat, wood chips, glucose). However, bacteria and fungi in mixed cultures have been shown to completely degrade PCP, with a pre exposition to PCP, the biodegradation capacity significantly increased."

In summary, this conclusion states that microbial degradation of PCP:

- While occurring in both environments, works faster in aerobic than anaerobic environments.
- Can occur with PCP as the substrate[1] (i.e., the food for bacteria to grow on), but acts much better when present with another substrate like wood chips or sugar.

[1] Although not stated in the article, the WDNR Guidance on natural attenuation for petroleum releases (WDNR 2014a) states clearly that petroleum hydrocarbons, like mineral spirits, are excellent substrate compounds for bacterial growth.

- Will completely degrade the PCP;
- That pre exposition to PCP (i.e., bacteria that have been exposed to PCP for some time and are acclimated to the presence of PCP) significantly increases the biodegradation capacity.

The Bosso (2014) article also summarizes the decay products reported by the articles they surveyed. Out of the 80 rows of studies presented by Bosso (2014), 15 did not report on decay products. Therefore, out of the 65 studies that did report on decay products:

- 29 of the studies reported chloride as the only decay product detected,
- 15 that reported only some form of hydroquinones or catechol[2] detected,
- 21 that reported some form of lower chlorinated phenol, some with a hydroquinone or catechol.

The 21 studies showing lower chlorinated phenols as a daughter product would indicate that the biodegradation in that study was via reductive dehalogenation (the replacement of a chlorine atom with a hydrogen atom). This is a similar process as typically occurs for chlorinated solvents. However, the majority of the studies showed different degradation processes, showing only chloride (29), a quinone or catechol[1] degradation product (15), for a total of 44 of the 65 studies that reported on daughter products. Therefore, the majority of PCP degradation studies showed degradation via a process other than reductive dehalogenation.

An important conclusion by Kao (2004) is that cleavage of the benzene ring can occur either before or after removal of the chlorine substituents, giving rise to a whole array of intermediates. These intermediates would typically not be included in routine analytical procedures. This degradation process would be similar to degradation of benzene, toluene, ethylbenzene, and xylenes (BTEX). This is a logical comparison, given the similarity of the basic chemical structure of PCP (i.e., a benzene ring) and the basic chemical structure of the BTEX compounds (i.e., also a benzene ring).

The typical process for demonstrating degradation of BTEX compounds does not include identifying the daughter products, because of the complexity of the analytical methods and the probable wide range of daughter products. As shown by these two studies-- the comprehensive review by Bosso (2014) and the site example by Kao (2004)-- the degradation of PCP can, and typically does fall into the same category as BTEX compounds, where identification of the daughter products is typically not feasible for the same reasons as for BTEX compounds.

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[2] Hydroquinones and catechols are similar to phenol in that they have a benzene ring, but have two or more oxygens attached to the benzene ring whereas the phenol and PCP has one oxygen attached to the benzene ring.

These conclusions from the literature are very important for assessing the natural attenuation of PCP at Wauleco. In summary, they are:

- PCP is a substrate (i.e., food source) and it is biodegraded while being consumed by the bacteria. Therefore, biodegradation is not dependent on another food source to sustain the bacterial growth, like required for biodegradation of chlorinated solvents. PCP will continue to biodegrade until the PCP is consumed. This will allow for biodegradation of PCP at Wauleco until the PCP is dissipated and will not rely on the presence of some other factor. This is unlike a chlorinated solvent site that relies on the presence of another substrate to maintain the biological activity needed for biological degradation.
- PCP will biodegrade more rapidly in environments where PCP has been present for some time where the biological population is acclimated to the presence of PCP. The PCP in groundwater at the Wauleco site has been present for many years and would have an acclimated biological population.
- PCP will degrade in either aerobic or anaerobic environments. This demonstrates that PCP will degrade both within the anaerobic environment present beneath the residual phase LNAPL to the southeast of the Wauleco property, and in the aerobic environment present to the northeast of the Wauleco property.
- PCP degradation typically will not result in commonly detectable organic compounds or toxic daughter products. This is the type of PCP degradation occurring at Wauleco -- in both the groundwater and in the groundwater extraction system's biological treatment system.
- Chloride is a daughter product from the biodegradation of PCP, however, the low concentrations are rarely discernible in a natural environment. This is particularly true in an urban environment such as the Wauleco site, where the variability in groundwater chloride concentrations from de-icing salt greatly exceeds the chloride concentration from PCP degradation, rendering chloride unusable as a marker to detect PCP degradation at Wauleco. This is described in the 2013 Annual Groundwater Monitoring Report (TRC, 2013).

The biodegradation properties of PCP described in these literature examples are directly comparable to the natural biodegradation of BTEX compounds occurring at numerous contamination sites across the state (i.e., any gasoline release site). Many of these sites have been closed, with residual phase LNAPL left in place, relying on natural attenuation to achieve compliance with groundwater standards in some reasonable period of time. These similarities are that:

- BTEX and PCP both biodegrade through its use as a substrate for naturally occurring biological growth,
- BTEX and PCP are both completely biodegraded in either aerobic or anaerobic environments,
- BTEX and PCP are both completely biodegraded with no discernible or toxic daughter products.

3.2 Penta Wood Products

The Penta Wood Products site, located in Siren, Wisconsin (Penta Woods), conducted detailed natural attenuation studies on the biodegradation of PCP at that site and reported on the results in 2017 (GHD, 2017). Selected segments from the 2017 report detailing the results are included in Appendix B). These studies included a microcosm study, using soil and groundwater from the site and a "BioTrap"[3] study. Among the objectives of these studies were to:

- Determine whether bacteria capable of degrading PCP are present at the Site,
- Demonstrate in-situ biodegradation of PCP using a BioTrap.

Results of the studies at Penta Woods concluded:

- "The results from the microcosm tests indicate that PCP and diesel range petroleum hydrocarbons (TPH (C9-C36)) are readily degradable under aerobic conditions and that PCP and TPH (C9-C36) are also degradable under anaerobic conditions; however, the anaerobic process is much slower." (see page 5 of the Penta Wood Report in Appendix B of this memo).
- "Overall, the data suggests that monitored natural attenuation (MNA) would be an effective treatment for the downgradient area, and biodegradation of PCP and TPH (C9-C36) is expected to occur at a moderate rate. MNA may be effective for the source area. The BioTrap and amended microcosm data show that PCP degradation does occur under anaerobic conditions; however, slower biodegradation rates are expected." (See page 6 of the Penta Wood Report in Appendix B of this memo).

These conclusions are consistent with the Bosso (2014) article discussed above.

3.3 Wauleco Groundwater Treatment System

Biodegradation of PCP at the Wauleco site has been accomplished for the duration of the groundwater extraction system within the biological fluid bed treatment system. Operation of this system includes addition of a small amount of ammonia-nitrogen (typically 0.9 mg/L to 1.4 mg/L) and phosphate (0.3 mg/L to 1.1 mg/L). The dissolved oxygen of the influent to the biological fluid bed is typically in the 2 mg/L to 3 mg/L range due to aeration occurring in the influent discharge into a surge tank prior to pumping into the biological fluid bed. Some bacterial inoculum is added to the carbon when the carbon is replaced and periodically during the life of the carbon. Degradation of PCP clearly occurs within the biological fluid bed given the life of the carbon before PCP breakthrough (i.e., greater than several years). While there are some low concentrations of 2,3,4,6 tetrachlorophenol in the effluent (i.e., about 10% of the PCP

[3] BioTrap – is a product from Microbial Insights, Inc. that is a passive sampler hung in a monitoring well to collect microbes growing in the environment for analysis to determine the types of microbes present.

concentration), this is also detected in the influent, at about the same percentage of PCP. Therefore, the 2,3,4,6 tetrachlorophenol appears to be a component of the original Penta solution and not a degradation product within the biological fluid bed.

Operational data demonstrates that the PCP is degrading, with no reductive dehalogenation decay products being generated.

3.4 Natural Attenuation Processes

Natural attenuation processes for groundwater contaminants include biodegradation, dispersion, dilution, sorption, volatilization; radioactive decay; and chemical or biological stabilization, transformation, or destruction of contaminants (U.S. EPA, 2002). The applicability of these processes at the Wauleco Site is as follows:

- **Biodegradation** – This is a focus of this Technical Memorandum, with general processes described in Section 3 and assessment of biodegradation at Wauleco specifically in Sections 5.3 through 5.5 and Section 6.
- **Dispersion** – Dispersion is the process by which groundwater mixes in an aquifer and can cause dilution of dissolved concentrations in groundwater. Dilution is a natural attenuation property in that it reduces the concentration, although it does not decrease the mass of contaminant present. This process is considered in Section 5.5, in the process of quantifying the PCP decay rate. Transverse dispersity, the property describing dispersion in a steady state plume, is typically estimated based on the size of the groundwater plume and can be estimated as about 5% of the length of the plume (Fetter, 2001).
- **Dilution** – The natural attenuation process of dilution in laminar flow through groundwater in a porous media occurs through dispersion, as it is the only mechanism for mixing water with different concentrations of a dissolved constituent. Dispersion, and therefore, dilution in the aquifer, is quantified by the property of dispersivity and groundwater velocity. For example, EPA (2002) guidance on quantifying the rate of natural attenuation uses an equation (as described in Section 5.4 of this Technical Memorandum) that considers dispersion, groundwater velocity, and adsorption. These properties are appropriate for PCP, because PCP does not have significant volatility and is not subject to radioactive decay. Dilution does occur at the point of groundwater discharge to a surface water body. So, while that process occurs at Wauleco, the focus of this assessment is the natural attenuation of PCP in groundwater. The rate of natural attenuation presented in Section 5.4 uses the Buscheck and Alcantar equation (as described by Newell, 2002) which considers the concentration declines through both dispersion and biodegradation. The concentration decline due to dispersion, using a typical dispersivity value recommended by Fetter (2001), is incorporated into the Buscheck and Alcantar equation when solving for the biodegradation rate. Therefore, dispersion and dilution are incorporated into the evaluation. If dispersion were sufficient to account for all of the concentration decline

observed in the NE Profile or the SE Profile, then the decay rate calculated in Section 5.4 would be zero. Conversely, if dispersion were set to zero in this equation, then the calculated decay rate would be greater than presented in Section 5.4. As such, dispersion and dilution are integrated into the evaluation.

- **Sorption** – Sorption of PCP on to soil can result in some natural attenuation of PCP. However, the natural organic content of the Wauleco soils is relatively low so that adsorption of PCP does not appear to be a significant factor. This is very clearly illustrated by the concentration change at well W16 shortly after discovery of the water main leak in July 2011. The PCP concentration at W16 spiked from non-detect to 3,000 ug/L then back to non-detect (TRC, 2018 Annual Groundwater Monitoring Report, see page 9). This demonstrates that sorption of PCP at Wauleco is not very significant. In addition, within a stable groundwater plume, any sorption capacity has already been consumed, so that migration would occur with no additional sorption. Therefore, sorption of PCP is not considered further as a factor of natural attenuation.
- **Volatilization or Radioactive Decay** – PCP is not sufficiently volatile for volatilization to be a significant natural attenuation factor. Nor does it decay by radioactive decay.
- **Chemical or Biological Stabilization, Transformation, or Destruction of Contaminants** – Chemical stabilization refers primarily to inorganic contaminants that are subject to precipitation due to changes in pH or redox (e.g., precipitation of hexavalent chromium under reducing conditions converting it to the insoluble trivalent chromium). PCP is somewhat subject to pH conditions in that its solubility increases with pH increases above about 7.0. However, this property does not appear to be significant within the natural pH fluctuation of 5 to less than 8.

In summary, the only two processes for natural attenuation of PCP in the groundwater at the Wauleco Site are dispersion and biodegradation. Therefore, assessment of the loss of PCP through time-concentration graphs, distance-concentration graphs, and the analyses associated with these trends are effective means to assess the natural attenuation of PCP.

Changes in PCP concentration may also occur due to changes in flow direction, resulting in groundwater flow originating from a location with higher or lower PCP concentrations. However, this is not a natural attenuation process and must be accounted for in a clear CSM of the groundwater flow system.

3.5 Summary

In summary:

- The literature demonstrates that natural biodegradation of PCP occurs through several biological processes under both aerobic and anaerobic environments with either PCP as the substrate, or co-metabolically with another substrate (e.g., a petroleum derivative like

mineral spirits constituents [WDNR, 2014a]). Degradation in the presence of another substrate is faster than without the other substrate.

- The studies at Penta Woods demonstrates both aerobic and anaerobic degradation of PCP, with faster degradation rates occurring in aerobic than anaerobic conditions. The demonstration of biological degradation of PCP at PentaWoods was similar to a BTEX site, where the primary reliance is on the reduction of PCP in groundwater and secondary reliance on the presence of biological activity.
- The site-specific Wauleco data from operation of the groundwater treatment system demonstrates that the PCP is degrading, with no reductive dehalogenation decay products being generated.
- Dispersion and biodegradation are the only two processes for natural attenuation of PCP in the groundwater at Wauleco. Dilution is not a natural attenuation process in the groundwater. However, changes in groundwater flow direction, may affect PCP concentrations and needs to be considered through a clear CSM of the groundwater flow system.

Section 4
Current Conceptual Site Models

This description of the current CSMs is presented to inform the selection of data representative of LNAPL, groundwater, and PCP migration appropriate for analysis based on lines of evidence to evaluate natural biodegradation addressed in EPA and WDNR Guidance The extent of LNAPL and PCP concentrations in LNAPL is briefly presented in Section 4.1 to illustrate the history of the LNAPL, as a source of PCP to groundwater.

The PCP in groundwater is briefly summarized in Sections 4.2 Groundwater CSM and 4.3 PCP Distribution to illustrate the relationship of PCP in groundwater to the location of the LNAPL. The purpose of these Sections is to select wells, and lines of wells appropriate for assessment of biodegradation in the groundwater. In particular, Sections 4.2 and 4.3 describe the groundwater extraction system's extent of capture and influence so that wells outside of this influence, but within the residual phase LNAPL area and within the PCP plume in groundwater, can be selected along migration pathways for use in assessing the biodegradation occurring outside the influence of the groundwater extraction system.

4.1 LNAPL Conceptual Site Model

The Conceptual Site Model at Wauleco consist of two media, the LNAPL CSM (LCSM), and the Groundwater CSM. The LCSM was described in detail in the December 2019 Residual Phase LNAPL Investigation Technical Memorandum (Residual Phase LNAPL Investigation Tech Memo; TRC, 2019). As described in the Residual Phase LNAPL Investigation Tech Memo:

- The amount of LNAPL within the immobile residual phase LNAPL zone has declined substantially from the original estimated volume. The original residual saturation, estimated as 17% based on testing in 1992, has reduced to an average of approximately 1.6%, or a reduction of 90.6% from the original residual saturation.
- The concentration of PCP within this immobile residual phase LNAPL has reduced from the original product of 5% to an average of 0.27% (weight by weight measurement), or a reduction of 94.6% from the original concentration.
- The distribution of PCP mass shows that the majority of the estimated PCP mass is in the vicinity of boring M01 (5,003 lbs. of PCP in LNAPL) with very low concentrations and mass of PCP in the rest of the residual phase LNAPL area (94 lbs. of PCP in LNAPL).
- The mass of PCP in LNAPL is somewhat geologically controlled in the boring M01 area, in that residual phase LNAPL is present in a seam of fine grained soils.

Figure 2 presents a cross section illustrating this LCSM and Figure 3 shows the extent of the residual phase LNAPL, in the areas around each of the cryogenic borings, including boring M01 referenced above.

4.2 Groundwater Conceptual Site Model

The Groundwater CSM is based on various conditions at and near the Wauleco Site, as described in the following subsections.

4.2.1 Hydrogeology

The hydrogeologic conditions at and downgradient of the Site is described to:

- Present the hydraulic conductivity, porosity, and the gradient on the water table, to calculate the groundwater velocity in Section 4.2.4 that will be used in Section 5 for estimating biodegradation rates.
- Describe the relationship to the Wisconsin River elevation to help respond to WDNR's comment regarding potential inflow from the River affecting PCP concentration in wells near the River.

The hydrogeology of the Wauleco Site is illustrated in the generalized cross section shown in Figure 2. The geology consists of a sand and gravel outwash deposit within the Wisconsin River bedrock valley. The sand and gravel outwash deposits extend to bedrock on the west side of the Wauleco Site. An extensive silt and clay unit is shown on Figure 2 to be present below the Wauleco site a short distance (10 ft. to 20 ft.) below the water table.

The hydraulic conductivity of the sand and gravel at the Wauleco Site has been tested through various single well slug tests and calibration of a groundwater flow model (Keystone, 1992). The single well hydraulic conductivity tests show a typical value of approximately 10.8 ft/day whereas pumping test results yielded a range between approximately 9 ft/day and 65 ft/day. The calibration of Keystone's groundwater flow model required a hydraulic conductivity of 20 ft/day to 120 ft/day, with an average of approximately 65 ft/day (Keystone, 1992, page 2-15).

This increase in the estimated hydraulic conductivity is typical when increasing the scale of the test from a single well (with the typical damage to the formation with drilling a monitoring well), to a pumping test, to a model that considers the larger scale of the site. The bulk hydraulic conductivity of a site increases with the larger scale assessments due to including more higher conductivity sand and gravel seams. Therefore, a

representative hydraulic conductivity associated with the scale of the plume, as described below, is on the order of 65 ft/day (2.3 x 10^{-2} cm/s).

Hydraulic conductivity of the silts and silty clay underlying the sand and gravel at W3A and W10A are estimated to be in the range of 0.12 ft/day (4 x 10^{-5} cm/s) to 0.49 ft/day (1.7 x 10^{-4} cm/s). This is based on field hydraulic conductivity tests at wells W-3B and W-10B (Keystone, 1986), and would represent the horizontal hydraulic conductivity, whereas, the vertical hydraulic conductivity would be expected to be 1/10th or less than these horizontal hydraulic conductivity values (i.e., 1 x 10-5 cm/s to 1 x 10-6 cm/s).

4.2.2 Surface Water

Surface water conditions are addressed in this section to assess impacts on groundwater flow and its influence on data selected to evaluate natural attenuation.

Groundwater flow at and around the Wauleco Site is naturally controlled, in large part, by the Wisconsin River east of the Site. The Rothschild Dam on the Wisconsin River (approximately 4 miles downstream) forms Lake Wausau (see Figure 1). Lake Wausau extends from the Rothschild Dam up to approximately the Thomas St. bridge. Operations at the Rothschild Dam have maintained a steady Lake Wausau pool level for the last 60 years (between elevations 1160.6 and 1160.8, (FERC 2016), with the exception of a short-term drawdown from October 4, 2016 until beginning to re-fill on November 15, 2016. The pool level for Lake Wausau extends to roughly the Thomas St. bridge east of Wauleco. The exact location of the upstream extent of the pool level probably varies through time with flow of the Wisconsin River, which is controlled in large part by operations of the dams and reservoirs on the Wisconsin River.

Upstream of Wauleco, the Wausau dam (located approximately 3,200 ft. upstream of Thomas St. as shown on Figure 1) creates a pool level upstream of the dam. The dam tail water elevation is dependent on river discharge. During a selected period of time[4] the tail water stage ranges between an elevation of 1163.84 at a flow of 1,896 cfs and 1167.33 at a flow of 14,000 cfs. This range in river stage at the Wausau Dam tail water would result in a river stage variation of about 0.5 ft. adjacent to well W10A. Therefore, there may be some bank storage, with flow parallel to, and down the slope of the River toward the Lake Wausau pool elevation. However, this should be short lived as the higher river stages are relatively short lived, especially in terms of groundwater flow rates. For example, at a 1 ft/day groundwater flow rate, inward flow from the River

[4] Stage data at the Wausau dam is displayed in real time on a web site and was recorded by TRC over several weeks to get a range of stage and flow rates. The web site is: https://accel.wisconsinpublicservice.com/environment/hydrodata.aspx

would only reach 30 ft. inland from the River over a month high water stage. While head changes are more rapid and may reach much further away from the River, flow occurs at a much slower rate.

4.2.3 Groundwater Flow Directions

Groundwater flow direction conditions are described because they effect what wells/lines of wells are appropriate to include in assessing if biodegradation of PCP is occurring.

Groundwater flow on and around the Wauleco Site has been monitored routinely since 1994 and intermittently since about 1986. The routine monitoring is reported in Annual Groundwater Monitoring Reports. The water table maps presented in the routine Annual Groundwater Monitoring Reports show that the water table has maintained very stable groundwater flow patterns. The July 2018 water table map, included in Figure 4, is representative of the current groundwater flow system. This map shows a groundwater gradient from the west toward the Wisconsin River, with a capture zone from the groundwater extraction system superimposed on this general flow direction. The groundwater extraction rate in July 2018 was approximately 22 gpm.

The non-pumping groundwater contours can be easily interpolated across the cone of depression formed by the groundwater extraction system. The interpreted, non-pumping water table contours are shown on Figure 4 as dotted contours. The limited cone of depression, outside of the capture zone, is expected based on the relatively high hydraulic conductivity of the aquifer, which would result in very small drawdowns at distance from the pumping center. Groundwater flow lines present outside this cone of depression (i.e., outside of the dotted contour lines showing the area affected by pumping) are unaffected by pumping. Well profiles along current groundwater flow lines, that are outside of the capture zone, and are virtually unaffected by current pumping include:

- **Northeast Profile** – From well DFOMW12 to W13 to W18. This profile is oriented along the current groundwater migration pathway from a source of PCP that is present along a line from wells W02 to DFOMW11 to DFOMW12. This line source of PCP, extending to the north of the residual phase LNAPL was apparently formed during a historical groundwater flow path causing some amount of PCP, and potentially a small amount of LNAPL (which is currently not detected), to move to the north of the main body of the residual phase LNAPL. The probable condition that caused this migration was during the July 1990 to July 1991 period in which 100% of the groundwater extraction system was recharged through the seepage near the former dip tank. This is also the time in which the PCP concentration

increased at well W02 (see graph in Appendix E, with the increase that began in January 1991).

The Northeast Profile line is well outside the capture zone and completely outside the cone of depression as shown on Figure 4. This profile is oriented along the current groundwater migration pathway from a source of PCP that is present along the centerline of the PCP concentration that was formed under different PCP migration conditions.

- **Northern, Line Source Profile** – A concentration distance profile is presented in Figure 13 from well W02 through DFOMW11, DFOMW12, then downgradient to well W28. As described in the Northeast Profile discussion, this line:
  - Follows a probable line source of PCP extending north of the main body of the residual phase LNAPL, that does not follow the current groundwater flow direction.
  - Then turns to follow the current groundwater flow direction between wells DFOMW12 and well W28.
- **Southeast Profile** – From well W41 to W27 to W11 to W21. This profile line is well outside the capture zone and is virtually outside the cone of depression, as shown on Figure 4. The only slight effect by the cone of depression is shown near W27.

Well profiles that are affected by pumping and/or by natural fluctuations in groundwater flow direction are:

- **Centerline Profile** – From W72 (upgradient of the Site and LNAPL footprint) to W01A to the extraction wells (as represented by the PCP concentration in the influent to the treatment system) to W03A to W10A. This profile extends through and then downgradient of the capture zone (see Figure 4).
- **Stagnation Zone Profile** – From W22 to W40/W40R to W26 to W29 to W32. This profile extends through the capture zone and through the stagnation zone downgradient of the capture zone (see Figure 4). The stagnation zone appears to dissipate between well W40 and W26, so that the gradients from W26 to W29 to W32 represents natural groundwater flow rates.

4.2.4 Groundwater Flow Rate

Groundwater flow rate conditions are described for use in the estimate of the PCP decay rate in Section 5.4.

Groundwater flow rates in the Wauleco Site vicinity are based on the bulk hydraulic conductivity of approximately 65 ft/day, as described in Section 4.2.1 – Hydrogeology, a total porosity of 39% (based on average porosity in data presented in Table 4 of the

Residual Phase LNAPL Investigation Tech Memo: TRC, 2019), with an estimated effective porosity of 30%, and the July 2018 water table map gradients in along the Southeast Profile and the Northeast Profile are shown in Table A.

Table A
Groundwater Velocity Calculations

LOCATION	WELL PAIR	GRADIENT	GROUNDWATER VELOCITY
Northeast	DFOMW12-W13	(1163.29-1161.89)/192 = 0.0073	1.6 ft/day
Northeast	W13-W-18	(1161.89-1161.21)/330 = 0.0021	0.45 ft/day
Southeast	W41 – W11	(1162.91-1161.15)/630 = 0.0028	0.61 ft/day
Southeast	W11 – W21	(1161.15-1160.95)/543 = 0.0004	0.08 ft/day
Northeast Average Flow Velocity			1.0 ft/day
Southeast Average Flow Velocity[(a)]			0.6 ft/day

Footnotes:

[(a)] The groundwater velocity from W11 to W21 is significantly lower than the upgradient velocity from W41 to W11. To maintain the conservation of flow through this area with this decline in velocity to occur, flow would have to divert around this area with a significant steepening of the gradient. However, this lower gradient through the area from W11 to W21, suggests that the hydraulic conductivity in this area is higher than the average value being used in this evaluation. Therefore, the average groundwater velocity for the southeast profile uses the estimate from the W41 to W11 area of 0.6 ft/day. While the same condition is seen between the W2-W13 segment and the W13-W28 segment, the difference is not extreme, so the average velocity is used.

4.3 PCP Distribution

Figures 5 and 6 illustrate the distribution of PCP concentrations from the July 2018 groundwater sampling event, with the time concentration graphs for selected wells added to Figure 6. The distribution of PCP in the groundwater is directly related to the distribution of LNAPL, as shown on these figures.

The peak PCP concentration in the Wauleco property monitoring wells is at well W33 (2,800 ug/L). However, the 3,000 ug/L contour line is wrapped around the central portion of the property based on the groundwater extraction system concentrations (i.e., the treatment system influent concentrations). These data are reported in the Quarterly Reports and are illustrated on Figure 7. The average concentration for 2018 was approximately 4,400 ug/L, with a range of 2,061 ug/L to 10,630 ug/L. This concentration on the property is related to the central portion of the LNAPL. As shown in the Residual Phase LNAPL Investigation Technical Memorandum (TRC, 2019) this is also the area with the majority of the remaining PCP mass within the LNAPL, containing a mass of 5,093 lbs PCP within the M01 boring area and only 94 lbs in the remaining area of LNAPL. These areas are shown on Figure 3.

The peak PCP isoconcentration contour, 3,000 ug/L, extends from on the Wauleco property to southeast of the property, in the vicinity of wells W41 (2,900 ug/L in July 2018) and W27 (5,200 ug/L in July 2018). The PCP concentration at these wells are related to the extent of LNAPL extending down to this area as well. However, concentrations drop dramatically downgradient of well W27. At well W11 the July 2018 PCP concentration was 120 ug/L and further downgradient, the concentration drops to non-detect at well W21. This flow path (i.e., W41 to W27 to W11 to W21) is well outside the current remediation system's capture zone as shown on Figures 4 and 5 and is a flow path that is virtually unaffected by the groundwater extraction system as shown by the probable non-pumping water table conditions shown on Figure 4. Therefore, this decline in PCP concentration along this flow line is occurring under natural, non-pumping conditions.

Northeast of the Wauleco property there is a peak of PCP at well DFOMW11 (4,100 ug/L in July 2018) and DFOMW12 (2,300 ug/L in July 2018). These concentrations occur in the vicinity of the northern extent of LNAPL, and are well outside the capture zone of the groundwater extraction system as shown on Figures 4 and 6. Downgradient of these wells, the concentration drops to low or non-detect concentrations at wells W13 (2.7 ug/L in July 2018), W28 (2.5 ug/L in July 2018), and W18 (<3.0 ug/L in July 2018). Therefore, this decline in PCP concentration along this flow line is occurring under natural, non-pumping conditions.

4.4 Summary

In summary:

- The LCSM has demonstrated that the residual phase LNAPL within its footprint at the Wauleco Site has:
  - Decreased in volume by 90.6% (i.e., by reduced residual saturation) and reduced in PCP concentration by 94.6%.
  - The majority of the remaining mass present within the LNAPL is in the boring M01 area (i.e., 5,003 lbs of PCP within the former dip tank area) which may be somewhat geologically controlled with the fine grained soils containing the bulk of the PCP in LNAPL.
  - The minority of remaining PCP mass (94 lbs) is present within the remaining residual phase LNAPL footprint.
- Groundwater flow is relatively stable over the decades of monitoring, with measurable changes due to changes in the Wauleco groundwater extraction system pumping rate, a large water main leak (repaired in July 2011) and drawdown of Lake Wausau in 2016.

- The capture zone of the current groundwater extraction system (at approximately 22 gpm) is superimposed on the natural groundwater flow and the extent of the capture zone and the cone of depression are clearly illustrated on the water table map.
- The Northeast Profile and the Southeast Profile lines are groundwater migration pathways that are well outside the current groundwater extraction system's capture zone and cone of depression where PCP behavior under natural groundwater flow can be assessed.

Section 5
Lines of Evidence for PCP Degradation at Wauleco

5.1 Lines of Evidence

The ability of PCP to naturally biodegrade is described in detail in Section 3 of this Technical Memorandum. Given the evidence in Section 3 that PCP can and does naturally biodegrade in the environment, the primary line of evidence for whether PCP naturally biodegrades at the Wauleco Site, as described by EPA (1998, page 6), is "*Historical ground water and/or soil chemistry data that demonstrate a clear and meaningful trend of decreasing contaminant mass and/or concentration over time at appropriate monitoring or sampling points."* The historical data trends are presented in this Section to describe the trends in decreasing PCP concentration.

The secondary line of evidence is to use geochemical data to "demonstrate indirectly the type(s) of natural attenuation processes active at the site, and the rate at which such processes will reduce contaminant concentrations to required levels." (EPA, 1998, page 6). The geochemical data is discussed in Section 6.

Trends of PCP concentration data are presented as time-concentration graphs for both the LNAPL (Section 5.2) and groundwater (Section 5.3), and in distance-concentration graphs for PCP in groundwater (Section 5.4). The geochemical conditions are presented in Section 6 and compared to the observed biodegradation described in Section 5.

5.2 Residual Phase LNAPL PCP Time-Concentration Trends

The LNAPL present beneath and around the Site is the primary source of PCP to groundwater. Therefore, the trend in the PCP concentration within this LNAPL is critical to the future trends in PCP in groundwater and the reliability that the historical trends will continue into the future. As such, the first time-concentration trend that is discussed is the PCP concentration within the remaining LNAPL.

5.2.1 Time-Concentrations Trends

As described in the Residual Phase LNAPL Investigation Tech Memo (TRC, 2019) both the volume of LNAPL has declined (by 90.6% to 94.3%[5]) and the PCP concentration

[5] The range in percent removal is when considering only the samples with current residual saturation (90.6%) or 94.3% when including samples with zero residual saturation in the interval where the laser induced fluorescence (LIF) detected LNAPL.

within the LNAPL has declined (by 94.6%), for a mass reduction of 99.5% (see TRC, 2019, page 15).

The evidence for reduction in the volume of residual phase LNAPL is the measured initial residual phase saturation of mineral spirits in the Wauleco soils of 17% and the measured residual phase saturation, through the 2019 analyses of residual saturation, of 1.6% saturation. The data relied upon for this reduction in the residual saturation is based on 127 soil samples, so this reduction is considered to be very reliable.

The evidence for reduction in the PCP concentration within the LNAPL consists of both samples of LNAPL analyzed for PCP through time and the 127 samples analyzed for PCP in the Residual Phase LNAPL Investigation Tech Memo (TRC, 2019). Table 1, similar to Table 1 in the Residual Phase LNAPL Investigation Tech Memo (TRC, 2019), illustrates the PCP decline through time in the mobile phase LNAPL. One additional point is added to Table 1, the 0.27% average PCP concentration in the residual phase LNAPL as presented in the Residual Phase LNAPL Investigation Tech Memo (TRC, 2019). In addition, the graph is presented on a log scale for the PCP concentration axis, because the decline is following the first order decay equation and is declining at a logarithmic rate. This allows projecting the trend line forward in time, illustrating the expected continued decline of PCP in the LNAPL.

5.2.2 Future Reliability of this Trend

As shown on the graph in Table 1, the average PCP in mobile phase LNAPL concentration is expected to decline to 0.1% in the next 10 years. This trend for mobile phase LNAPL is expected to be similar for residual phase LNAPL. This logarithmic decline is occurring with a half-life of approximately 10 years[6], so that the PCP concentration in LNAPL is expected to halve every 10 years.

The future reliability of any trend is an important consideration discussed in the WDNR Guidance. Therefore, each line of evidence is followed by an evaluation of the future reliability of the trend discussed. The historical decline in PCP concentration in the mobile phase LNAPL is clearly illustrated in the graph in Table 1. However, the data is quite variable, due to the fact that the analyses of PCP in mobile phase LNAPL is a difficult matrix for analysis and each sample would have been diluted by several hundred times, reducing the accuracy of the resulting number. However, this is not the case for the final value, 0.27%, which is the average of numerous samples. Therefore,

[6] The decay rate is shown on the trend line on the graph on Table 1 as -2e-04 day^{-1}, or -.073 yr^{-1}. This equates to a half-life of 9.4 years.

the trend in concentration from 5% in the original product to 0.27% in the remaining residual phase LNAPL in 2019 is a reliable, qualitative trend.

The causes for this decline in PCP concentration within the mobile phase LNAPL are:

- The probable greater solubility of PCP in groundwater than the mineral spirits constituent's solubility in water, resulting in reduction of PCP concentrations in the LNAPL.
- The concentration gradient between PCP in the LNAPL and PCP in groundwater.

This reduction in the PCP concentration in the LNAPL is expected to continue, as it is based on the chemical properties of PCP and mineral spirits. The only reason for this trend to slow down or stop is if the PCP concentrations in groundwater were to increase, reducing the concentration gradient from the LNAPL to groundwater. This second factor, continued reduction of PCP concentration in groundwater, is the subject of the following sections of this Technical Memorandum.

5.3 Groundwater PCP Time-Concentration Trends

5.3.1 Groundwater Extraction Wells - On-Site Within Capture Zone:

Groundwater Extraction Wells: The primary data set for groundwater quality on the Wauleco property is from the extraction wells' influent to the treatment system. These data are from the field laboratory and show the flow weighted average from the capture zone. As shown on the time-concentration graph (Figure 7) the data are quite variable, but the trend line while pumping at 42 gpm showed a distinct decline in influent concentration from roughly 10,000 ug/L to roughly 5,000 ug/L. This is also during a time when the mobile phase LNAPL was removed from the area of capture, greatly reducing the volume of LNAPL and mass of PCP. The reduction to 22 gpm pumping rate appears to have resulted in variations around a constant average PCP influent concentration, at roughly 4,700 ug/L. This average PCP influent concentration indicates that the dissolution rate of PCP from the LNAPL to the underlying flowing groundwater is at a roughly steady state condition.

Future Reliability of this Trend: The recent, range of PCP concentration in the extracted groundwater (at roughly 4,700 ug/L) is expected to continue under pumping conditions. If the groundwater extraction system were shut down, it is expected that PCP concentrations in the area would remain in the same range, but the average would

probably rise somewhat. This projection is based on the LNAPL and groundwater flow condition, including:

- The effectiveness of the capture zone at 22 gpm in capturing the groundwater flow occurring within the width of the extraction wells' spacing (i.e., the 22 gpm pumping rate is capturing approximately the natural flow through this area). This results in no dilution from groundwater flowing from outside the LNAPL footprint.
- The effectiveness of the capture zone also results in a minimal increase in groundwater velocity directly upgradient of the extraction wells. Therefore, the groundwater velocity would not reduce significantly with elimination of pumping, and, therefore, the same contact time between LNAPL and the underlying groundwater would occur, resulting in a similar groundwater concentration as during pumping conditions.

Therefore, the average PCP concentration in the vicinity of the extraction wells is not expected to increase substantially when the groundwater extraction system is shut down.

5.3.2 Well W03A - On-Site Within Capture Zone

Well W03A: W03A is located on the Wauleco property, within the groundwater extraction system's capture zone and within the LNAPL footprint. As shown on Figure 6, the PCP concentration at W03A was very high (>10,000 ug/L) for most of the 1990s. Then mobile phase LNAPL appeared in 1997 and was present through 2009. No groundwater samples were collected from wells with mobile phase LNAPL. When the mobile phase LNAPL was bailed from the well in 2010, no more mobile phase LNAPL returned, demonstrating that the mobile phase LNAPL present had been trapped in the well, probably for several years. When subsequent groundwater samples were collected in January 2010, the PCP concentration was 3,700 ug/L. It has since declined to and varied around approximately 300 ug/L to 700 ug/L. The current trend appears to be stable in this range. Nearby well W17 has shown a similar trend in PCP through time, dropping from 1,000 ug/L in 2004 when mobile phase LNAPL disappeared at this location to less than 100 ug/L since 2017.

The PCP trend at well W03A (see time concentration graphs in Appendix E) does not show any apparent seasonal trend. Rather, the trend is generally downward, with the historical peaks being as often in the winter as in the spring/summer/fall. Therefore, the PCP concentration at this well does not illustrate decreases due to spring, summer, or fall rainfall recharge nor concentration increases in winter.

Future Reliability of this Trend: The stability of PCP concentrations at W03A, between 300 ug/L to 700 ug/L, appears to be stable under the current pumping conditions. The

concentration may rise somewhat under non-pumping conditions, but as described above for the extraction wells, this rise is expected to be relatively small.

5.3.3 Northeast of Site – Outside of Capture Zone

The time-concentration graphs for the key wells northeast of Wauleco are shown on Figure 6 on the PCP isoconcentration map. Table B summarizes the observations for the key wells in this area.

Table B
Summary of Time Concentration Trends
Northeast of Site – Outside of Capture Zone

WELL	LOCATION WITH RESPECT TO LNAPL AND CAPTURE ZONE	INITIAL CONCENTRATION AND DECLINE HISTORY	BEHAVIOR WITH REDUCED PUMPING	WILL TREND CONTINUE WITH TERMINATION OF PUMPING?
DFOMW11	Outside capture zone & outside of LNAPL. Center of lobe of PCP north of property.	~5,000 ug/L in 2010/2011 with fluctuating decline to 240 ug/L in 2019.	Limited history prior to reduced pumping.	LNAPL is not present in this area to act as a source, so decline in concentration is expected to be maintained, and likely continue to decrease.
W02	Outside capture zone & inside of LNAPL	Increased to 25,000 ug/L, mobile phase LNAPL present between 2000 and 2009. Since bailing out LNAPL in 2010, concentrations have declined to 260 ug/L in 2019 (similar to DFOMW11).	Reduced pumping had little to no effect on continued decline.	Source of PCP from LNAPL may increase the PCP concentration somewhat, but minimal change expected based on no expected change in groundwater velocity.
W13	Outside capture zone. Downgradient of LNAPL	1,500 ug/L in 1992, declining to non-detect (ND) in 2005.	No change, continued at ND.	Rapid degradation between upgradient LNAPL and W13 is expected to continue, resulting in continued <10 ug/L (see discussion in Section 5.4.1 Northeast Profile Distance-Concentration Trend).

Table B
Summary of Time Concentration Trends
Northeast of Site – Outside of Capture Zone

WELL	LOCATION WITH RESPECT TO LNAPL AND CAPTURE ZONE	INITIAL CONCENTRATION AND DECLINE HISTORY	BEHAVIOR WITH REDUCED PUMPING	WILL TREND CONTINUE WITH TERMINATION OF PUMPING?
W18	Outside capture zone downgradient of LNAPL	Declined from 10,000 ug/L (W18) in 1992 to <100 ug/L and ultimately <10 ug/L by approximately 2002	No change.	Downgradient of W13 (See W13 expectation).
W28	Outside capture zone and outside of LNAPL	Declined 4,000 ugL (W28) in 1992 to <100 ug/L and ultimately <10 ug/L by 2001	No change.	Expected to maintain a non-detect result, with occasional <10 ug/L with rapid degradation in area (see W13 expectation).

The PCP concentration throughout the seasons at these wells (see time concentration graphs in Appendix E) do not illustrate decreases due to spring, summer, or fall rainfall recharge nor increases in winter. Specific observations are as follows:

- **DFOMW11** – Peak concentrations occurred in July 2010 (4,800 ug/L), July 2011 (5,000), June 2012 (4,200), July 2014 (5,800), July 2015 (5,300), and July 2018 (4,100), with much lower concentrations (i.e., 580 ug/L to 2,200 ug/L) in the spring or winter months.
- **W-02** – The annual variations in PCP concentrations are dwarfed by the changes occurring through multiple years, indicating that seasonal variations are small relative to the overall downward trend through multiple years.
- **W-02** – The annual variations in PCP concentrations are dwarfed by the changes occurring through multiple years, indicating that seasonal variations are small relative to other effects, such as movement of mobile phase LNAPL in the early years or decline in the source of PCP in later years.
- **W-13** – Like W02, seasonal variations in the early years were small compared to the overall concentration decline, typically to non-detect.
- **W-18** – Like W-02 and W-13, the early variations in PCP concentration were dwarfed by the overall concentration decline, typically to non-detect.
- **W-28** – This well had significant variations in PCP concentration prior to declining to typically less than 10 ug/L around 2000. The peak concentrations occurred in March 1988 (10,000), April 1989 (3,670), May 1990 (4,460), May and June 1991 (both 4,600), and December 1992 (6,640). After 1992, concentrations dropped nearly

continuously to typically less than 10 ug/L. Therefore, the PCP concentration at this well does not illustrate decreases due to spring, summer, or fall rainfall recharge nor increases in winter due to a lack of rainfall recharge.

Future Reliability of These Trends: The source of PCP to these wells appears to be the dissolved phase PCP in the vicinity of DFOMW11 or the northern extent of the LNAPL that is present in the vicinity of W02. These wells have been outside of the groundwater capture zone since at least 2011, after beginning to reduce the extraction rate to 22 gpm, so that it is expected that the groundwater quality in this area has equilibrated to natural flow through the area. Therefore, the future reliability of these trends is strong based on continuing the same groundwater flow conditions (i.e., groundwater flow rate) and general water quality that has been occurring over the last several years.

5.3.4 Southeast of Site – Outside of Capture Zone

The time-concentration graphs for the key wells southeast of Wauleco are shown on Figure 6 on the PCP isoconcentration map. Table C summarizes the observations for the key wells in this area.

Table C
Summary of Time Concentration Trends
Southeast of Site – Outside of Capture Zone

WELL	LOCATION WITH RESPECT TO LNAPL AND CAPTURE ZONE	INITIAL CONCENTRATION AND DECLINE HISTORY	BEHAVIOR WITH REDUCED PUMPING	WILL TREND CONTINUE WITH TERMINATION OF PUMPING?
W41 and W27	Outside capture zone & adjacent to LNAPL. W41 is somewhat upgradient of the LNAPL and W27 is downgradient of the LNAPL.	Both wells have generally similar history: ~15,000 ug/L in 1990s (a little later for well W27), declining to 1,000 ug/L to 9,000 ug/L through 2009 (W27 is typically a little higher, being the downgradient of the LNAPL).	No apparent change.	Both of these wells are outside the capture zone, and adjacent to remaining LNAPL. Therefore, no short term change in concentration is expected.

Table C
Summary of Time Concentration Trends
Southeast of Site – Outside of Capture Zone

WELL	LOCATION WITH RESPECT TO LNAPL AND CAPTURE ZONE	INITIAL CONCENTRATION AND DECLINE HISTORY	BEHAVIOR WITH REDUCED PUMPING	WILL TREND CONTINUE WITH TERMINATION OF PUMPING?
W11	Downgradient of LNAPL at W41 & W27 and outside of capture zone.	Up to 2,500 ug/L in early 1990s, declined to <600 ug/L through 2010. An increase from 2011 through 2014 attributed to the Wausau water main leak.	No change, although the 2011 to 2012 period is superimposed on changes due to the Wausau water main leak.	Degradation between LNAPL, near W27 & W41, and W11 is expected to continue, resulting in <200 ug/L (see Section 5.4.2 Southeast Profile Distance-Concentration Trend).
W21	Outside capture zone downgradient of LNAPL at W27 & W41 and downgradient of W11.	Declined from occasional detects >10 ug/L (up to 180 ug/L) in pre-2002 to always <10 ug/L, and typically ND.	No change.	Degradation along W41 to W21 flow path is expected to continue, resulting in <10 ug/L at W21.

Well W41 is the only well out of this group with multiple samples throughout the year (see time concentration graphs in Appendix E) to be able to discern seasonal trends, as wells W11, W21, and W27 have been on annual monitoring programs for most of their history. Well W41 has had a series of PCP concentration peaks and valleys, with the peaks occurring in June 1993 (32,000 ug/L), July 1997 (18,000), January 2000 (7,800), January 2001 (7,600), January 2003 (7,200), July 2004 (5,900), July 2005 (5,900), July 2008 (6,500), April 2012 (7,600). There does not appear to be a seasonal predominance for these peaks in concentration.

Future Reliability of These Trends: The source of PCP to these wells is the LNAPL in the vicinity of wells W41 and W27. Therefore, the concentration at wells W41 and W27 are expected to continue their stable trend, fluctuating between 1,000 ug/L to 9,000 ug/L. Like the wells northeast of the Site, these wells to the southeast of the Site have been outside of the groundwater capture zone since reduction of the extraction rate began in 2011, so that it is expected that the groundwater quality in this area has equilibrated to natural flow through the area. Therefore, the future reliability of these trends is strong based on continuing the same groundwater flow conditions (i.e., groundwater flow rate) and general water quality as has been occurring over the last several years.

5.3.5 East of Site – Outside of Capture Zone

The wells east of the Site are in a different set of conditions than either northeast or southeast of the Site. This area has:

- residual phase LNAPL present across much of the area, which acts as a source of PCP to groundwater,
- potential interactions from the River, depending on groundwater levels and river stage.

Well W10A: Well W10A is a key well monitoring the Site, in that it is located downgradient of the Site, is within the LNAPL footprint, and is nearest to the River. As shown on Figure 4, the water table map and Figure 5, the PCP isoconcentration map, well W10A is located outside and north of the capture zone, but may be downgradient of an area on the edge of the extraction system's cone of depression[7]. Therefore, groundwater flow upgradient of well W10A is only minimally affected by the groundwater extraction system.

The initial PCP concentration at well W10A was in the 15,000 ug/L range in the early 1990s, probably directly related to the presence of residual phase LNAPL, with PCP concentration at about 3.2% (see 1986 LNAPL PCP concentration in TRC, 2019). The decline in PCP concentration between 1996 to 1998 is potentially due to the recirculation of treated groundwater through the seepage bed at a rate of 100% of the extraction rate for over a year leading up to June 1991[8]. During the period 2000 to 2008 the PCP concentration was generally stable in the 5,000 ug/L to 8,000 ug/L range.

Since July 2008, PCP concentration has steadily declined at well W10A to 610 ug/L in July 2019. This decline is not due to the reduction in pumping from 42 gpm to 22 gpm, as the concentration decline started before the reduction in pumping and continued after the reduction.

Because Well W10A is located near the River, it was suggested that the decline in PCP concentration could be due to river water inflow to the groundwater, with subsequent dilution of the PCP in the groundwater at W10A. If river water inflow were causing

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[7] Note: A pumping system's cone of depression typically extends beyond the pumping system's capture zone and includes an area where the head and groundwater velocity are affected by pumping, but the groundwater is not captured by the pumping system.

[8] Note: Groundwater extraction system began in Jan. 1988, with Phase I seepage bed starting in 1st quarter 1988, and Phase II seepage bed in Nov. 1989. Reduction from 100% recirculation to 50% recirculation in June 1991, then to 3 gpm in February 1992, then discontinuation of recirculation in late 1993.

decreasing PCP concentrations, we would expect the following conditions to coincide with decreasing PCP conditions at W10A:

- Local groundwater flow inward from the river near W10A, and
- Water chemistry parameters indicating aerobic groundwater conditions at W10A.

The following data were used to assess these conditions:

- The history of groundwater gradients near the river based on the head at the closest wells, and
- Other general water chemistry parameters.

Groundwater Gradients: Appendix C includes an evaluation of groundwater gradients through time for four groups of wells along the River. As shown in this evaluation, the gradient in the vicinity of, and upstream of W10A, is primarily toward the River, especially upstream of W10A. Upstream (i.e., north) of W10A shows groundwater flow toward the river at almost every measurement date, whether pumping at 42 gpm or 22 gpm. This is shown by the gradient defined by wells W28-W18-W13 (see Triangle A in Figure C-2). The hydraulic gradient defined by wells W10A, W17, and W18[9] (see Triangle B in Figure C-2) indicates that groundwater flow is typically toward the River except during pumping at 42 gpm (from January 1999 through March 11, 2011), when the gradient was typically toward the southwest, indicating flow may have occurred from the River into the groundwater during this time. However, the PCP concentration at W10A was relatively stable (i.e., between 4,500 ug/L and 8.800 ug/L, see PCP time-concentration graph on Figure 6) during most of this time (i.e., from July 2001 to July 2008), indicating that even if inflow from the river were occurring, it did not result in decreasing PCP concentrations. The decrease in PCP concentrations at W10A has been occurring since 2010, during which time the local groundwater flow has been predominantly toward the River. Based on these data, potential inflow from the River has not affected PCP concentrations at well W10A.

General Water Chemistry: Appendix D contains an evaluation of the general water chemistry that are presented primarily for the secondary line of evidence for natural attenuation. However, these parameters are also useful in evaluating whether inflow from the River has occurred and affected water quality at well W10A. If there were inflow of water from the River affecting the water quality at W10A, the redox conditions at this well would be aerobic, with very low dissolved iron and manganese. As shown in Appendix D, dissolved manganese and dissolved iron have consistently been

[9] A triangle of wells is the minimum number to determine groundwater flow direction (i.e., from geometry, 3 points are needed to define a plane).

detected at concentrations greater than 875 ug/L and 2,000 ug/L, respectively, since analyses of the Monitored Natural Attenuation (MNA) parameters became routine in January 2014. These constituents demonstrate that the groundwater is strongly anaerobic. In addition, the nitrate-N and nitrate+nitrite-N results from 1987 to the present show primarily non-detectable concentrations, also indicating anaerobic conditions. The anaerobic conditions at W10A present a strong line of evidence that inflow from the River is not occurring, and, therefore, is not the cause of decreasing PCP concentrations at well W10A.

An evaluation of the seasonal trend in PCP concentration at W10A is limited to the time frame 1987 to June 1994 when the well was sampled more than once per year, which has been its frequency since June 1994 (see time concentration graphs in Appendix E). Between 1987 and 1994 the peaks in PCP concentration occurred in March 1988 (13,500 ug/L), July 1989 (15,200), May 1991 (29,800), October 1991 (16,500), and June (17,000). However, the lows between these peaks did not typically occur in January, rather, many of the January events saw a minor peak, with the low PCP concentration present in November and April events. The data reveals no seasonal trend apparent in dissolved PCP concentration at well W10A.

In summary, declining PCP concentrations at well W10A are not due to inflow from the River or a change in groundwater flow so that flow is not originating from the LNAPL. Therefore, the only reason for the decline in PCP is due to biodegradation of PCP occurring in the aquifer.

5.3.6 Other Wells East of the Site – Inside or Downgradient of Capture Zone

Table D summarizes the observations for the key wells in this area.

Table D
Summary of Time Concentration Trends
East of Site – Inside or Downgradient of Capture Zone

WELL	LOCATION WITH RESPECT TO LNAPL AND CAPTURE ZONE	INITIAL CONCENTRATION AND DECLINE HISTORY	BEHAVIOR WITH REDUCED PUMPING	WILL TREND CONTINUE WITH TERMINATION OF PUMPING?
W22	In stagnation zone of capture zone & within LNAPL	Initially 10,000+ ug/L and mobile phase LNAPL has declined to consistently between 1,400 ug/L and 8,000 ug/L since 2005.	Consistent PCP concentrations before and after pumping rate reductions. See footnote 1 below. regarding 2010 and 2011 period.	Consistent PCP concentrations are expected after shut down due to the overlying LNAPL.

Table D
Summary of Time Concentration Trends
East of Site – Inside or Downgradient of Capture Zone

WELL	LOCATION WITH RESPECT TO LNAPL AND CAPTURE ZONE	INITIAL CONCENTRATION AND DECLINE HISTORY	BEHAVIOR WITH REDUCED PUMPING	WILL TREND CONTINUE WITH TERMINATION OF PUMPING?
W26	Downgradient of capture zone and outside of, but adjacent to LNAPL footprint.	15,000+ ug/L through 2000. Following pumping at 42 gpm the concentration declined to about 4,600 ug/L and started declining slowly to <500 ug/L and as low as <10 ug/L.	Continued the decline in PCP without an affect by the reduction in pumping rates.	Concentrations are expected to remain low, with occasional spikes when flow is to the south, from the adjacent LNAPL.
W29	Outside of capture zone and outside of, but near the LNAPL footprint.	5,000+ ug/L until 1990 with a rapid decline to <5,000 ug/L in 1990 with the onset of groundwater extraction and further reduction to <100. A recent increase to 6,600 ug/L may have coincided with flow from the nearby LNAPL, resulting in a shorter flow path from LNAPL to this well, resulting in an increase in PCP concentration.	Coincided with a potential effect from the water main leak, so difficult to determine whether the increase in 2011 and 2012 was associated with reduction in pumping rate.	Future concentration is dependent on whether flow direction is between LNAPL and well. Terminating pumping may shift flow slightly south, so that flow may more often come a shorter distance to LNAPL, resulting in higher PCP concentrations than when flow is from the west, which is a longer distance from upgradient LNAPL.

Footnotes:

(1) Well W22 was strongly influenced by a water main leak at the corner of Thomas St. and Cleveland Ave., roughly 200 feet upgradient (see more discussion in the 2011 Annual Groundwater Monitoring Report). The leak was discovered and repaired in June 2011. The PCP concentrations from January 2010 through October 2011 dropped to less than 100 ug/L.

These wells east of the Site, either within or adjacent to the LNAPL are strongly affected by the direction of groundwater flow, which can, at times, have a southerly component of flow that will shorten the flow distance between the LNAPL footprint and each well, reducing the distance and time for natural degradation of PCP between the source and the subject monitoring well, resulting in variable PCP concentrations in these wells.

However, as described in Section 5.4 PCP Distance Concentration Trends, the PCP will degrade further downgradient from these areas.

Well W10A is located to the north of the capture zone and W29 is located downgradient of the capture zone. A comparison of the PCP time-concentration trends at these two wells is shown on Figure 6. As described in Section 5.3.5, the trend of PCP at W10A is not affected by pumping and has shown a decline in PCP since 2010 that is not associated with any other influence besides biodegradation of PCP. Since 2010 the PCP concentration at W29 has fluctuated. First, in 2011 and 2012 there was a small spike in PCP concentration at W29 (from less than 100 ug/L to 1,700 ug/L and 1,800 ug/L in 2011 and 2012, respectively). This appears to be attributed to the water main leak that was discovered and repaired in July 2011. Subsequently, the PCP concentration dropped to less than 100 ug/L in 2013 and 2014. In 2015 and 2016 concentrations spiked again, up to 6,600 ug/L in 2016. As described above, this spike in 2015-2016 may be due to a shorter flow distance from the LNAPL. Both of these spikes occurred while PCP concentration at W10A was declining. However, the sources of the changes at W10A and W29 between 2011 and 2016 are unrelated. This is supported by the observation over the last three years (2016 through 2019) where both wells have been declining.

The PCP concentration throughout the seasons at these wells (W22 and W26) do not illustrate decreases due to spring, summer, or fall rainfall recharge nor increases in winter (see time concentration graphs in Appendix E). Well W29 has been monitored annually since 1991, so no seasonal evaluation is possible. Specific observations are as follows:

- **W22** – There has been no seasonal trend apparent in PCP concentration at well W22, rather, the trend since 2005 (when mobile phase LNAPL was last present) has been relatively steady, except for:
  - The period of 2010 to 2011 during and shortly after the nearby water main leak described in Table D, footnote 1.
  - The period from July 2018 through July 2019 when PCP concentrations dropped from 5,200 ug/L to 13 ug/L.
- **W26** – As described in Table D, the proximity of residual phase LNAPL to well W26 results in PCP fluctuations due more to small changes in groundwater flow direction than in seasonal rainfall recharge.

5.4 PCP Distance Concentration Declines

The EPA guidance document on estimating and use of decay rates (Newell et. al. 2002) states that calculating distance-concentration rate constant is a positive indication that attenuation (i.e., biodegradation) is occurring downgradient of the source area (see Newell, 2002, at

page 10). This distance concentration approach is applied to two groundwater flow lines outside of the current groundwater capture zone. As described in Section 4.2 Groundwater Conceptual Site Model under Groundwater Flow Directions, these flow lines are characterized as the Northeast Profile and the Southeast Profile.

In addition to applying this approach to groundwater flow lines downgradient of a source area, the plume must be at steady state (Newell, 2002). The PCP concentration within the source areas of these profiles are generally at steady state concentration. Although the PCP concentration within the LNAPL is declining, the PCP concentrations in groundwater within the LNAPL footprint are generally steady state. This is illustrated in the distance-concentration graphs (Figures 8 through 11), where the concentrations for the years 2010 through 2019 are very similar, illustrating similar concentration-distance profiles through time. This is especially true for the Northeast and Southeast Profiles, which are outside the capture zone and unaffected by pumping at the Site. The one exception to this is the Northeast Profile for 2019. The PCP concentration at DFOMW12 is significantly lower in 2019 than in prior years, suggesting that the plume in 2019 is declining, and not in steady state conditions. However, the average trend line minimizes this date, so, overall, the trend can be considered in steady state.

5.4.1 Northeast Profile

The Northeast Profile consists of wells DFOMW12 to W13 to W18. The concentration-distance graph for this profile is shown in Figure 9 for PCP concentrations from July 2010 through July 2019, with an average trend line. This graph illustrates the decline in PCP in the short distance, and short time of travel, between wells DFOMW12 and W13. The only date when this decline between wells DFOMW12 and W13 was not carried forward to downgradient well W18 was in July 2011, when W18 was at a higher concentration. However, the trend returned the next year, so the cause of the increase in PCP at W18 in 2011 was very short-lived. The source of this increase is unknown, but may have been the water main leak that was discovered and repaired in July 2011.

Table 2 is an estimate of the biological decay rate using the method of Buscheck and Alcantar, as described by Newell (2002). Data used in this analysis is the groundwater velocity calculation for this profile, from Table A, and the slope of the concentration-distance trend line. The trend line is in the form of the first-order decay equation (i.e., $C/C_0=e^{-kt}$), where the slope is the k value. For this profile, the slope is 0.043. The decay rate calculation in Table 2 shows that degradation of PCP in the Northeast Profile is occurring at a decay rate of 0.062 day^{-1} or a half-life of 11 days. This can be viewed as the PCP concentration drops by ½ every 11 day flow distance.

As described in Section 6 Geochemical Data, the NE area is aerobic to slightly anaerobic. This high decay rate in an aerobic environment is consistent with the observations in the

literature (as described in Section 3.1 Literature Review) and supported by observations at a Wisconsin BRRTS Site, Penta Wood Products (as described in Section 3.2).

Future Reliability of this Trend – This trend has been occurring for at least the last 16 years since decline of the PCP concentration at W13 and has been occurring under the lower extraction rate (22 gpm) since June 2012. This groundwater profile is along a groundwater flowline that is outside of the capture zone and is only minimally affected by drawdown of the extraction system. Therefore, this trend is very reliable, certainly under the current pumping system, and is expected to be reliable under non-pumping conditions as well.

5.4.2 Northern, Line Source Profile

The Northern, Line Source Profile extends from well W02 through DFOMW11 and DFOMW12 then to well W28. The profile from W02 through DFOMW12 **does not** follow the current groundwater flow direction, as described in Section 4.2.3. Rather, this segment follows what appears to be a source of PCP along the line extending from W02 to DFOMW12 (i.e., a "Line Source") that acts as a source of PCP to groundwater flowing below this line toward the northeast and east. The segment from DFOMW12 to W28 **does** follow the current groundwater flow direction.

5.4.3 Southeast Profile

The Southeast Profile consists of wells W41 to W27 to W11 to W21 and occurs outside of the groundwater capture zone in an area with no or very minimal drawdown from the extraction system. The concentration-distance graph for this profile is shown in Figure 10 for PCP concentrations from July 2010 through July 2019, with an average trend line. This graph illustrates the decline in PCP in this distance, and time of travel, between wells W27 (downgradient of the LNAPL source) and W21. The profiles from 2010 through 2019 show a consistent pattern from the edge of the LNAPL source (well W27) to well W21. The PCP concentration at well W41 is somewhat variable, but it is located in an upgradient or sidegradient position with respect to the LNAPL source, so it is not an integral part of the concentration-distance analysis and is not incorporated into the trend line. Well W21 is consistently less than 10 ug/L, showing substantial decline from well W11 that ranges from 97 ug/L to 660 ug/L over this time frame. The 5 ug/L at well W21 in July 2019 sneaks above the other trend lines, but is still very consistent with the prior trend lines. This small detect merely indicates that the profile is truly downgradient of the PCP source and that groundwater migration is occurring in this direction, helping to validate the analysis.

The decay rate estimate, shown in Table 2, for the Southeast Profile is 0.008 day^{-1} or a half-life of 86 days.

As described in Section 6 Geochemical Data, the SE area is strongly anaerobic in the vicinity of the residual phase LNAPL (i.e., near wells W41 and W27) and becomes progressively more aerobic at wells W11 and W21. The lower decay rate in an anaerobic environment is consistent with the observations in the literature (as described in Section 3.1 Literature Review) and supported by observations at a Wisconsin BRRTS Site, Penta Wood Products (as described in Section 3.2). **Future Reliability of this Trend** – This trend has been occurring for at least the last 26 years, since the decline of the PCP concentration at W11 occurred in 1993 and has been occurring under the lower extraction rate (22 gpm) since June 2012. This groundwater profile is along a groundwater flowline that is outside of the capture zone and is only minimally affected by drawdown of the extraction system. Therefore, this trend is very reliable, certainly under the current pumping system, and is expected to be reliable under non-pumping conditions as well.

5.4.4 Stagnation Zone Profile

The Stagnation Zone Profile shown on Figure 4 is located directly downgradient of the capture zone. W22 is just outside the capture zone and certainly within the stagnation zone downgradient of the capture zone. Further downgradient the groundwater velocity will return to natural flow, so that between the W29 to W32 segment, it is expected that natural flow is re-established. The distance-concentration profile between W40 and W29 on Figure 11 is quite variable through time, which is explained below. However, the primary observation from this distance-concentration graph is that the decline in PCP between wells W29 and W32 is steep (i.e., a large decline during many dates) and is consistent through time. On only one date is there a detect of 5 ug/L at well W32, which is evidence that well W32 is in fact downgradient of the PCP plume.

This variability in this Stagnation Zone Profile distance-concentration graph is probably due to small changes in groundwater flow and the presence of the profile being parallel to the LNAPL footprint. During periods when there is even a small component of flow from the north to the southeast, the PCP concentration would be higher, due to a shorter distance (and shorter time) for degradation of PCP between the LNAPL and either well W26 or W29. When flow is more to the east, PCP degradation would have a greater distance and time for degradation to occur. In addition, if there is a component of flow from the south, groundwater reaching well W26 has a much longer flow from the LNAPL source of PCP to groundwater. In this situation, the W26 concentration would be expected to be lower than normal. Therefore, a second graph is presented on Figure 11,

showing selected dates when the concentration at well W26 is relatively high and the concentration at well W29 is relatively low. These dates (July 2006, 2008, 2009, 2013, and 2017) may reflect migration directly east from the LNAPL shown at W22 and W40.

Future Reliability of this Trend – The reliability of the trend between W29 and W32 is strong, in that this decay has been occurring for the duration of monitoring at the site (i.e., since before 1990). As shown on the full data set graph in Figure 11, there is a large degree of fluctuation in concentrations at W26 and W29, probably due to small changes in groundwater flow direction. It is expected that this trend (i.e., the large variability) will continue due to the proximity of the LNAPL to these wells. More importantly, and very reliable is the PCP concentration decline as migration away from the LNAPL occurs.

5.4.5 Centerline Profile

The Centerline Profile is shown on Figure 4 to be from upgradient to through the center of the Wauleco property and then to the east through well W10A. The distance-concentration graph for this profile (see Figure 12) illustrates the increase from upgradient of the plume to the concentration in the center of the Site, as shown by the extraction system's influent concentration to the treatment system. The decline in PCP downgradient of the extraction system wells, at W03A is likely due to both biodegradation and lower PCP concentrations within the LNAPL near W03A than in the center of the Site. This is consistent with the observation in the Residual Phase LNAPL Investigation Tech Memo that concluded the remaining mass of PCP within LNAPL near the former dip tank was greater than in other areas of the LNAPL footprint. The increase in PCP concentration at W10A is probably due to the PCP concentration in LNAPL near W10A being somewhat greater than near W03A. However, concentration at W10A for the dates shown on Figure 12 has been continuously declining, so that this trend is moving in the direction of becoming a continuous decrease from on the Site. However, in the meantime, this profile simply reflects the probable PCP concentration within the residual phase LNAPL nearest to the wells and cannot be used for estimating biodegradation rates, as this method cannot be used within an area with a source of PCP to groundwater.

Reliability of this Trend – The trend shown on the Centerline Profile distance-concentration graph will probably continue in the pattern shown in Figure 12 while the LNAPL remains a continuing source of PCP to groundwater. However, it is expected that the PCP concentration at W10A will continue to decline, given that is has been declining since 2001 and that it is outside the capture zone of the groundwater extraction system.

5.5 Summary

In summary the time concentration graphs demonstrate that PCP is declining in most locations across the Site. These include the following:

- Wells located on-Site and inside the capture zone:
  - PCP in the extracted groundwater on-Site has declined within the LNAPL footprint. While the PCP concentration is quite variable in the extraction wells, the PCP in the extracted water from below the LNAPL has declined through time until the reduction in pumping rate to 22 gpm, when the concentration has generally stabilized. This trend, while variable, is expected to continue as the PCP concentration within the LNAPL is continuing to decline as well (see Section 5.3.1).
  - Even wells inside the capture zone and within the LNAPL footprint, like W03A and W17 have shown dramatic declines (see Section 5.3.2).
- Wells located outside the capture zone:
  - Wells outside the capture zone, northeast and southeast of the Site and downgradient of the LNAPL (e.g., W02, W13, W28, and W18 to the northeast and W11 and W21 to the southeast) have shown declining PCP concentrations in areas where groundwater flow has been continuously from the remaining LNAPL footprint. These declines in PCP concentration are not due to changes in flow direction. Therefore, the primary source of these declines is due to biodegradation of PCP, and is expected to continue (see Section 5.3.3).
  - Well W10A, located outside of the capture zone, within the LNAPL footprint, near the River, has shown a significant decline in PCP concentration. This decline is demonstrated to not be associated with any other influence, such as flow from the River into the aquifer. Rather, the primary explanation for this decline is biodegradation of PCP in the aquifer (see Section 5.3.5).
  - Concentration-Distance trends along the Northeast Profile and Southeast Profile are along naturally occurring flow lines, outside the capture zone and outside the cone of depression. Analysis of these trend lines considers the effect of dispersion, which is the only other potential cause for a decline in concentration. These trends demonstrate that there is a strong and consistent declining trend away from the LNAPL that can only be explained by biodegradation of the PCP (see Section 5.4.1 NE Profile and Section 5.4.2 SE Profile).
- Wells located adjacent to the LNAPL:
  - Wells adjacent to the LNAPL southeast of the Site (wells W41 and W27) have declined from historic highs, but have stabilized in a range of 1,000 ug/L to 9,000 ug/L, due to the continued source of PCP from the LNAPL (see Section 5.3.4).

- Wells in areas adjacent to remaining LNAPL where slight variations in flow direction may result in flow from a distant LNAPL source to flow from a nearby LNAPL source (e.g., W26 and W29) have varying PCP concentrations (see Section 5.3.6).

- The biological decay rates for PCP is greater in the Northeast Profile (11 day half-life) than in the Southeast Profile (86 day half-life) (see Section 5.4).
- These trends along the Northeast and Southeast Profiles are outside the zones of capture and cone of depression (as described in Section 4.2.3), have been occurring for many years, since at least 2012 with the reduction in pumping, and are expected to continue even with shut down of the extraction system (see Section 5.4).
- None of the wells monitored with seasonal sampling for PCP indicate variations in PCP concentrations suggestive of dilution due to spring, summer, or fall rainfall recharge. Rather, their general multi-year PCP concentration declines dwarf any potential seasonal changes and/or there are as many peaks in summer as in winter revealing no seasonality patterns.

In summary, the reductions in PCP concentration, where not due to variations in flow distance from a source of LNAPL (like at W26 and W29), evidence substantial declines in PCP concentration that are due to biodegradation of PCP.

Section 6
Geochemical Data

The secondary line of evidence for natural attenuation in the EPA Guidance is to use geochemical data to "demonstrate *indirectly the type(s) of natural attenuation processes active at the site, and the rate at which such processes will reduce contaminant concentrations to required levels*." (EPA, 1998, page 6). In general, this is asking whether the geochemical conditions are consistent with, and would allow for, degradation of the constituents of concern. For example, biodegradation of chlorinated solvents (e.g., tetrachloroethene or PCE) is known to occur under anaerobic conditions. The purpose of the geochemical evaluation for chlorinated solvents would be to determine whether anaerobic conditions are present in areas where PCE is shown to be declining. In the case of PCP, it is shown to biodegrade under both aerobic and anaerobic conditions but occurs at a faster rate under aerobic conditions (see discussion in Section 3).

Appendix D presents an evaluation of the geochemical conditions at the Site. As shown on Table D-1, the background groundwater quality (at wells W08 and W01A) are aerobic to weakly anaerobic whereas downgradient locations in close proximity to LNAPL (e.g., W10A, W22, W27, and W40) are all strongly anaerobic, indicating a large amount of biological activity consuming the oxygen and nitrate and mobilizing manganese and iron for electron acceptors.

The degradation of PCP in the Northeast Profile is degrading at a faster rate (at a decay rate of 0.06 day^{-1} or a half-life of 11 days) than in the Southeast Profile (at a decay rate or half-life of 0.0081 day^{-1} or half-life of 86 days). The geochemical data and evaluation in Appendix D demonstrate that the Northeast Profile is in aerobic or slightly anaerobic (i.e., nitrate reducing) conditions and that the Southeast Profile is strongly anaerobic near the LNAPL source (at and downgradient of well W27), turning to progressively more aerobic at wells W11 and W21.

The differences in the geochemical conditions in the Northeast Profile (generally aerobic) and the Southeast Profile (generally anaerobic) are probably due to the position of the residual phase LNAPL with respect to the groundwater flow directions. The Northeast Profile is on the northern portion of the residual phase LNAPL area, with the typical groundwater flow direction to the northeast (see Figure C-1). However, as shown on Figure C-2, there are occasions during some times of the year when there is a more southerly flow direction (shown as a gradient over 90 degrees on Triangle A and over 135 degrees on Triangle B on Figure C-2). These variations in flow direction show that groundwater from north of the residual phase LNAPL, containing a higher dissolved oxygen content, can occasionally move into the area, creating more aerobic conditions than flow from under the residual phase LNAPL area.

In contrast, the Southeast Profile is located south of the residual phase LNAPL area. Therefore, the occasional southern component of groundwater flow, shown on Triangles B (Figure C-2) and C (Figure C-3), cause more flow from the residual phase LNAPL area to flow to the south, affecting wells along the Southeast Profile. This water would be strongly anaerobic. Therefore, the occasional changes in groundwater flow direction would result in more anaerobic water in the Southeast Profile, promoting the anaerobic conditions from the predominantly upgradient residual phase LNAPL area (i.e., near well W41).

These flows from the north into the Northeast Profile and the Southeast Profile are very short term, as shown in the gradient direction graphs in Appendix C. However, they may have a longer term effect on the redox conditions (promoting aerobic conditions in the Northeast Profile and anaerobic conditions in the Southeast Profile) during the time frame of the predominant flow path in these two profiles.

These geochemical conditions are consistent with the decay rates in these two profiles, showing fast degradation in the aerobic conditions in the Northeast Profile and somewhat slower degradation in the anaerobic conditions in the majority of the Southeast Profile. The change from anaerobic to aerobic or weakly anaerobic in the downgradient portion of the Southeast Profile is beneficial and would tend to increase the degradation rate in this portion of the profile.

Section 7
Findings and Conclusions

7.1 Findings

Key findings presented in this Technical Memorandum include the following:

- **Literature Review of Biodegradation Mechanisms:** Numerous publications demonstrate that PCP is biodegradable with naturally occurring bacteria and is similar to biodegradation of BTEX in that it occurs under aerobic or anaerobic conditions with typically no readily observable daughter products. This biodegradation occurs:
  - With PCP as the sole substrate but occurs more quickly when another biodegradable substrate is present (Bosso, 2014) (e.g., a petroleum hydrocarbon like mineral spirits, WDNR, 2014a).
  - Under aerobic or anaerobic conditions, although degradation occurs faster aerobically,
  - To completely remove PCP from the environment, with typically the only degradation product being low concentrations of chloride ions (which may not be detectable in an urban environment). This degradation is similar to BTEX compounds, in that BTEX degradation has no observable decay products and biodegradation relies on demonstrating reduction of the constituents and the presence of biological activity.
  - At the Penta Woods Products Site, in Siren Wisconsin, with demonstrated PCP biodegradation similar to the literature experience, i.e., complete degradation, no observable degradation products, faster in aerobic than aerobic conditions, but occurring in both. They also concluded that this biodegradation would support an MNA remedy for the site.
  - At the above ground treatment system at Wauleco, again with no observable degradation products.
  - The only natural attenuation processes occurring that can reduce the PCP concentration in groundwater at the Wauleco site are dispersion and biodegradation. Other natural attenuation processes, volatilization, radioactive decay, and chemical or biological stabilization do not occur with PCP, and sorption of PCP is not a significant transport factor at Wauleco. Dilution, which would occur with discharge to surface water bodies, does not occur in groundwater except by dispersion.

- **Current Conceptual Site Models:**
  - The LCSM describes conditions of the remaining residual phase LNAPL as:
    - Having been reduced by 90.6% from the original residual saturation.
    - Having a reduction in the average PCP concentration within the LNAPL of 94.6%.
    - The presence of a majority of the remaining PCP being within the vicinity of the former dip tank area and geologically confined in tighter soils
  - Groundwater flow along the Northeast and Southeast Profile lines are flow lines outside the zones of capture and cone of depression formed by the groundwater extraction system. Therefore, flow along these profile lines occur under natural conditions, unaffected by pumping on the Site.
  - Seasonal trend evaluations of the PCP in groundwater at wells with multiple samples collected per year shows that either the overall declines in PCP through multi-year trends dwarf any seasonal changes or that the peaks and valleys in PCP concentration are not specific to a particular season. Therefore, there is no indication that rainfall recharge results in significant dilution during the spring, summer, or fall seasons.
- **Lines of Evidence for PCP Degradation at Wauleco:**
  - EPA guidance (Newell et. al. 2002) states that calculating distance-concentration rate constant is positive in indicating that attenuation (i.e., biodegradation) is occurring downgradient of the source area.
  - The distance-concentration trend analysis at Wauleco considers biodegradation and dispersion, the only natural attenuation processes for PCP in the groundwater at the Wauleco Site and quantifies that biodegradation of PCP is occurring with half-lives of 11 days along the Northeast Profile and 86 days along the Southeast Profile.
  - The time-concentration trends support that biodegradation is occurring, showing significant declines in concentration along the Northeast and Southeast Profiles as well as within and downgradient of the capture zone; for example, at W10A, where significant PCP reductions have been occurring since 2008, with no influence from inflow of River water or changes in groundwater flow.
  - The reductions in PCP concentration through time and with distance downgradient of the residual phase LNAPL are reliable and are expected to continue, with or without pumping along the Northeast and Southeast Profile lines as these are outside the influence of the extraction system.
  - Within the influence of the extraction system, concentration reductions are also expected to continue during pumping, but there may be some concentration increase after shutdown of the extraction system. However, natural biodegradation of PCP will continue to occur under non-pumping conditions.

- Site-specific testing that necessary bacteria are present at the Wauleco site has not been performed. However, consistent with EPA Guidance, this is not a requirement to demonstrate whether natural attenuation (NA) is occurring. The EPA Guidance states that historical data (EPA line of evidence #1) and data characterizing the nature and rates of natural attenuation processes (EPA line of evidence #2) should be presented for all sites. Where data from lines of evidence 1 and 2 need additional support, then data from microcosm studies (EPA line of evidence #3) may also be necessary. For Wauleco, lines of evidence 1 and 2 do not need additional support. Nevertheless, Wauleco will perform a study to assess the presence of necessary bacteria.

- **Geochemical Data:**
  - The geochemical data at Wauleco demonstrate that the aerobic to weakly anaerobic groundwater upgradient of Wauleco is strongly affected by biological activity as it migrates across the Site, resulting in consumption of electron acceptors (i.e., decrease in nitrate and increases in dissolved manganese and iron).
  - Geochemical conditions in the Northeast Profile line are generally aerobic to weakly anaerobic, which promotes the high PCP degradation rate shown in the Northeast Profile.
  - In contrast, the geochemical conditions along the Southeast Profile are strongly anaerobic in the western portion of the profile (e.g., well W27) and turning to progressively more aerobic at downgradient wells W11 and W21. This is consistent with the lower PCP degradation rate shown in the Southeast Profile.
  - The higher PCP degradation rate in the aerobic NE Profile and slower PCP degradation rate in the anaerobic SE Profile are consistent with the literature findings, as described in Section 3.1, and at a Wisconsin BRRTS site, Penta Wood Products, as described in Section 3.2.

7.2 Conclusions

The conclusions from this analysis are:

- That PCP at Wauleco is degrading via biological degradation methods that are well accepted and described in the literature and as has been demonstrated at the Penta Woods site in Wisconsin. The PCP degradation at Wauleco is faster in an aerobic area (the NE Profile Line) than an anaerobic area (SE Profile Line), which is also consistent with conditions documented at other sites in the literature and at Penta Woods. The biodegradation at Wauleco will very reliably continue to occur under the current pumping conditions and will also continue to occur under non-pumping conditions.
- 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 directions.

- Monitoring wells W26 and W29 are a special case because they are located adjacent to the LNAPL footprint but are not consistently downgradient of the LNAPL. Therefore, these wells are subject to small changes in groundwater flow directions that result in differences in flow distances from the LNAPL and, hence, fluctuations in PCP concentrations at these wells. However, as shown on the Stagnation Zone Profile concentration-distance graph (Figure 11), these intermittent PCP concentrations are degraded before reaching the next downgradient well.

Section 8
References

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Table 1
Historical PCP Concentration in Mobile Phase LNAPL
Wauleco, Inc.
Wausau, Wisconsin

SAMPLE DATE	DATE OF ANALYSIS	METHOD	ANALYSIS RESULT	
			mg/L	% w/w
Jun-86	Jun-86	Total[1]		3.23%
26-Aug-96	26-Aug-96	TCLP[2]	11,700	1.46%
16-Feb-98	16-Feb-98	TCLP[2]	25,000	3.13%
2-Sep-99	2-Sep-99	TCLP[2]	11,000	1.38%
23-Oct-00	23-Oct-00	TCLP[2]	5,400	0.68%
12-Oct-01	12-Oct-01	TCLP[2]	5,000	0.63%
19-Sep-02	19-Sep-02	TCLP[2]	4,200	0.53%
23-Jul-03	23-Jul-03	TCLP[2]	10,000	1.25%
7-Mar-05	7-Mar-05	TCLP[2]	1,400	0.18%
18-Jul-05	18-Jul-05	TCLP[2]	1,572	0.20%
22-Oct-06	22-Oct-06	TCLP[2]	6,200	0.78%
10-Nov-08	10-Nov-08	TCLP[2]	790	0.10%
8-Jul-09	8-Jul-09	TCLP[2]	530	0.07%
3-Sep-10	3-Sep-10	TCLP[2]	<1,000	<0.13%
Samples collected over	18-Apr-18	Total[3]	7700	0.96%
time in 2017 and 2018	5/11/2018	TCLP[4]	430	0.05%
Average Cryogenic Sampling	6/8/2019	Total Cryogenic Sampling	2160	0.27%

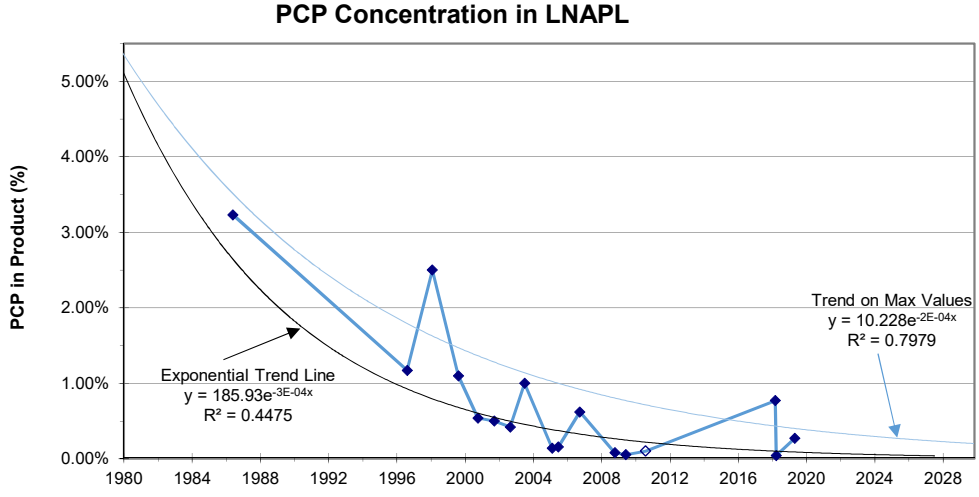
Footnotes:

[1] Analysis presented in Keystone, 1986 Site Characterization Report, September 5, 1986, Appendix D, Table 2

[2] Rader Environmental samples of recovered product prior to destruction analyzed by TCLP methods.

[3] Alpha Analytical total analysis using GC/MS-SIM 8270-SIM

[4] CT Labs TCLP Analysis



All analyses by TCLP, except as noted as total.
Open symbol indicates non-detect, plotted at detection limit.

Details on 1986 Keystone sample analyses and average for PCP Concentrations in product

WELL	PCP CONCENTRATION IN PRODUCT (%)	AREA OF SITE
W-7	3.10%	Central portion of product extent
W-4	3.30%	Central portion of product extent
W-5	3.30%	Central portion of product extent
Average of wells W-7, W-4, and W-5	3.23%	Representative of product being recovered
W-15	1.10%	Upgradient extent of product, in the SE lobe of product
W-6	0.30%	Upgradient extent of product

Table 2
Biodegradation Decay Rate Calculation
Wauleco, Inc.
Wausau, Wisconsin

Objective:
Estimate PCP Decay Rate in groundwater

Methods:
1. Estimate decay rate using groundwater concentration profile logarithmic decay curve and converting the slope of the decay curve to the decay rate using the Buscheck and Alcantar (1995) methods.

2. Estimate mass decay rate based on 1st order decay equation and dissolved phase PCP concentration declines along groundwater flow lines.

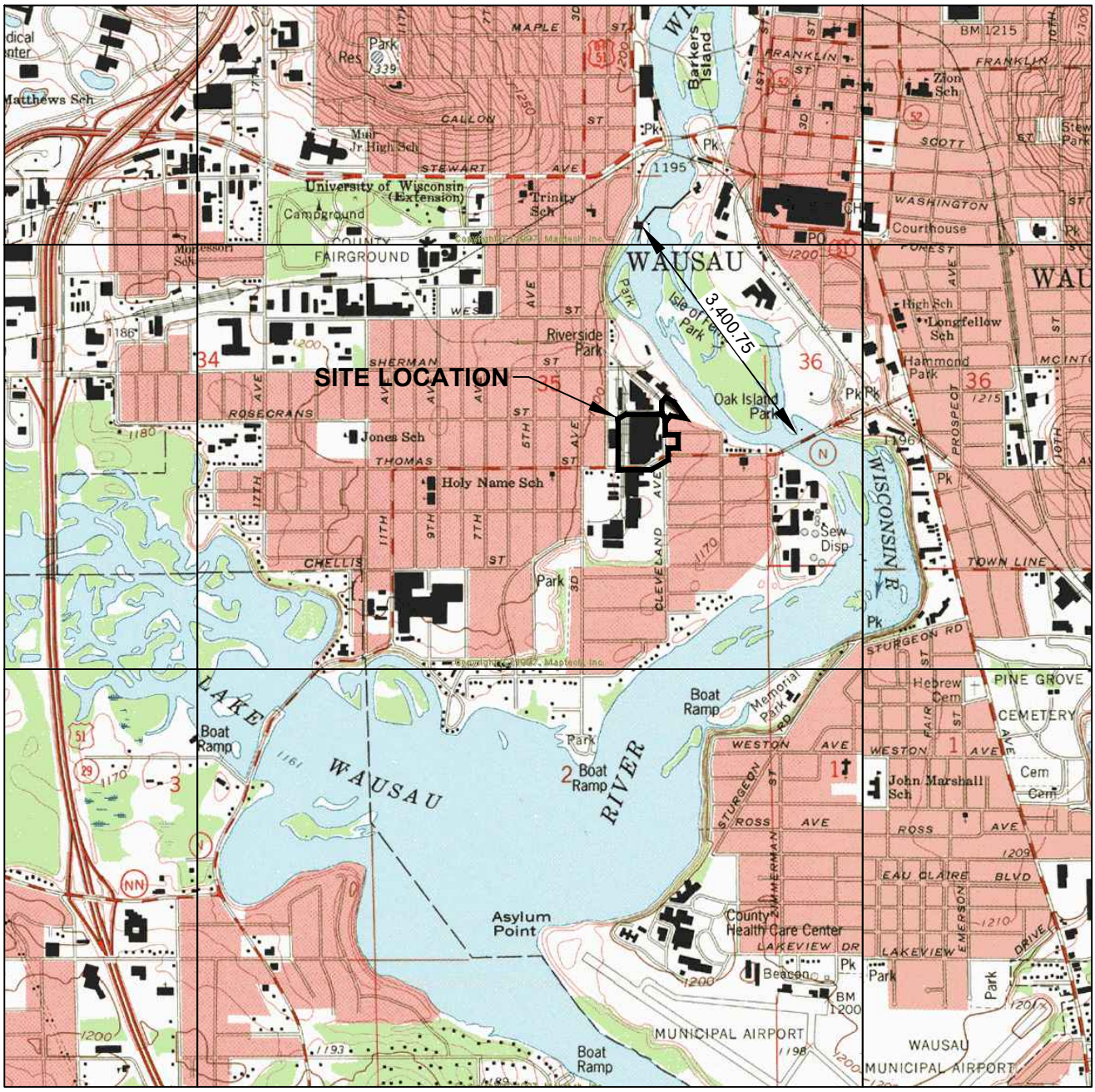
	Northeast Profile	**Southeast Profile**
1. Decay Rate Estimate		
Slope from Respective Distance Concentration Graphs	0.043	0.009
Groundwater Velocity (Vc) (ft/day)	1.0	0.6
Dispersivity (ft) (5% of plume length of 200 ft. in Northeast (Profile and 1,100 ft. in Southeast Profile)	10	55
Decay rate (l) see note 1 for method. (day^{-1})	0.0615	0.0081
Half Life (days)	11	86

1. Calculation of decay rate from Buscheck and Alcantar (1995) as in 1998 EPA Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Groundwater. EPA/600R/R-98/128.

$$\lambda = \frac{v_c}{4\alpha_x}\left(\left[1+2\alpha_x\left(\frac{k}{v_x}\right)\right]^2 - 1\right) \qquad \text{eq. C.3.32}$$

Where:
λ = first-order biological rate constant
v_c = retarded contaminant velocity in the x-direction
α_x = dispersivity
k/v_x = slope of line formed by making a ln-linear plot of contaminant concentration versus distance downgradient along flow path

Prepared by: K. Quinn 1/15/2020
Checked by: L. Auner 1/20/2020



WISCONSIN

QUADRANGLE LOCATION

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

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PROJECT:

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LINES OF EVIDENCE OF PCP DEGRADATION
WAUSAU, WISCONSIN

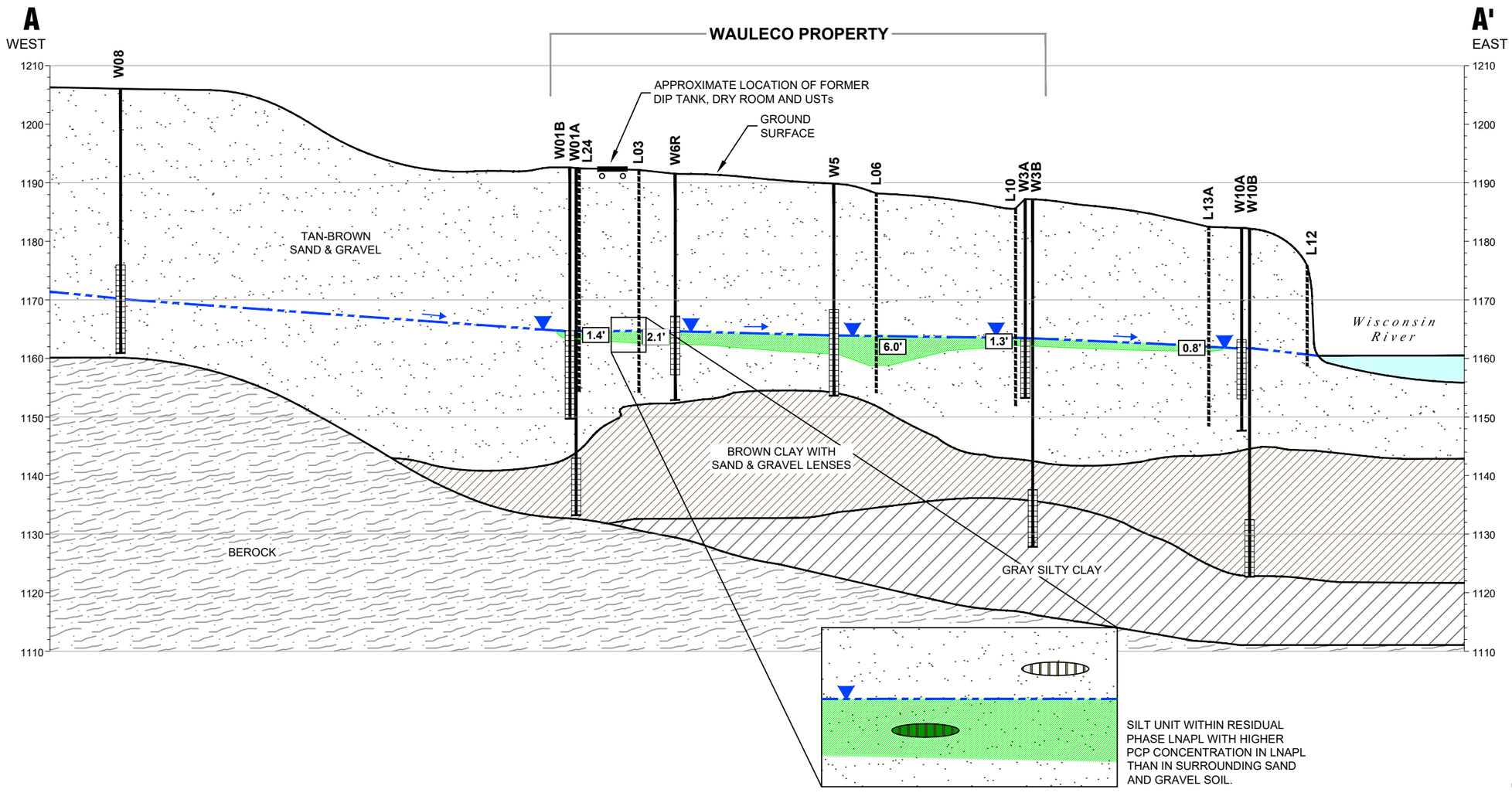
TITLE:

SITE LOCATION MAP

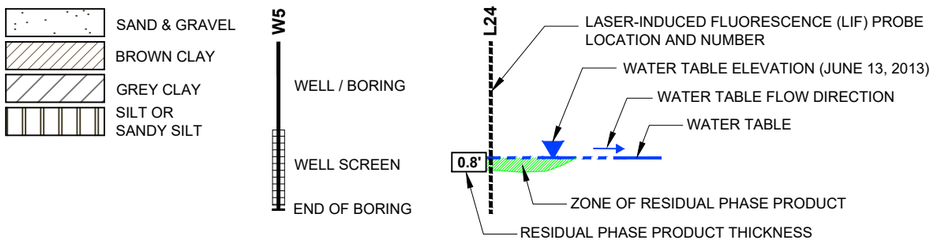
DRAWN BY:	T. FIEBRANZ
CHECKED BY:	K. QUINN
APPROVED BY:	B. IVERSON
DATE:	MARCH 2020
PROJ. NO.:	189597.0009 PH 3 TASK 1
FILE:	189597.0009.03.dwg

FIGURE 1

GENERALIZED GEOLOGIC CROSS-SECTION A-A'



LEGEND



NOTES

1. LIF SURVEY PERFORMED IN 2013 AND 2015.
2. GENERALIZED GEOLOGIC CROSS-SECTION BASED ON 1985 EDER ASSOCIATES CROSS-SECTION.

PROJECT:
WAULECO, INC.
LINES OF EVIDENCE OF PCP DEGRADATION
WAUSAU, WISCONSIN

TITLE:
CURRENT LNAPL CONCEPTUAL SITE MODEL

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CHECKED BY:	K. QUINN		
APPROVED BY:	B. IVERSON		**FIGURE 2**
DATE:	MARCH 2020		

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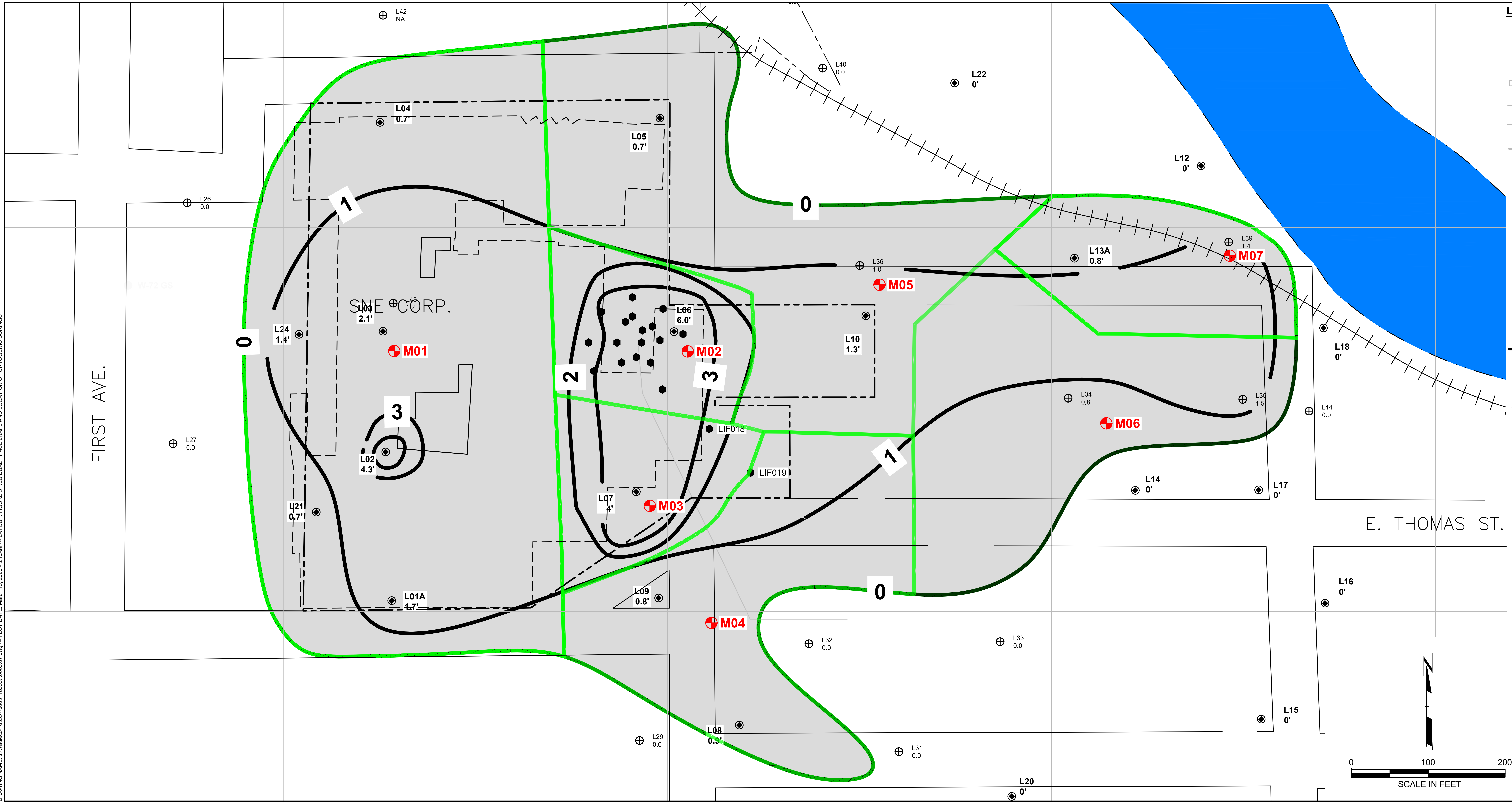
FILE NO.: 189597.0009.06.dwg

11:7 -- ATTACHED XREFS: -- ATTACHED IMAGES:
DRAWING NAME: J:\Waulleco\189597\0009\ 189597.0009.06.dwg -- PLOT DATE: March 13, 2020 - 5:16AM -- LAYOUT: FIGURE 12 CURRENT LNAPL CONCEPTUAL SITE MODEL

Version: 2017-03-03



LEGEND

W17 — MONITORING WELL LOCATION AND NUMBER

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)

-2003 & 2005 LIF LOCATIONS (LIF001-LIF017 SHOWN BUT NOT LABELED)

L22 0' — - 2013 LIF LOCATION - THICKNESS OF LIF RESPONSE

L24 1.4' — -2015 LIF PROBE LOCATION -THICKNESS OF LIF RESPONSE

M06 — CRYOGENIC RESIDUAL PHASE LNAPL INVESTIGATION BORING LOCATIONS

AREAS REPRESENTED BY CRYOGENIC BORINGS M01 THROUGH M07

0 — ESTIMATED THICKNESS OF RESIDUAL PHASE LNAPL

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. LASER INDUCED FLUORESCENCE (LIF) SURVEY COMPLETED JUNE 2-13, 2015 AND JUNE 11-13, 2013 BY TRC AND COLUMBIA TECHNOLOGIES, INC.
3. LIF RESULTS FROM 2003 AND 2005 WERE ALSO USED TO ESTIMATE THE THICKNESS OF RESIDUAL PHASE LNAPL.
4. THICKNESS OF LIF RESPONSE AT L06 WAS CORRECTED FROM AUGUST 25, 2015 MEMORANDUM, WAULECO: EXTENT OF RESIDUAL PHASE LNAPL/2015 LIF SURVEY, FIGURE 1, LIF SURVEY RESULTS.

PROJECT:
WAULECO, INC.
LINES OF EVIDENCE OF PCP DEGRADATION
WAUSAU, WISCONSIN

TITLE:
RESIDUAL PHASE LNAPL AND LOCATION OF CRYOGENIC BORINGS

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

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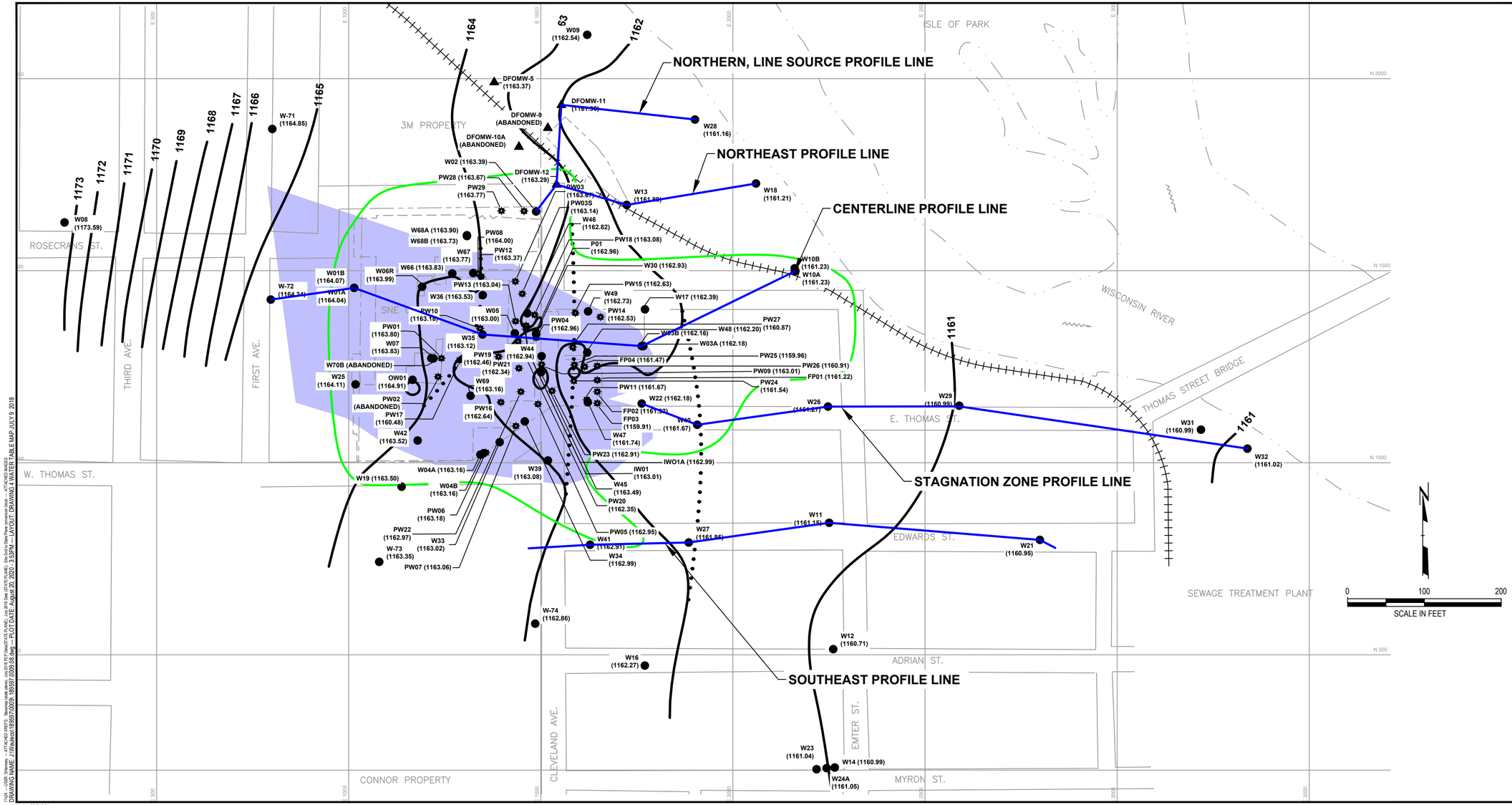

LEGEND

- 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
- 1161 — WATER TABLE ELEVATION CONTOUR. DOTTED CONTOUR SHOWS INTERPRETED NON-PUMPING GROUNDWATER ELEVATION.
- OUTLINE OF RESIDUAL PHASE LNAPL
- DISTANCE - CONCENTRATION PROFILE LINES

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. WATER ELEVATIONS OBTAINED BY TRC ON JULY 9, 2018. ON THIS DATE, THE PUMPING RATE OF THE GROUNDWATER EXTRACTION SYSTEM WAS APPROXIMATELY 22.5 GPM
3. 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.

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LINES OF EVIDENCE OF PCP DEGRADATION
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TITLE:
WATER TABLE MAP JULY 9 2018

DRAWN BY:	T. FIEBRANZ	PROJ NO.:	189597.0009 PH 3 TASK 1
CHECKED BY:	K. QUINN		
APPROVED BY:	B. IVERSON		**FIGURE 4**
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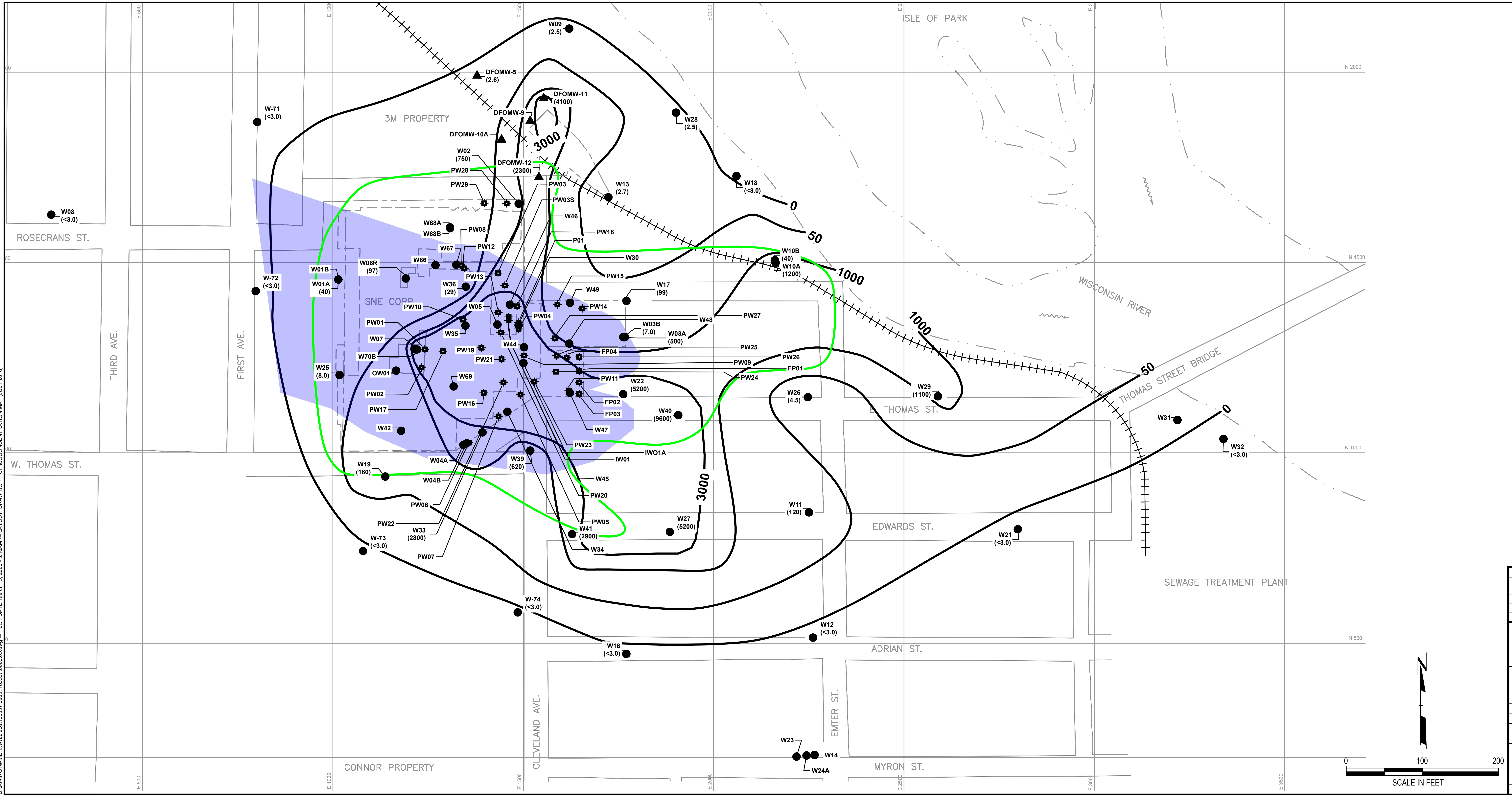

LEGEND

- 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

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	APP'D.

PROJECT:

WAULECO, INC.
LINES OF EVIDENCE OF PCP DEGRADATION
WAUSAU, WISCONSIN

TITLE:

PCP ISOCONCENTRATION MAP (JULY 2018)

DRAWN BY:	T. FIEBRANZ	PROJ NO.:	189597.0009 PH 3 TASK 1
CHECKED BY:	K. QUINN		
APPROVED BY:	B. IVERSON		FIGURE 5
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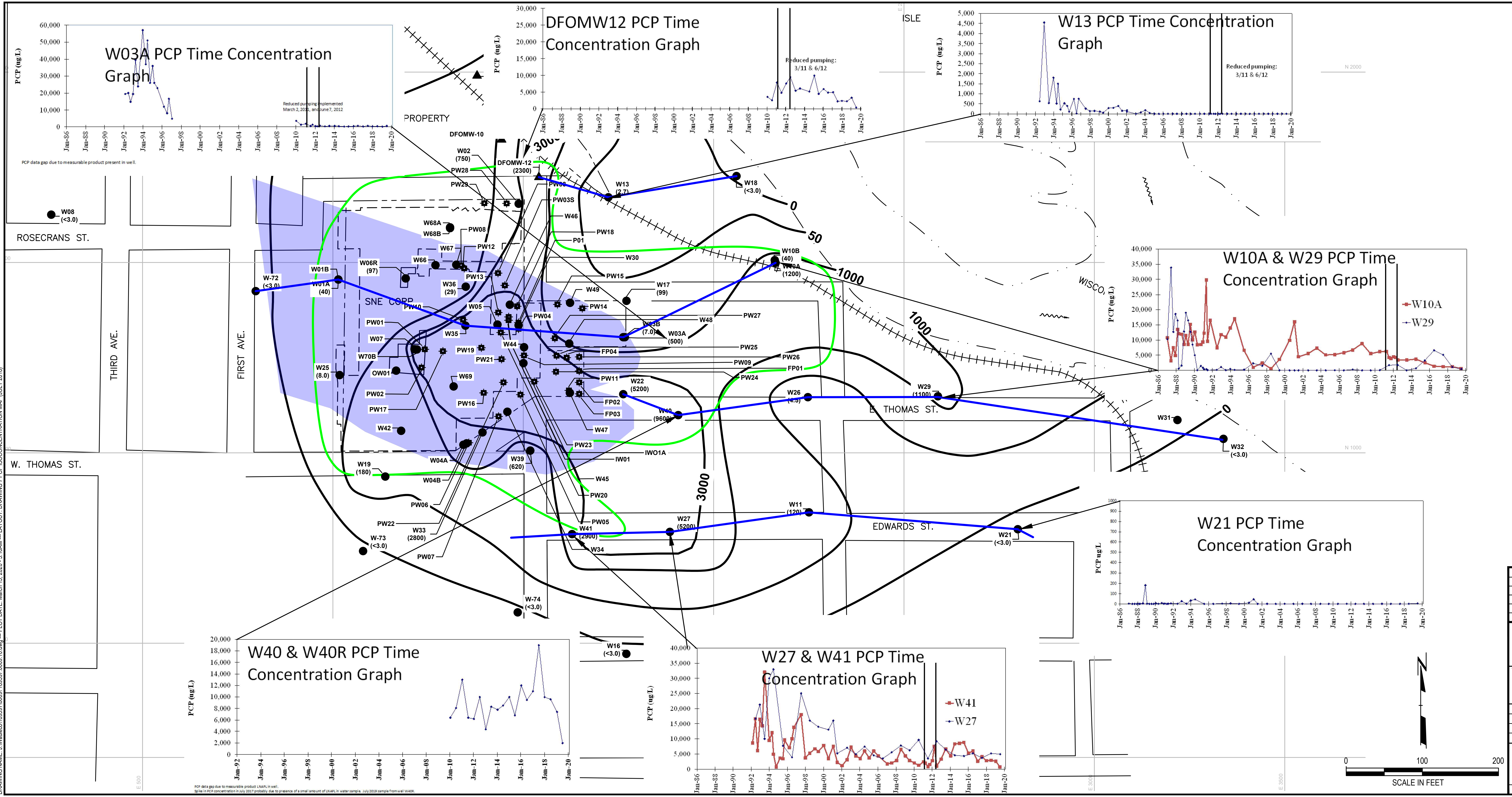

LEGEND

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

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.
LINES OF EVIDENCE OF PCP DEGRADATION
WAUSAU, WISCONSIN

TITLE:
PCP GRAPHS ON ISOCONCENTRATION MAP (JULY 2018)

DRAWN BY:	T. FIEBRANZ	PROJ NO.:	189597.0009 PH 3 TASK 1
CHECKED BY:	K. QUINN		**FIGURE 6**
APPROVED BY:	B. IVERSON		
DATE:	MARCH 2020		

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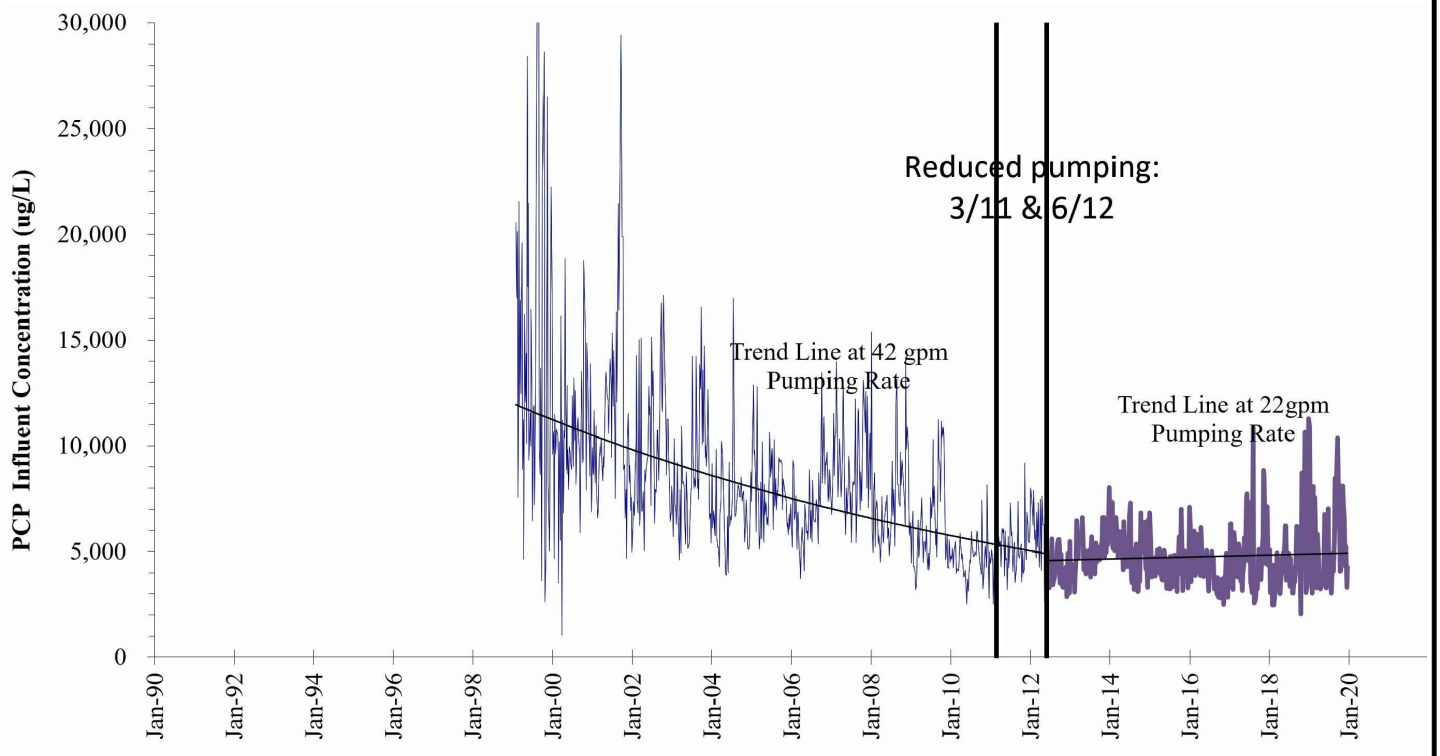
FILE NO.: 189597.0009.10.dwg



PROJECT:	**WAULECO, INC.** **LINES OF EVIDENCE OF PCP DEGRADATION** **WAUSAU, WISCONSIN** **WAUSAU, WISCONSIN**
TITLE:	**PCP CONCENTRATION** **EXTRACTION WELLS INFLUENT** **TO TREATMENT SYSTEM**
DRAWN BY: T. FIEBRANZ	PROJ NO.: 189597.0009 PH 3 TASK 1
CHECKED BY: K. QUINN	**FIGURE 7**
APPROVED BY: B. IVERSON	
DATE: MARCH 2020	

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Lowell, MA 01854
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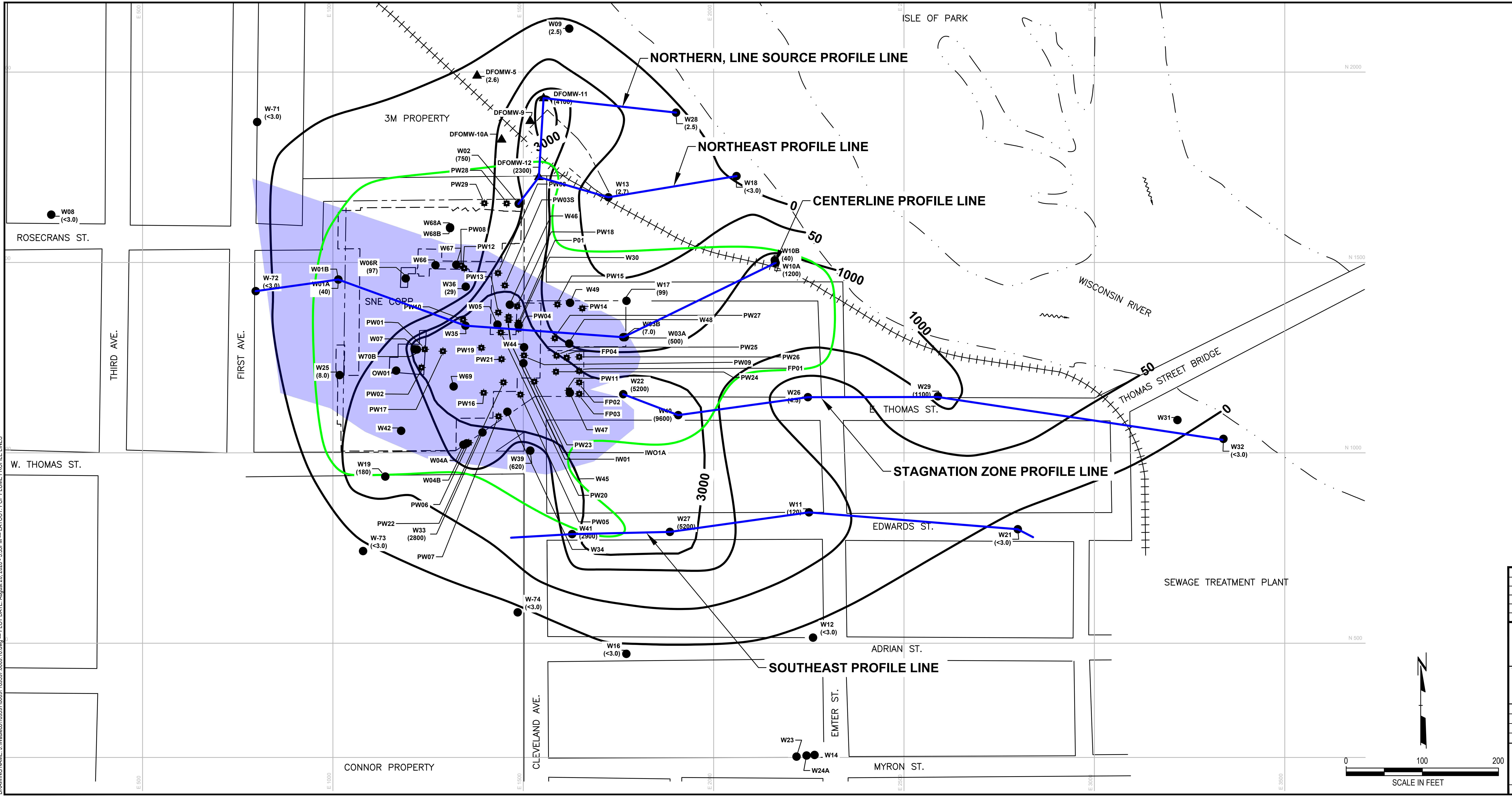
FILE NO.: 189597.0009.0013.dwg



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

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	APP'D.

PROJECT:

WAULECO, INC.
LINES OF EVIDENCE OF PCP DEGRADATION
WAUSAU, WISCONSIN

TITLE:

PCP PLUME PROFILE LINES

DRAWN BY:	T. FIEBRANZ	PROJ NO.:	189597.0009 PH 3 TASK 1
CHECKED BY:	K. QUINN		**FIGURE 8**
APPROVED BY:	B. IVERSON		
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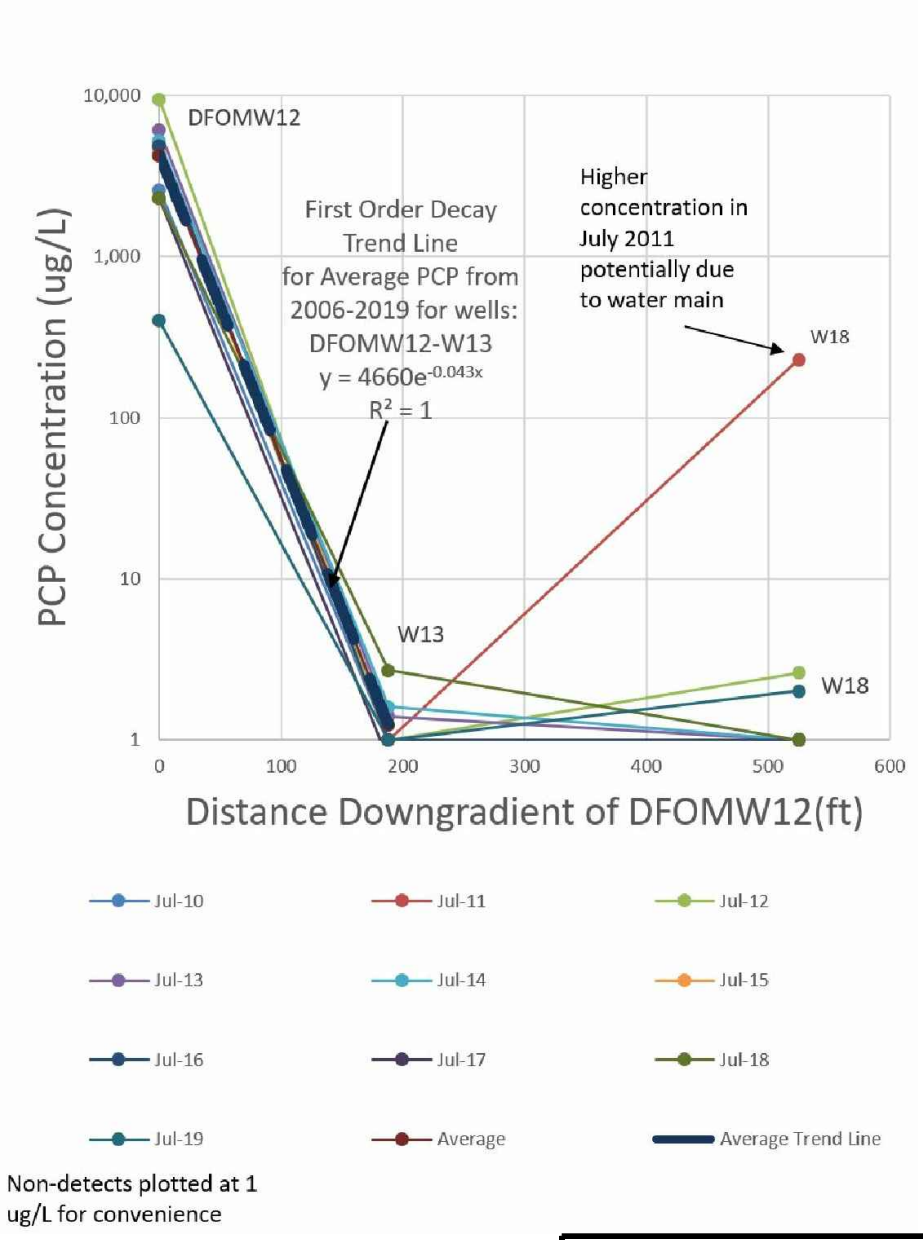

Non-detects plotted at 1 ug/L for convenience

PROJECT:	WAULECO, INC. LINES OF EVIDENCE OF PCP DEGRADATION WAUSAU, WISCONSIN WAUSAU, WISCONSIN
TITLE:	NORTHEAST PROFILE DISTANCE - CONCENTRATION GRAPH
DRAWN BY: T. FIEBRANZ	PROJ NO.: 189597.0009 PH 3 TASK 1
CHECKED BY: K. QUINN	FIGURE 9
APPROVED BY: B. IVERSON	
DATE: MARCH 2020	

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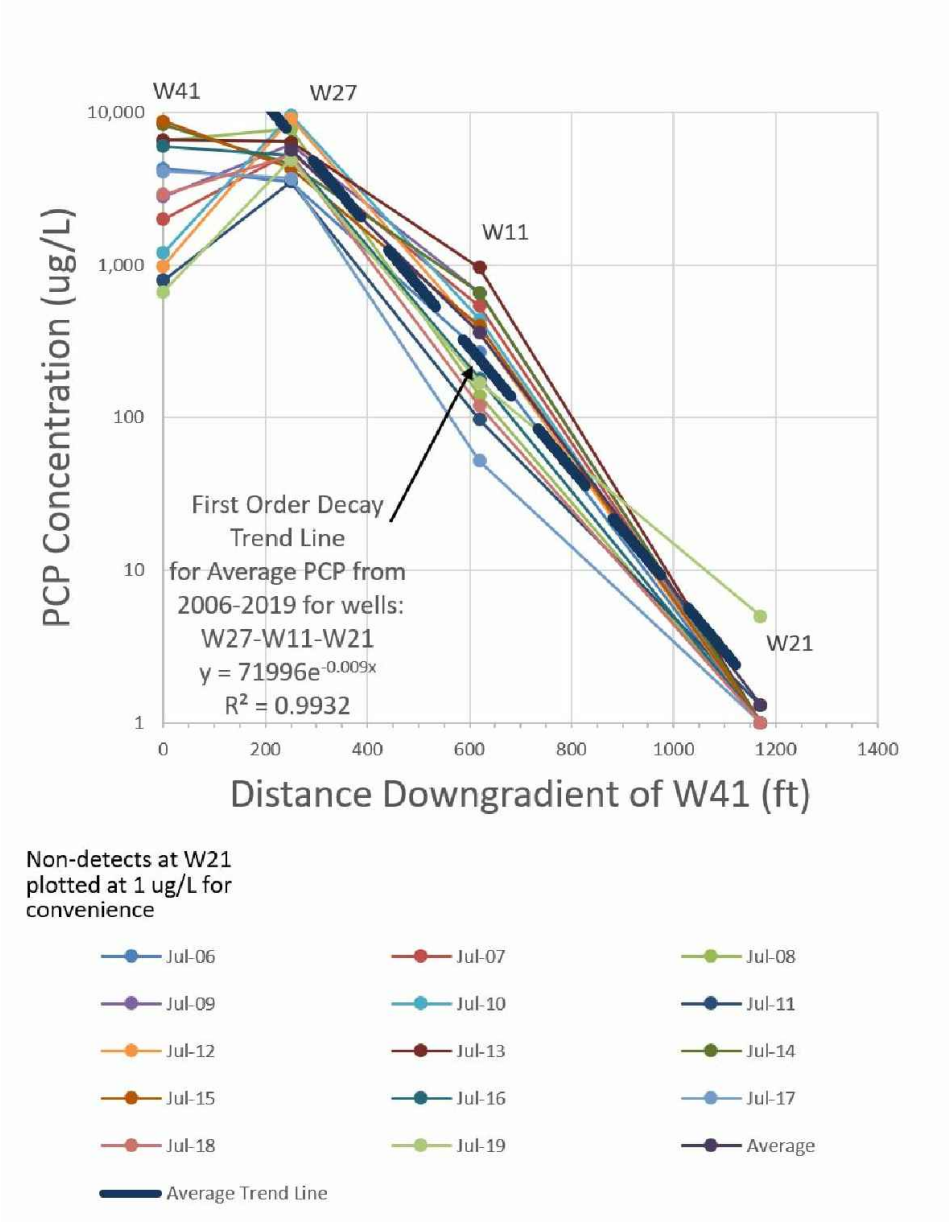

PROJECT:	**WAULECO, INC.** **LINES OF EVIDENCE OF PCP DEGRADATION** **WAUSAU, WISCONSIN** **WAUSAU, WISCONSIN**
TITLE:	**SOUTHEAST PROFILE** **DISTANCE - CONCENTRATION GRAPH**

DRAWN BY:	T. FIEBRANZ	PROJ NO.:	189597.0009 PH 3 TASK 1
CHECKED BY:	K. QUINN		**FIGURE 10**
APPROVED BY:	B. IVERSON		
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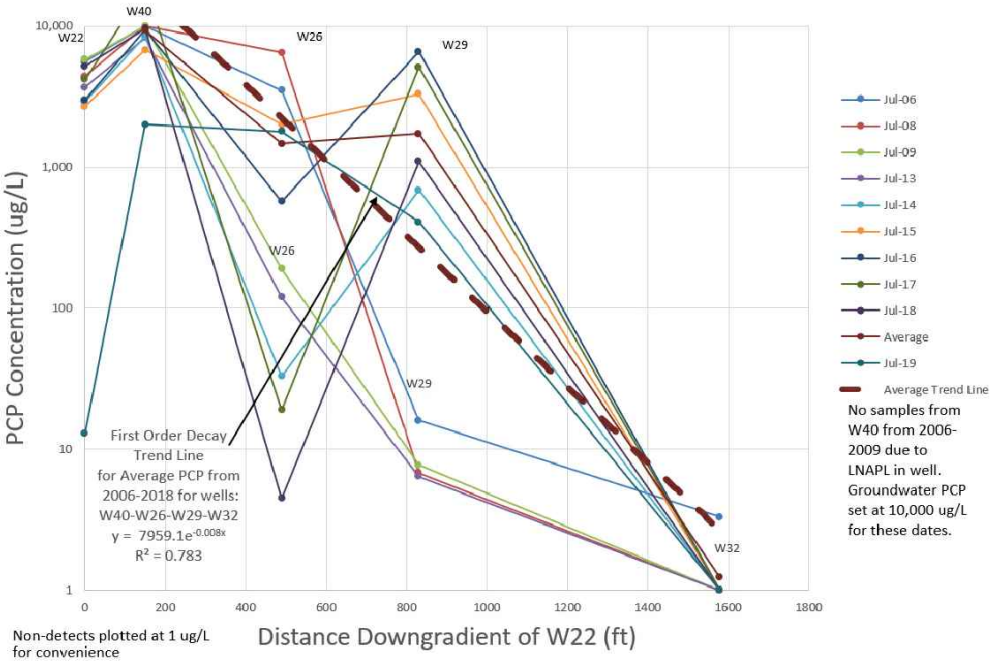
2006 Through 2019



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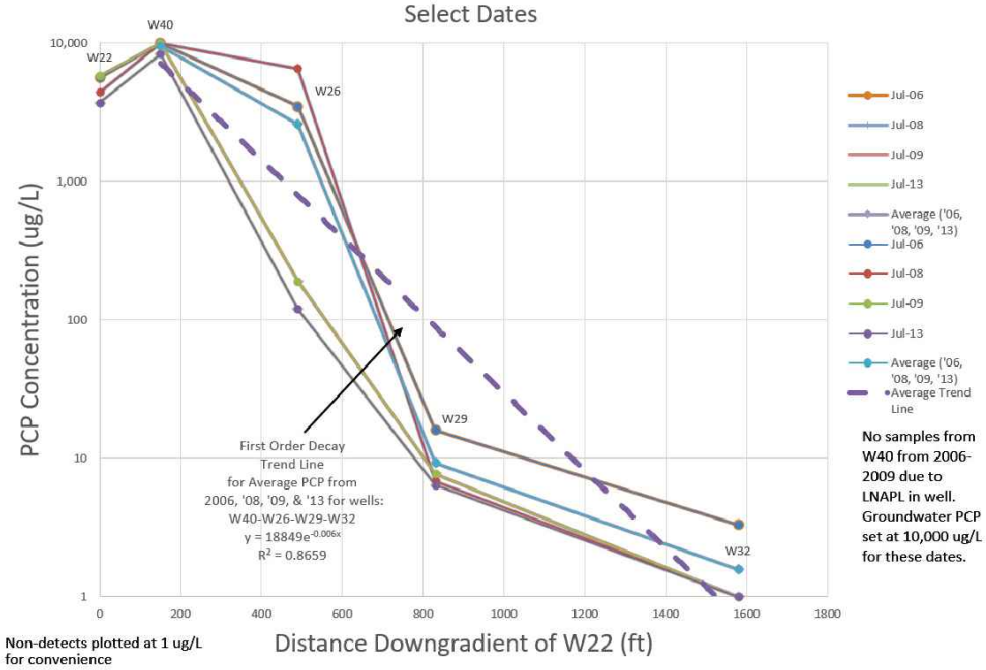

PROJECT:	**WAULECO, INC.** **LINES OF EVIDENCE OF PCP DEGRADATION** **WAUSAU, WISCONSIN** **WAUSAU, WISCONSIN**
TITLE:	**STAGNATION ZONE PROFILE** **DISTANCE - CONCENTRATION GRAPH**

DRAWN BY:	T. FIEBRANZ	PROJ NO.:	189597.0009 PH 3 TASK 1
CHECKED BY:	K. QUINN		
APPROVED BY:	B. IVERSON		**FIGURE 11**
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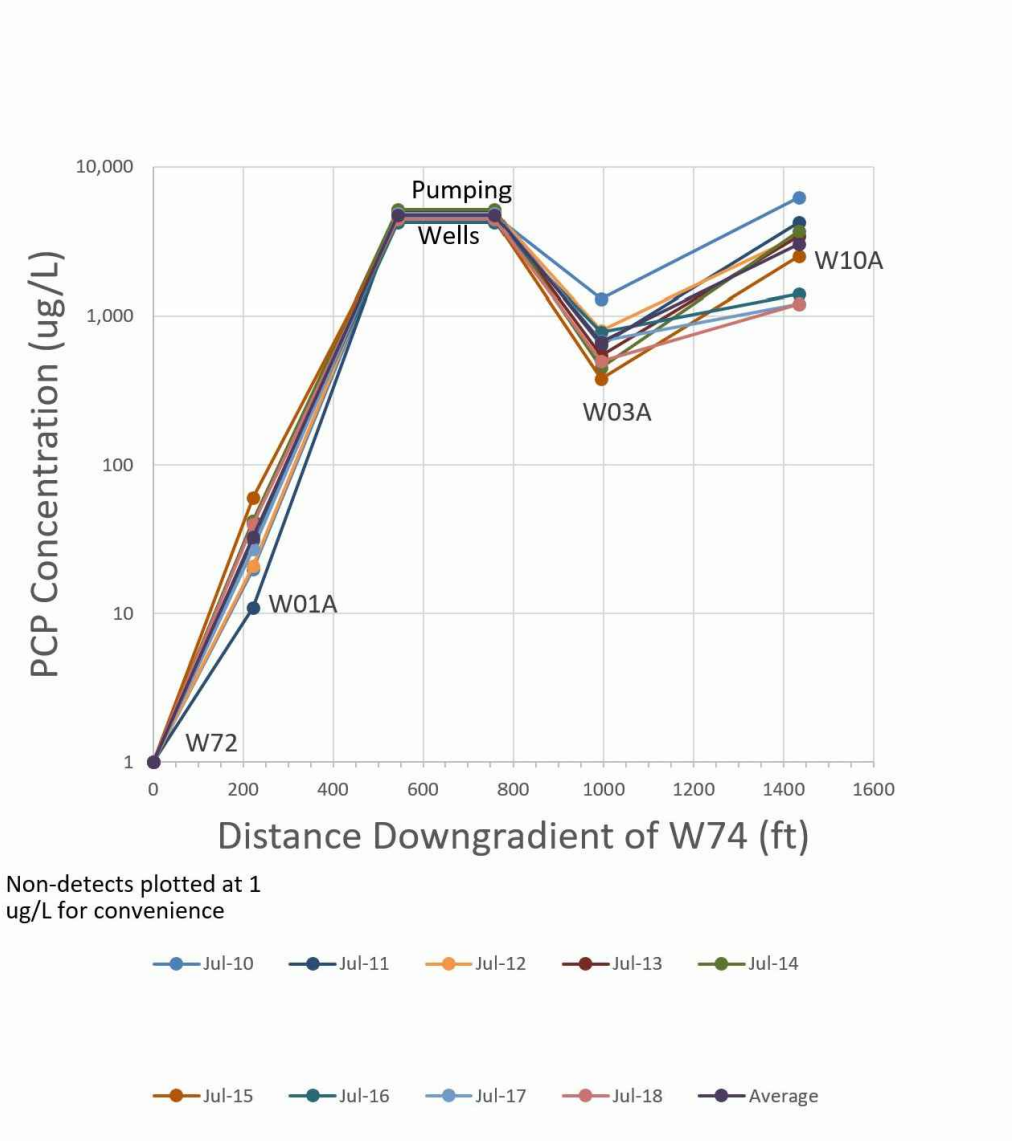

PROJECT:	**WAULECO, INC.** **LINES OF EVIDENCE OF PCP DEGRADATION** **WAUSAU, WISCONSIN** **WAUSAU, WISCONSIN**
TITLE:	**CENTERLINE PROFILE** **DISTANCE - CONCENTRATION GRAPH**
DRAWN BY: T. FIEBRANZ	PROJ NO.: 189597.0009 PH 3 TASK 1
CHECKED BY: K. QUINN	**FIGURE 12**
APPROVED BY: B. IVERSON	
DATE: MARCH 2020	

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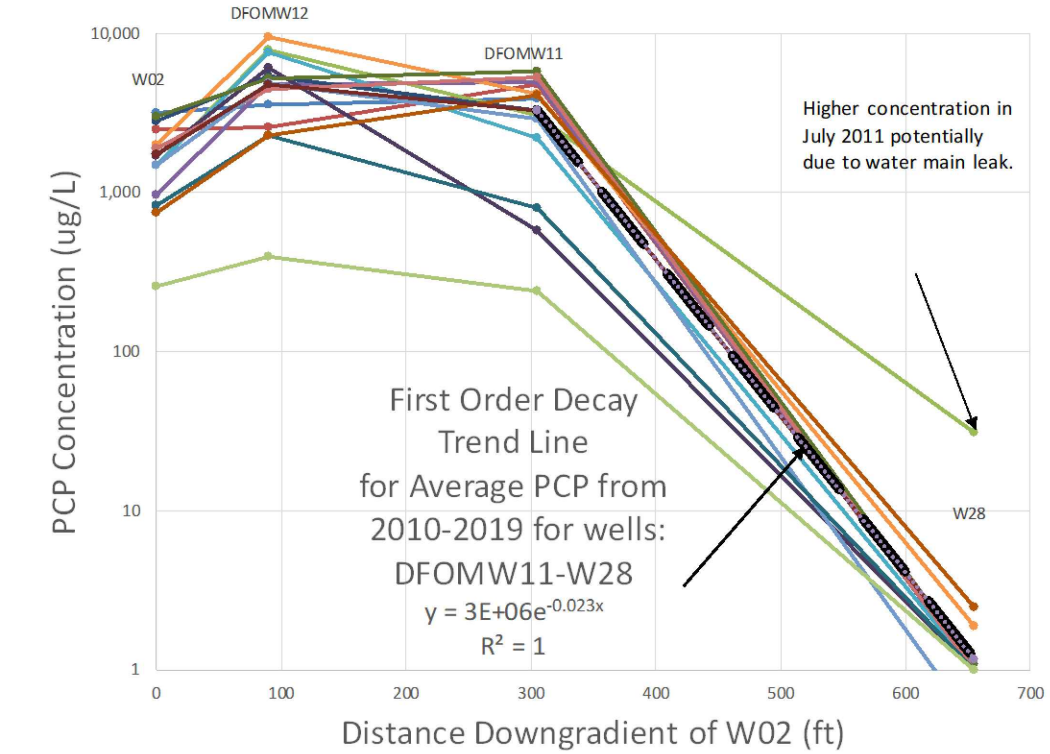

Non-detects plotted at 1 ug/L for convenience

18-Jan-10 | 15-Jul-10 | 25-Jan-11 | 20-Jul-11
18-Jan-12 | 9-Jul-12 | 7-Jan-13 | 8-Jul-13
16-Jul-14 | 8-Jul-15 | 7-Jul-16 | 13-Jul-17
12-Jul-18 | 11-Jul-19 | Average | Average Trend Line

PROJECT:	**WAULECO, INC.** **LINES OF EVIDENCE OF PCP DEGRADATION** **WAUSAU, WISCONSIN** **WAUSAU, WISCONSIN**
TITLE:	**NORTHERN LINE SOURCE PROFILE DISTANCE - CONCENTRATION GRAPH**
DRAWN BY: T. FIEBRANZ	PROJ NO.: 189597.0009 PH 3 TASK 1
CHECKED BY: K. QUINN	**FIGURE 13**
APPROVED BY: B. IVERSON	
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Appendix A
Bosso (2014) Article on Biodegradation of PCP

Rev Environ Sci Biotechnol (2014) 13:387–427
DOI 10.1007/s11157-014-9342-6

REVIEWS

A comprehensive overview of bacteria and fungi used for pentachlorophenol biodegradation

Luciano Bosso · Gennaro Cristinzio

Published online: 15 June 2014

Abstract Pentachlorophenol (PCP) is an extremely dangerous worldwide pollutant due to its high toxicity towards all organisms. It has been introduced into the environment mainly as a wood preservative, biocides and from the bleaching of paper or tissues. The use of PCP indiscriminate has led to the contamination of water and soil systems. Many countries have specific regulations, guidelines or procedures for the management and disposal of PCP but the most common methods are: adsorption with activate carbons, incineration in an approved and secure area, closed in sealed containers and biological degradation. PCP depletion can occur either by abiotic processes such as: absorption, volatilization and photo degradation or by biotic degradation. One of the main studies focused on remediation using plants, animals and microbial communities. Aerobic and anaerobic microorganisms can degrade PCP under a variety of conditions and at different PCP concentrations. Bacterial strains such as *Pseudomonas* sp., *Sphingomonas* sp., *Arthrobacter* sp., *Mycobacterium* sp., *Flavobacterium* sp., *Serratia* sp. and *Bacillus* sp., and fungal cultures as *Trametes* sp., *Phanerochaete* sp., *Anthracophyllum* sp., *Armillaria* sp., *Bjerkandera* sp., *Ganoderma* sp., *Lentinula* sp., *Penicillium* sp, *Trichoderma* sp., *Rhizopus* sp. and *Plerotus* sp. showed various rates and extent of PCP degradation. This review focuses on PCP degradation by various aerobic and anaerobic microorganisms with emphases on the biological and chemical aspects. Furthermore we will analyze intermediate products, processes and enzymes involved in the degradation of PCP in different environmental conditions and at various PCP concentrations.

Keywords Bioremediation · Chlorophenols · Microbial community · Microorganisms · Soil · PCP · Environmental contaminants · Biotechnology

Abbreviations

PCP	Pentachlorophenol
PCA	Pentachloronanisole
PH	Phenol
CP	Chlorophenol
DCP	Dichlorophenol
TCP	Trichlorophenol
TeCP	Tetrachlorophenol
CHYQ	6-Chlorohydroxyquinol
CHQ	Chlorohydroquinone
DCHQ	Dichlorohydroquinone
TriCHQ	Trichlorohydroquinone
TCHQ	Tetrachlorohydroquinone
DECB	2-Chloro-1,4-benzenediol
DCBQ	2,6-Dichloro-1,4-benzoquinone
TeCBQ	Tetrachloro-1,4-benzoquinone
TCC	Tetrachlorocatechol

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1 Introduction

Pentachlorophenol (PCP) is an artificial semivolatile organochlorine compound abundantly used as a low cost biocide in agricultural and industrial purposes. High PCP use has produced high levels of environmental contamination. In fact its presence has been detected in air, soil, lakes, rivers, basins, snow, sediments, rainwater, drinking water, aquatic organisms, plants, fungi, bacteria, eggs, in mammals milk, blood, adipose tissue and urine (ATSDR 2001; EPA 2008).

A low-cost method to remove PCP is biological depletion such as microbial degradation (McAllister et al. 1996; Gadd 2001; Singh 2006; Rubilar et al. 2008; Field and Sierra-Alvarez 2008; Juwarkar et al. 2010).

PCP degradation is a process that can be completed through three ways: oxygenolysis, hydroxylation or reductive dehalogenation (McAllister et al. 1996; Field and Sierra-Alvarez 2008).

Several microorganisms showed excellent ability at tolerating and removing PCP, a few examples are as follow: to avoid its toxicity by excluding it from the cell; by converting it into a non-toxic compound or by using PCP as the sole source of carbon (McAllister et al. 1996). The capacity to transform PCP into less toxic product, depends on environmental conditions including, water content (Seech et al. 1991), temperature (Valo et al. 1985), pH level and the organic matter (Cea et al. 2005), humic substances (Rüttimann-Johnson and Lamar 1997), oxygen and electron acceptors (D'Angelo and Reddy 2000).

Under anaerobic conditions, bacteria can transform PCP with reductive dehalogenation, where the chlorine atoms are sequentially replaced by hydrogen atoms until it is completely transformed into phenol (PH), benzoate, acetate, carbon dioxide and methane (Mohn and Tiedje 1992). Reducative dechlorination has been observed in many soils, sediments and sewage sludge (D'Angelo and Reddy 2000). Mikesell and Boyd (1986) showed the complete reductive dechlorination of PCP by combined activities of indigenous bacteria. Fungi and bacteria can transform PCP by incorporating one or two oxygen atoms from the diatomic O_2 using the structure of the contaminant in an aerobic condition through the oxygenase process. This process allows the destruction of the aromatic ring and the subsequent formation of CO_2 though a slow aerobic transformation; especially for the highly chlorinated compounds such as PCP (Reddy and Gold 2000). This is due to the fact that the aromatic ring is deficient in electrons and less susceptible to electrophilic attack by O_2 (Sahm et al. 1986). On the other hand, this may also be done by means of hydroxylation reactions which convert PCP into other compounds such as tetrachlorohydroquinone (TCHQ) (Xun and Orser 1991; Crawford et al. 2007; Xun et al. 2010) by replacing the chlorine atom with a hydroxide, converting the PCP into intermediate products (Vijay et al. 2000; Crawford et al. 2007; Xun et al. 2010). Other degradation processes of PCP, using either fungus or bacteria, can be achieved through methylation (McAllister et al. 1996; Gadd 2001). It occurs mainly in co-metabolism which is the occurrence of specific enzymatic reactions which aren't involved but not precisely targeted for this function. In fact many fungi have been shown to detoxify PCP by methylation using a specific lignin-degrading system, existing to serve other functions such as degradation of wood components such as lignin and cellulose (McAllister et al. 1996; Gadd 2001; Rubilar et al. 2008). Using reactions catalyzed by PH-oxidases such as laccases and peroxidases, fungi are able to make the primary PCP transformation into pentachloroanisole (PCA) (McAllister et al. 1996; Gadd 2001; Singh 2006; Rubilar et al. 2008). PCA is a less toxic form of PCP and because it is a compound with a more lipophilic composition, which passes through the cellular membrane and quickly bioaccumulated by microorganisms. The last process to remove PCP exploit the adsorption into the biomass of fungi and bacteria (living or dead). It has been found that some microbial cultures have a particular affinity in binding PCP (Ahmaruzzam 2008; Rubilar et al. 2012) i.e. the adsorption takes place thanks to the charge attraction between PCP and microorganisms biomass. The principal degradation pathways, genes and associated enzymes involved in the detoxification mechanism against recalcitrant organic compounds like PCP for bacteria and fungi, have been widely studied especially in recent papers and historical reviews (Crawford et al. 2007; Rubilar et al. 2008; Xun et al. 2010; Carvalho et al. 2011; Yadid et al. 2013; Copley et al. 2013).

However, numerous studies have demonstrated that under aerobic conditions, PCP can be efficiently reduced until a complete mineralization (Reddy and

Gold 2000; Pointing 2001; Leontievsky et al. 2002; Walter et al. 2004; Crawford et al. 2007; Field and Sierra-Alvarez 2008; Rubilar et al. 2008; Xun et al. 2010).

In this paper we will focus on the degradation of PCP by bacteria and fungi, analyzing primarily the species for a biological and chemical profile. Secondly we will explore the intermediate products, processes and enzymes involved in PCP degradation in microbiological culture media, soils, sludge and sediments in different environment conditions following the extensive review of McAllister et al. (1996).

2 PCP degradation by bacteria

In recent years, many bacterial strains isolated from every medium (soil, water, plant and animal) have been found useful in playing an important role in the tolerance, degradation and mineralization of PCP (McAllister et al. 1996; Field and Sierra-Alvarez 2008). While PCP tolerance is one of the main variables and can be a good starting point for the selection of useful strains for PCP degradation, the more interesting and, albeit, important aspect is the capacity of the microorganisms to degrade and mineralize PCP to CO_2, Cl^{-1} and H_2O (Crawford et al. 2007; Xun et al. 2010). When considering all bacteria, the genus most recently studied that may have the best possible potential in regards to bioremediation are: *Pseudomonas* sp., *Flavobacterium* sp., *Nocardioides* sp., *Novosphingobium* sp., *Desulfitobacterium* sp., *Mycobacterium* sp., *Sphingomonas* sp., *Kokuria* sp., *Bacillus* sp., *Serratia* sp. and *Acinetobacter* sp. (Table 1). All these bacteria may be isolated from different substrates.

2.1 The genus *Acinetobacter*

Acinetobacter is a genus of Gram-negative bacteria. Within this genus there are a diverse group of organism that range from human pathogens to environment. This bacterium is non-motile, containing multiple compounds that exhibit an oxidative capacity to arrive to a final mineralization (e.g. CPs) (see McAllister et al. 1996). These characteristics are used in various biotechnological applications, including bioremediation.

2.1.1 *PCP degradation in microbiological culture media*

Acinetobacter sp. ISTPCP-3, isolated by Sharma et al. (2009), was capable of degrading PCP. Optimum growth condition for the bacterial strains in the presence of PCP was investigated with varying pH levels, initial PCP concentrations and temperatures. The results indicated elevated PCP degradation between pH levels of 6.5 and 7.5. The optimum condition for the maximum degradation of PCP was at pH level 7.0 degrading 95 % of 50 mg L^{-1} PCP. The bacterial strains were able to completely degrade PCP at all concentrations lower than 100 mg L^{-1} within 48 h. At 200 mg L^{-1} PCP, the degradation was incomplete. The optimum growth temperature for the strain was at 30 °C. It degraded 50 mg L^{-1} PCP after only 24 h. *Acinetobacter* sp. ISTPCP-3 was able to use PCP through an oxidative process with ortho ring-cleavage producing 2,3,5,6-TCHQ and 2-chloro-1,4-benzenediol (DCBE). Sharma and Thakur (2008) isolated two bacterial strains identified as *Escherichia coli* PCP1 and *Acinetobacter* sp. PCP3. The ability of these two bacterial strains to effectively degrade PCP was observed with an emphasis on the growth and utilization of PCP. During the experiment the parameters were 96 h at 30 °C, with pH levels between 7.2 and 7.4, all being in a mineral medium supplemented with 100 mg L^{-1} PCP. Utilization of PCP was higher in *Acinetobacter* sp. PCP3 which exemplified the capacity to utilize PCP, more than 20 % within 6 h, while *E. coli* PCP1 utilized only 10 %. However, the most significant result observed in this study was the utilization of more than 80 % of PCP by *Acinetobacter* sp. PCP3. After 96 h only *E. coli* PCP1 had used almost 60 %. The release of intermediate products such as TCHQ, 2,3,4,6-TeCP and DCBE were more present in *Acinetobacter* sp. PCP3 than *E. coli*.

2.2 The genus *Bacillus*

Bacillus is another genus capable of PCP degradation under aerobic conditions (Field and Sierra-Alvarez 2008). Bacteria belonging to *Bacillus* sp. have great skills in metabolizing various industrial pollutants, many of which are complex organic compounds. It is a model organism for laboratory and field studies as it is one of the best understood bacteria in terms of ecology

Table 1 Degradation of PCP by bacteria

Species	Condictions	PCP	Degradation	Dechlorination	Mineralization	Degradation product(s)	Degradation time	References
Sphingomonas chlorophenolica RA2	Soil	30–100 mg Kg^{-1}	NR	NR	+	Chloride ions	1–7 months	Miethling and Karlson (1996)
Mycobacterium chlorophenolicum PCP1	Soil	30–100 mg Kg^{-1}	NR	NR	+	Chloride ions	1–7 months	
Arthrobacter sp. ATCC 33790	Liquid	145 mg L^{-1}	NR	80–100 %	NR	NR	108 days	Edgehill (1996)
Flavobacterium gleum	Liquid	100 ppm	25 %	NR	NR	TeCP	4 days	Yueb and Ward (1996)
Pseudomonas sp.	Liquid	100 ppm	20 %	NR	NR	TeCP	4 days	
Agrobacterium radiobacter	Liquid	100 ppm	60 %	NR	NR	TeCP	4 days	
Mixed colture	Liquid	100 ppm	80 %	NR	NR	TeCP	4 days	
Sphingomonas sp. P5	Liquid	37–168 μM	+	NR	+	Chloride ions	1 day	Rutgers et al. (1997)
Sphingomonas chlorophenolica RA-2	Liquid	250–300 mg L^{-1}	100 %	NR	+	TCHQ; TriCHQ; 2,6-DCHQ	NR	McCarthy et al. (1997a, b)
Pseudomonas sp. SR3	Soil	175 ppm	50–65 %	NR	+	Chloride ions	56 days	Pfender et al. (1997)
Flavobacterium sp. ATCC53874	Soil	125 ppm	50–65 %	NR	+	Chloride ions	4 days	
Pseudomonas sp. Bu34	Liquid	1,000–4,000 mg L^{-1}	75–90 %	NR	+	NR	30–57 days	Lee et al. (1998)
Mycobacterium chlorophenolicum PCP1	Soil	22 mg Kg^{-1}	5–50 %	NR	NR	Chloride ions	60 days	Combrisson and Jocteur Monrozier (1999)
Sphingomonas sp. K6	Liquid	0.2–2 mg L^{-1}	100 %	NR	+	Chloride ions	30 days	Männistö et al. (1999)
Spingomonas sp. K101	Liquid	0.2–2 mg L^{-1}	100 %	NR	+	Chloride ions	30 days	
Sphingomonas sp. K74	Liquid	0.2–2 mg L^{-1}	60 %	NR	+	Chloride ions	30 days	
Nocardioides sp. K44	Liquid	0.2–2 mg L^{-1}	–	NR	–	Chloride ions	–	
Nocardioides sp. K103	Liquid	0.2–2 mg L^{-1}	–	NR	–	Chloride ions	–	
Candidatus comitans K112	Liquid	0.2–2 mg L^{-1}	100 %	NR	+	Chloride ions	28 days	
Pseudomonas amygdali K104	Liquid	0.2–2 mg L^{-1}	60 %	NR	+	Chloride ions	NR	

Table 1 continued

Species	Condictions	PCP	Degradation	Dechlorination	Mineralization	Degradation product(s)	Degradation time	References
Pseudomonas sp.	Liquid	0.405–0.474 mg L^{-1}	90–100 %	NR	NR	NR	28–361 days	Schmidt et al. (1999)
Desulfitobacterium frappieri PCP-1	Liquid	1–100 mg L^{-1}	NR	99–100 %	NR	TCP; TCP; PH	30 days	Tartakovsky et al. (1999)
Arthrobacter sp. ATCC 33790	Liquid	50–120 mg L^{-1}	+	NR	+	Chloride ions	5 months	Hamid Mollah and Grant Allen (1999)
Sphingomonas sp. UG30	Liquid	30 mg L^{-1}	65 %	NR	NR	Chloride ions	12–18 days	Alber et al. (2000)
Sphingomonas sp. UG31	Soil	100–500 mg Kg^{-1}	+	NR	NR	Chloride ions	12–18 days	
Sphingomonas sp. UG32	Liquid	100–500 mg Kg^{-1}	+	NR	NR	Chloride ions	12–18 days	
Saccharomonospora viridis	Liquid	10 mg L^{-1}	100 %	NR	NR	TCHQ; TeCBQ	10 days	Webb et al. (2001)
Novosphingobium sp. MT1	Liquid	5 % PCP of a mixture	100 %	NR	NR	NR	150 h	Tiirola et al. (2002)
Serratia marcescens TE1	Liquid	100 mg L^{-1}	52 %	NR	NR	CHQ; DCHQ; TCHQ	96 h	Shah and Thakur (2002)
Serratia marcescens TE2	Liquid	100 mg L^{-1}	59 %	NR	NR	CHQ; DCHQ; TCHQ	96 h	
Pseudomonas fluorescens TE3	Liquid	100 mg L^{-1}	+	NR	72 %	CHQ; DCHQ; TCHQ	72–96 h	
Serratia marcescens TE4	Liquid	100 mg L^{-1}	68 %	NR	NR	CHQ; DCHQ; TCHQ	96 h	
Pseudomonas sp. IST103	Liquid	0.1 g L^{-1}	+	70 %	NR	NR	96 h	Gautam et al. (2003)
Pseudomonas sp. IST103	Soil	0–1,000 mg L^{-1}	+	NR	NR	TCHQ	45 days	
Pseudomonas veronii PH-05	Liquid	0.3 mM	30 %	NR	NR	TCC	100 h	Nam et al. (2003)
Sphingobium chlorophenolicum ATCC 39723	Liquid	0.3/3 mM	80 %	NR	NR	tetrachlorobenzoquinone	4 days	Dai and Copley (2004)
Pseudomonas gladioli M-2196	Liquid	3 μL L^{-1}	+	NR	NR	TCC	4 days	Nakamura et al. (2004)
Pseudomonas gladioli M-2196	Soil	2.5 μL L^{-1}	10 %	NR	NR	TCC	28 days	
Desulfitobacterium hafniense	Liquid	0.0013–0.364 g L^{-1}	60 %	NR	NR	TCP	225 days	Lanthier et al. (2005)

Table 1 continued

Species	Condictions	PCP	Degradation	Dechlorination	Mineralization	Degradation product(s)	Degradation time	References
Sphingomonas chlorophenolica	Liquid	100–800 mg L^{-1}	+	+	NR	NR	46–165 h	Yang et al. (2005)
Sphingomonas chlorophenolica RA2	Soil	0–300 ppm	+	NR	+	NR	3 weeks	Colores and Schmidt (2005)
Pseudomonas mendocina NSYSU	Liquid	20–320 mg L^{-1}	+	NR	NR	2,4,6-TCP; 2, 4-DCP; 4-CP; 2-CP	12–20 days	Kao et al. (2005)
Bacillus cereus ITRC S_6	Liquid	300 mg L^{-1}	62.75 %	NR	NR	NR	144–168 h	Chandra et al. (2006)
Serratia marcescens ITRC S_9	Liquid	300 mg L^{-1}	86.6 %	NR	NR	NR	144–168 h	
Dehalococcoides sp. CBDB1	Liquid	20 μM	NR	100 %	NR	3,5-DCP; 3,4-DCP; 2,4-DCP; 3-CP	1 week	Adrian et al. (2007)
Arthrobacter sp. ATCC33790	Soil	95.43–521 mg Kg^{-1}	+	NR	NR	NR	56 days	Pu and Cutright (2007)
Flavobacterium sp. ATCC 21918	Soil	263–539 mg Kg^{-1}	+	NR	NR	NR	56 days	
Mixed colture (ATCC33790 + ATCC 21918)	Soil	262–539 mg Kg^{-1}	+	NR	NR	NR	56 days	
Serratia marcescens ITRC S_7	Liquid	300 mg L^{-1}	90.33 %	+	NR	TCHQ; CHYQ	168 h	Singh et al. (2007)
Escherichia coli PCP1	Liquid	100 mg L^{-1}	60 %	NR	NR	TCHQ; DCBQ; 2,3,4,6-TeCP	96 h	Sharma and Thakur (2008)
Pseudomonas aeruginosa PCP2	Liquid	100 mg L^{-1}	15–65 %	NR	NR	TCHQ; DCBQ; 2,3,4,6-TeCP	6–96 h	
Acinetobacter sp. PCP3	Liquid	100 mg L^{-1}	80 %	NR	NR	TCHQ; DCBQ; 2,3,4,6-TeCP	96 h	
Pseudomonas fluorescens	Liquid	200 μM	+	NR	NR	PH	4 days	Lin et al. (2008)
Bacillus sp. ITRC S_8	Liquid	50.31 mg L^{-1}	+	+	NR	2-CP; TCHQ	168 h	Singh et al. (2008)
Serratia marcescens ITRC S_9	Liquid	50.31 mg L^{-1}	+	+	NR	2-CP; TCHQ	168 h	
Mixed colture (ITRC S_8 + ITRC S_9)	Liquid	50.31 mg L^{-1}	94 %	+	NR	2-CP; TCHQ	168 h	

Table 1 continued

Species	Condictions	PCP	Degradation	Dechlorination	Mineralization	Degradation product(s)	Degradation time	References
Sphingobium chlorophenolicum ATCC 39723	Liquid	100 μM	100 %	NR	+	TCHQ; TriCHQ; 2,6-DCHQ	1–4 h	Huang et al. (2008)
Pseudomonas testosteroni CCM 7350	Soil	10–100 mg L^{-1}	+	NR	NR	NR	7–24 days	Sejáková et al. (2009)
Sphingomonas chlorophenolica PCP-1	Liquid	160 mg L^{-1}	100 %	NR	NR	Chloride ions	25 h	Yang and Lee (2008)
Bacillus cereus ITRC S_6	Liquid	50.3 mg L^{-1}	90–100 %	NR	NR	TCHQ; CHYQ; 2, 4, 6-TCP	168 h	Chandra et al. (2009)
Serratia marcescens ITRC S_7	Liquid	50.3 mg L^{-1}	85–100 %	NR	NR	TCHQ; CHYQ; 2, 4, 6-TCP	168 h	
Mixed colture (ITRC S_6 + ITRC S_7)	Liquid	50.3 mg L^{-1}	+	NR	NR	TCHQ; CHYQ; 2, 4, 6-TCP	144 h	
Bacillus cereus (DQ002384)	Liquid	300 mg L^{-1}	62.75 %	+	NR	TCHQ; CHYQ; 2,3,4,6-TeCP	168 h	Singh et al. (2009)
Serratia marcescens (AY927692)	Liquid	300 mg L^{-1}	85.5 %	+	NR	TCHQ; CHYQ; 2,3,4,6-TeCP	168 h	
Serratia marcescens (DQ002385)	Liquid	300 mg L^{-1}	90.33 %	+	NR	TCHQ; CHYQ; 2,3,4,6-TeCP	168 h	
Mixed colture	Liquid	300 mg L^{-1}	93 %	+	NR	TCHQ; CHYQ; 2,3,4,6-TeCP	168 h	
Acinetobacter sp. ISTPCP-3	Liquid	20–250 mg L^{-1}	+	NR	NR	2,3,5,6-TCHQ; DCBE	24–48 h	Sharma et al. (2009)
Pseudomonas stutzeri CL7	Liquid	50–600 mg L^{-1}	90–95 %	NR	+	Chloride ions	120 h	Karn et al. (2010°)
Bacillus megaterium CL3	Liquid	50–600 mg L^{-1}	80–100 %	+	NR	Chloride ions	168 h	Karn et al. (2010b)
Bacillus pumilus CL5	Liquid	50–600 mg L^{-1}	80–100 %	+	NR	Chloride ions	168 h	
Bacillus thuringensis CL11	Liquid	50–600 mg L^{-1}	80–100 %	+	NR	Chloride ions	168 h	
Mixed colture (CL3 + CL5 + CL11)	Liquid	100 mg L^{-1}	80–100 %	+	NR	Chloride ions	168 h	
Kokuria sp. CL2	Liquid	100 mg L^{-1}	100 %	+	NR	Chloride ions	24–168 h	Karn et al. (2011)
Bacillus sp.	Liquid	500 μg ml^{-1}	56 %	NR	NR	Chloride ions	48 h	Tripathi et al. (2011)

Table 1 continued

Species	Condictions	PCP	Degradation	Dechlorination	Mineralization	Degradation product(s)	Degradation time	References
Pseudomonas testosteroni CCM 7350	Soil	10–100 mg L^{-1}	+	NR	NR	NR	7–21 days	Vítková et al. 2011
Mixed colture	Liquid	5–40 mg L^{-1}	15–100 %	+	+	Chloride ions	100 h	Huang et al. (2012)
Brevibacterium casei TVS-3	Liquid	1,000 mg L^{-1}	82 %	+	NR	Chloride ions	168 h	Verma and Singh (2013)

NR not reported; + denotes positive observation; − denotes no response

and biology. *Bacillus* strains can make a pivotal impact on residual life of PCP.

2.2.1 PCP degradation in microbiological culture media

Karn et al. (2010b) isolated by secondary pulp and paper industry sludge, *Bacillus* sp. Strains were capable of PCP bioremediation. The strains were isolated and identified as: *Bacillus megaterium* CL3, *Bacillus pumilus* CL5 and *Bacillus thuringensis* CL11. The microorganisms were used in PCP degradation in a mineral medium where the initial PCP concentration was 100 mg L^{-1}. The effect of PCP on the growth and degrading ability of *B. megaterium* CL3, *B. pumilus* CL5 and *B. thuringensis* CL11 were also examined at different concentrations: 50, 100, 200, 400, and 600 mg L^{-1} PCP. The degradation capability test was studied with varying parameters such as pH level and temperature. In mineral mediums all three isolates were able to grow and utilize PCP. Degradation of PCP increased with time for all of the isolates. The strains were able to completely remove PCP from the medium within 168 h. All these isolates were able to degrade more than 90 % of PCP at 400 mg L^{-1}. Strains *B. megaterium* CL3 and *B. thuringensis* CL11 were able to remove up to 80 % when grown at 600 mg L^{-1} PCP, compared to that of strain *B. pumilus* CL5, which was able to remove 91 % of PCP at the same concentration. These results show that all *Bacillus* isolates have the ability to degrade PCP at high concentrations. All the isolates removed 90 % of PCP between pH levels of 7.5 and 8.5 while PCP removal efficiency significantly decreased when the initial pH level of the medium was 9.5. When testing diverse temperatures the removal of PCP was less efficient at 25 °C than at 30 °C or 37 °C for the three bacteria species. Tripathi et al. (2011) collected 42 isolates from a treated tannery effluent in India, one being, identified as *Bacillus* sp. was tolerant at 500 μg ml^{-1} PCP. The results clearly indicate that there was a concomitant increase in bacterial growth during 0–48 h with as much as 56 % of PCP simultaneously biodegraded after 48 h of incubation. Singh et al. (2009) isolated and studied *Bacillus cereus* DQ002384 in regards to PCP degradation, individually and in mixed cultures with two *Serratia marcescens* strains, AY927692 and DQ002385. In an optimized growth condition, mixed cultures were

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found to be able to degrade up to 93 % of PCP at concentrations of 300 mg L^{-1}, on the other hand, when used individually *B. cereus* DQ002384 only degraded 62.75 % of PCP. Many intermediate products of PCP degradation were detected, such as TCHQ. Chandra et al. (2006) isolated a PCP degrading bacterial strain known as *B. cereus* ITRC S_6. The degradation and bacterial growth were performed in flasks containing 300 mg L^{-1} PCP and 1 % glucose. The bacteria showed good tolerance and growth with PCP reaching the stationary phase after only 144 h of incubation. But, it showed no growth in absence of glucose; thus, indicating that PCP degradation was the resultant of co-metabolism. *Bacillus cereus* ITRC S_6 degraded about 62.75 % of PCP during 168 h of incubation. In any case *B. cereus* ITRC S_6, alone or in mixed cultures was used by Chandra et al. (2009) for the treatment of pulp and paper mill effluent with contaminated levels of 50.3 ± 1 mg L^{-1} PCP, with all studies being conducted within 168 h. The PCP reductions from the pulp and paper mill effluent using the bacterial strains were quite impressive, emphasizing their ability to synergically interact. In fact, *B. cereus* ITRC S_6 when used in mixed cultures degraded 90 and 100 %. In both cases the capacity to degrade PCP was very high. Only when there was a synergic interaction was PCP completely degraded. When using the effluent samples to confirm the ability to degrade PCP by the varied bacteria strains the products 2,4,6-TCP, TCHQ, ChLO and TriCH were found.

2.3 The genus *Desulfitobacterium*

Desulfitobacterium genus was discovered in the last decade and can dehalogenate organic compounds through reductive dehalogenation. They are versatile microorganisms, strictly anaerobic bacteria, that can be used with a wide variety of electron acceptors, such as nitrate, sulfite, metals, humic acids, and man-made or naturally occurring halogenated organic compounds (van Elsas et al. 1997).

2.3.1 PCP degradation in engineered systems

Tartakovsky et al. (1999) studied the PCP degradation capacity of *Desulfitobacterium frappieri* PCP-1, adding it to a mixed bacteria community in an anaerobic bioreactor. There were two initial concentrations used: 1 and 100 mg L^{-1} PCP. While the incubation time was: 30 and 60 days. PCP removal efficiency was 99 % and the dechlorination efficiency was 90.5 %. PH and TCP were observed as dechlorination intermediate products. *D. frappieri* PCP-1 transformed PCP to TCP, and TCP to PH followed by PH-mineralization, which was caused most likely by indigenous microorganisms. Lanthier et al. (2005) developed a PCP-degrading, methanogenic fixed-film reactor, by using broken granular sludge. This consortium acclimated to increasing concentrations of PCP. After 225 days of acclimation, the reactor was performing at a very high level. They reached a PCP-degrading rate of 1.173 μM day^{-1}, with a PCP degradation efficiency of approximately 60 %. Only TCP was observed as an intermediate product. PCR species-specific primers highlighted a significant presence of *Desulfitobacterium hafniense* in the biofilm test during the reactor acclimation phase. *D. hafniense* cells were scattered in the biofilm and they accounted for 19 % of the community. These results suggest that the presence of PCP-dehalogenating *D. hafniense* in the biofilm was crucial for the performance of the reactor.

2.4 The genus *Flavobacterium*

Flavobacterium sp. are generally communal bacteria rod-shaped Gram-negative that live in soil and water (van Elsas et al. 1997). This genus includes economically disastrous animal pathogens (especially in freshwater fish) and environmental bacteria. Some species of *Flavobacterium* are capable of degrading PCP and other similar compounds (McAllister et al. 1996).

2.4.1 PCP degradation in soil, sediment and sludge

Pfender et al. (1997) used *Flavobacterium* sp. ATCC 53874 in a laboratory-scale bioremediation project in a soil microcosm amended with 125 ppm PCP. Over 50 % of the available PCP was quickly mineralized from soil by *Flavobacterium* sp. ATCC53874 within 4 days. While after 42 days of incubation, about 65 % of PCP was mineralized. *Flavobacterium* sp. ATCC 21918 in a mixed culture (in combination with *Arthrobacter* sp. ATCC 33790) was used by Pu and Cutright (2007) to evaluate the PCP biodegradation in two different field soils, from Columbia (CO) and New

Mexico (NM). The soils were incubated for 56 days. In the CO soil, with the presence of *Flavobacterium* sp. ATCC 21918 as well as the mixed culture, the initial concentrations were at 539 mg PCP kg^{-1} soil. After 56 days in CO soil the biodegradation efficiencies were 12 % and 25 % for both *Flavobacterium* sp. ATCC 21918 and the mixed culture. In NM soil, the initial concentration was 262.26 mg PCP kg^{-1} soil for *Flavobacterium* sp. ATCC 21918 and also in the mixed culture. The biodegradation in soil was 79.2 % and 98.2 % for *Flavobacterium* sp. ATCC 21918 and the mixed culture. Finally, in the NM soil, the indigenous bacteria were also biostimulated using 2 ml of a nutrient mineral solution, but in every case PCP degradation was 60 %. Naturally, in order to ensure there were enough nutrients and terminal electron acceptor, every two weeks, the supernatants in the biostimulation experiment, were replaced with their respective fresh medium solution.

2.5 The genus *Kocuria*

Kocuria, previously classified into the genus *Micrococcus* (which are closely related phylogenetically but differ in some chemotaxonomic properties) is a common bacteria which is widespread. This organism is an strictly aerobic, Gram-positive coccus occurring in tetrads with a majority of strains being non-pathogenic (van Elsas et al. 1997).

2.5.1 PCP degradation in microbiological culture media

Karn et al. (2011) used *Kokuria* sp. CL2 in the PCP degradation in a mineral medium batch culture at 100 mg L^{-1} PCP. During the 168 h. *Kokuria* sp. CL2 utilized 55, 95 and 100 % after 24, 96 and 168 h of incubation. During the course of the bacterial treatment, PCP was mineralized and the liberation of an inorganic chloride ion into the culture medium was observed. The concentration of the chloride ion increased as the degradation of PCP continued. This study showed that the removal efficiency of PCP by *Kokuria* sp. CL2 is very effective and can be used in the degradation of PCP which is contained in pulp and paper mill waste often released into the environment. In the same work *Kokuria* sp. CL2 was used also in the PCP degradation in sludge. Flasks were incubated for 336 h. Tolerance and degradation tests was carried out at 100 mg L-1 PCP. The *Kokuria* sp. CL2 strain was capable of mineralizing PCP. It was able to remove up to 58.64 % PCP from the sludge.

2.6 The genus *Mycobacterium*

The bacteria belonging to this genus are aerobic, non-motile (except for only one species which) and Gram-positive microorganisms that includes pathogens known to cause serious diseases in mammals. The mycobacteria have been observed to grow in a fungus-like when cultured in liquid medium i.e. this alludes the suffix *myco*. Thanks to its enzymes, this genera is widely used also in bioremediation studies. These enzymes include essentially some dioxigenase, dehydrogenase and hydrolase compounds and have been successfully used in the degradation of toxic substances as well as pyrene (Liang 2010).

2.6.1 PCP degradation in soil, sediment and sludge

A strain widely used in PCP degradation, mostly in soil, is *Mycobacterium chlorophenolicum* (*Rhodococcus chlorophenolicus*). It is a Gram-positive bacterium non-motile and well-performing at biodegrading (van Elsas et al. 1997). Furthermore *M. chlorophenolicum* in some cases can exhibit cyclic change in morphology from coccus to rod in the presence of a contaminant like PCP (Häggblom et al. 1988). Miethling and Karlson (1996) studied mineralization of 30 and 100 mg PCP Kg^{-1} soil using also *Mycobacterium chlorophenolicum* PCP1. They compared the activity of soil without inoculation, determining its natural capacity of PCP mineralization. Non inoculated soil completely mineralized 30 mg PCP Kg^{-1} soil within 7 months, but showed little to no degradation activity at 100 mg PCP Kg^{-1} soil in the same time period (less than 2 %). At 30 mg PCP Kg^{-1} soil, inoculated with *M. chlorophenolicum* PCP1 increased the mineralization slightly over what the indigenous bacterial activity produced. At 100 mg PCP Kg^{-1} soil only 27 % was mineralized within 7 and a half months. The mineralization of PCP in sterile and non-sterile soil microcosm with or without the addition of *M. chlorophenolicum* PCP-1 was examined by Combrisson and Jocteur Monrozier (1999). In this case the soil used in the study, never had a contamination of PCP. The soil microcosms were incubated with 22 mg PCP Kg^{-1} soil for 60 days. Only 5 % of the PCP was

mineralized in the sterile soil with or without *M. chlorophenolicum* PCP-1. About 50 % of PCP was mineralized in a non-sterile soil with or without the bacterium strains. These results suggest that the PCP was not easily accessible to *M. chlorophenolicura* PCP-1 and that PCP mineralization could only occur if the operation was in a microbial consortium.

2.7 The genus *Pseudomonas*

Microorganisms widely studied are *Pseudomonas* sp., ubiquitous bacteria with good potential in bioremediation. There are many species of the genus *Pseudomonas* with the capability to use many chlorophenols i.e. Chlorophenol (CP), Dichlorophenol (DCP) and Trichlorophenol (TCP) as carbon and energy sources under aerobic conditions including PCP (McAllister et al. 1996; Field and Sierra-Alvarez 2008). Almost all strains of this genus that are analyzed in the following studies were isolated from PCP contaminated sites (soil, secondary sludge of pulp and paper mill, aquifer sediments, tannery effluent, groundwater) showing a good capacity of PCP tolerance and degradation at very high concentrations of the contaminant.

2.7.1 PCP degradation in microbiological culture media

The most efficient strain of *Pseudomonas* sp. that was able to remove PCP was isolated by Lee et al. (1998) in Korea. The isolate *Pseudomonas* sp. Bu34 degraded almost 75 % of 4,000 mg L^{-1} (it appears to be the highest concentration tested) PCP after 57 days and about 90 % of 1,000 and 2,000 mg L^{-1} PCP during 30 days of incubation. Toxicity test (comparing acclimated and non-acclimated cells, where acclimated ones insinuate previous contact with PCP) showed in the non-acclimated, the cell number of strain Bu34 decreased although within 24 h increasing culture amount of PCP from 75 to 4,000 mg L^{-1}. In the acclimated strain the toxic effect did not appear until concentrations of 1,000–4,000 mg L^{-1} PCP. In fact, in acclimated experiments the number of cells of *Pseudomonas* sp. Bu34 considerably increased, achieving stationary phase within 10 days. The results obtanined with this *Pseudomonas* strains were extraordinary. Therefore, for the authors, strain Bu34 may have some of the characteristics of a "superbug" which could degrade very high concentrations of PCP. Karn et al. (2010a) isolated *Pseudomonas stutzeri* CL7 that was able to utilize 90 % of PCP at concentrations between 50 and 600 mg L^{-1} PCP. More than 95 % PCP degradation was recorded exceeding at 200 mg L^{-1} PCP. The isolate completely mineralized PCP after 120 h of incubation showing good growth in relation to simultaneous liberation of chloride ions. The initial concentration of the chloride ion was 200 mg L^{-1} but during 160 h it reached values of 500 mg L^{-1}. Furthermore, the growth of *P. stutzeri* CL7 was significantly reduced increasing the concentration of PCP. The possible explanation for reduction in degradation of PCP by the bacterial strain CL7 might be due to decreased activity of the degrading enzymes. Kao et al. (2005) isolated *Pseudomonas mendocina* NSYSU by analyzing its capacity to degrade PCP, changing pH levels and temperature. The results showed that PCP was rapidly removed after only 12 days at any of the following PCP concentrations: 20, 40, 80 and 100 mg L^{-1} PCP. The concentration of 150 mg L^{-1} PCP, demonstrated a complete depletion after 18 days. No PCP removal was detected at concentrations of 320 mg L^{-1} PCP within the 20 days of incubation. The analysis indicated that the optimal capacity of degradation for *P. mendocina* NSYSU includes the following conditions: slightly acidic ($6 < pH < 7$), aerobic and relatively moderate ambient temperature (20 °C < temperature < 30 °C). Finally, in microcosm experiments the following PCP degradation products were recovered: 2,4,6-TCP; 2,4-DCP; 4-CP and 2-CP. Shah and Thakur (2002) tested *Pseudomonas fluorescens* TE3 in degradation test at a concentration of 100 mg L^{-1} PCP. Bacterial strains grew in the PCP within the first 72 h, while later declined and degraded 72 % of PCP after 96 h which resulted in the highest release of chloride. The degradation of PCP *P. fluorescens* TE3 was conducted by using the oxidative process as indicated by the accumulation of degradation products such as chlorohydroquinone (CHQ), dichlorohydroquinone (DCHQ) and TCHQ. All which are intermediary metabolites used by the bacterial strain. Finally *P. fluorescens* TE3 showed a greater capability to degrade all the intermediate compounds, but the maximum utilization was only 70 % of TCHQ. Sharma and Thakur (2008) used *Pseudomonas aeruginosa* PCP2 for PCP degradation monitoring growth and use of 100 mg L^{-1} PCP. The isolate used more than 15 % within 6 h and 65 % in 96 h. Gautam et al.

(2003) used *Pseudomonas* sp. IST103 in PCP degradation at 0.1 g L^{-1}. It utilized PCP continuously until a maximum of 70 % after 96 h. The PCP utilization was also supported by chloride release and ring cleavage. Bacterial strains Gram-negative, identified as *Pseudomonas* sp. were used from Yueb and Ward (1996) in which degradation was monitored at 100 ppm PCP, individually and in mixed cultures with other bacteria (*Flavobacterium gleum* and *Agrobacterium radiobacter*). PCP degradation using only *Pseudomonas* sp. was 20 %, while in the mixed culture was 80 %. Nam et al. (2003), in Korea, isolated a new strain for PCP degradation in the *Pseudomonas*' genus. It was identified as *Pseudomonas veronii* PH-05. The amount of PCP decreased in time, with a gradual increase in cell density. PCP's initial concentration of 0.3 mM decreased to 0.21 mM. About 30 % of PCP was bio-transformed to metabolic intermediates such as tetrachlorocatechol (TCC). After 72 h of incubation, about 12 % of PCP had been converted into TCC. A strain of *P. fluorescens* was found in South Africa by Lin et al. (2008) and was found to be able to degrade 200 μM PCP after only 4 days. Optimal PCP degradation conditions of *P. fluorescens* were at a pH level of 7 and a temperature of 30 °C. The supplementation of 1 % glucose stimulated the growth of the microorganism and enhanced the ability to utilize PCP from the effluent sample. The authors did not show values or percentages of the PCP that was degraded. In Finland, a contaminated site was found with 1 mg L^{-1} PCP, Männistö et al. (1999) isolated and tested seventeen bacteria strains for PCP degradation. From these bacteria, the authors isolated *Pseudomonas amygdali* K104. They evaluated the PCP degradation when the compound was alone or in a mixed solution which contained: 80 % 2,3,4, 6-tetrachlorophenol (2,3,4, 6-TeCP) and approximately 20 % PCP. The initial concentration of PCP was 2 mg L^{-1}, while the 2,3,4,6-TeCP was: in the first test 0.2 mg L^{-1} PCP. In second test only 1 mg L^{-1} PCP was used. About 60 % of PCP was degraded while in a mixed solution with 2,3,4, 6-TeCP at both concentrations. When PCP was alone, *P. amygdali* K104 did not degrade the contaminate. Nakamura et al. (2004) used a particular genes encoding system to contrive PCP-degrading enzymes from *Sphingomonas chlorophenolicum* (Dai and Copley 2004). They introduced this gene into the chromosome of *Pseudomonas gladioli* M-2196, which achieved the transformation of a strain with the ability to degrade PCP to a maximum concentration of 3 μL PCP. This strain degraded more than 80 % PCP within 4 days. TCC was the metabolite in PCP degradation.

2.7.2 PCP degradation in engineered systems

Using indigenous microorganisms, specifically *Pseudomonas* sp., and nutrient amendments (essentially N and P), Schmidt et al. (1999) evaluated the PCP degradation in four batch Pyrex carboys (reactors). The reactors were fitted with an air hose and difftmer stone and aerated with compressed air. The study had the objectives of determining the rate and extent of PCP removal in conditions with unamended or amended mediums with N and P. The first phase of the experiment was conducted during 28 days of incubation with a PCP initial concentration of 0.405 mg L^{-1}. In the second phase, PCP degradation was evaluated after 32 days 361 of incubation with a PCP initial concentration at 0.474 mg L^{-1}. In all cases, PCP decreased from the initial concentration up to $<$0.002 mg L^{-1} PCP with the exception of the abiotic control. The results showed how PCP removal is positively affected by the presence of N and P.

2.7.3 PCP degradation in soil, sediment and sludge

Gautam et al. (2003) isolated *Pseudomonas* sp. IST103 from the effluent sediment of paper mill in India. The strain was tested in two sets of soil microcosms containing 20 and 40 % moisture, each having the following PCP concentrations: 0, 10, 100, 500, and 1,000 mg L^{-1}. *Pseudomonas* sp. showed significant use of PCP, about 80 %, with higher cell growth after 45 days, the highest being when PCP was applied up to levels of 100 mg L^{-1} and a concentration of 20 % moisture. At 40 % moisture about 70 % of PCP was used. Inhibitory effects on the growth of the bacterial strain and PCP utilization were seen at 500 and 1,000 mg L^{-1} PCP for both moistures. Finally, a qualitative analysis with HPLC showed that TCHQ was the metabolite of PCP degradation in soil microcosms *Pseudomonas* sp. SR3 was used by Pfender et al. (1997) in a laboratory-scale bioremediation of soil microcosm in a bottle amended with 175 ppm PCP. Over 50 % of the available PCP was quickly mineralized from the soil within 4 days. After 42 days of incubation, about 65 % of PCP was

mineralized. Nakamura et al. (2004) modified a chromosome of *Pseudomonas gladioli* M-2196 using particular genes encoding PCP-degrading enzymes from *Sphingomonas chlorophenolicum* described by Dai and Copley (2004) transforming bacterium into a strain with the ability to degrade PCP in a soil microcosm. In the soil, the degradation capacity of PCP was much lower. In fact, in soil after 28 days of incubation the degradation was of only 10 % with an initial concentration of 50 μM. Sejáková et al. (2009) studied PCP contaminated soils' ability to degrade autochthonous microorganisms and the effects of bioaugmentation brought about by the bacterial strain *Pseudomonas testosteroni* CCM 7530. The biodegradation of PCP was performed in soil (Fluvisol, Chernozem, and Regosol) with the presence of *P. testosteroni* CCM 7530 as well as without additional bioaugmentation. The biodegradation of PCP in soil was carried out under laboratory conditions using the real soil with an initial PCP concentration of 10 and 100 mg PCP kg^{-1} soil. For each experiment, three sets of soil samples were used and analyzed after 7, 17 and 24 days. The soil samples with concentrations of 10 mg PCP kg^{-1} soil revealed higher degradation in comparison to the soil with 100 PCP mg kg^{-1} soil where the degradation was already observed within 7 days. The biodegradation of PCP in the bioaugmented soils evaluated after 24 days depended on other factors such as the addition of sorbent, initial PCP concentration, and above all the soil type. In bioaugmented Regosol and Fluvisol with a concentration of 10 mg PCP kg^{-1} soil, about 72–74 % degradation was noted, while with Chernozem only 57 %. Biodegradation of PCP in soils with 100 mg PCP kg^{-1} soil was remarkably lower (49 % Regosol, 39 % Chernozem and 34 % Fluvisol). These PCP degradation values although interesting, were significantly lower in comparison to the same soils not inoculated with *P. testosteroni* CCM 7530 but amended with an organo-mineral complex or lignite. Bioaugmentation of the soil by external microorganisms with a PCP degradation capability did not ensure higher levels of pollutant being degraded. In spite of the microbial activity resulted in biotransformation of PCP into certain toxic substances, probably lower chlorinated phenols that are more soluble than PCP, and therefore more toxic to present biota. The results of this study demonstrated that the effect of tested sorbents, organic matter and lignite, and inoculum addition on PCP concentration depended markedly on soil type. In some cases, the positive effect of bioaugmentation or addition of sorbent on the effectiveness of biodegradation process was disputable because the standard toxicity test on a plant model indicated the increase of soil ecotoxicity after termination of the biodegradation process. Vítková et al. (2011) studied the degradation capacity of autochthonous microorganisms and the effect on bioaugmentation by the bacterial strain *Pseudomonas testosteroni* CCM 7530 and the biostimulation with lignite in a PCP contaminated soil. The biodegradation experiments with PCP were performed in soils (Fluvisol, Chernozem, and Arenosol) with the presence of an inoculum of *P. testosterone* CCM 7530 also without additional bioaugmentation. The biodegradation of PCP in soil was carried out under laboratory conditions at concentrations of 10 and 100 mg PCP kg^{-1} soil. For each experiment, three sets of soil samples were used and analyzed after 7, 14, and 21 days of incubation. The soil samples with concentrations of 10 mg PCP kg^{-1} soil revealed higher degradation in comparison to soils with concentrations of 100 mg PCP kg^{-1} soil, especially chernozem where the highest degradation was 78 % in non-amended soil and 55 % in lignite-amended soil. Biodegradation of PCP in bioaugmented soils was evaluated after 21 days. It depended on the soil type and the presence of lignite. The lignite exhibited significant improvement of degradation, about 20 % in each soil type, except for chernozem and aerosol at 10 mg kg^{-1} PCP, where the degradation was 29 and 55 %. In general, the degradation of PCP was higher with the bioaugmentation conditions, mainly in chernozem and in lignite-amended soil. The degradation efficiency order is as follows: chernozem, fluvisol and arenosol. It can be concluded that lignite shows its protective effects thank to high efficiency of sorption ability of toward PCP, but for the most part only for the non-degrading autochthonous micro flora.

2.8 The genus *Serratia*

Serratia is a common genus of aerobic rod-shaped bacterium (optionally anaerobic) that has provided possible biotechnological approaches to clean up polluted environments contaminated by PCP in axenic condition (Abo-Amer 2011). The most common

species of the genus is *Serratia marcescens*, an human pathogens that may causes nosocomial infections.

2.8.1 PCP degradation in microbiological culture media

Singh et al. (2009) confronted the synergistic PCP biodegradation of a microbial consortium composed by *Serratia marcescens* AY927692, *Serratia marcescens* DQ002385 and *Bacillus cereus* DQ002384 against the effectiveness of this bacterium alone. All experiments were carried out after 168 h in an optimized condition for growth of bacteria (at 30 ± 1 °C, pH 7.0 ± 0.2, 120 r.p.m.) and at different environmental conditions, i.e., temperature (20, 30 and 37 °C), pH (6.0, 7.0 and 9.0) and aeration rate (50, 120 and 200 r.p.m.). The Initial concentration was 300 mg L^{-1} PCP. In an optimized condition for growth, the mixed culture was found to be able to degrade up to 93 % of PCP, compared to a single *S. marcescens* strain AY927692 as well as DQ002385 which degraded PCP at percentages of 85.50 % and 90.33 %. Mixed cultures degraded 62.75 % of PCP at 20 °C and 83.33 % at 37 °C; 70 % at pH 6 and 75.16 % at pH 9; 73.33 % at 50 rpm and 91.63 % at 200 rpm. However *S. marcescens* AY927692 was more skilled in PCP degradation in mixed cultures than when used alone at the following conditions: 50 rpm, 20 and 37 °C, pH 6 and 9. Many intermediate products of PCP degradation were analyzed. The identification of TCHQ suggested that the degradation occurred through dechlorination. The consortia showed better overall removal efficiencies than the single strains that used PCP as a carbon and energy source. Another strain of *Serratia marcescens* ITRC S_7 (Singh et al. 2007) was used in PCP tolerance and degradation experiments. It was found to be able to degrade up to 90.33 % at 300 mg L^{-1} PCP with a simultaneous release of a chloride ion. Bacterial dechlorination occurred in mineral mediums when in the presence of glucose as an additional carbon and energy source, within 168 h of incubation. In the absence of glucose the bacterium was unable to utilize PCP, indicating the process of co-metabolism. Finally the metabolites obtained from the degradation of PCP were TCHQ and 6-chlorohydroquinol. *Serratia marcescens* ITRC S_9 and a mixed culture with *Bacillus* sp. ITRC S_8 were used by Singh et al. (2008) in experiments dealing with PCP degradation of pulp paper mill effluent collected in India containing 50.31 mg L^{-1} PCP. The degradation studies were performed in batch cultures composed from pulp and paper mill effluent samples, 1 % glucose, 0.5 % peptone and bacteria in individual or combined coltures. Mixed coltures degraded PCP up to 94 % with the simultaneous release of a chloride ion which was limited at 1,200 mg L^{-1} after 168 h, emphasizing bacterial dechlorination in the medium. In same time span as well as, individually, the bacteria strains released chloride ions below 800 mg L^{-1}. Furthermore, in mixed culture the strains showed a growth and degradation of PCP at higher efficiencies than when alone. The final analysis with high performance liquid chromatography (HPLC) of pulp paper mill effluent degradation products showed the formation of 2-CP and TCHQ. From pulp and paper mill effluent sludge samples, collected in India, Chandra et al. (2006) isolated *Serratia marcescens* ITRC S_9. PCP degradation and growth exams were tested at 300 mg L^{-1} PCP and 1 % glucose. *S. marcescens* ITRC S_9 showed tolerance and growth with PCP reaching the stationary phase after 144 h of incubation. In addition, the bacterium strain did not grow in the absence of glucose; thus, it indicated that PCP degradation is the result of co-metabolism. *S. marcescens* ITRC S_9 degraded 86.6 % of PCP degradation after 168 h of incubation. The PCP-degrading bacterial strains, *S. marcescens* ITRC S_7 and mixed cultures with *Bacillus cereus* ITRC S_6 were used by Chandra et al. (2009) for the treatment of pulp and paper mill effluent contaminated by many substances including 50.3 ± 1 mg L^{-1} PCP. The bacteria were incubated in flasks with: contaminated effluent samples, 1.0 % glucose, 0.5 % peptone and 1 ml of bacterial culture (individual and mixed). The reduction of PCP effects from pulp paper mill effluent by the bacterial strains was remarkable, in respects to their synergic action. *S. marcenscens* ITRC S_7 and mixed cultures degraded 85 and 100 %. The use of effluent samples was to confirm the ability of bacteria strains to degrade PCP, and then evaluate the intermediate metabolites. The substances in the flasks consisted of: TCHQ, 2-CP, 6-chlorohydroxyquinol (CHYQ) and 2, 4, 6-TCP. Shah and Thakur (2002) isolated three different strains capable of degrading PCP. They were identified as *Serratia marcescens* (TE1, TE2 and TE4). Degradation potential at 100 mg L^{-1} PCP was investigated in terms of growth, ring cleavage, chloride release and PCP utilization. Three

strains of *S. marcescens* TE1, TE2 and TE4, after 96 h, were able to utilize 52, 59 and 68 %. During this carbon use an amount of chloride had accumulated in the culture broth. The degradation of PCP by bacterial strains was conducted through an oxidative process as indicated by accumulation of degraded products such as CHQ, DCHQ and TCHQ. All intermediary metabolites were used by the bacterial strains. In fact after 96 h *S. marcescens* TE1 was able to utilize 63 % of CHQ. However, the amount of PCP utilized by this strain was 42 %. *S. marcescens* TE2 was able to remove 62 % of DCHQ and 43 % of PCP. *S. marcescens* TE4 had a greater capability to degrade all of the compounds, but the maximum utilization of PCP was 68 %. *S. marcescens* T4, T1 and T2 were able to degrade 50 % and, 65 % of TCHQ.

2.9 The genus *Sphingomonas*

Sphingomonas genus was separated from Pseudomonas by Yabuuchi et al. (1990) and it is often used in a number of bioremediation experiments especially that of CPs (McAllister et al. 1996; Field and Sierra-Alvarez 2008). *Sphingomonas* sp. in some cases showed a rapid mineralization of PCP thanks to the remarkable ability to break down hydrocarbon bonds.

2.9.1 *PCP degradation in microbiological culture media*

Dai and Copley (2004) used the Gram-negative *Sphingobium chlorophenolicum* ATCC 39723, obtained from the American Type Culture Collection, to improve the degradation of PCP using the genome shuffling method (Patnaik et al. 2002). PCP final concentrations that were used in the experiment were: 0.3 and 3 mM PCP. They obtained several strains of *S. chlorophenolicum* and all microorganisms were able to degrade and tolerate PCP much better than that of the wild type. They tested PCP degradation of the strains in different conditions of contaminant exposure and when bacteria cells were pre-exposed to 50 μM of PCP, both strains, including the wild type, ended up degrading PCP at a higher rate after genome shuffling. The Mutant and wild type strains at a concentration of 0.3 mM PCP, both did a very good job at degrading PCP, although when they added 3 mM PCP to the medium only the mutant strain had the ability to grow and degrade PCP. During the experiments tetrachloro-1,4-benzoquinone (TeCBQ) was found. McCarthy et al. (1997a) isolated from highly PCP contaminated soil, a strain of *Sphingomonas chlorophenolica* RA-2. The isolate degraded 100 % of PCP at 250 and 300 mg L^{-1} PCP. The principal products of degradation were TCHQ, trichlorohydroquinone (TriCHQ) and 2,6-DCHQ. The final pathway has yet to be defined but it is known that this bacteria can mineralized PCP to CO_2, H_2O, and Cl^- (McCarthy et al.1997b). *Sphingomonas* sp. UG30 was used in PCP mineralization and degradation by Alber et al. (2000). In an vitro experiment the authors analyzed the mineralization capacity at 30 mg L^{-1} PCP, adding ammonium phosphate or ammonium nitrate as a nitrogen source. The Optimum PCP degradation of about 65 % occurred using ammonium phosphate. Rutgers et al. (1997) showed that PCP affects the growth rate of *Spingomonas* sp. P5 This bacterium uses PCP uniquely as the source of carbon and energy. This experiment was conducted in a continuous liquid culture with on-line measurement and control of the substrate concentration. A Specific growth rate, showed a maximum value of 0.142 ± 0.004 h^{-1} at a set-point of PCP concentrations between 37 and 168 μM. At PCP concentrations above 168 μM, the growth rate decreased by inhibition. Further studies of the degradation of PCP with *Spingomonas chlorophenolicum* ATCC 39723 were conducted by Huang et al. (2008). The wild type and mutant strains (PcpF) were tested in PCP remediation. In the PcpF strain, they added an orf19 gene which was able to produce great quantities of Glutathionyl–Hydroquinone Lyase, enzyme which is very useful in PCP degradation. The *S. chlorophenolicum* ATCC 39723 wild type and PcpF were cultured in the mineral medium with as well as without glutamate induced with 100 μM PCP and incubated until PCP mineralization. The wild type and PcpF completely degraded 100 μM PCP within 40 min in the presence of glutamate. Contrarily, bacteria grew without glutamate, wild type cells degraded 100 μM PCP in 1 h while, the PcpF only needed 4 h to complete the degradation of PCP. The PcpF strain was more sensitive to PCP's toxicity and had a significant decrease PCP degradation rate, due to the accumulation of the GS-hydroquinone. Thus, PcpF played an important role in PCP degradation and converted the GS-hydroquinone conjugates back to the intermediates of PCP degradation pathways. In addition to *Pseudomonas* sp., Männistö et al. (1999)

isolated three other strains that could degrade PCP from *Sphingomonas* sp. (isolates K6, K101, and K74). The PCP degradation capacity was evaluated when the compound was alone or in a mixed solution that contained 80 % 2,3,4,6-TeCP and 20 % PCP. When PCP was present in mixed solutions with 2,3,4,6-TeCP, it completely was degraded by strain *Sphingomonas* sp. K101. Contritely the strain *Spingomonas* sp. K74 partially degraded about 60 %. When PCP was alone at 2 mg L^{-1}, only *Sphingomonas* sp strain. K6 degraded PCP completely in less than 30 days. This indicates that the degradation of PCP in *Sphingomonas* sp. K101 and K74 may have been induced by 2,3,4,6-TeCP.

Evidently, PCP is a compound which is too toxic for these strains and they are able to degrade PCP through co-metabolism. On the other hand, isolate K6 degraded PCP by itself, but not in the mixture solution with 2,3,4,6-TeCP.

2.9.2 PCP degradation in engineered systems

Spinghomonas chlorophenolica, notoriously has been able to degrade and dechlorinate PCP which was used by Yang et al. (2005) in batch reactor experiments. The authors showed how a PCP pre exposition of *S. chlorophenolica* increased the ability to degrade the contaminant. In fact at the initial PCP concentration of 380 mg L^{-1}, the *S. chlorophenolica* completely degraded the PCP within 45.6 h, whereas increasing the PCP concentration from 560 to 720 mg L^{-1}, it efficiently decreased PCP to 34.7 and 58.9 % during 165 h of incubation. On the other hand, without a pre-exposition between the organism and PCP, the contaminant was removed completely within 89.2 h at 250 mg L^{-1} PCP. However, the removal efficiency rose to 89 %, after 110.8 h, at 400 mg L^{-1}. If the initial PCP concentration was increased above 600 mg L^{-1}, *S. chlorophenolica* could not degraded PCP.

Strains of *Sphingomonas* sp. UG30 were used by Alber et al. (2000) in soil perfusion bioreactors in PCP degradation tests. The authors analyzed the PCP mineralization capacity of the bacterium in a glucose medium with ammonium phosphate or ammonium nitrate as a nitrogen source. In this experiment, bioreactors were used at three different PCP concentrations 100, 225 and 500 mg PCP Kg^{-1} soil. The first two concentrations obtained degradation of 80 % and 99 %. At 500 mg PCP Kg^{-1} soil there was no degradation. Another test was conducted by Yang and Lee (2008) using a pure PCP-degrading bacterium strain, identified as *Sphingomonas chlorophenolica* PCP-1, isolated from PCP-contaminated soils in Taiwan. This bacterium was tested in a batch reactor with contaminated water by 160 mg L^{-1} PCP. Depletion of the PCP and the chloride release were measured at different bacterial biomasses (0.14, 0.28, 0.42 and 0.54 g L^{-1}). The results indicated that at 160 mg L^{-1} PCP was completely degraded within 25 h under different bacterial biomass' (dry weight) in the groundwater. It was evident that while the biomass of the bacteria increased, the degradation of PCP decreased and ca. 110 mg L^{-1} chloride was released by each bacterial concentration within the same period of time.

2.9.3 PCP degradation in soil, sediment and sludge

Alber et al. (2000) used *Sphingomonas* sp. UG30 in PCP mineralization and degradation in statically incubated soil. *Sphingomonas* sp. UG30 was tested at three different PCP concentrations (100, 225 and 500 mg PCP Kg^{-1} soil) during 22 days of incubation. For the first two concentrations the results showed a degradation of 25 and 65 %. At 500 mg PCP Kg^{-1} soil there was no degradation. *Sphingomonas chlorophenolica* RA2 was used by Colores and Schmidt (2005) in a microcosm soil contaminated with the following concentrations: 0, 10, 50, 100, or 300 ppm PCP. *S. chlorophenolica* RA2 degraded only 10 % in the soil contaminated with 10 ppm PCP, reaching 30 % after three weeks of incubation. No degradation was noted at any other concentrations. Miethling and Karlson (1996) studied PCP mineralization in a sample of soil from Denmark with levels of 30 and 100 mg PCP Kg^{-1} soil after inoculation with *S. chlorophenolica* RA2. They compared the result with the activity of the same soil without inoculation, determining its natural capacity for PCP mineralization. None of the inoculated soils completely mineralized 30 mg PCP Kg^{-1} soil within 7 months but showed little to no degradation activity at 100 mg Kg^{-1} in the same time period (less than 2 %). In soil inoculated with 30 mg PCP Kg^{-1} soil, *S. chlorophenolica* RA2 reduced the mineralization time drastically to only 1 month. At 100 mg Kg^{-1}, mineralization was slower because of the high PCP toxicity but approached completion within 7 and a half months. The inhibition could have

been overcome by addition of sawdust (1 g Kg^{-1} soil), which was shown to increase the mineralization rate (Miethling and Karlson 1996).

2.10 Other genera

Other bacteria showed a good tolerance and ability to degrade PCP, even at low initial concentrations. They were rarely studied in the presence of PCP, although it would be useful to consider them for future research.

2.10.1 PCP degradation in microbiological culture media

Verma and Singh (2013) have isolated a bacteria identified as *Brevibacterium casei* (TVS-3) able to degrade 1,000 mg L^{-1} PCP. The bacterium degraded 72 % PCP within 168 h at pH 7.5 and 35 °C temperature. After 168 h *B. casei* showed maximum PCP utilization of 720 mg L^{-1} and released 900 mg L^{-1} chloride ions. Finally *B. casei* carried out the maximum depletion of PCP, about 82 %, at pH 8.0 and 35 °C within 168 h. The predominant Gram-negative bacterial strain, identified as *Agrobacterium radiobacter,* was used by Yueb and Ward (1996) in PCP degradation tests, individually and in combination with *Pseudomonas* sp. After 4 days of incubation at 100 ppm PCP, the capacity of PCP degraded by individual isolates was lower than observed when the strains were combined. In fact, *A. radiobacter* and the mixed culture degraded 60 and 80 %, respectively. Finally, the mass spectrum analysis showed that a principal metabolite of PCP degradation produced by *Pseudomonas* sp. and *A. radiobacter* was tetrachlorophenol (TeCP). From mushroom compost, Webb et al. (2001) isolated a strain known as *Saccharomonospora viridis* which was tested to degrade at concentrations of 10 mg L^{-1} PCP. The experiment was carried out after 10 days of incubation but within only eight days all of the PCP was degraded. The authors highlighted that *S. viridis* does not possess the ability to degrade PCP but rather transform it into other compost. They proposed this initial pathway for PCP transformation: PCP → TCHQ → TeCBQ. When the PCP concentration was above 20 mg L^{-1} it resulted in being too toxic for *S. viridis*. Adrian et. al (2007) used PCP as electron acceptors with *Dehalococcoides* sp. (strains 195 and CBDB1) demonstrating that this bacterium could produce a reductive dechlorination of the compounds. Only strains *Dehalococcoides* sp. CBDB1 dechlorinated PCP completely and quite rapidly, within 1 week of incubation. PCP dechlorination produced a mixture of 3,5-DCP, 3,4-DCP, 2,4-DCP, 3-CP and 4-CP, indicating that several degradation pathways were catalyzed. Männistö et al. (1999) isolated and tested the degradation capabilities of PCP with strains *Nocardioides* sp. (isolates K44 and K103) and *Candidatus comitans* K112. In these experiments, Männistö et al. evaluated the PCP degradation capacity when the compound was independent or mixed in a solution which contained 80 % 2,3,4,6-TeCP and about 20 % PCP. The mixed solution of 2,3,4,6-TeCP, PCP ended up completely degraded by strains. When PCP was independent at 2 mg L^{-1}, the strain *C. comitans* K112 degraded the PCP completely within the 28 days of incubation. *Nocardioides* sp. K44 and K103 did not degrade the PCP when alone, although they did degraded it completely when mixed with 2,3,4,6-TeCP. *Novosphingobium* sp. MT1 is a bacteria which was isolated in contaminated water and sand in Finland, presenting a mixture of CP contaminant (Tiirola et al. 2002). The substrate was spiked four times with a CPs mixture containing 2,4,6,TCP, 2,3,4,6-TeCP, and PCP, which was the approximate ratios as in the influent groundwater where the bacterium was isolated. *Novosphingobium* sp. MT1 strain showed a good PCP degradation capacity. It completely degraded the PCP after the first spiking (about 150 h) which continued to have a very high level of degradation even after the other three spikings.

2.10.2 PCP degradation in engineered systems

Arthrobacter sp. was used in PCP degradation reactor tests in engineered systems. Edgehill (1996) isolated and used *Arthrobacter* sp. ATCC 33790 in a biofilm culture. After 108 days, about 85 % of PCP was dechlorinate from an initial concentration of 145 mg L^{-1} PCP. Using a mixed culture containing *Arthrobacter* sp. in a cyclic batch bioreactor with non-sterile conditions, Hamid Mollah and Grant Allen (1999), showed the strains belonging to this genus can degrade and mineralize PCP. There were two PCP concentration used in the experiments: 50 and 120 mg L^{-1}. Both concentrations were subjected to complete degradation of PCP in a five-month span. Anaerobic bacteria *Actinomycetes* sp., *Streptacidiphilus* sp., aerobic

Rhodococcus erythropolis, *Amycolatopsis* sp. and *Gordonia* sp. were found to be tolerant and able to degrade PCP in contaminated effluent in a biocathode by Huang et al. (2012). The biocathode use microorganisms as catalysts to transfer electrons from the cathode to another electron acceptor, provide an alternative to catalysts of the oxygen reduction. These bacteria, in a mixed culture, were tested at different initial PCP concentrations (5, 10, 20, 30 and 40 mg L^{-1}) with the variable of time being 100 h of incubation. Under PCP concentrations of 20 mg L^{-1}, the PCP was completely degraded within the 100 h. While at 30 and 40 mg L^{-1} only 15 and 50 % respectively. The maximum PCP degradation rate in the biocathode was 0.263 ± 0.05 mg/L-h (51.5 mg g^{-1} VSS-h) with 60.6 % reduction of PCP from 31.2 ± 2.1 to 12.3 ± 2.1 mg L^{-1} after 3 days. The abiotic control showed a PCP loss of 10.6 %, due to the chemical reduction, adsorption, measurement errors and diffusion through the membrane into the anode chamber. Chloride accumulated in the solution was in proportion to the PCP removed, demonstrating microbial dechlorination. At an initial PCP concentration of 30 mg L^{-1}, chloride ions were produced after 72 h, while there was less Cl^- released at 40 mg L^{-1} PCP. Probably due to the inhibition of microorganisms at this concentration. At a high temperature of 50 °C and pH level of 6 the PCP degradation improved. Principal PCP degradation product obtained by the experiments were: TCHQ, TriCHQ and 2,6-DCHQ. The specific role of the individual microorganism was not analyzed but in the overall they were able to mineralize PCP in the biocathode.

3 Critical and relevance aspect in PCP degradation by bacteria

3.1 PCP degradation in microbiological culture media

The PCP biodegradability in microbiological culture media has been considered in many works, being the initial step for a whatever bioremediation work. The majority of these studies are focused on metabolism of single organisms, mixed cultures and degradation pathways under different conditions (pollutant concentration, temperature, pH level and moisture). The Gram-negative *Brevibacterium casei* (Verma and Singh 2012) was the most resistant bacterium able to degrade very high PCP concentrations. This bacterium may be potentially useful in PCP bioremediation processes, but despite that, in bibliography there is only one study of PCP depletion. It might be interesting to increase the knowledge on the interaction between this bacterium and PCP. In many studies on bioremediation *Pseudomonas* sp. and *Sphingomonas* sp. are certainly the most tolerant to PCP in respects to bacteria which degrade this compound. These two genera have showed excellent capacity also in other pollutant degradation and several of the new genetic studies are focused on them. They are able to remove very high initial concentrations of PCP. A large number of species belonging to Pseudomonas sp. has been widely used. *Pseudomonas fluorescens*, versatile bacteria with biocontrol properties, has really paved the way for considerable possibilities in bioremediation strategy. To protect the roots of some plant species against parasitic fungi and bacteria (Haas and Défago 2005). Specific considerations must be thought for *Pseudomonas aeruginosa*. In fact, this bacterium, despite the ability to degrade PCP (although slowly), has some negative attributes like species that can cause diseases in animals (including humans) and in plants (He et al. 2004). Definitely the most innocuous genus listed is *Pseudomonas* (e.g. *P. mendocina*, *P. stutzeri* and *P. veronii*). As for the second genus, *Sphingomonas chlorophenolica* RA-2 is the species that appears in most of the works. *Pseudomonas* sp. and *Sphingomonas* sp. are able to mineralize PCP and the intermediate products of the PCP biodegradation are: TCHQ (Shah and Thakur 2002; McCarthy et al. 1997a), TCC (Nam et al. 2003), CPs (Kao et al. 2005) and CHQs (Shah and Thakur 2002; McCarthy et al. 1997a) (Table 1). This bacteria has shown excellent results which have been highlighted *Bacillus* sp. Which has the ability to degrade PCP very quickly, either individually or when used in mixed cultures with other *Bacillus* or with microorganisms of other genera. However, a very important point to remember is that in the *Bacillus* genus, there is *Bacillus cereus* which is responsible for a minority of food-borne illnesses, causing severe nausea, vomiting and diarrhea (Kotiranta et al. 2000). Others strains of genus which are reported in this review appear to be extremely weak parasites. The intermediate products of the PCP biodegradation for *Bacillus* sp. are TCHQ (Singh et al. 2009). *Serratia marcescens* was often

isolated from many sites highly contaminated with PCP showing a formidable ability in the degradation of PCP caused by a reductive dechlorination. The intermediate products of PCP biodegradation for *Serratia* sp. are TCHQ and CHQs (Shah and Thakur 2002, Singh et al. 2008, 2009). If it is a naturally occurring reaction, it has the potential to be applied to treat PCP contaminated sites e.g. pulp, paper and mill effluent. Although it has lower possible uses in the bioagumentation processes because it's nature as a human pathogen, it has been linked responsible for infections in the urinary tract and skin (Hejazi and Falkiner 1997). When used in a mixed culture (e.g. Bacillus sp.) showed a good synergistic effect, which increased the percentage of the PCP degraded. *Arthobacter* sp. also showed a high level of tolerance and degradation even though the best performance of PCP removal was only in mixed cultures. The genus *Nocardioides* was not able to degrade PCP when alone, but only in the presence of another pollutant despite low initial concentrations. *Candidatus comitans* and *Escherichia coli* showed a slow capacity in degrading PCP opposed to *Acinetobacter* sp., *Dehalococcoides* sp., *Kokuria* sp. and *Novosphingobium* sp. which showed a higher capacity at tolerating and degrading to PCP, as well as being quicker than most other strains. *Saccharomonospora viridis* degraded at a low PCP concentrations, and was not able to mineralize PCP values of 20 mg L^{-1} which are already toxic to these species.

3.2 PCP degradation in engineered systems

In engineered systems there were few studies available on the biological treatment of PCP. This system in a short time could result an interesting practical system for bioremediation of PCP-contaminated water and soil samples (Table 1); although in some cases they can result very expensive. *Sphingomonas chlorophenolica* was the best microorganism in dealing with PCP degradation in the batch reactor tests. It was able to remove very high concentrations of PCP very quickly. The tolerance threshold of to this pollutant appears to be around 500 mg Kg^{-1} without pre exposition to PCP. At this value the degradation capacity seem to be inefficient. Moreover, with a PCP pre-contact, *S. chlorophenolica* can tolerate and degrade concentrations around of 750 mg Kg^{-1} (Yang et al. 2005; Lanthier et al. 2005). *Arthrobacter* sp. showed a high capacity in regards to PCP degradation but it was rather slow (Edgehill 1996). *Desulfitobacterium* sp., which is one of the most versatile strains, could possibly be one of the best candidates (having analyzed both species) for developing the bioremediation processes (Tartakovsky et al. 1999). This bacterium produced TCP as intermediate products of PCP biodegradation. Mixed cultures generally mineralized concentrations of PCP at values of no more than 20 mg L^{-1}. This ability to completely mineralize the PCP and its metabolites, depends essentially on the type of microbial consortium and their synergistic effects. In presence of amendments such as N and P, the ability to degrade PCP, the bacteria strains greatly increase (e.g. *S. chlorophenolica* and *Pseudomonas* sp.).

3.3 PCP degradation in soil, sediment and sludge

Physical, chemical, and biological studies on PCP biodegradation in contaminated soil, effluent and sludges represent a fundamental topic on which much research has been focused (Table 1). Various bacteria have been employed to remove PCP, and obtain complete mineralization. Inoculation with PCP degraders may, in some cases, be the only way for microbial cleanup of contaminated sites. However, the success of PCP bioremediation is affected by several factors, including the species of microorganisms and site properties (soil characteristics, environmental conditions, pollutants amount and etc.). When contaminants such as PCP enter the soil or water they are subjected to a high number of bio-chemicals processes (Castillo and Bárcenas 1998; McAllister et al. 1996; Field and Sierra-Alvarez 2008). A contaminant may be lost at different rates and at different phases (Stokes et al. 2006). The Remaining fraction present in the soil is not completely available to all organisms, because they can be sequestered by organic and inorganic compounds. Fluvisol, Chernozem, and Arenosol studied by Vítková et al. (2011) showed a different ability in PCP bioremediation. It was widely noted that a good success of decontamination and detoxification depends mainly on the amount of organic soil matter and other parameters as seen also by Scelza et al. (2008). Naturally the presence of particular microorganisms can facilitate the speed and quality of remediation in many contaminants. The species belonging to genus *Spingomonas* and *Pseudomonas* showed an excellent

ability to metabolize PCP also at very high initial concentrations; even at 500 mg PCP Kg^{-1} soil was inhibited. Exactly as in microbiological liquid media, the genus *Pseudomonas* biodegrades PCP into TCHQ. PCP mineralization can in many cases be increased and accelerated (also in less time), above all when there are in the optimal conditions for growth. *Mycobacterium chlorophenolicum* and *Kokuria* sp. showed a high level of tolerance and degradation of PCP. Finally, *Flavobacterium* sp. had the ability to degrade and mineralize high PCP concentrations but this capacity was dependent on the type of soil in which it was tested and the presence of other microorganisms. In fact this genus in the interaction with other microorganisms often tends to be overwhelmed.

4 PCP degradation by fungi

Only during the last forty years PCP fungal bioremediation received some consideration (McAllister et al. 1996; Gadd 2001; Pointing 2001; Singh 2006; Field and Sierra-Alvarez 2008; Rubilar et al. 2008). Currently degradation of PCP by Ascomycetes, Basidiomycetes, Deuteromycetes and Zygomycetes has been widely studied (McAllister et al. 1996; Gadd 2001; Singh 2006). Ascomycetes showed good results as the disappearance of PCP occurs both in PH-oxidase and oxidase ways. Basidiomycetes deplete PCP moderately, irrespective of high producers of PH-oxidase. Unlike bacteria, fungi do not normally use PCP as source of carbon or energy. PCP degradation is not the consequence of specific enzymes used for these functions. In fact, in fungi, this process occurs through co-metabolic reactions using fungal enzymes, which generally are slotted for other purposes. The biodegradation capacity of some fungi for PCP has shown that they can tolerant very high concentrations such as 500–1,000 mg L^{-1} PCP. Even if fungi are not completely efficient in respect to PCP degradation in a liquid culture or soil (McAllister et al. 1996; Gadd 2001). The Fungi groups most commonly used in experiments of PCP degradation are Basidiomycetes agents of White and Brown-rot. Among these, the genus *Phanerochaete*, *Anthracophyllum* and *Trametes* have received more attention due to their better results, even when a number of other groups of fungi were tested in PCP degradation: Zygomycetes, Ascomycetes and Deuteromycetes (Table 2).

4.1 The genus *Anthracophyllum*

Over the last ten years, white-rot fungi widely used in bioremediation experiments is *Anthracophyllum discolor*. It is a Chilean fungus from Patagonia which has showed an excellent capacity in bioremediation versus several toxic compounds such as PCP and Polycyclic aromatic hydrocarbon (PAH) (Diez 2010). In this genus, which is widespread in tropical regions, there are only ten species until now known.

4.1.1 PCP degradation in microbiological culture media

Tortella et al. (2008) evaluated *Anthracophyllum discolor* Sp4 capacity in the biodegradation of some CP compounds which included PCP at 25 mg L^{-1}. After 15 days of incubation, *A. discolor* Sp4 degraded 96 % of PCP. PCP degradation by fungi in a liquid medium was correlated only by the ligninolytic enzyme production. Maximum production of manganese peroxidase was detected in *A. discolor* Sp4 between 3 and 6 days of incubation while, a high concentration of lignin peroxidase was produced between 6 and 9 days of incubation. Laccase was not detected. *A. discolor* Sp4 was the strain that presented the highest manganese and lignin peroxidase production, being also superior when compared to the control fungus *P. chrysosporium* CECT-2798.

4.1.2 PCP degradation in soil, sediment and sludge

During the last few years *Anthracophyllum discolor* has been widely used in PCP degradation in soil. Rubilar et al. (2011) investigated the bioremediation capacity in Chilean andisol soil contaminated with 250 and 350 mg PCP Kg^{-1} soil using a strain of *A. discolor*. The fungus strain was used in experiments as free and immobilized in wheat grains (a lignocellulosic material). At initial PCP concentrations of 250 and 350 mg Kg^{-1} soil, immobilized *A. discolor* removed 80 and 93.2 %. In the biotic controlled soil only 50 and 62.6 % of PCP was removed at levels of 250 and 350 mg PCP Kg^{-1} soil. This difference in PCP removal could be due to the synergistic effects occurring between fungi and autochthonous microorganisms. In the sterile soil without fungus (the abiotic control), for both PCP concentrations tested the amount of pollutant removed was 40 %, mainly due to the previously mentioned characteristics

Table 2 Degradation of PCP by fungi

Species	Condictions	PCP	Degradation	Dechlorination	Mineralization	Degradation product(s)	Degradation time	References
Phanerochaete chrysosporium ATCC 42725	Soil	100 μg g^{-1}	+	NR	NR	PCA; 2,3,4,6-tetrachloroanisole	4 weeks	Leštan and Lamar (1996)
Phanerochaete sordida HHB-8922-Sp	Soil	100 μg g^{-1}	92 %	NR	NR	PCA; 2,3,4,6-tetrachloroanisole	4 weeks	
Irpex lacteus ATCC 11245	Soil	100 μg g^{-1}	82 %	NR	NR	PCA; 2,3,4,6-tetrachloroanisole	4 weeks	
Bjerkandera adusta ATCC 62023	Soil	100 μg g^{-1}	86 %	NR	NR	PCA; 2,3,4,6-tetrachloroanisole	4 weeks	
Trametes versicolor MD-277	Soil	100 μg g^{-1}	86 %	NR	NR	PCA; 2,3,4,6-tetrachloroanisole	4 weeks	
Lentinula edodes LE2	Soil	200 mg Kg^{-1}	50–70 %	NR	NR	NR	21 days	Okeke et al. (1996)
Phanerochaete chrvsosporium BKM 1767	Soil	200 mg Kg^{-1}	75–85 %	NR	NR	NR	21 days	
Phanerochaete chrysosporium BMK-F-1767	Liquid	250 mg L^{-1}	72–95 %	NR	NR	Chloride ions	3 days	Aiken and Logan (1996)
Lentinula edodes LE2	Soil	200 mg Kg^{-1}	35 %	+	10 %	PCA	10 weeks	Okeke et al. (1997)
Phanerochaete sordida	Soil	175 ppm	+	NR	+	PCA	56 days	Pfender et al. (1997)
Trametes versicolor	Liquid	25 mg L^{-1}	>99 %	NR	NR	NR	12 h	Pallerla and Chambers (1998)
Armillaria gallica 1039	Liquid	25 mg L^{-1}	+	NR	NR	NR	7 days	Chiu et al. (1998)
Armillaria gallica 1057	Liquid	25 mg L^{-1}	+	NR	NR	2-methyl-1,3 benzenediol; 6-phenyl-dodecane	7 days	
Armillaria mellea M51	Liquid	25 mg L^{-1}	+	NR	NR	2-methyl-1,3 benzenediol	7 days	
Ganoderma lucidum HK-1	Liquid	25 mg L^{-1}	+	NR	NR	2-methyl-1,3 benzenediol; 1-octyl-benzene	7 days	
Lentinula edodes L54	Liquid	25 mg L^{-1}	+	NR	NR	NR	7 days	
Lentinula edodes L67	Liquid	25 mg L^{-1}	+	NR	NR	NR	7 days	
Lentinula edodes L68	Liquid	25 mg L^{-1}	+	NR	NR	1-chloro-3-methoxy-benzene	7 days	

Table 2 continued

Species	Condictions	PCP	Degradation	Dechlorination	Mineralization	Degradation product(s)	Degradation time	References
Phanerochaete chrysosporium M1	Liquid	25 mg L^{-1}	+	NR	NR	3,3-dimethyl-cyclohexanol	7 days	
Pleurotus pulmonarius PL-27	Liquid	25 mg L^{-1}	+	NR	NR	NR	7 days	
Polyporus sp. Cv-1	Liquid	25 mg L^{-1}	+	NR	NR	1-chloro-3-methoxy-benzene	7 days	
Volvariella volvacea V34	Liquid	25 mg L^{-1}	+	NR	NR	benzenediol	7 days	
Trametes versicolor PRL 572	Soil	996 μg g^{-1}	29 %	NR	+	PCA; 2,3,4,6-tetrachloroanisole	42 days	Tuomela et al. (1999)
Coriolus versicolor	Liquid	50–200 ppm	+	NR	NR	NR	72 h	Ullah and Evans (1999)
Gloeophyllum striatum DSM 9592	Liquid	5 μM	10 %	NR	NR	NR	19 days	Fahr et al. (1999)
Gloeophyllum striatum DSM 9592	Liquid	5 μM	10 %	NR	NR	NR	19 days	
Phanerochaete chrysosporium IFO 31249	Liquid	30 mg L^{-1}	72.6 %	NR	NR	NR	15 days	Ryu et al. (2000)
Trametes sp. KFCC 10941	Liquid	30 mg L^{-1}	64 %	NR	NR	NR	15 days	
Pleurotus sp. KFCC 10943	Liquid	30 mg L^{-1}	70.33 %	NR	NR	NR	15 days	
Coriolus versicolor FPRL-28A	Liquid	50–100 ppm	75–100 %	NR	NR	NR	2–24 h	Ullah et al. (2000)
Phanerochaete chrysosporium OGC101	Liquid	100 M	10–90 %	+	+	NR	30 h	Reddy and Gold (2000)
Rhizopus nigricans	Liquid	12.5–500 mg L^{-1}	+	NR	NR	NR	6–8 days	Tomasini et al. (2001)
Rhizopus nigricans	Liquid	12.5 mg L^{-1}	60–100 %	NR	NR	NR	24–120 h	Cortés et al. (2002)
Trichoderma harzianum 2023	Liquid	10 ppm	+	NR	NR	NR	9 days	Rigot and Matsumura (2002)
Absidia fusca	Liquid	100 mg L^{-1}	35–40 %	NR	NR	NR	4 days	Guiraud et al. (2003)

Table 2 continued

Species	Condictions	PCP	Degradation	Dechlorination	Mineralization	Degradation product(s)	Degradation time	References
Mucor ramosissimus IM 6203	Liquid	10 mg L^{-1}	+	NR	NR	2,3,5,6-TCHQ	7 days	Szewczyk et al. (2003)
Abortiporus biemmis HR145	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	Walter et al. (2003)
Oudemansiella australis HR345	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Peniophora sacrata HR226	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Peniophora sacrata HR235	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Peniophora sacrata HR240	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Peniophora sacrata HR241	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Rigidoporus catervatus HR316	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Stereum fasciatum HR348	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Trametes sp. HR192	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Trametes sp. HR196	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Trametes sp. HR197	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Trametes versicolor HR131	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Trametes versicolor HR154	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Trametes versicolor HR160	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Trametes versicolor HR275	Liquid	50 mg L^{-1}	100 %	NR	NR	NR	42 days	
Trametes versicolor HR277	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Trametes versicolor HR445	Liquid	50 mg L^{-1}	+	NR	NR	NR	42 days	
Pleurotus pulmonarius	Liquid	2–100 ppm	60–90 %	NR	NR	TCHQ; TCP	2 days	Law et al. (2003)

Table 2 continued

Species	Condictions	PCP	Degradation	Dechlorination	Mineralization	Degradation product(s)	Degradation time	References
Agrocybe perfecta CCB161	Soil	1,180–1,278 mg Kg^{-1}	78 %	NR	+	PCA; Chloride ions	90 days	Machado et al. (2005)
Trametes villosa CCB176	Soil	1,180–1,278 mg Kg^{-1}	58 %	NR	+	Chloride ions	90 days	
Trametes villosa CCB213	Soil	1,180–1,278 mg Kg^{-1}	58 %	NR	+	PCA; Chloride ions	90 days	
Psilocybe castanella CCB444	Soil	1,180–1,278 mg Kg^{-1}	64 %	NR	+	Chloride ions	90 days	
Peniophora cinerea CCB204	Soil	1,180–1,278 mg Kg^{-1}	43 %	NR	+	PCA; Chloride ions	90 days	
Penicillium camemberti	Liquid	0.001 M	+	NR	NR	Chloride ions	21 days	Taseli and Gokcay (2005)
Phanerochaete chrysosporium BKM-F-1767	Soil	100 mg Kg^{-1}	90 %	NR	NR	NR	60 days	Jiang et al. (2006)
Tramtes versicolor HR131	Soil	1,000 mg Kg^{-1}	>90 %	NR	NR	PCA	1–2 years	Walter et al. (2005)
Anthracophyllum discolor	Soil	100–300 mg Kg^{-1}	50–100 %	NR	NR	PCA; TCHQ	28 days	Rubilar et al. (2007)
Bjerkandera adusta ATTC 90940	Soil	100–300 mg Kg^{-1}	>80 %	NR	NR	PCA; TCHQ	28 days	
Byssochlamys fulva	Liquid	12.5–25 mg L^{-1}	20 %	NR	NR	NR	8 days	Scelza et al. (2008)
Anthracophyllum discolor Sp4	Liquid	25 mg L^{-1}	96 %	NR	NR	NR	15 days	Tortella et al. (2008)
Lenzites betulina Ru-30	Liquid	25 mg L^{-1}	80 %	NR	NR	NR	15 days	
Inonotus sp. Sp2	Liquid	25 mg L^{-1}	<50 %	NR	NR	NR	15 days	
Stereum sp. Ru-24	Liquid	25 mg L^{-1}	<50 %	NR	NR	NR	15 days	
Phanerochaete chrysosporium CECT-2798	Liquid	25 mg L^{-1}	72 %	NR	NR	NR	15 days	
Galerina patagònica Sp3	Liquid	25 mg L^{-1}	88 %	NR	NR	NR	15 days	
Stereum hirsutum Sp1	Liquid	25 mg L^{-1}	<50 %	NR	NR	NR	15 days	
Stereum hirsutum Ru-104	Liquid	25 mg L^{-1}	<50 %	NR	NR	NR	15 days	

Table 2 continued

Species	Condictions	PCP	Degradation	Dechlorination	Mineralization	Degradation product(s)	Degradation time	References
Tramtes hirsuta Ru-008	Liquid	25 mg L^{-1}	0 %	NR	NR	NR	15 days	
Trametes versicolor Ru-107	Liquid	25 mg L^{-1}	0 %	NR	NR	NR	15 days	
Trametes versicolor Ru-0030	Liquid	25 mg L^{-1}	0 %	NR	NR	NR	15 days	
Amylomyces rouxii	Liquid	12.5 mg L^{-1}	50–100 %	NR	NR	NR	120 h	Montiel-González et al. (2009)
Laetiporus cincinnatus	Liquid	50 ppm	+	NR	NR	NR	30 days	Ramesh and Pattar (2009)
Trametes versicolor	Liquid	50 ppm	96.14 %	NR	NR	NR	30 days	
Fomes fomentarius	Liquid	50 ppm	+	NR	NR	NR	30 days	
Ganoderma aplanutum	Liquid	50 ppm	+	NR	NR	NR	30 days	
Pleurotus ostreatus	Liquid	50 ppm	+	NR	NR	NR	30 days	
Chrysonilia sitophila DSM 16514	Liquid	1–20 mg L^{-1}	+	NR	NR	CHQ	50–60 days	Carvalho et al. (2009)
Mucor plumbeus DSM 16513	Liquid	1–20 mg L^{-1}	+	NR	NR	NR	50–60 days	
Trichoderma longibrachiatum DSM 16517	Liquid	1–20 mg L^{-1}	+	NR	NR	DCBQ	50–60 days	
Cladosporium herbarum	Liquid	1–20 mg L^{-1}	+	NR	NR	DCBQ	50–60 days	
Penicillium glabrum DSM 16516	Liquid	1–20 mg L^{-1}	+	NR	NR	CHQ	50–60 days	
Penicillium olsonii DSM 16515	Liquid	1–20 mg L^{-1}	+	NR	NR	NR	50–60 days	
Eupenicillium hirayamae	Liquid	1–20 mg L^{-1}	+	NR	NR	NR	50–60 days	
Penicillium brevicompactum	Liquid	1–20 mg L^{-1}	+	NR	NR	NR	50–60 days	
Penicillium glandicola	Liquid	1–20 mg L^{-1}	+	NR	NR	CHQ	50–60 days	
Penicillium variabile	Liquid	1–20 mg L^{-1}	+	NR	NR	CHQ	50–60 days	
Penicillium diversum	Liquid	1–20 mg L^{-1}	+	NR	NR	NR	50–60 days	
Penicillium decumbens	Liquid	1–20 mg L^{-1}	+	NR	NR	DCBQ	50–60 days	
Penicillium janczewskii	Liquid	1–20 mg L^{-1}	+	NR	NR	CHQ	50–60 days	

Table 2 continued

Species	Condictions	PCP	Degradation	Dechlorination	Mineralization	Degradation product(s)	Degradation time	References
Penicillium corylophilum	Liquid	1–20 mg L^{-1}	+	NR	NR	CHQ	50–60 days	
Penicillium adametzii	Liquid	1–20 mg L^{-1}	+	NR	NR	TeCBQ	50–60 days	
Penicillium fennelliae	Liquid	1–20 mg L^{-1}	+	NR	NR	NR	50–60 days	
Penicillium restrictum	Liquid	1–20 mg L^{-1}	+	NR	NR	NR	50–60 days	
Mucor ramonissimus IM 6203	Liquid	10 mg L^{-1}	90 %	NR	NR	2,3,5,6-TCHQ; pentachloromethoxybenzene	240 h	Szewczyk and Dlugoski (2009)
Anthracophyllum discolor	Soil	250 mg Kg^{-1}	93.6 %	NR	NR	NR	28 days	Cea et al. (2010)
Trametes pubescens CBS 696.94	Liquid	30 mg L^{-1}	77 %	NR	NR	Chloride ions	13 days	González et al. (2010)
Pleurotus pulmonarius CCB19	Liquid	25 mg L^{-1}	20–70 %	NR	NR	NR	96 h	de Souza et al. (2011)
Anthracophyllum discolor	Soil	250–350 mg PCP Kg^{-1}	80–93 %	NR	NR	NR	28 days	Rubilar et al. (2011)
Phanerochaete chrysosporium CECT-2798	Soil	250–350 mg PCP Kg^{-1}	65–79 %	NR	NR	NR	28 days	
Rhizopus oryzae ENHE	Liquid	12.5–25 mg L^{-1}	85–88 %	NR	NR	NR	48–72 h	León-Santiestebán et al. (2011)
Mucor plumbeus DSM 16513	Liquid	15–18.8 μM	+	+	+	TriCHQ; TCHQ	4 days	Carvalho et al. (2011)
Trametes pubescens CBS 696.94	Liquid	15 mg L^{-1}	41 %	NR	NR	NR	8 h	Gaitan et al. (2011)
Paraconiothyrium variabile	Liquid	20 mg L^{-1}	0 %	NR	NR	NR	9 days	Forootanfar et al. (2012)

NR not reported; + denotes positive observation; − denotes no response

of the Chilean Andisols which have particularly efficient sorbents for CPs (Cea et al. 2005). Rubilar et al. (2007) carried out a series of laboratory-based studies to determine the range of PCP concentration in soils (100, 250 and 350 mg PCP Kg^{-1} of soil) which could be degraded in slurry soil flasks by *A. discolor*. The fungus isolate degraded all PCP but only at the initial concentration of 100 mg PCP Kg^{-1} soil, while for the other two concentrations 250 and 350 mg PCP Kg^{-1} soil around 50 % of the contaminant was recovered. In *A. discolor*, PCP degradation metabolites were evaluated using GC/MS analysis. The main reaction of PCP degradation was methylation with the production of PCA. The second reaction identified was hydroxylation in the form of TCHQ, which is then methylated to generate tetrachloro-1, 4-dimethoxybenzene, followed by successive dechlorination reactions to form 2,5-dichloro-1, 4-dimethoxybenzene and 2-chloro-1, 4-dimethoxybenzene. A series of demethoxylation, carboxylation, reduction, and methylation reactions were conducted to form 3,4-dimethoxybenzaldehyde and then the formation of CO_2 for the complete mineralization. Cea et al. (2010) reported a bioaugmentation essay with *A. discolor* in a soil contaminated with PCP and evaluated its impact on the microbial soil community. In this experiment three types of microcosm soils (contaminated with 250 mg PCP Kg^{-1} soil) were created: fresh soil, fresh soil plus wheat straw and fresh soil plus wheat straw inoculated with *A. discolor*. Laccase and Manganese peroxidase activity were higher in the presence of the white-rot fungus while the PCP that was removed after 28 day of incubation was 93.6 % for the fresh soil plus wheat straw and about 87 % in the fresh soil plus wheat straw inoculated with *A. discolor*.

4.2 The genus *Mucor*

Members of the genus *Mucor* were extensively studied in human and plant pathology. It is found commonly in soil, especially on rotten vegetable matter, and has showed discrete ability in directing contaminant degradation such as PCP.

4.2.1 PCP degradation in microbiological culture media

Szewczyk et al. (2003) tested the growth and degradation abilities of fifteen fungal strains isolated from contaminated areas, in the presence of compounds such as PCP. Among these strains there was *Mucor ramosissimus* IM 6203 that in the PCP degradation process released an intermediate product of 2,3,5,6-TCHQ. The PCP degradation by *M. ramosissimus* IM 6203 was improved significantly in a medium with overworked oil where only 8.3 % of PCP and 4.3 % of 2,3,5,6-TCHQ were found after 7 days of incubation, starting from 10 mg L^{-1}PCP. Szewczyk and Dlugoski (2009) evaluated PCP degradation and metabolites formed in cultures of *Mucor ramosissimus* IM 6203 with different optimized mediums. After 240 h, 90 % of PCP was removed from an initial concentration of 10 mg L^{-1} and the following metabolites were identified: 2,3,5,6-TCHQ and some anisoles such as pentachloromethoxybenzene. Carvalho et al. (2011) detected the capacity of *Mucor plumbeus* DSM 16513 to remove PCP in a liquid culture and its pathway of PCP degradation using liquid chromatography coupled with quadruple time-of-flight mass spectrometry. In PCP degradation experiments the presence or absence of glucose was a very important variable. When glucose was added to the culture, all PCP was removed during 4 days of incubation. While, after 60 days without glucose only 80 % was degraded. The pathway of PCP degradation exhibited the presence of TCHQ, TriCHQ and phase II-conjugated metabolites. Carvalho et al. (2009) studied the co- and direct metabolism of PCP using *Mucor plumbeus* DSM 16513 in experiments of percentage of PCP decay, under co-metabolic conditions with PCP concentration being between 5 and 15 mg L^{-1}. *M. plumbeus* DSM 16513 was able to degrade PCP while at 15 mg L^{-1} the strains failed *the* biotransformation of PCP. Experiments of PCP decay in fungal cultures under metabolic conditions showed that *M. plumbeus* DSM 16513 removed 85 % of PCP up to 5 mg L^{-1} (maximum value tested).

4.3 The genus *Penicillium*

In the genus *Penicillum* there are ubiquitous soil fungi widespread in all the world. It is commonly present wherever organic material is available. During the last 30 years, *Penicillium* spp. have demonstrated remarkable ability to degrade different xenobiotic compounds and could be potentially interesting for the development of bioremediation processes for pollutant transformation/mineralization.

4.3.1 PCP degradation in microbiological culture media

Carvalho et al. (2009) studied the co- and direct metabolism of PCP by *Penicillium* fungal strains isolated from the cork-colonizing community. The species that were isolated are: *P. glabrum* DSM 16516, *P. olsonii* DSM 16515, *Eupenicillium hirayamae* (anamorph state of *P. hirayamae*), *P. brevicompactum*, *P. glandicola*, *P. variabile, P. diversum*, *P. decumbens*, *P. janczewskii*, *P. corylophilum*, *P. adametzii*, *P. fennelliae* and *P. restrictum*. In experiments of mycelium growth and PCP decay (%) under co-metabolic conditions with PCP concentrations being between 5 and 20 mg L^{-1}, all fungi were able to degrade PCP. But the maximum capacity to remove PCP, at 20 mg L^{-1}, was detected only by *P. brevicompactum*, *P. olsonii* DSM 16515 and *P. janczewskii,* with PCP decay of *56 and 59 %*. Experiments dealing with PCP decay in fungal cultures under metabolic conditions showed that *P. glandicola* and *P. janczewskii* removed PCP up to 5 mg L^{-1} (maximum value tested) for a result of 34, 67, and 85 %. Only in co-metabolic conditions was produced and identified a PCP metabolic intermediate. CHQ was recovered in the cultures of *P. corylophilum*, *P. glabrum* DSM 16516, *P. glandicola*, *P. janczewskii* and *P. variabile*; 2,6-dichloro-1,4-benzoquinone (DCBQ) in the cultures of *P. decumbens*; and finally TeCBQ in the cultures of *P. adametzii*. Taseli and Gokcay (2005) isolated and studied a *Penicillium camemberti* for its ability to degrade PCP as well as other chlorinated compounds. The batch experiments were conducted in shake flasks using PCP as a co-substrate and *P. camemberti* removed around 56 % PCP. Experiments in shake flasks, produced 86 % of the PCP removal after 21 days.

4.4 The genus *Phanerochaete*

The genus *Phanerochaete* is a widespread group of saprophytic and wood decay fungi. It is a secondary decomposer of both hardwood and softwood. This ability has generated much interest in bioremediation process as an environmentally benign alternative to chemical bleaching. It has been shown to detoxify PCP by the methylation process using its lignin-degrading enzymes (McAllister et al. 1996; Gadd 2001; Field and Sierra-Alvarez 2008). This fungus has shown extensive and rapid conversion of PCP in other compounds.

4.4.1 PCP degradation in microbiological culture media

Aiken and Logan (1996) studied the degradation of 250 mg L^{-1} PCP by *Phanerochaete chrysosporium* BMK-F-1767 in a static flask culture using ammonium lignosulphonates (waste product of the papermill industry) as a carbon and nitrogen source. When ammonium lignosulphonates was used as the nitrogen source, PCP removal was 75 %. When ammonium lignosulphonates was used as a carbon source, PCP removal was 72 %. When *P. chrysosporium* BMK-F-1767 grew on a nitrogen-limited glucose ammonia medium, it removed 95 % of PCP. Ryu et al. (2000) investigated the roles and activity of lignin peroxidase, manganese peroxidase and laccase in biodegradation of 30 mg L^{-1} PCP using *Phanerochaete chrysosporium* IFO 31249. After 15 days *P. chrysosporium* FO 31249 showed a value of PCP degradation of 72.6 %. The lignin peroxidase and manganese peroxidase activities were not detected in the culture broth of *P. chrysosporium* containing PCP. These results presumed the inhibition on production of enzyme by PCP. These data indicated that PCP was degraded under non-ligninolytic conditions. Chiu et al. (1998) studied the tolerance, bio-sorption and biodegradation capacity (these last two activities in relation to the fungi biomass dry wet) in the presence of 25 mg L^{-1} PCP for *Phanerochaete chrysosporium* M1. The tolerance to PCP was very high for *P. chrysosporium* M1 as well as the bio-sorption and biodegradation capacity. Tortella et al. (2008) carried out a study on the ability of *Phanerochaete chrysosporium* CECT-2798 in a biodegradation test with 25 mg L^{-1} PCP. After 15 days of incubation, *P. chrysosporium* degraded 72 % of PCP. Reddy and Gold (2000) evaluated intermediate products involved in PCP degradation with *Phanerochaete chrysosporium* OGC101. After 30 h of incubation, 10 % of PCP was degraded in a nitrogen-limited medium while 90 % of PCP was degraded into optimum nutrient conditions. The pathways for the degradation of PCP were elucidated by the characterization of the fungal metabolites and oxidation products generated by purified lignin peroxidase and manganese peroxidase. The oxidative dechlorination reaction of PCP produced TeCBQ. The quinone was subsequently reduced to tetrachlorodihydroxybenzene, that with another four successive reductive dechlorinations produced 1,4-hydroquinone

and latter formed 1,2,4-trihydroxybenzene. Alternatively TeCBQ was converted to 2,3,5-trichlorotrihydroxybenzene, which undergoes successive reductive dechlorinations, which produced 1,2,4-trihydroxybenzene. Finally, 1,2,4-trihydroxybenzene in each of the pathways was ring-cleaved, with subsequent degradation to CO_2.

4.4.2 PCP degradation in soil, sediment and sludge

The genus most frequently used for PCP remediation in soil, sediment and sludge has been *Phanerochaete*. Rubilar et al. (2011) investigated the bioremediation capacity in Chilean andisol soil contaminated with 250 and 350 mg PCP Kg^{-1} soil using *Phanerochaete chrysosporium* CECT-2798. The fungus strain was incorporated as free and immobilized in wheat grains, a lignocellulosic material. At PCP concentration of 250 and 350 mg PCP Kg^{-1}soil, *P. chrysosporium* CECT-2798 removed 65 and 79 %. In the controlled soil with wheat grains only 50 and 62.6 % were removed at 250 and 350 mg PCP Kg^{-1} soil. In the sterile soil without fungus (the abiotic control), for both PCP concentrations 250 and 350 mg PCP Kg^{-1} soil the amount of pollutant removed was 40 %, probably due to the Chilean Andisols, which are particularly efficient sorbents for CPs, mainly the allophane–ferrihydrite associations with organic matter (Cea et al. 2005). Pfender et al. (1997) used in a laboratory-scale bioremediation in a soil microcosm in a bottle amended with 175 ppm PCP, as well as two bacteria other than just *Phanerochaete sordida*. Over 35 % of the available PCP was transformed into PCA after 56 days, while only 10 % was mineralized. Okeke et al. (1996) determined the temperature, soil moisture potential and initial pH levels might influence the transformation of PCP by *Phanerochaete chrysosporium* BKM 1767. This fungal strain showed the highest levels of degradation, about 75 % at 25 °C and with the soil pH level of 4.0. On the other hand, the extent of PCP degradation related to soil moisture content was higher for *P. chrysosporium* BKM 1767, about 85 %. Jiang et al. (2006) investigated the reduction of PCP in contaminated soil inoculating it with free and immobilized *Phanerochaete chrysosporium* BKM-F-1767. Parallel beakers were adopted with the same components of soil, yard waste, straw, bran for aerated composting and 100 mg PCP Kg^{-1} soil. In the soil inoculated with *P. chrysosporium* BKM-F-1767 (free and immobilized), 90 % of the PCP was removed during the 60 days of incubation, while in the same time span the controlled soil which was without inoculation only degraded 50 % of PCP. Leštan and Lamar (1996) evaluated the PCP degradation in a soil microcosm of *Phanerochaete chrysosporium* ATCC 42725 and *Phanerochaete sordida* HHB-8922-Sp. After 4 weeks of incubation in a soil artificially contaminated with 100 μg g^{-1} PCP and inoculated with a 3 % pelleted fungal inoculums, both fungi showed a good capacity to convert PCP to PCA. *P. sordida* HHB-8922-Sp removed 92 % of the PCP at initial concentrations. PCP methylation was reported for *P. chrysosporium* ATCC 42725 and *P. sordida* HHB-8922-Sp, which transformed PCP to PCA.

4.5 The genus *Pleurotus*

Pleurotus is a genus that includes some eaten mushrooms that are found in both tropical and temperate climates throughout the world. It has been used widely in mycoremediation of pollutants such as petroleum, PAH and CPs (Gadd 2001; Singh 2006).

4.5.1 PCP degradation in microbiological culture media

Law et al. (2003) used a spent compost of oyster mushroom *Pleurotus pulmonarius* in PCP degradation and biodegradation processes analysis of the xenobiotic compound. With only 5 % of spent compost mushroom of *P. pulmonarius* 88.9 % was removed (18.8 % biosorption and 70.1 % biodegradation) of 2 mg L^{-1} PCP. Further increases in the amount of fungi showed no improvement in the total removal efficiency. For concentrations ranging from 10 to 100 mg L^{-1} PCP, the trends of *P. pulmonarius* to remove PCP efficiently was between 60 % and 80 %. PCP degradation with *P. pulmonarius* involves dechlorination, methylation, carboxylation and ring cleavage with abundant release especially of TCHQ and TCP. Ryu et al. (2000) investigated the roles and trend of lignin peroxidase, manganese peroxidase and laccase in the biodegradation of 30 mg L^{-1} PCP using *Pleurotus* sp. KFCC 10943. After 15 days of incubation *Pleurotus* sp. KFCC 10943 showed low enzymatic activity but degraded 70.33 % of PCP. Chiu et al. (1998) detected biodegradation capacity in the presence of 25 mg L^{-1} PCP for *Pleurotus*

pulmonarius PL-27. The strain M51 and *P. pulmonarius* PL-27 showed the highest degradative capacity, it being 13 and 10 mg PCP for a gram of mycelium dry wet. Chloroanisols were PCP breakdown intermediates (Table 2). de Souza et al. (2011) investigated PCP removal and adsorption by *Pleurotus pulmonarius* CCB19 in submerged cultures, formed with basal or corn cob medium, in the presence and absence of laccase. When PCP was added at a final concentration of 25 mg L^{-1}, the laccase production considerably increased and 70 % of PCP was removed after 96 h. Instead with low laccase activity the removal of PCP was less than 20 %. The amount of PCP adsorbed in the mycelial mass was about 10 % whether it was obtained in the corn cob medium with laccase or in the basal cultures without laccase. Ramesh and Pattar (2009) tested the biodegradation ability of PCP by *Pleurotus ostreatus*. The fungal strain showed a peak in laccase activity after 30 days of incubation which produced the highest amount of PCP removed. In a static culture *P. ostreatus* degraded 100 % of 50 ppm PCP during 30 days of incubation.

4.6 The genus *Rhizopus*

Rhizopus is a common saprophytic fungi on plants and specialized as animal parasites. A few species of *Rhizopus* are known to cause disease in humans as well as *Rhizopus oryzae.* It is the principal cause of zygomycosis. They are found on a wide variety of organic substrates.

4.6.1 PCP degradation in microbiological culture media

Cortés et al. (2002) studied PCP degradation in a solid-state culture with a strain of *Rhizopus nigricans*. This fungus displayed high growth tolerance in the presence of PCP (up to 100 mg L^{-1}) and degraded 60 % within 24 h and 100 % after 120 h of 12.5 mg L^{-1}PCP. Tomasini et al. (2001) found a strain of *R. nigricans* able to adsorb and degrade PCP in a submerged culture. They found that *R. nigricans* adsorbed PCP and that its adsorption capacity was higher when they increased the PCP concentration in a liquid medium. The biomass of *R. nigricans* adsorbed between 0.004 and 0.15 mg PCP mg mycelium $^{-1}$. Moreover the fungus completely removed 12.5 mg L^{-1} of PCP within 6 and 8 days with a mycelium age of 48 and 96 h. León-Santiestebán et al. (2011) described PCP absorption in a nylon fiber in which *Rhizopus oryzae* ENHE was immobilized. Various immobilization techniques were evaluated but, those that produced more biomasses were: cultures with nylon cubes that contained PCP at an equilibrium concentration and nylon at an equilibrium concentration amended with 14 mg PCP g^{-1} nylon. Two initial PCP concentrations of 12.5 and 25 mg L^{-1} were tested. In both cultures, PCP removal was similar: after 48 h in the cultures with 12.5 mg L^{-1} PCP 88.6 % of contaminate was removed and in cultures with 25 mg L^{-1} PCP, 85.7 % was removed. In 72 h for both concentrations the fungus immobilized in nylon 100 % of PCP.

4.7 The genus *Trametes*

Members of the genus *Trametes* were extensively studied for interesting activity in medicine and plant pathology. The aggressive white rot fungi which is world spread, thanks to its enzymes it could be an excellent candidate for direct PCP degradation (McAllister et al. 1996; Gadd 2001; Field and Sierra-Alvarez 2008).

4.7.1 PCP degradation in microbiological culture media

Ullah and Evans (1999) analyzed the ability of *Coriolus versicolor* to deplete PCP comparing inoculated and un-inoculated wheat husk incubated with 200 ppm PCP. In a second experiment they detected PCP degradation by wheat husk inoculated with *C. versicolor* increasing concentrations from 50 to 200 ppm PCP. When wheat husk was inoculated, the PCP was completely removed at any concentration after 72 h. While when the authors used un-inoculated wheat husk only 70 % of 200 ppm PCP was depleted. Walter et al. (2003) evaluated nine *Trametes* sp. strains with the potential for bioremediation of 50 mg L^{-1} PCP. The fungi were identified as: *Trametes* sp. HR192, *Trametes* sp. HR196, *Trametes* sp. HR197, *Trametes versicolor* HR131, *Trametes versicolor* HR154, *Trametes versicolor* HR160, *Trametes versicolor* HR275, *Trametes versicolor* HR277 and *Trametes versicolor* HR445. The PCP remaining in the liquid fraction after 42 days of stationary incubation was evaluated and the highest degradation capacity

was found to be in *Trametes versicolor* HR275 where 100 % of PCP was removed. In correlation to PCP degradation they also detected the presence and production of laccase. For the genera *T. versicolor* the laccase activity was high and the enzyme production varied with time. González et al. (2010) tested the white-rot fungi *Trametes pubescens* CBS 696.94 in CPs bioremediation and between various compounds used PCP. The experiments were carried out with an initial PCP concentration of 30 mg L^{-1} and in the absence or presence of supplemented glucose to obtain a final concentration of 1.75 g L^{-1}. After 13 days of incubation there were no differences in PCP degradation with or without glucose and chloride. In both cases 77 % of PCP was degraded. Ryu et al. (2000) used lignin peroxidase, manganese peroxidase and laccase by *Trametes* sp. KFCC 10941 in the biodegradation of 30 mg L^{-1} PCP. After 15 days of incubation *Trametes* sp. KFCC 10941 showed higher enzymatic production, above all with that, of laccase and manganese peroxidase but, on other hand, only 64 % of PCP was degraded. Tortella et al. (2008) used *Tramtes hirsuta* Ru-008, *Trametes versicolor* Ru-107 and *Trametes versicolor* Ru-0030 in a biodegradation test with PCP at an initial concentration of 25 mg L^{-1}. All other fungi degraded the PCP under 50 %, while for three strains, *T. versicolor* (Ru-0030 and Ru-008) and *T. hirsuta* Ru-008, PCP caused an inhibitory effect on growth and enzymatic production. Gaitan et al. (2011) using laccase produced by white-rot fungus *T. pubescens* CBS 696.94 evaluated the PCP degradation capacity in a shake flask. Two laccase iso enzymes with different molecular weights were isolated and identified. After 8 h of reaction, 41 % of 15 mg L^{-1} PCP was removed in a mixture with other CPs. Ramesh and Pattar (2009) tested in vitro the biodegradation capacity of PCP with *T. versicolor*. The fungus isolates showed a peak in laccase activity after 30 days of incubation which resulted in the highest amount of PCP removed. In a static culture studies *T. versicolor* degraded 96.14 % of PCP within 30 days of incubation.

4.7.2 PCP degradation in engineered systems

Pallerla and Chambers (1998) investigated the capacity of *T. versicolor* to degrade 25 mg L^{-1} PCP after 12 h of incubation in continuous polyurethane immobilized fungal fluidized bed bioreactor. *T. versicolor* degraded about 99 % of PCP after 12 h. Ullah et al. (2000) used a system of different solid substrates to grow *Coriolus versicolor* FPRL-28A. They evaluated laccase activity and the removal of PCP from aqueous effluent. Substrates included wood chips, cereal grain, wheat husk and wheat bran. Higher activity of laccase occurred with wheat husk and wheat bran. Laccase in wheat husk and wheat bran cultures removed 75–80 % of 50 ppm PCP within 24 h, all the way to 100 % after 120 h. in a 5-1 stirred tank reactor with wheat pellets uninoculated and inoculated with *C. versicolor* FPRL-28A was tested for the removal capacity of 100 ppm PCP after 30 days. The inoculated pellet removed 90 % PCP during 100 min while uninoculated, during the same period of time removed only 50 %.

4.7.3 PCP degradation in soil, sediment and sludge

Excellent results in PCP degradation in soil were shown by *Trametes.* The ability of the Brazilian basidiomycetes to degrade PCP in soils recovered from areas contaminated with organochlorine industrial residues was studied by Machado et al. (2005). *Trametes villosa* CCB176 and *Trametes villosa* CCB213 were tested for tolerance and degradation at high PCP concentrations in soil. Fungi were inoculated into the soil containing 1,278 mg PCP Kg^{-1} soil supplemented with gypsum and sugar, which the authors evaluated for the PCP depletion percentages. *T. villosa* CCB213 reduced 58 % the PCP present in the contaminated soil after 90 days of incubation. Both *Trametes* strains mineralized PCP with the successive production of chloride ions during growth, indicating dehalogenation of the molecule and the conversion of PCP to PCA. Walter et al. (2005) used an isolate of *Tramtes versicolor* HR131 in field-scale bioremediation of PCP. They devised an engineered soil cell to develop biopiles for fungi bioremediation of aged PCP-contaminated soil from a former timber treatment site. The soil cells were engineered to allow: forced aeration, irrigation, leachate collection, monitoring of temperature and soil humidity. PCP degradation and fungal survival were monitored at regular intervals for 2 and a half years. The PCP field remediation using *T. versicolor* HR131 declined from 1,000 mg PCP Kg^{-1} soil to 100 mg PCP Kg^{-1} soil within one year. Decreasing at 4 mg PCP Kg^{-1} soil in two years. At the end of the experiment there was little PCA detected, confirming earlier findings that PCA

may not be an intermediate metabolite of PCP transformation by *T. versicolor* HR131. Leštan and Lamar (1996) detected the fate of PCP in soil microcosm inoculated by *Trametes versicolor* MD-277. In the soil artificially contaminated with 100 μg g^{-1} of PCP and inoculated with 3 % pelleted fungal inoculums, *T. versicolor* MD-277 transformed PCP to PCA after 4 weeks degrading 86 % of PCP. Tuomela et al. (1999) investigated the fate of PCP in autoclaved soil supplemented with straw and inoculated with the white-rot fungus *Trametes versicolor* PRL 572. This strain during 42 days of incubation mineralized about 29 % of the PCP and at the end of experiment only trace amounts of PCA and 2,3,4,6-tetrachloroanisole were detected.

4.8 The genus *Trichoderma*

Trichoderma is a genus common in soil with interesting capabilities as potential bioremediator for environmental cleanup and as biological control agent versus numerous plant diseases. All these properties are possible thank to production of extracellular enzymes (Tripathi et al. 2013).

4.8.1 PCP degradation in microbiological culture media

Carvalho et al. (2009) studied the PCP degradation using *Trichoderma longibrachiatum* DSM 16517 in experiments of mycelium growth and percentage of PCP decay, under co-metabolic conditions with PCP concentration being between 5 and 15 mg L^{-1}. *T. longibrachiatum* DSM 16517 was able to degrade PCP while at 15 mg L^{-1} the strains failed biotransformation of PCP. Rigot and Matsumura (2002) using *Trichoderma harzianum* 2023 evaluated PCP degradation at 10 ppm as an initial concentration. After 9 days of incubation PCP was entirely and quickly converted to PCA.

4.9 Other genera

4.9.1 PCP degradation in microbiological culture media

Guiraud et al. (2003) studied the bioremediation capability of PCP by *Absidia fusca* detecting the performance of two strains isolated from different environment. After 4 days of incubation the strain1 and strain 2 degraded 41 and 33 %, respectively, of 100 mg L^{-1} PCP. Walter et al. (2003) evaluated a pool of 367 white-rot fungi, native to New Zealand, which are usable in PCP bioremediation. After several tests, some isolates were screened for PCP degradation (50 mg L^{-1} PCP) in vitro. The fungi identified were: *Abortiporus biemmis* HR145, *Oudemansiella australis* HR345, *Peniophora sacrata* HR226, *Peniophora sacrata* HR235, *Peniophora sacrata* HR240, *Peniophora sacrata* HR241, *Rigidoporus catervatus* HR316 and *Stereum fasciatum* HR348. PCP remaining in the liquid fraction after 42 days of stationary incubation was evaluated and had a high degradation capacity for all strains of *Peniophora sacrata* (100 % PCP removed). The correlation to PCP degradation was detected also in the presence and production of laccase. The genera *P. sacrata* laccase activity was high at different points in the experiments. Chiu et al. (1998) detected the tolerance, bio-sorption and biodegradation capacity (these last two activities were in relation to fungi biomass' dry wet) in the presence of 25 mg L^{-1} PCP for various fungi as *Armillaria gallica* 1039, *Armillaria gallica* 1057, *Armillaria mellea* M51, *Ganoderma lucidum* HK-1, *Lentinula edodes* L54, *Lentinula edodes* L67, *Lentinula edodes* L68,, *Polyporus* sp. Cv-1 and *Volvariella volvacea* V34. The tolerance was higher for *A. gallica* 1039, *A. gallica* 1057 and *A. mellea* M51 while any or all strains tolerated 100 mg L^{-1} PCP. *Polyporus* sp. Cv-1 possessed the greatest biosorption capacity, about 31 mg PCP for a gram of mycelium dry wet. Chloroanisols were PCP breakdown intermediates for almost all fungi. Tortella et al. (2008) carried out the first report on the ability of several indigenous wood-rotting fungi from Chile to produce hydrolytic and ligninolytic enzymes during the biodegradation of some of the xenobiotic compounds like PCP. Strains were identified and used in laboratory tests on the biodegradation with concentrations of 25 mg L^{-1} PCP. The Fungi used were: *Lenzites betulina* Ru-30, *Inonotus* sp. Sp2, *Stereum* sp. Ru-24, *Galerina patagònica* Sp3, *Stereum hirsutum* Sp1 and *Stereum hirsutum* Ru-104. After 15 days of incubation *L. betulina* Ru-30 and *G. patagònica* degraded PCP by 80 and 88 %. All other fungi degraded the PCP under 50 %, while the strains *S. hirsutum* Ru-104 PCP caused an inhibitory effect on the growth and enzymatic production. PCP degradation by fungi in a liquid

medium has been correlated with that of ligninolytic enzyme production. In fact, manganese peroxidase was detected in all strains tested. *L. betulina* Ru-30 and *G. patagònica* produced a maximum activity of manganese peroxidase (20 U L^{-1}) between 6 and 12 days of incubation. Lignin peroxidase was produced in *S. hirsutum* Ru-104, and *G. patagònica* Sp3 between 6 and 9 days of incubation (12 and 18 U L^{-1}, respectively). Laccase was never detected. Scelza et al. (2008) used *Byssochlamys fulva* in PCP removal experiments in a liquid medium with 12.5 and 25 mg L^{-1} PCP. The isolates of *B. fulva* degraded 20 % of both PCP concentrations during 8 days of incubation. Montiel-González et al. (2009) used the plasmids pVELipA and pTAAMnP1 (Stewart et al. 1996), containing lignin peroxidase and manganese peroxidase cDNA, recovered by white rot fungi *Phanerochaete chrysosporium*, for the transformation of *Amylomyces rouxii*. Sixty-nine *A. rouxi* elements were obtained, but only two were chosen for testing PCP removal in a submerged culture because they showed the highest peroxidase activity: CTL4 (lignin-peroxidase) and CTM5 (manganese-peroxidase). CTL4 and CTM5 removed 95 % of 12.5 mg L^{-1} PCP, compared with only 55 % of the *A. rouxii* wild type after 120 h of incubation. After 144 h of incubation, two of the elements were able to remove 100 % of the initial PCP, whereas the original strain removed only 49 %. Carvalho et al. (2009) studied the co- and direct metabolism of PCP using these fungal strains: *Chrysonilia sitophila* DSM 16514, and *Cladosporium herbarum*. In experiments of mycelium growth and percentage of PCP decay, under co-metabolic conditions with PCP concentration being between 5 and 15 mg L^{-1}, both fungi were able to degrade PCP while at 15 mg L^{-1} the strains failed to biotransform PCP. Finally, CHQ was recovered in the cultures of *C. sitophila* DSM 16514 and DCBQ in the cultures of *C. herbarum*. Ramesh and Pattar (2009) tested in vitro the biodegradation capacity of PCP by five selected isolates of white-rot fungi: *Laetiporus cincinnatus*, *Fomes fomentarius*, *Ganoderma applanatum*. All fungi showed a peak in laccase activity after 30 days of incubation which produced the highest amount of 50 ppm PCP removed. In a static culture study *F. fomentarius* degraded a high amount of PCP during 30 days of incubation, about 96.14 %. Fahr et al. (1999) evaluated brown rot fungi *Gloeophyllum striatum* (strains DSM 9592–DSM 10335) and *Gloeophyllum trabeum* WP 0992 to determinate PCP degradation using radioactively labeled compounds ([U-^{14}C] PCP). The strains *G. striatum* DSM 9592 and DSM 10335 were tested in a liquid medium contaminated with 5 μM of PCP, but after 19 days of incubation only 10 % of $^{14}CO_2$ was liberated showing a very slow degradation capacity. Forootanfar et al. (2012) studied the ability of the ascomycete *Paraconiothyrium variabile* to eliminate PCP and other CPs in submerged culture medium. The fungal strain was not able to remove 20 mg L^{-1} and PCP minimized the radial and biomass growth.

4.9.2 PCP degradation in soil, sediment and sludge

Among other fungi used in PCP degradation in soil we mentioned Machado et al. (2005) who analyzed the capacity of some basidiomycetes to degrade PCP in soils recovered from areas contaminated with organochlorine industrial residues. Three of the fungi isolated from different ecosystems were tested for tolerance and degradation to high PCP concentrations in soil. The Fungi identified were: *Agrocybe perfecta* CCB161, *Psilocybe castanella* CCB444 and *Peniophora cinerea* CCB204. These species were inoculated into soil containing 1,278 mg PCP Kg^{-1} soil supplemented with gypsum and sugar and the authors then evaluated PCP depletion percentages. *P. cinerea* CCB204, *P. castanella* CCB444 and *A. perfecta* CCB161 reduced the PCP present in the contaminated soil by 43, 64 and 78 %. All the fungi mineralized PCP, although principally P. *cinerea* CCB204 produced chloride ions during growth in the soil containing PCP, indicating dehalogenation of the molecule. Conversion of PCP to PCA was observed after only 90 days of incubation in the soils inoculated with *A. perfecta* CCB161 and *P. cinerea* CCB204. Rubilar et al. (2007), using a strain of *Bjerkandera adusta* ATTC 90940, carried out a series of PCP remediation laboratory-based studies in slurry soil flasks at initial PCP concentrations of 100, 250 and 350 mg PCP Kg^{-1} soil. *B. adusta* ATTC 90940 degraded PCP no matter the initial PCP concentration and in all cases only 25 mg PCP Kg^{-1} of soil remained. Okeke et al. (1996) determined the temperature, soil moisture potential and initial pH levels might influence the transformation of PCP by *Lentinula edodes* LE 2. this fungus showed the highest levels of degradation (about 75 %) at 25 °C and with the soil pH level at

4.0. The extent of PCP degradation related to soil moisture content by *L. edodes* LE 2 was under 50 %. using the same strain *L. edodes* LE 2, Okeke et al. (1997) evaluated bioremediation treatment in sterilized and non-sterilized soils contaminated with PCP and inoculated primarily with just the fungus strain and then with *L. edodes* LE2 together with a natural soil micro flora. When *L. edodes* LE2 was used independently, the rate of PCP removal was rapid for the initial 4 weeks and 99 % of PCP was biotransformed after 10 weeks. In a mixed culture, PCP biotransformation by *L. edodes* LE2 was slower, only 79 % of the PCP was depleted after 10 weeks. PCP and PCA in the soils after 10 weeks were completely eliminated in the sterilized soil with only *L. edodes* LE2, while PCA was still detected in the soils with the mixed micro flora and *L. edodes* LE2. Dechlorination and mineralization of the xenobiotic compound were detected in the presence by *L. edodes* LE2 but the dechlorination efficiency was greater with *L. edodes* LE2 (29.50 %) than when fungi were used in a mixed culture (22.40 %). Other products were detected from biotransformation of the PCP such as: TeCA and TeCP during the first 4 weeks in both sterilized and non-sterilized soils. Leštan and Lamar (1996) evaluated the PCP degradation in the soil microcosm of organisms such as *Irpex lacteus* ATCC 11245 and *Bjerkandera adusta*. After 4 weeks of incubation in a soil artificially contaminated with 100 μg PCP g^{-1} soil and inoculated with 3 % pellet fungal inoculums, both fungi showed a good capacity to transform PCP to PCA. Besides *I. lacteus* ATCC 11245 and *B. adusta* ATCC 62023 removed 82 and 86 % of PCP.

5 Critical and relevance aspect in PCP degradation by fungi

5.1 PCP degradation in microbiological culture media

PCP degradation by fungi in a liquid culture has been investigated in a high number of experiments. In these studies, PCP biodegradation focused on the capacity to deplete PCP using a single fungal strain or a mixed culture. Furthermore, the degradation intermediates products under different conditions and concentrations were analyzed (Table 2). In the liquid cultures, several species of fungi were used, but the genus *Trametes, Phanerochaete* and *Anthracophyllum* were surely the most used in this years and capable to degrade PCP (Table 2). These fungi had a capability to remove PCP at any initial concentration (5–300 mg L^{-1}) as also for white-rot fungi (Chiu et al. 1998; Law et al. 2003; Tortella et al. 2008). The presence of enzymes like laccase, lignin peroxidase and manganese peroxidase were correlated with higher capacities for PCP degradation. Usually, when fungi increase their enzymatic activity, they showed a higher degradation of PCP (Ryu et al. 2000; Walter et al. 2003; Tortella et al. 2008). When white-rot fungi was inoculated in wood chips or in wheat i.e. lignocellulosic materials, there was a good performance in PCP degradation (Ullah et al. 2000). Lignocellulosic materials are used as vehicles of fungal inoculation in soil or liquid mediums (McAllister et al. 1996) as well as when fungi are used in nitrogen limitations (Aiken and Logan 1996). Less effective were Deuteromycetes and Zygomycetes fungi that only tolerated low PCP concentrations (<20 mg L^{-1}), although in a few cases demonstrating a good degradation capacity versus PCP (Cortés et al. 2002; Carvalho et al. 2009). Any type of fungi class that was used in PCP degradation, when the pH level tended to reach acid values, the amount of PCP removed increased considerably (Mathialagan and Viraghavan 2009; Rubilar et al. 2012).

5.2 PCP degradation in soil, sediment and sludge

Despite the great ability by some fungi to degrade PCP, there are not many studies on PCP degradation in soil, sediment and sludge (Table 2). In the few works that we have found there are the same species used in vitro and all the fungi analyzed fall within the white-rot fungi group. Moreover, little is actually known about the pathway of PCP removal in soil, while, the principal processes that have been conducted on soil contaminated with PCP l are known as volatilization, adsorption, leaching and degradation (CCME 1997). The white-rot fungi of the genus *Phanerochaete, Anthracophyllum* and *Trametes* are surely the most frequently used as well as being the most efficient microorganism in PCP bioremediation experiments in soil; as stated in the previous paragraph. The principal compounds products by PCP biodegradation for the three fungi was PCA (Leštan and Lamar 1996; Tuomela et al. 1999; Machado et al. 2005; Rubilar

et al. 2007). They were very adept at degrading large amounts of PCP (250–1,000 mg Kg^{-1} soil) as well as *Lentinula edodes* LE2 ($\sim$200 mg Kg^{-1}), *Agrocybe perfecta* CCB161, *Psilocybe castanella* CCB444 and *Peniophora cinerea* CCB204 ($\sim$1,278 mg Kg^{-1}). The remaining fungi analyzed were able to degrade PCP only at an initial concentration of <100 mg Kg^{-1} soil. All the fungi that were analyzed in these studies exploited lignin peroxidase, manganese peroxidase and laccase were able to degrade PCP and convert it essentially into PCA or TCHQ through dehalogentation and dechlorination (McAllister et al. 1996; Cea et al. 2005; Field and Sierra-Alvarez 2008; Rubilar et al. 2011) (Table 2). The ability for PCP degradation was increased when the fungi were incorporated in lignocellulosic materials such as wheat grains (Rubilar et al. 2011), transformed into pellets (Leštan and Lamar 1996), adjusting the pH levels (4–5.5) and temperatures (25 °C) (Okeke et al. 1996; Rubilar et al. 2011) or by inserting a contaminated soil gypsum and sugar (Machado et al. 2005). An important role in the removal of PCP is that of the indigenous microorganisms. These last, in some experiments have showed a natural ability to degrade or adsorb PCP in soil at high percentages (<50 %) (Okeke et al. 1996; Cea et al. 2005; Rubilar et al. 2011). For example, in the Chilean andisols 40 % of PCP was removed due to their specific characteristics and ability to sorbent PCP and other CPs, all thanks to their allophane–ferrihydrite association with organic matter (Cea et al. 2005). Organic matter shows a very good efficiency at adsorbing PCP (Scelza et al. 2008) afterwards degraded by autochthonous microorganisms (Okeke et al. 1996).

6 Concluding remarks

How quickly, completely and efficiently PCP is degraded, depends on microorganism biodiversity and environmental conditions. Several bacteria and fungi have the capability to biodegrade PCP and they have been isolated from a variety of environments: industrial sewage (Szewczyk et al. 2003; Machado et al. 2005), contaminated soils, and effluent and fresh water sediments (Shah and Thakur 2002; Chandra et al. 2006, 2009; Lin et al. 2008). PCP degradation, mineralization, adsorption and dechlorination by microorganisms in different conditions are summarized in Tables 1 and 2. Analyzing all the works of PCP bioremediation, we have found that aerobic microorganisms have been shown to be highly efficient at degrading and mineralizing at higher PCP concentrations, more than the anaerobic microorganisms. Furthermore, bacteria showed the greatest efficiency, in regards to, degradation of PCP, whereas the fungi demonstrated lower capability and efficiency. In addition, all the bacteria and fungi, in a pure or mixed culture, act much better when used with an amendment (wheat, wood chips, glucose). However, bacteria and fungi in mixed cultures have been shown to completely degrade PCP, with a pre exposition to PCP, the biodegradation capacity significantly increased. Pellets, immobilizing cells and engineered systems also were much more efficient in the degradation of PCP. Finally, exploiting local biodiversity with the biostimulation of the microbial community with compost or soil improvers, increases the capacity to degrade PCP significantly in time (Alber et al. 2000; Puglisi et al. 2009).

In this review, numerous genus of bacteria were studied that can utilize PCP as carbon and energy sources such as: *Pseudomonas* sp., *Flavobacterium* sp., *Mycobacterium* sp., *Sphingomonas* sp., *Kokuria* sp., *Bacillus* sp., *Serratia* sp. and *Arthrobacter* sp. PCP biodegradation by these bacteria is well established and most of the studies have evaluated the metabolism and co-metabolism with unsubstituted or substituted PCP as the primary substrate. The main strategies and processes used with bacteria in the degradation of PCP release intermediate products such as TCHQ, TriCHQ, CHQ, DCHQ, TCC, CHYQ and 2,3,4,6-TeCP (McAllister et al. 1996; Shah and Thakur 2002; Sharma and Thakur 2008; Field and Sierra-Alvarez 2008; Singh et al. 2008, 2009; Chandra et al. 2009). Several Gram-negative and positive bacterial strains were used in PCP remediation, even if, Gram-negative strains seem to be more efficient in the tolerance and degradation of PCP (McAllister et al. 1996; Field and Sierra-Alvarez 2008). This happens because these bacteria are able to exclude PCP from the cell and due to the presence of lipopolysaccharide in the cell wall (Izaki et al. 1981). Aerobic and anaerobic biodegradation of PCP by the bacterial strains has been demonstrated in field and laboratory works. As well as the genes that produce useful enzymes for PCP degradation in *Flavobacterium* sp. ATCC39723 which have been characterized and cloned in Escherichia

coli, granting the latter with the ability to degrade PCP (McAllister et al. 1996). In fact, the characteristics of PCP degradative enzymes can be improved by engineering methods to move forward their potential in the bioremediation strategy and in industrial applications. Cloning in *Pseudomonas gladioli* genes of *Sphingomonas chlorophenolicum* (Dai and Copley 2004) and some genes *of Phanerochete chryosporum* in *Amylomyces rouxi* (Stewart et al. 1996), these authors observed significant improvements in the rate and capacity of these organisms to degrade PCP. Therefore cloning the genes that useful in the degradation of PCP into the indigenous microorganisms that, for example, don't have the capacity to degrade contaminants could overcome some problems related to introduction into soils or other mediums producing new "exotic" organisms. However little is yet known on the potential pathogenic effect that any species can produce versus other microorganisms, like plants and animals. There are some species such as *Pseudomonas aeruginosa* and *Bacillus cereus* that for example are very dangerous for human health (He et al. 2004; Kotiranta et al. 2000). This fact may greatly limit their use in the bioremediation strategy.

Fungi as well are very useful as PCP degraders, especially the genus: *Phanerochaete, Anthracophyllum, Agrocybe, Lentinula* and *Trametes.* Different genus or families of fungi exhibit tolerances and degradation capacities to PCP. Furthermore, fungal strains are generally less efficient than bacteria in PCP degradation and only in only a few cases are they able to completely mineralize PCP. In addition, fungi adsorb PCP on the mycelium, leaving intact the contaminant. However, the fungi strains, thanks to their excellent enzymatic pool, can break down PCP in a molecule, making them more biovailable to be degraded by other microorganisms (McAllister et al. 1996; Pointing 2001). Unlike bacteria, fungi are not capable of using PCP as a source of carbon. PCP degradation is not a direct consequence of fungal metabolism, but rather of a co-metabolic process. They have enzymes useful in degrading wood components such as lignin o cellulose i.e. PH-oxidase, laccase lignin and manganese peroxidase, which are capable of breaking down PCP molecules (McAllister et al. 1996; Pointing 2001). For fungi, the main strategies and processes in the degradation of PCP release intermediate products such as PCA, TCHQ, TCP, CHQ, DCBQ and TriCHQ (Leštan and Lamar 1996; Machado et al. 2005; Carvalho et al. 2009; Rubilar et al. 2009; Carvalho et al. 2011). While for white or brown-rot fungi, a future in bioremediation is definitely possible, because they are not dangerous to humans or animals; although some species such as *Armillaria mellea* can cause serious diseases in many plants i.e. if there is a bioremediation intervention in an agricultural field. Therefore, it also depends on the context in which the fungi isolates will be used. For some deuteromycetes and zigomycetes species, like some bacteria, little is yet known on their potential pathogenic effects on other microorganisms or versus plants and animals; for example the species *Rhizopus oryzae* that can cause oral or cerebral mucormycosis. PCP toxicity is well known fact, especially for some organisms (Crosby 1981); the toxicity of the degradation intermediate products is still not well documented. In fact, microbial metabolism of contaminants such as PCP may produce, in some cases, toxic metabolites (McAllister et al. 1996; Field and Sierra-Alvarez 2008).

Improving the biodiversity of the microorganisms present in a medium, a pollutant compound can be completely and more quickly mineralize. In fact it is important to remember that increasing microbial biodiversity, we increase the possibility to have organisms capable to degrade the contaminant or its intermediates products. Microorganisms use essentially oxygenases and hydroxylase that insert O_2 and $-$OH into the compound prior to ring cleavage or using reductive dechlorination, which eliminate a Cl-group at the compounds ring, which is essential to the intermediate metabolites in an aerobic pathway of PCP degradation (McAllister et al. 1996; Field and Sierra-Alvarez 2008). But in bioremediation strategy it is equally important to know the environmental conditions (physical and chemical properties of the sites) and physico-chemical characteristics of the contaminant (Providenti et al. 1993). It is essential to know as well the relation and interaction between microbial consortium—environmental conditions—toxic compounds, since this will allow the researcher to obtain high performances in the bioremediation process i.e. to achieve degradation, bioaccessibility and bioavailability of PCP, some factors such as aeration, moisture, content of the organic matter, microbial biodiversity, temperature and pH level, soil improvers and compost could optimize PCP degradation (Providenti et al. 1993; McAllister et al. 1996;

Scelza et al. 2008; Puglisi et al. 2009; Cea et al. 2010; Juwarkar et al. 2010).

7 Future perspectives

All the information occurs in this review can be used to push forward the recent bioremediation technological advances such as "omic" based technique (genomics, proteomics and metabolomics).

Understudying thoroughly as the microorganisms can tolerate, degrade and mineralize some pollutants as PCP, represent the first step to realize an excellent remediation processes/studies. Reviewing the case studies showed in this work could further increase the experiments to extend the knowledge of genes encoding the metabolites useful in PCP degradation. Until now the molecular aspect of PCP degradation has received little attention (Orser and Lange 1994; Juwarkar et al. 2010; Villemur 2013; Carvalho et al. 2013; Copley et al. 2013). Moreover the genetic manipulation can offer a means of engineering microorganism to deal with PCP that may be present in a contaminated site (Villemur 2013). Other experiments should be carried out about how PCP effect microbial cell. little is known about how PCP can influence not only microorganisms community but also more specifically cellular processes, production of toxins, cyclic changes in morphology, lipid membrane components, biomass growth, enzymatic activity, sporulation and reproduction capacity. Another interesting focal point worthwhile to examine is the microbial interaction in relation to the PCP. It could increase the possibility to use allochthonous microorganisms in bioremediation processes. This point is currently highly debated because many authors believe that to insert a microorganism, for example in a different soil, generates a turnover in microbial community. This is absolutely true, but the soil, like the water and air, is a dynamic system constantly changing and allochthonous microorganisms are continuously transported by wind, animal and rain in different areas even thousands of miles away.

It is very important to know the interaction of all the factors dealing the bioremediation process as well as reforming and restructuring the strategy in which contaminated sites are processed and effectively decontaminated. In this way we can move the world forward to produce a safer environment for human, animal and plant communities.

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Appendix B
Penta Woods – Microcosm and BioTrap Study Memorandum

Final

Quarterly Report

April through June 2017

Penta Wood Products Superfund Site

Wisconsin Department of Natural Resources

GHD | 1801 Old Highway 8 Northwest Suite 114 St. Paul Minnesota 55112 USA

086165| Report No 15 | July 14, 2017

workers. The residential well and onsite water supply well locations are shown on Figure 3.1. The samples were analyzed for PCP, BTEX, and naphthalene. The well purging and sampling data are summarized in Table 2.2. The residential well sample analytical data are summarized in Table 3.1. Copies of the laboratory reports and data validation are included in Appendix C. Historical residential and onsite water supply well PCP data are included in Appendix A.

3.1 Residential Well Sample Analytical Data

PCP was detected in residential well RW1 at a concentration of 0.015 µg/L and was detected in the onsite water supply well at a concentration of 0.020 µg/L (0.022 µg/L – duplicate) (Table 3.1), which are less than the PAL (0.1 µg/L) and ES (1 µg/L). PCP was not detected in the remaining residential wells. Naphthalene and BTEX were not detected in any of residential wells or the onsite water supply well (Table 3.1). These results are similar with historical data. Copies of the laboratory reports and the data validation memo are presented in Appendix C.

4. Microcosm and BioTrap Study

Microcosm and BioTrap studies were conducted in accordance with the Remediation System Shutdown Pilot Study Work Plan (GHD; November 13, 2015). The objectives of the microcosm study were to gather the data necessary to:

- Determine whether natural attenuation of PCP is occurring at the Site
- Determine whether natural attenuation is occurring under aerobic conditions, anaerobic conditions, or both
- Determine a Site-specific biodegradation rate for PCP

The objectives of the BioTrap study were to gather the data necessary to:

- Determine whether bacteria capable of degrading PCP are present at the Site
- Demonstrate in situ biodegradation of PCP using a BioTrap

A technical memorandum presenting the results of the studies is included in Appendix D.

The results from the microcosm tests indicate that PCP and diesel range petroleum hydrocarbons (TPH(C_9-C_{36})) are readily degradable under aerobic conditions and that PCP and TPH(C_9-C_{36}) are also degradable under anaerobic conditions; however, the anaerobic process is much slower. The addition of emulsified vegetable oil (EVO) to optimize anaerobic conditions appears to increase the biodegradation rate of PCP. Based on the half-lives measured in the microcosms, the cleanup time for the aerobic area under aerobic conditions would be 6.3 months. The cleanup time for the anaerobic area without EVO enhancement would be 66 months (5.5 years). These estimated cleanup times assume that LNAPL is not present and there is no ongoing source of contamination.

These conclusions are supported by the data from the BioTraps. In the BioTraps deployed in the downgradient area in wells MW9 and EW11S, the dominant class of organisms, the Proteobacteria degraded PCP and incorporated it into the biomass at a moderate rate. In the source area in wells MW20 and MW29, the BioTrap data appears to indicate that well MW20 may be in a

transitional zone where some aerobic and some anaerobic processes are occurring. Although the BioTrap from MW20 contained the anaerobic Fimicutes, which were the dominant class of organisms in MW29, Proteobacteria were the dominant class of organisms in MW20, and the rate of incorporation of PCP into biomass was similar to the aerobic wells. In MW29, which was likely highly anaerobic, the Fimicutes dominated, and slower incorporation of PCP into biomass was observed.

No mineralization of PCP (i.e., degradation into carbon dioxide) was observed in the BioTrap study; however, the BioTraps were deployed for only 32 days, which may not be long enough for mineralization of PCP to occur.

Overall, the data suggests that monitored natural attenuation (MNA) would be an effective treatment for the downgradient area, and biodegradation of PCP and TPH(C_9-C_{36}) is expected to occur at a moderate rate. MNA may be effective for the source area. The BioTrap and amended microcosm data show that PCP degradation does occur under anaerobic conditions; however, slower biodegradation rates are expected. Analysis of the unamended anaerobic microcosms after more time has elapsed would provide additional information about the rates that can be expected. Additional anaerobic microcosm testing may be performed after 24 and 36 months.

5. Waste Management and Disposal

Historical hazardous waste disposal is summarized in Appendix A. GHD continues to collect and containerize PPE and other waste produced during sampling events onsite.

5.1 Sodium Hydroxide

Sodium hydroxide remained stored in an above ground tank at the Site after remediation system decommissioning was completed in January 2016. On March 20, 2017, 5,000 gallons of sodium hydroxide were removed from the Site, as documented in the Quarterly Report – January through March 2017 (GHD; May 3, 2017). On April 7, 2017, the remaining 200 gallons of sodium hydroxide were removed from the Site and transported to the Advanced Waste Services of Wisconsin, ChemWorks Treatment Facility located in Milwaukee, Wisconsin under Profile CHE1000137125 for reclamation and reuse as part of the facility treatment operations. Waste disposal documentation including the bill of lading (BL2790504000) and waste profile is provided in Appendix E.

5.2 Ferric Sulfate

Ferric sulfate remained stored in an above ground tank at the Site after remediation system decommissioning was completed in January 2016. On April 7, 2017, 2,759 gallons of ferric sulfate were removed from the Site and transported to the EQ Illinois Facility located in Harvey, Illinois under Profile CHE1000137125 for disposal. Waste disposal documentation including the uniform waste profile, hazardous waste manifest (001251251VES), and certificate of disposal is provided in Appendix E.

Appendix D
Microcosm and BioTrap Study Memorandum

Memorandum

May 16, 2017
Revised July 7, 2017
Revised July 10, 2017

To:	Brian Sandberg	Ref. No.:	086165
From:	Sophia Dore/Christa Bucior/adh/5	Tel:	716-205-1978
cc:	Timothy Ree		

Subject: **Evaluation of the Potential for Natural Attenuation of Pentachlorophenol Treatability Study**
Penta Wood Products Superfund Site, Siren, Wisconsin

1. Introduction

Pentachlorophenol (PCP) and diesel fuel are present in groundwater at the Penta Wood Products Superfund Site, located in Siren, Wisconsin. Light Non-Aqueous Phase Liquid (LNAPL) present in some wells. The PCP concentrations range from 1,000 micrograms per liter (µg/L) (in the LNAPL wells) to 10 µg/L in the former release area. The remediation system operation was temporarily shut down at the Site for conducting a pilot study to evaluate whether monitored natural attenuation (MNA) will be an effective remedial action at the Site. A microcosm study and a BioTrap study were performed as lines of evidence that MNA is occurring at the Site. This memorandum contains the results of the microcosm and BioTrap studies. The studies were conducted in general accordance with the Remediation System Shutdown Pilot Study Work Plan (GHD, November 2015) Microcosm Study.

2. Microcosm Study

2.1 Objectives

The objectives of this microcosm laboratory study were to gather the data necessary to:

i) Determine whether natural attenuation of PCP is occurring at the Site

ii) Determine whether natural attenuation can occur under aerobic conditions, anaerobic conditions, or both

iii) Determine a Site-specific degradation rate for PCP

2.2 Sample Acquisition

The microcosm study was conducted using samples of soil and groundwater collected from the Site during drilling and well installation activities in November and December 2015. Four gallons of groundwater from the aerobic zone and 4 gallons of groundwater from the anaerobic zone were collected separately along with

5 pounds of soil from the aerobic zone and 5 pounds of soil from the anaerobic zone. The aerobic zone samples were collected at borehole SB1. The anaerobic zone samples were collected at well MW29. Borehole SB1 and well MW29 are shown on Figure 1. The soil samples from the aerobic and anaerobic zones and the groundwater from the aerobic zone were received by the GHD Innovative Technology Group (ITG) laboratory in Niagara Falls, New York on December 3, 2015. An additional four gallons of groundwater from the anaerobic zone were received on April 22, 2016.

2.3 Task 1: Initial Characterization

Upon arrival at the laboratory, the samples were analyzed for the following parameters to provide a characterization of baseline conditions for the study:

Groundwater

i) pH

ii) PCP

iii) Diesel range petroleum hydrocarbons (TPH[C_9-C_{36}])

iv) Ammonia-nitrogen

v) Orthophosphate-phosphorus

vi) Total and dissolved iron and manganese (groundwater)

Soil

i) pH

ii) PCP

iii) Diesel range petroleum hydrocarbons

iv) Ammonia-nitrogen

v) Orthophosphate-phosphorus

vi) Percent Moisture

vii) Percent Solids

viii) Total iron and manganese (soil)

The results from the initial analysis of groundwater SB1, the groundwater from the aerobic area, showed 87 µg/L of PCP and 0.176 milligram per liter (mg/L) of TPH(C_9-C_{36}). The pH was in the neutral range at 6.72, ammonia-nitrogen was below the analytical detection limit, and orthophosphate-phosphorus was present at 1.85 mg/L. Total iron was present at 27,600 µg/L and dissolved iron at 1,010 µg/L. Total manganese was present at 4,480 µg/L and dissolved manganese at 3,340 µg/L. These ratios of total to dissolved iron and manganese are consistent with the aerobic conditions known to exist in the area from which this sample was collected.

The results from the initial analysis of groundwater MW29, the groundwater from the anaerobic area, showed 1,430 µg/L of PCP and 1,540 mg/L of TPH(C_9-C_{36}). The pH was again in the neutral range at 6.71, ammonia-nitrogen was below the analytical detection limit, and orthophosphate-phosphorus was present at 1.45 mg/L. Total iron was present at 10,500 µg/L and dissolved iron was present at 270 µg/L. Total manganese was present at 2,530 µg/L and dissolved manganese at 2,350 µg/L. The manganese results are typical of anaerobic conditions; however, the dissolved iron concentration is lower than would be expected. These data are summarized in Table 1.

The results from the initial analysis of soil SB1, the soil sample collected from the aerobic area, showed 0.502 milligram per kilogram (mg/kg) of PCP and TPH(C_9-C_{36}) below the analytical detection limit. The pH of the soil was 7.14, ammonia-nitrogen was below the analytical detection limit, and orthophosphate-phosphorus was present at 27.8 mg/kg. The soil contained 6,880 mg/kg of total iron and 79.9 mg/kg of total manganese.

The results from the initial analysis of soil MW29, the soil sample collected from the anaerobic area, showed 61.0 mg/kg of PCP and 153 mg/kg of TPH(C_9-C_{36}). The pH of the soil was 6.65, ammonia-nitrogen was below the analytical detection limit, and orthophosphate-phosphorus was present at 20.5 mg/kg. The soil contained 8,330 mg/kg of total iron and 94.6 mg/kg of total manganese. These data are summarized in Table 2.

2.4 Task 2: Aerobic Microcosm Tests

Microcosms were set up to assess the potential for natural attenuation of PCP and petroleum hydrocarbons under aerobic conditions using soil and groundwater from borehole SB1. Forty grams of soil were placed in serum bottles along with 200 milliliters (mL) of groundwater.

The following treatments were performed:

1. Soil and groundwater only (biotic control)
2. Soil, groundwater, oxygen
3. Soil/sand, groundwater, oxygen, and sodium azide (abiotic control)

After 0, 3, 6, and 12 months, duplicate microcosms for each treatment were to be sacrificed and analyzed for PCP and petroleum hydrocarbons in the soil and groundwater. After 3 months, treatment of 94 percent of the PCP was observed in the microcosms that contained soil and groundwater, and TPH(C_9-C_{36}) was removed to non-detect levels. Ninety-five percent treatment of PCP was observed in microcosms that received oxygen. TPH(C_9-C_{36}) was also removed to non-detect levels in these microcosms. These data suggest that natural attenuation is effective for treatment of PCP and TPH in the aerobic zone of the Site. These data are summarized in Tables 3 and 4.

After 6 months, PCP and TPH(C_9-C_{36}) were not detected in any of the biological microcosms. These data show that natural attenuation is effective for treatment of PCP and TPH in the aerobic zone of the Site. These data are summarized in Tables 5 and 6.

PCP and TPH(C_9-C_{36}) concentrations did not decrease in the sodium azide treatment samples (abiotic control), which confirms that the decreased concentrations in the soil and groundwater (biotic control) and soil, groundwater, and oxygen treatments are due to biological degradation.

Since both PCP and TPH(C_9-C_{36}) had been reduced to non-detect levels, no further analyses of these microcosms were performed. The data were used to calculate first order rate constants and half lives for PCP. Under aerobic conditions, the half life for PCP was 0.7 month. These calculations are shown in Attachment A. Since TPH was removed to non-detect levels at the 3-month sampling event, the half life for TPH could not be calculated.

2.5 Task 3: Anaerobic Microcosm Tests

Microcosms were set up to assess the potential for natural attenuation of PCP and TPH(C_9-C_{36}) under anaerobic conditions using soil and groundwater collected from well MW29. Microcosms were set up in the anaerobic hood. Forty grams of soil were placed in serum bottles along with 200 mL of groundwater.

The following treatments were performed:

1. Soil and groundwater only (biotic control)
2. Soil, groundwater, and emulsified vegetable oil (EVO)
3. Soil/sand, groundwater, and sodium azide (abiotic control)

After 0, 3, 6, and 12 months, duplicate microcosms for each treatment were to be sacrificed and analyzed for PCP in the soil and groundwater.

After 3 months, no reduction in the concentration of PCP was observed in any of the microcosms. An increase in the aqueous concentration of PCP was observed in some of the microcosms, which is likely associated with PCP partitioning out of the soil into the groundwater. Treatment of TPH(C_9-C_{36}) was observed in all microcosms. In microcosms containing soil and groundwater, 37 percent removal of TPH(C_9-C_{36}) was observed and 30 percent removal of TPH(C_9-C_{36}) was observed in the microcosms that received EVO. These data suggest that anaerobic biodegradation of the TPH has occurred; however 3 months is not enough time for anaerobic biodegradation of PCP to occur. These data are shown in Tables 7 and 8.

After 6 months, 35 percent removal of PCP was observed in the microcosms that received EVO. No removal of PCP was observed in any of the other microcosms, and the increases in aqueous PCP combined with decreases in soil PCP were again observed suggesting that PCP is partitioning out of the soil. Treatment of TPH(C_9-C_{36}) was again observed in all microcosms. In microcosms containing soil and groundwater, treatment of TPH(C_9-C_{36}) had increased to 42 percent, and 51 percent removal of TPH(C_9-C_{36}) was observed in the microcosms that received EVO. These data suggest that after 6 months some anaerobic degradation of the PCP has occurred in microcosms where anaerobic conditions were optimized with EVO. Anaerobic degradation of the TPH is continuing but appears to be slow. These data are shown in Tables 9 and 10.

After 12 months, 93 percent removal of PCP and 64 percent removal of TPH(C_9-C_{36}) were observed in the microcosms that received EVO. Seventy percent removal of PCP and 56 percent removal of TPH(C_9-C_{36}) were observed in the microcosms that contained soil and groundwater only. Eighty-seven percent removal of PCP and 52 percent removal of TPH(C_9-C_{36}) observed in the azide control samples suggest that after 12 months, the azide is no longer suppressing microbial activity. These data suggest that anaerobic degradation of PCP and TPH is occurring, both in the microcosms where anaerobic conditions were optimized and in the unamended microcosms. Biodegradation of the PCP appears to have taken more than 6 months to start, but once started, biodegradation is proceeding fairly rapidly. Biodegradation of the TPH(C_9-C_{36}) continues to proceed slowly. These data are shown in Tables 11 and 12. Additional testing may be performed after 24 and 36 months.

The data were used to calculate first order rate constants and half lives for PCP. Under anaerobic conditions, the half life for PCP under unenhanced conditions was 5.5 months and with the addition of EVO was 2.9 months. The half life for TPH(C_9-C_{36}) under unenhanced conditions was 9.6 months and with the addition of EVO was 8.5 months. These calculations are shown in Attachment A.

3. BioTrap Study

BioTrap samplers are passive sampling tools that collect microbes over time for the purpose of better understanding biodegradation potential. BioTraps contain Bio-Sep® beads that are 2-4 millimeters (mm) in diameter and are made of Nomex® and powdered activated carbon (PAC). When a BioTrap sampler is deployed in a monitoring well, the beads absorb contaminants and nutrients present in the aquifer and become colonized by microorganisms. Once recovered from a monitoring well, Deoxyribose Nucleic Acid (DNA) and Ribose Nucleic Acid (RNA), or phospholipid fatty acids (PLFA) can be extracted from the beads for analysis to evaluate the microbial community. Most microbes prefer to be attached to a surface rather than free floating. The BioTrap provides a large surface area for the microbes to colonize and form biofilms. BioTrap samplers can be "baited" with various amendments or compounds to answer Site-specific questions and screen remedial alternatives. For example, BioTraps can be baited with specific contaminants of concern, such as PCP. They can also be baited with ^{13}C labeled compounds (stable isotope probing) to demonstrate conclusively that biodegradation is occurring.

3.1 Objectives

The objectives of the BioTrap study were to gather the data necessary to:

i) Determine whether bacteria capable of degrading PCP are present at the Site

ii) Demonstrate in situ biodegradation of PCP using a BioTrap

3.2 BioTrap Study

BioTraps baited with ^{13}C labelled PCP were obtained from Microbial Insights. During April and May 2016, they were installed in two wells in the source area (wells MW20 and MW29) and two wells in the downgradient area (wells MW9 and EW11S). The BioTraps were left in place for 32 days. After 32 days, the BioTraps were retrieved and analyzed for the following:

- ^{13}C PCP concentration
- PLFA
- Stable isotope probing
- Dissolved ^{13}C inorganic carbon

A copy of the laboratory report is included in Attachment B.

3.2.1 ^{13}C Pentachlorophenol Concentration

An attempt to quantify ^{13}C PCP in the BioTraps after deployment was made; however, the phenol group on the PCP has been found to chemisorb to the beads. Therefore, quantitative extraction of the PCP was not possible, and it was not possible to compare the concentration of PCP after the BioTraps were retrieved from the wells to the initial concentration of PCP in the BioTraps.

3.2.2 Phospholipid Fatty Acids

The biomass collected in the BioTraps was analyzed for PLFA. The biomass in the four BioTraps was similar with the source area. BioTraps from wells MW20 and MW29 having counts of 3.8×10^5 cells per bead and 1.9×10^6 cells per bead, respectively. BioTraps from downgradient wells MW9 and EW11S had counts of 2.3×10^6 cells per bead and 1.1×10^6 cells per bead, respectively.

The PLFA analysis showed that the dominant class of organism in the well MW20 BioTrap was Proteobacteria, which are fast growing gram negative bacteria, which utilize many carbon sources and adapt quickly to a variety of environments. The dominant class of organism in the well MW29 BioTrap was Firmicutes, which are anaerobic fermenting bacteria. The well MW20 BioTrap also contained Firmicutes.

The dominant type of organism in both downgradient wells MW9 and EW11S was the Proteobacteria with very low percentage of Firmicutes. These data show that anaerobic bacteria were dominant in well MW29 and also present in well MW20 but not present in the downgradient wells MW9 and EW11S. This is consistent with the source area being anaerobic while the downgradient area is more aerobic.

3.2.3 Stable Isotope Probing

Stable isotope probing demonstrated that ^{13}C was incorporated into the microbial biomass. The ^{13}C enriched biomass was between 1.1 and 2.0×10^4 cells per bead for wells MW9, MW29, and EW11S and 2.2×10^3 cells per bead for well MW20.

The ratio between the heavier and lighter isotopes is expressed as a delta value (δ). The δ value is calculated according to the following equation:

$\delta(‰) = (R(sample)/R(standard)-1)X1000$

R= ratio of heavy to light isotope

This ratio was calculated for the PLFA to determine the extent to which they were enriched in ^{13}C. The average $\delta^{13}C$ values for the PLFA in wells MW9 and EW11S, as well as well MW20, ranged from 257 to

360 percent, which is in the moderate range indicating a moderate incorporation of ^{13}C-labeled PCP into microbial biomass. The average $\delta^{13}C$ value for well MW29 was 94 percent, which is in the low range indicating low incorporation of ^{13}C-labeled PCP into microbial biomass. Well MW29 had the greatest concentration of Firmicutes, which are anaerobic bacteria and a lower concentration of Proteobacteria, which are bacteria that can utilize a wide range of carbon sources. It is possible that Proteobacteria have a greater capacity to degrade PCP than Firmicutes.

3.2.4 Dissolved ^{13}C Inorganic Carbon

$\delta^{13}C$ value for dissolved inorganic carbon was also measured in the BioTraps. If inorganic carbon was enriched in ^{13}C, it would indicate that complete mineralization of the PCP to carbon dioxide (CO_2) had occurred. The natural abundance of ^{13}C is approximately 1 percent, and the percent ^{13}C in the inorganic carbon in the four BioTraps ranged from 1.08 to 1.09, which is very close to the natural abundance. The $\delta^{13}C$ values ranged from -21 to -14 percent, which are near background levels; therefore, it appears that little to no PCP mineralization occurred during the 32 days in which the BioTraps were in place. PCP mineralization could have occurred if the BioTraps had been left in place for a longer duration.

The BioTrap data are summarized in Table 13. The Microbial Insights laboratory report is included in Attachment B.

4. Summary

Aerobic Microcosms

- After 3 months, PCP was reduced by 94-95 percent, and TPH(C_9-C_{36}) was reduced to non-detect levels in the biological microcosms.
- After 6 months, both PCP and TPH(C_9-C_{36}) were reduced to non-detect levels in the biological microcosms.
- The addition of oxygen to the microcosms did not increase biodegradation rates.
- Under aerobic conditions, the half life for PCP was 0.7 month. Since TPH was removed to non-detect levels at the 3-month sampling event, the half life for TPH could not be calculated.
- These data show that natural attenuation under aerobic conditions is effective for treatment of the PCP and TPH(C_9-C_{36}) in the aerobic area of the Site.

Anaerobic Microcosms

- After 3 months, no treatment of PCP was observed in any of the microcosms; however, treatment of 37 percent of the TPH(C_9-C_{36}) was observed in the unamended microcosms.
- After 6 months, treatment of PCP was not observed in the unamended microcosms; however, 35 percent removal of PCP was observed when anaerobic conditions were optimized by the addition of EVO.
- Treatment of TPH(C_9-C_{36}) was observed in both the unamended and amended microcosms.

- After 12 months, 93 percent removal of PCP and 64 percent removal of TPH(C_9-C_{36}) were observed in the microcosms that received EVO; and 70 percent removal of PCP and 56 percent removal of TPH(C_9-C_{36}) were shown in the unamended microcosms.
- Removal was observed in the azide kill controls, suggesting that after 12 months, azide is no longer suppressing microbial activity.
- Under anaerobic conditions, the half life for PCP under unenhanced conditions was 5.5 months and with the addition of EVO was 2.9 months. The half life for TPH(C_9-C_{36}) under unenhanced conditions was 9.6 months and with the addition of EVO was 8.5 months. Additional treatment of PCP and TPH(C_9-C_{36}) is expected over time.

BioTraps

- Total biomass in the BioTraps ranged from 3.8 x 10^5 cells per bead to 2.3 x 10^6 cells per bead.
- The PLFA analysis showed that the dominant class of organism in the downgradient well BioTraps and the BioTrap from well MW20, which is located in the source area, was Proteobacteria, which are fast growing gram negative bacteria that utilize many carbon sources. The dominant class of organism in the well MW29 BioTrap (source area) was Firmicutes, which are anaerobic fermenting bacteria. The well MW20 BioTrap also contained Firmicutes.
- Stable isotope probing demonstrated that ^{13}C was incorporated into the microbial biomass.
- The average $\delta^{13}C$ values for the PLFA in wells MW9 and EW11S and well MW20 ranged from 257 to 360 percent, which indicate a moderate rate of incorporation of ^{13}C-labeled PCP into microbial biomass. The average $\delta^{13}C$ value for well MW29 was 94 percent, which indicates a low rate of incorporation of ^{13}C-labeled PCP into microbial biomass.
- Mineralization of ^{13}C labeled PCP into CO_2 was not observed during the 32-day BioTrap study period.

5. Conclusions

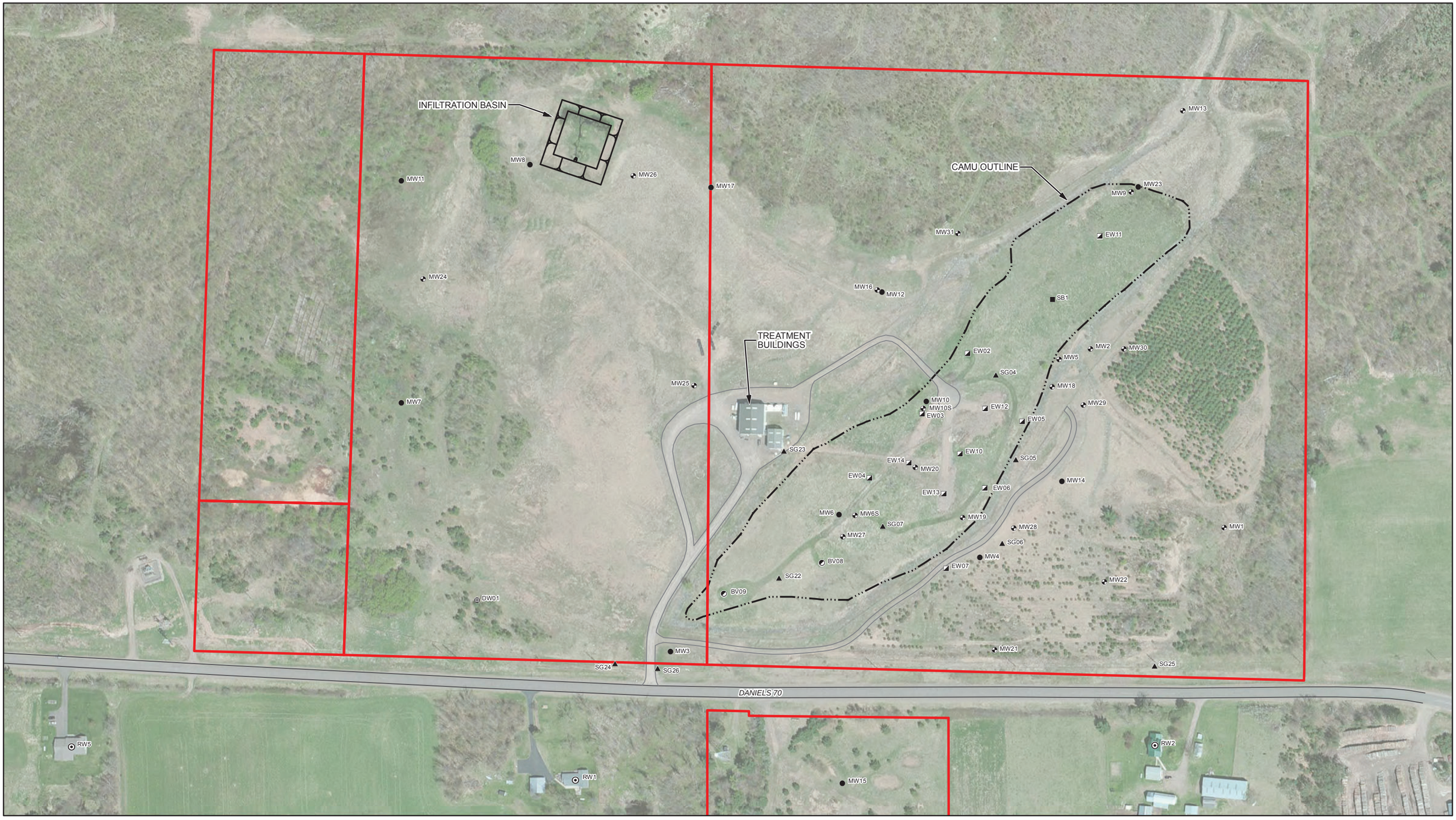
The results from the microcosm tests indicate that PCP and TPH(C_9-C_{36}) are readily degradable under aerobic conditions and that PCP and TPH(C_9-C_{36}) are also degradable under anaerobic conditions; however, this process is much slower. The addition of EVO to optimize anaerobic conditions appears to increase the biodegradation rate of PCP. Based on the half lives measured for the microcosms, the cleanup time for the aerobic area under aerobic conditions would be 6.3 months and for the anaerobic area without enhancement would be 66 months (5.5 years).

These conclusions are supported by the data from the BioTraps. In the BioTraps deployed in the downgradient area in wells MW9 and EW11S, the dominant class of organisms, the Proteobacteria, degraded PCP and incorporated it into their biomass at a moderate rate. In the source area in wells MW20 and MW29, the BioTrap data appears to indicate that well MW20 may be in a transitional zone where some aerobic and some anaerobic processes are occurring. Although the BioTrap from MW20 contained the anaerobic Firmicutes, which were the dominant class of organisms found in MW29, Proteobacteria were the

dominant class of organisms in MW20, and the rate of incorporation of PCP into biomass was similar to the aerobic wells. In MW29, which was likely highly anaerobic, the Firmicutes dominated, and slower incorporation of PCP into biomass was observed.

No mineralization of PCP (i.e., degradation into CO_2) was observed in the BioTrap study; however, the BioTraps were deployed for only 32 days, which may not be long enough for mineralization of PCP to occur.

Overall, it appears that MNA would be an effective treatment for the downgradient area, and biodegradation of PCP and TPH(C_9-C_{36}) is expected to occur at a moderate rate. MNA may be effective for the source area. The BioTrap and amended microcosm data show that PCP degradation does occur under anaerobic conditions; however, slower biodegradation rates are expected. Analysis of the unamended anaerobic microcosms after more time has elapsed would provide additional information about the rates that can be expected. Additional anaerobic microcosm testing may be performed after 24 and 36 months.



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LEGEND

- EXTRACTION WELL NEST
- UNCONFINED MONITORING WELL
- SEMICONFINED MONITORING WELL
- BIOVENTING WELL
- SOIL GAS WELL NEST
- WATER SUPPLY WELL
- RESIDENTIAL WELL
- APPROXIMATE CAMU LIMIT
- SITE PARCEL BOUNDARY

PENTA WOOD PRODUCTS SUPERFUND SITE
SIREN, WISCONSIN
EVALUATION OF THE POTENTIAL FOR NATURAL ATTENUATION OF PCP

086165-03-16
Jun 28, 2017

SITE PLAN

FIGURE 1

GIS File: I:\GIS\Projects\6-chars\08----\0861--\086165\086165-MEMO\086165-03(MEMO005)\086165-03(MEMO005)GIS-SP001.mxd

Table 1

Initial Groundwater Characterization Analytical Data - Microcosm Study
Penta Wood Products Superfund Site
Siren, Wisconsin

Parameters	Date Analyzed / Units	12/4/2015 SB1	4/28/2016 MW29
General Chemistry			
pH	S.U.	6.72	6.71
Ammonia-Nitrogen	mg/L	< 1.0	< 1.0
Orthophosphate-Phosphorus	mg/L	1.85	1.45
Semi-Volatile Organic Compounds			
Pentachlorophenol	µg/L	87	1430
Total Petroleum Hydrocarbons			
TPH(C_9-C_{36})	mg/L	0.176	1540
Total Metals			
Iron	µg/L	27600	10500
Manganese	µg/L	4480	2530
Dissolved Metals			
Dissolved Iron	µg/L	1010	270
Dissolved Manganese	µg/L	3340	2350

Notes:

< - Compound not detected above the reporting limit
S.U. - Standard units
µg/L - Micrograms per liter

Table 2

Initial Soil Characterization Analytical Data - Microcosm Study
Penta Wood Products Superfund Site
Siren, Wisconsin

Parameters	Date Analyzed / Units	12/3/2015 SB1	12/3/2015 MW29
General Chemistry			
pH	S.U.	7.14	6.65
Ammonia-Nitrogen	mg/kg	ND	ND
Orthophosphate-Phosphorus	mg/kg	27.8	20.5
Percent Moisture	%	7.77	4.45
Percent Solids	%	92.2	95.6
Semi-Volatile Organic Compounds			
Pentachlorophenol	mg/kg	0.502	61
Total Petroleum Hydrocarbons			
TPH(C_9-C_{36})	mg/kg	< 50	153
Total Metals			
Iron	mg/kg	6880	8330
Manganese	mg/kg	79.9	94.56

Notes:

ND - Not detected
< - Compound not detected above the reporting limit
J - Estimated value
S.U. - Standard units
mg/kg - Milligrams per kilogram
% - Percent

Table 3

Aerobic Biostudy SB1 Groundwater Analytical Data (3-Month Period) - Microcosm Study
Penta Wood Products Superfund Site
Siren, Wisconsin

			3-Month Period		
	Date Analyzed	**1/11/2016**	**4/11/2016**	**4/11/2016**	**4/11/2016**
Parameters	**Units**	**Start of Microcosm Study**	**Soil and Groundwater**	**Soil, Groundwater, and Oxygen**	**Soil, Groundwater, Oxygen, and Azide**
Semi-Volatile Organic Compounds					
Pentachlorophenol	µg/L	289 / 302	9.29 J / < 50	3.10 J / < 50	362 / 282
Total Petroleum Hydrocarbons					
TPH(C_9-C_{36})	mg/L	4.61 / 5.10	< 0.5 / < 0.5	< 0.5 / < 0.5	4.45 / 4.28
Removal of Pentachlorophenol	%		94.2	95.2	-8.96
Removal of TPH(C_9-C_{36})	%		41.5	41.5	4.41

Notes:

< - Compound not detected above the reporting limit
µg/L - Micrograms per liter
mg/L - Milligrams per liter
J - Estimated value
All samples were analyzed in duplicate.

Table 4

Aerobic Biostudy SB1 Soil Analytical Data (3-Month Period) - Microcosm Study
Penta Wood Products Superfund Site
Siren, Wisconsin

			3-Month Period		
Parameters	**Date Analyzed** / **Units**	**1/11/2016 Start of Microcosm Study**	**4/11/2016** Soil and Groundwater	**4/11/2016** Soil, Groundwater, and Oxygen	**4/11/2016** Soil, Groundwater, Oxygen, and Azide
Semi-Volatile Organic Compounds					
Pentachlorophenol	mg/kg	0.087 J / 0.094 J	< 0.1 / < 0.1	< 0.1 / < 0.1	< 0.1 / < 0.1
Total Petroleum Hydrocarbons					
TPH(C_9-C_{36})	mg/kg	< 50 / < 50	< 50 / < 50	< 50 / < 50	< 50 / < 50

Notes:

J - Estimated value
< - Compound not detected above the reporting limit
mg/kg - Milligrams per kilogram
All samples were analyzed in duplicate.

Table 5

Aerobic Biostudy SB1 Groundwater Analytical Data (6-Month Period) - Microcosm Study
Penta Wood Products Superfund Site
Siren, Wisconsin

Parameters	Date Analyzed / Units	1/11/2016 Start of Microcosm Study	6-Month Period 8/1/2016 Soil and Groundwater	8/1/2016 Soil, Groundwater, and Oxygen	8/1/2016 Soil, Groundwater, Oxygen, and Azide
Semi-Volatile Organic Compounds					
Pentachlorophenol	µg/L	289 / 302	< 50 / < 50	< 50 / < 50	92.7 / 110
Total Petroleum Hydrocarbons					
TPH(C_9-C_{36})	mg/L	4.61 / 5.10	< 0.5 / < 0.5	< 0.5 / < 0.5	< 0.5 / < 0.5
Removal of Pentachlorophenol	%		91.5	91.5	65.7
Removal of TPH(C_9-C_{36})	%		41.5	41.5	41.5

Notes:

< - Compound not detected above the reporting limit
µg/L - Micrograms per liter
mg/L - Milligrams per liter
J - Estimated value
% - Percent
All samples were analyzed in duplicate.

Table 6

Aerobic Biostudy SB1 Soil Analytical Data (6-Month Period) - Microcosm Study
Penta Wood Products Superfund Site
Siren, Wisconsin

			6-Month Period		
Parameters	**Date Analyzed** / **Units**	**1/11/2016 Start of Microcosm Study**	**8/1/2016** Soil and Groundwater	**8/1/2016** Soil, Groundwater, and Oxygen	**8/1/2016** Soil, Groundwater, Oxygen, and Azide
Semi-Volatile Organic Compounds					
Pentachlorophenol	mg/kg	0.087 J / 0.094 J	< 0.1 / < 0.1	< 0.1 / < 0.1	< 0.1 / < 0.1
Total Petroleum Hydrocarbons					
TPH(C_9-C_{36})	mg/kg	< 50 / < 50	< 50 / < 50	< 50 / < 50	< 50 / < 50

Notes:

J - Estimated value
< - Compound not detected above the reporting limit
mg/kg - Milligrams per kilogram
All samples were analyzed in duplicate.

Table 7

Anaerobic Biostudy MW29 Groundwater Analytical Data (3-Month Period) - Microcosm Study
Penta Wood Products Superfund Site
Siren, Wisconsin

Parameters	Date Analyzed / Units	5/6/2016 Start of Microcosm Study	3-Month Period 8/3/2016 Soil and Groundwater	3-Month Period 8/3/2016 Soil, Groundwater, and EVO	3-Month Period 8/3/2016 Soil, Groundwater, Oxygen, and Azide
Semi-Volatile Organic Compounds					
Pentachlorophenol	µg/L	2460 / 1580	8900 / 9600	3250 / 1240	8600 / 7900
Total Petroleum Hydrocarbons					
TPH(C_9-C_{36})	mg/L	464 / 501	224 / 224	470 / 308	430 / 428
Removal of Pentachlorophenol	%		<1	<1	<1
Removal of TPH(C_9-C_{36})	%		37.3	29.7	9.19

Notes:

µg/L - Micrograms per liter
mg/L - Milligrams per liter
EVO - Emulsified Vegetable Oil
% - Percent
< - Less than value listed
All samples were analyzed in duplicate.

Table 8

Anaerobic Biostudy MW29 Soil Analytical Data (3-Month Period) - Microcosm Study
Penta Wood Products Superfund Site
Siren, Wisconsin

			3-Month Period		
Parameters	**Date Analyzed** / **Units**	**5/6/2016 Start of Microcosm Study**	**8/3/2016** Soil and Groundwater	**8/3/2016** Soil, Groundwater, and EVO	**8/3/2016** Soil, Groundwater, Oxygen, and Azide
Semi-Volatile Organic Compounds					
Pentachlorophenol	mg/kg	23.3 / 38.1	3.60 / 2.63	3.20 / 1.68	< 0.1 / < 0.1
Total Petroleum Hydrocarbons					
TPH(C_9-C_{36})	mg/kg	919 / 2370	1250 / 1440	932 / 983	1400 / 1660

Notes:

< - Compound not detected above the reporting limit
mg/kg - Milligrams per kilogram
EVO - Emulsified Vegetable Oil
All samples were analyzed in duplicate.

Table 9

Anaerobic Biostudy MW29 Groundwater Analytical Data (6-Month Period) - Microcosm Study
Penta Wood Products Superfund Site
Siren, Wisconsin

Parameters	Units	Date Analyzed: 5/6/2016 Start of Microcosm Study	6-Month Period: 11/15/2016 Soil and Groundwater	11/15/2016 Soil, Groundwater, and EVO	11/15/2016 Soil, Groundwater, Oxygen, and Azide
Semi-Volatile Organic Compounds					
Pentachlorophenol	µg/L	2460 / 1580	15000 / 17800	1010 / 1610	6100 / 6500
Total Petroleum Hydrocarbons					
TPH(C_9-C_{36})	mg/L	464 / 501	105 / 237	149 / 264	295 / 213
Removal of Pentachlorophenol	%		<1	35.30	<1
Removal of TPH(C_9-C_{36})	%		42.3	51.0	25.40

Notes:

µg/L - Micrograms per liter
mg/L - Milligrams per liter
EVO - Emulsified Vegetable Oil
% - Percent
< - Less than value listed
All samples were analyzed in duplicate.

Table 10

Anaerobic Biostudy MW29 Soil Analytical Data (6-Month Period) - Microcosm Study
Penta Wood Products Superfund Site
Siren, Wisconsin

Parameters	Units	Date Analyzed: 5/6/2016 Start of Microcosm Study	6-Month Period: 11/15/2016 Soil and Groundwater	6-Month Period: 11/15/2016 Soil, Groundwater, and EVO	6-Month Period: 11/15/2016 Soil, Groundwater, Oxygen, and Azide
Semi-Volatile Organic Compounds					
Pentachlorophenol	mg/kg	23.3 / 38.1	18.3 / 22.4	11.0 / 10.5	4.69 / 9.53
Total Petroleum Hydrocarbons					
TPH(C_9-C_{36})	mg/kg	919 / 2370	1400 / 1360	1010 / 838	1950 / 1350

Notes:

< - Compound not detected above the reporting limit
mg/kg - Milligrams per kilogram
EVO - Emulsified Vegetable Oil
All samples were analyzed in duplicate.

Table 11

Anaerobic Biostudy MW29 Groundwater Analytical Data (12-Month Period) - Microcosm Study
Penta Wood Products Superfund Site
Siren, Wisconsin

Parameters	Date Analyzed / Units	5/6/2016 Start of Microcosm Study	6-Month Period: 5/8/2017 Soil and Groundwater	6-Month Period: 5/8/2017 Soil, Groundwater, and EVO	6-Month Period: 5/8/2017 Soil, Groundwater, Oxygen, and Azide
Semi-Volatile Organic Compounds					
Pentachlorophenol	µg/L	2460 / 1580	872 / 353	69 / 205	182 / 344
Total Petroleum Hydrocarbons					
TPH(C_9-C_{36})	mg/L	464 / 501	194 / 169	169 / 208	173 / 141
Removal of Pentachlorophenol	%		70	93.20	87
Removal of TPH(C_9-C_{36})	%		55.6	63.5	52.20

Notes:

µg/L - Micrograms per liter
mg/L - Milligrams per liter
EVO - Emulsified Vegetable Oil
% - Percent
All samples were analyzed in duplicate.

Table 12

Anaerobic Biostudy MW29 Soil Analytical Data (12-Month Period) - Microcosm Study
Penta Wood Products Superfund Site
Siren, Wisconsin

			6-Month Period		
Parameters	**Date Analyzed** / **Units**	**5/6/2016** **Start of Microcosm Study**	**5/8/2017** **Soil and Groundwater**	**5/8/2017** **Soil, Groundwater, and EVO**	**5/8/2017** **Soil, Groundwater, Oxygen, and Azide**
Semi-Volatile Organic Compounds					
Pentachlorophenol	mg/kg	23.3 / 38	12 / 11.2	7.1 / 6.54	1.04 / 0.67
Total Petroleum Hydrocarbons					
TPH(C_9-C_{36})	mg/kg	919 / 2370	903 / 819	501 / 359	1070 / 1090

Notes:

mg/kg - Milligrams per kilogram
EVO - Emulsified Vegetable Oil
All samples were analyzed in duplicate.

Table 13

Bio-Trap Analytical Data
Penta Wood Products Superfund Site
Siren, Wisconsin

Parameters	Sample Date: Units	5/23/2016 MW9	5/23/2016 EW11S	5/23/2016 MW20	5/23/2016 MW29
Biomass and ^{13}C Incorporation					
Total Biomass	Cells/bead	2,280,000	1,100,000	380,000	1,920,000
^{13}C Enriched Biomass	Cells/bead	19,800	14,500	2,170	11,200
Average PLFA $\delta^{13}C$	‰	257	360	276	94
Maximum PLFA $\delta^{13}C$	‰	435	1192	399	232
^{13}C Mineralization					
Inorganic Carbon $\delta^{13}C$	‰	-17	-14	-21	-20
$\%^{13}C$	%	1.09	1.09	1.08	1.08
Community Structure (% Total PLFA)					
Firmicutes	%	0.7	2.68	16.17	52.88
Proteobacteria	%	63.6	65.59	49.44	31.17
Anaerobic Metal Reducers	%	0.18	1.02	6.32	0
Actinomycetes	%	0.34	0.36	1.48	4.4
General	%	34.29	29.85	25.96	11.56
Eukaryotes	%	0.88	0.52	0.64	0

Notes:

δ13C - Del Carbon 13
PLFA - Phospholipid Fatty Acids
‰ - Parts per thousand
% - Percent

Attachment A
Biodegradation Rates

Attachment A – Biodegradation Rates

Based on the treatability study, the half lives under the different conditions tested are shown in the table below. Since no target concentration exists for total petroleum hydrocarbons (TPH), 10 milligrams per liter (mg/L) were used to calculate a treatment time. Please note that these half lives assume that non-aqueous phase liquid (NAPL) is not present and that there is no ongoing source.

	First Order Degradation Rate Constant	Half Life	Initial Concentration	Target Concentration	Time to Reach Target
PCP Aerobic Conditions – not enhanced	0.948	0.7 month	295 µg/L	1 µg/L	6.3 months
PCP Aerobic Conditions - O_2 added	0.948	0.7 month	295 µg/L	1 µg/L	6.3 months
TPH Aerobic Conditions	n/a	Too fast to measure			
PCP Anaerobic Conditions – not enhanced	0.126	5.5 months	2,020 µg/L	1 µg/L	66 months
PCP Anaerobic Conditions – enhanced with EVO	0.238	2.9 months	2,020 µg/L	1 µg/L	34.8 months
TPH Anaerobic Conditions - not enhanced	0.072	9.6 months	483 mg/L	10 mg/L	57.6 months
TPH Anaerobic Conditions - not enhanced with EVO	0.082	8.5 months	483 mg/L	10 mg/L	51 months

Appendix C
Groundwater Gradient Analysis

Appendix C
Gradient Analysis

Local hydraulic gradient directions were calculated for four locations along the Wisconsin River east of the Site. Each location corresponds to a set of three wells from which groundwater head measurements were used to calculate the gradient direction. Each set of three wells is referred to as a triangle group with letter designations A-D, as listed below and shown in Figure C-1:

- **Triangle A:** W-28, W-18, W-13
- **Triangle B:** W18, W10A, W17
- **Triangle C:** W10A, W29, W26
- **Triangle D:** W29, W21, W11

The dataset used to calculate groundwater flow directions was developed from the historical set of head measurements for the site from 1994 through the present, including the dates for which head measurements were available for all wells included in the analysis. These head measurements were calculated to take free product into account if present (applies only to W17). The resulting dataset had nearly monthly measurements for 1995-1998 and quarterly measurements for most of the following years.

Hydraulic gradient directions were calculated for each triangle group for each date included in the dataset using the EPA 3PE interactive spreadsheet tool for evaluating hydraulic gradients.[1] Gradient direction results are reported as angles measured clockwise from north, where 0^0 is north, 90^0 is east, 180^0 is south, and 270^0 is west. Results of the gradient direction analysis for each triangle group are summarized in Table C-1. Graphs of gradient direction vs. time for each triangle group are shown on Figures C-2 and C-3. Changes in the pumping rate of the groundwater extraction system are marked as vertical lines on these graphs.

The hydraulic gradient directions for each triangle group were also plotted on a rose diagram and posted on a map at the center of each triangle of wells to illustrate the variation in groundwater flow directions for that area (Figure C-1). Rose diagrams are circular histogram plots used to show the frequency of directional data – in this case, groundwater flow directions. The data shown on the rose diagrams was down-sampled to include only one measurement per quarter (using January, April, July, and October data when possible, and filling in gaps with other months if needed) to avoid overrepresentation of the years with monthly measurements. The measurement dates included in the wind roses are indicated in Table C-1 and include one

[1] Beljin, M., R. Ross, and S. Acree. 3PE: A Tool for Estimating Groundwater Flow Vectors. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-14/273, 2014. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NRMRL&direntryid=287064

measurement per quarter from 1994 Q4 through 2019 Q2 (except 2019 Q2 data due to abandonment of wells W26 and W29).

Results of this assessment include the following:

- As shown in Figures C-2 and C-3, there are no major trends in flow direction through time for the triangle groups A, C, and D. The flow direction in Triangle A is typically to the northeast (around 45^0), toward the River. The flow directions for Triangle C and Triangle D fluctuate between roughly east and south (about 90^0 to 180^0), with no clear pattern over time or correlation with pumping rates.
- As shown in Figure C-2, the flow direction in Triangle B (wells W18-W10A-W17) appears to be affected by changes in the pumping rate: when the pumping rate is 42 gpm, flow is generally towards the southwest (~220^0 to 265^0), and when the pumping rate is 22 or 29 gpm, flow is generally to the northeast (~50^0- 90^0), suggesting that flow in this area is towards the Site when pumping rates are higher and towards the River when pumping rates are lower. For example, during the time frame of 2010 through current, from shortly before the reduction in pumping, the graph for Triangle B in Figure C-2, there are only six, widely spaced dates, when flow was not towards the river. The rose diagram on Figure C1 shows this bipolar distribution of flow directions for Triangle B.

Table C-1
Groundwater Elevation Data and Calculated Gradient Directions
Wauleco Project Site
Wausau, Wisconsin

DATE	HYDRAULIC HEAD (ft amsl)									HYDRAULIC GRADIENT DIRECTION (° clockwise of N)				INCLUDE IN ROSE DIAGRAMS?
	W10A	W11	W13	W17	W18	W21	W26/ W26R	W28	W29/ W29R	A	B	C	D	
11/29/1994	1160.96	1160.83	1161.25	1161.24	1161.00	1160.74	1160.90	1161.01	1160.85	49	79	132	147	Yes
12/29/1994	1161.00	1160.83	1161.20	1161.13	1160.99	1160.54	1160.90	1161.03	1160.70	61	59	103	105	
1/31/1995	1160.94	1160.71	1161.11	1160.98	1160.99	1160.69	1160.87	1161.02	1160.53	67	137	87	8	Yes
2/28/1995	1160.87	1160.67	1161.07	1160.90	1160.93	1160.65	1160.77	1160.90	1160.45	33	147	93	7	
3/31/1995	1160.85	1160.79	1161.13	1161.05	1160.90	1160.69	1160.87	1160.78	1160.55	22	88	73	22	
4/28/1995	1160.94	1160.84	1161.18	1161.05	1160.93	1160.74	1160.90	1160.88	1160.54	34	57	82	18	Yes
5/31/1995	1161.03	1160.92	1161.25	1161.22	1161.03	1160.81	1160.97	1160.99	1160.61	35	65	85	19	
6/30/1995	1160.89	1160.74	1161.08	1161.08	1160.92	1160.65	1160.78	1160.86	1160.69	27	80	133	95	
7/28/1995	1160.80	1160.80	1161.16	1161.17	1160.82	1160.69	1160.87	1160.76	1160.71	35	70	56	72	Yes
8/30/1995	1162.40	1161.98	1163.26	1163.10	1162.69	1161.83	1162.02	1162.67	1162.06	43	102	185	155	
9/30/1995	1161.16	1161.02	1161.72	1162.06	1161.25	1160.87	1161.02	1161.17	1160.91	35	74	134	79	
10/31/1995	1161.36	1161.16	1161.88	1162.20	1161.42	1161.02	1161.32	1161.38	1161.07	40	72	85	87	Yes
11/30/1995	1161.18	1160.98	1161.60	1161.86	1161.17	1160.82	1161.02	1161.08	1160.92	34	64	142	112	
12/29/1995	1161.17	1160.90	1161.54	1161.63	1161.25	1160.59	1161.00	1161.30	1160.96	60	81	165	146	
1/31/1996	1161.29	1160.82	1161.62	1161.56	1161.39	1160.52	1161.12	1161.44	1161.06	64	99	157	160	Yes
2/29/1996	1161.14	1160.65	1161.39	1161.26	1161.18	1160.34	1161.00	1161.22	1160.95	61	96	157	163	
3/28/1996	1161.41	1161.34	1162.03	1156.99	1161.38	1161.18	1161.34	1161.36	1161.17	44	246	98	55	
4/30/1996	1162.12	1161.75	1162.72	1158.05	1162.14	1161.46	1161.76	1162.15	1161.64	47	245	158	112	Yes
5/30/1996	1161.22	1161.14	1161.73	1157.36	1161.14	1160.83	1161.16	1161.04	1160.91	36	247	89	78	
6/27/1996	1161.60	1161.51	1162.36	1157.96	1161.46	1161.08	1161.45	1161.38	1161.12	40	249	101	65	
7/31/1996	1161.18	1161.13	1161.81	1157.27	1161.27	1160.88	1161.17	1161.20	1160.98	38	243	79	91	Yes
8/28/1996	1161.04	1160.95	1161.51	1166.90	1161.08	1160.76	1160.99	1160.99	1160.83	34	66	93	88	
9/24/1996	1160.92	1160.85	1161.34	1161.47	1160.99	1160.71	1160.89	1160.92	1160.79	34	77	92	107	
10/28/1996	1160.99	1160.90	1161.32	1161.28	1161.06	1160.78	1160.93	1161.07	1160.87	49	88	125	122	Yes
11/19/1996	1161.60	1161.21	1161.86	1161.52	1161.83	1160.63	1161.32	1162.04	1161.45	165	177	202	153	
2/27/1997	1161.12	1160.89	1161.38	1161.12	1161.19	1145.20	1161.00	1161.25	1160.98	74	167	169	138	Yes
3/31/1997	1163.22	1161.94	1163.61	1162.22	1164.07	1162.15	1162.20	1164.54	1162.86	192	198	209	193	
4/24/1997	1161.36	1161.15	1161.88	1162.42	1161.30	1160.90	1161.19	1161.23	1160.99	38	60	119	87	Yes
5/28/1997	1161.07	1160.91	1161.46	1161.64	1161.08	1160.77	1160.97	1161.04	1160.87	39	67	125	119	
6/27/1997	1160.97	1160.82	1161.35	1161.47	1160.98	1160.70	1160.89	1160.91	1160.79	34	67	117	122	
7/17/1997	1161.01	1160.81	1161.35	1161.49	1161.10	1160.72	1160.91	1161.11	1160.83	49	83	133	147	Yes
8/27/1997	1160.82	1160.71	1161.16	1161.21	1160.88	1160.61	1160.77	1160.85	1160.70	39	79	114	132	
9/30/1997	1160.95	1160.80	1161.32	1161.40	1161.01	1160.70	1160.90	1160.93	1160.84	31	78	119	152	
10/29/1997	1161.01	1160.86	1161.39	1161.31	1161.03	1160.74	1160.94	1160.93	1160.85	30	71	116	133	Yes
11/25/1997	1160.97	1160.75	1161.26	1161.03	1161.03	1160.71	1160.86	1161.00	1160.81	38	130	151	168	
12/30/1997	1160.89	1160.70	1161.07	1160.86	1160.95	1160.66	1160.77	1160.99	1160.80	75	183	193	173	
1/30/1998	1160.81	1160.69	1161.00	1160.66	1160.81	1160.65	1160.78	1160.82	1160.75	50	245	125	168	Yes
2/27/1998	1161.11	1161.05	1161.49	1161.09	1161.09	1160.93	1161.08	1161.15	1161.02	58	311	103	122	
3/24/1998	1160.88	1160.79	1161.08	1161.01	1160.81	1160.67	1160.86	1160.74	1160.77	31	28	88	128	
4/27/1998	1161.02	1160.90	1161.33	1161.43	1160.97	1160.69	1160.97	1160.89	1160.83	33	55	95	115	Yes
5/27/1998	1160.90	1160.79	1161.17	1161.41	1160.93	1160.70	1160.88	1160.89	1160.81	36	71	91	147	
6/23/1998	1161.07	1160.84	1161.26	1161.36	1160.87	1160.67	1160.92	1160.76	1160.80	30	23	133	123	
7/31/1998	1160.80	1160.78	1161.19	1161.26	1160.82	1160.61	1160.90	1160.72	1160.76	31	69	46	131	Yes
8/25/1998	1160.87	1160.78	1161.12	1161.22	1160.93	1160.68	1160.91	1160.88	1161.07	31	81	270	174	
9/29/1998	1160.79	1160.65	1161.08	1161.15	1160.79	1160.72	1160.92	1160.76	1160.84	39	65	28	200	
11/30/1998	1160.74	1160.55	1161.05	1160.78	1160.78	1160.69	1160.88	1160.66	1160.80	24	131	27	210	Yes
12/29/1998	1160.68	1160.64	1160.79	1160.47	1160.75	1160.55	1160.74	1160.77	1160.74	92	219	359	164	
1/27/1999	1160.74	1160.73	1160.72	1160.28	1160.71	1160.65	1160.74	1160.74	1160.82	158	251	256	165	Yes
4/30/1999	1161.22	1161.21	1161.67	1160.59	1161.14	1160.89	1161.17	1161.10	1161.13	41	257	133	122	Yes
7/22/1999	1160.98	1160.97	1161.57	1160.87	1161.00	1160.80	1160.99	1160.96	1170.86	41	229	256	183	Yes
10/21/1999	1160.69	1160.62	1160.92	1160.50	1160.74	1160.61	1160.66	1160.77	1160.64	59	224	140	171	Yes
1/10/2000	1160.66	1160.63	1160.76	1160.02	1160.66	1160.55	1160.59	1160.74	1160.61	117	245	195	122	Yes
4/19/2000	1160.64	1160.61	1160.78	1159.68	1160.65	1160.62	1160.61	1160.72	1160.64	96	244	219	198	Yes
7/17/2000	1160.99	1160.92	1161.47	1160.77	1160.97	1160.78	1160.96	1160.97	1160.85	46	254	91	101	Yes
10/18/2000	1160.63	1160.61	1160.93	1160.12	1160.61	1160.51	1160.60	1160.64	1160.40	53	249	84	26	Yes
1/29/2001	1160.71	1160.61	1160.77	1159.46	1160.68	1160.66	1160.65	1160.82	1160.70	145	247	215	210	Yes
4/12/2001	1162.76	1161.68	1162.57	1160.28	1163.45	1162.06	1161.94	1164.04	1162.65	198	223	216	201	Yes
7/9/2001	1160.86	1160.76	1161.11	1160.33	1160.84	1160.69	1160.80	1160.86	1160.75	51	249	132	129	Yes
10/15/2001	1160.85	1160.79	1161.07	1160.15	1160.87	1160.80	1160.81	1160.93	1160.82	72	243	193	198	Yes
1/14/2002	1160.99	1160.81	1161.12	1159.97	1160.86	1160.78	1160.87	1161.01	1160.82	100	257	153	150	Yes
4/17/2002	1162.61	1161.82	1163.24	1160.77	1162.89	1162.01	1162.03	1163.09	1162.26	99	232	200	203	Yes
8/5/2002	1160.97	1160.95	1161.34	1158.86	1161.27	1160.84	1160.93	1161.00	1160.87	358	233	111	79	Yes
10/10/2002	1161.76	1161.59	1162.37	1161.33	1161.85	1161.48	1161.61	1161.99	1161.59	69	228	171	137	Yes
1/13/2003	1160.87	1160.79	1161.15	1160.19	1160.99	1160.75	1160.83	1160.95	1160.81	32	230	149	155	Yes
4/23/2003	1162.23	1161.97	1162.96	1161.25	1162.18	1161.82	1162.00	1162.37	1161.93	66	250	160	121	Yes
7/16/2003	1160.87	1160.81	1161.20	1160.44	1160.87	1160.75	1160.87	1160.91	1160.79	55	245	76	115	Yes
10/13/2003	1160.81	1160.77	1161.01	1160.34	1160.83	1160.78	1160.78	1160.86	1160.80	59	241	210	198	Yes
1/20/2004	1160.79	1160.72	1160.91	1160.14	1160.79	1160.76	1160.74	1160.86	1160.75	100	245	190	257	Yes
4/9/2004	1161.49	1161.36	1162.06	1161.27	1161.49	1161.23	1161.37	1161.56	1161.31	55	245	149	111	Yes
7/12/2004	1161.09	1161.03	1161.52	1161.35	1161.05	1160.87	1161.02	1161.05	1160.91	46	52	110	77	Yes
10/6/2004	1160.85	1160.79	1161.08	1160.51	1160.87	1160.75	1160.80	1160.89	1160.79	53	240	167	137	Yes

Table C-1
Groundwater Elevation Data and Calculated Gradient Directions
Wauleco Project Site
Wausau, Wisconsin

DATE	HYDRAULIC HEAD (ft amsl)									HYDRAULIC GRADIENT DIRECTION (° clockwise of N)				INCLUDE IN ROSE DIAGRAMS?
	W10A	W11	W13	W17	W18	W21	W26/ W26R	W28	W29/ W29R	A	B	C	D	
1/19/2005	1160.92	1160.82	1161.22	1160.37	1160.95	1160.81	1160.85	1161.01	1160.88	64	240	201	179	Yes
4/20/2005	1161.17	1161.01	1161.34	1160.79	1161.33	1161.00	1161.00	1161.33	1161.05	46	214	195	177	Yes
7/18/2005	1160.74	1160.72	1161.09	1160.58	1160.71	1160.63	1160.72	1160.70	1160.65	44	263	91	75	Yes
10/18/2005	1160.93	1160.90	1161.28	1161.11	1160.95	1160.83	1160.90	1160.93	1160.86	42	76	115	94	Yes
1/16/2006	1160.81	1160.73	1160.98	1159.92	1160.90	1160.73	1160.75	1160.94	1160.80	92	236	215	184	Yes
4/19/2006	1160.91	1160.89	1161.11	1160.25	1160.87	1160.79	1160.88	1160.88	1160.83	49	251	108	91	Yes
7/17/2006	1160.79	1160.73	1161.09	1160.33	1160.81	1160.67	1160.73	1160.84	1160.71	54	241	158	115	Yes
10/5/2006	1160.85	1160.81	1161.16	1160.60	1160.88	1160.78	1160.82	1160.90	1160.81	51	235	158	137	Yes
1/11/2007	1160.98	1161.03	1161.35	1160.76	1160.94	1160.87	1161.13	1160.95	1160.96	48	262	42	106	Yes
4/19/2007	1160.92	1161.01	1161.22	1160.97	1160.79	1160.77	1161.12	1160.77	1160.87	43	358	44	93	Yes
7/9/2007	1160.79	1160.82	1161.28	1161.01	1160.81	1160.66	1160.95	1160.81	1160.77	46	74	42	117	Yes
10/17/2007	1160.84	1160.84	1161.32	1161.26	1160.89	1160.76	1160.83	1160.87	1160.78	43	76	87	77	Yes
1/25/2008	1160.82	1160.70	1160.92	1159.95	1160.80	1160.71	1160.78	1160.85	1160.73	84	247	117	198	Yes
4/29/2008	1161.74	1161.61	1162.66	1162.76	1161.44	1161.22	1161.57	1161.44	1161.26	46	42	106	65	Yes
7/18/2008	1160.92	1160.82	1161.48	1160.97	1160.87	1160.71	1160.89	1160.87	1160.77	46	14	90	105	Yes
10/13/2008	1160.71	1160.63	1160.92	1159.78	1160.67	1160.62	1160.73	1160.73	1160.66	66	249	62	175	Yes
1/20/2009	1160.84	1160.66	1160.80	1160.05	1160.74	1160.63	1160.84	1160.81	1160.72	135	257	76	171	Yes
4/24/2009	1160.95	1160.87	1161.40	1160.34	1160.87	1160.76	1160.95	1160.84	1160.80	42	257	76	88	Yes
7/28/2009	1160.82	1160.70	1161.34	1160.40	1160.73	1160.63	1160.85	1160.75	1160.68	48	265	67	119	Yes
10/20/2009	1160.71	1160.70	1161.14	1160.43	1160.74	1160.65	1160.69	1160.76	1160.68	49	236	149	110	Yes
1/13/2010	1160.83	1160.75	1161.07	1160.77	1160.88	1160.67	1160.77	1160.89	1160.75	50	198	158	137	Yes
4/22/2010	1160.79	1160.76	1161.11	1160.73	1160.80	1160.67	1160.76	1160.80	1160.71	46	231	108	95	Yes
7/12/2010	1161.01	1160.92	1161.68	1162.42	1160.99	1160.73	1160.94	1160.93	1160.79	40	64	101	83	Yes
10/8/2010	1161.43	1161.43	1162.36	1163.75	1161.07	1160.93	1161.34	1160.97	1160.94	41	52	88	60	Yes
1/12/2011	1161.06	1160.90	1161.53	1161.41	1161.13	1160.78	1160.97	1161.17	1160.89	53	84	130	133	Yes
4/5/2011	1161.49	1161.42	1162.12	1163.42	1161.39	1161.17	1161.39	1161.29	1161.20	37	61	104	67	Yes
7/26/2011	1161.01	1161.12	1162.38	1163.55	1161.07	1160.77	1161.07	1160.97	1160.79	41	67	65	62	Yes
10/24/2011	1160.92	1160.92	1161.65	1161.82	1160.94	1160.77	1160.92	1160.90	1160.80	42	67	76	73	Yes
1/16/2012	1160.77	1160.75	1161.20	1160.79	1160.80	1160.67	1160.78	1160.83	1160.72	51	142	68	112	Yes
4/27/2012	1160.85	1160.85	1161.40	1161.26	1160.87	1160.74	1160.84	1160.85	1160.77	43	70	84	79	Yes
7/24/2012	1160.91	1160.85	1161.56	1161.89	1160.99	1160.76	1160.88	1160.96	1160.81	42	73	99	106	Yes
10/17/2012	1160.85	1160.88	1161.34	1161.34	1160.93	1160.83	1160.90	1160.91	1160.97	43	80	294	170	Yes
1/15/2013	1160.77	1160.78	1161.10	1160.77	1160.79	1160.73	1160.79	1160.80	1160.75	48	165	53	91	Yes
4/24/2013	1162.17	1161.80	1163.08	1163.11	1162.47	1161.72	1161.81	1162.55	1162.08	56	94	212	176	Yes
7/15/2013	1161.29	1161.23	1162.30	1163.40	1161.18	1160.95	1161.24	1161.13	1160.96	43	61	86	61	Yes
10/22/2013	1160.99	1160.94	1161.63	1162.04	1161.01	1160.83	1160.95	1160.95	1160.84	40	67	96	65	Yes
1/20/2014	1160.93	1160.86	1161.28	1161.05	1160.98	1160.75	1160.88	1161.01	1160.83	53	102	125	120	Yes
4/25/2014	1162.00	1161.68	1162.70	1162.91	1162.04	1161.49	1161.69	1162.08	1161.64	50	69	169	125	Yes
7/17/2014	1161.08	1161.03	1161.73	1162.36	1161.05	1160.83	1161.08	1161.01	1160.87	42	63	76	73	Yes
10/20/2014	1161.42	1161.36	1162.43	1163.26	1161.33	1161.07	1161.33	1161.31	1161.11	45	61	98	68	Yes
1/21/2015	1161.55	1161.26	1162.03	1161.95	1161.61	1161.19	1161.36	1161.68	1161.32	59	79	166	161	Yes
4/27/2015	1161.16	1161.13	1161.76	1161.81	1161.07	1160.96	1161.14	1161.05	1160.99	44	53	83	71	Yes
7/14/2015	1161.14	1161.09	1161.82	1162.36	1161.14	1160.95	1161.11	1161.09	1160.98	41	65	88	74	Yes
10/30/2015	1160.97	1160.93	1161.51	1161.48	1161.07	1160.89	1160.96	1161.06	1160.93	44	83	94	137	Yes
1/25/2016	1161.34	1161.15	1161.87	1161.99	1161.43	1161.08	1161.21	1161.45	1161.13	49	78	142	119	Yes
4/26/2016	1161.70	1161.39	1162.25	1162.69	1162.01	1161.27	1161.45	1162.04	1161.49	56	94	188	161	Yes
7/19/2016	1160.98	1160.94	1161.66	1162.05	1160.98	1160.78	1160.98	1160.94	1160.81	42	65	76	72	Yes
10/10/2016	1159.07	1159.43	1160.69	1161.19	1159.06	1158.61	1159.22	1158.88	1158.67	39	65	63	63	Yes
1/9/2017	1161.23	1160.83	1161.62	1161.10	1161.47	1160.93	1161.11	1161.55	1161.12	95	182	184	199	Yes
4/24/2017	1162.38	1161.93	1163.58	1163.67	1162.19	1161.66	1161.96	1162.21	1161.80	47	52	156	102	Yes
7/7/2017	1161.46	1161.36	1162.62	1163.50	1161.36	1161.12	1161.49	1161.38	1161.13	47	61	72	61	Yes
10/12/2017	1161.15	1161.12	1162.18	1162.41	1161.22	1160.99	1161.15	1161.21	1161.03	45	70	76	82	Yes
1/8/2018	1161.25	1161.05	1161.65	1161.20	1161.41	1161.04	1161.14	1161.47	1161.17	67	176	194	181	Yes
4/20/2018	1161.35	1161.27	1161.89	1161.52	1161.46	1161.26	1161.32	1161.48	1161.32	49	117	179	178	Yes
7/9/2018	1161.23	1161.15	1161.89	1162.39	1161.21	1160.95	1161.27	1161.16	1160.99	41	64	69	73	Yes
10/18/2018	1161.90	1161.81	1163.13	1164.37	1161.79	1161.42	1161.81	1161.73	1161.46	43	61	90	65	Yes
1/4/2019	1161.34	1161.23	1161.98	1162.00	1161.31	1161.10	1161.27	1161.30	1161.16	45	61	110	97	Yes
7/3/2019	1161.68	1161.65	1163.17	1165.20	1161.66	1161.29	1161.68	1161.55	1161.37	41	65	76	75	Yes

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Checked by: L. Hoerning, 1/16/2020

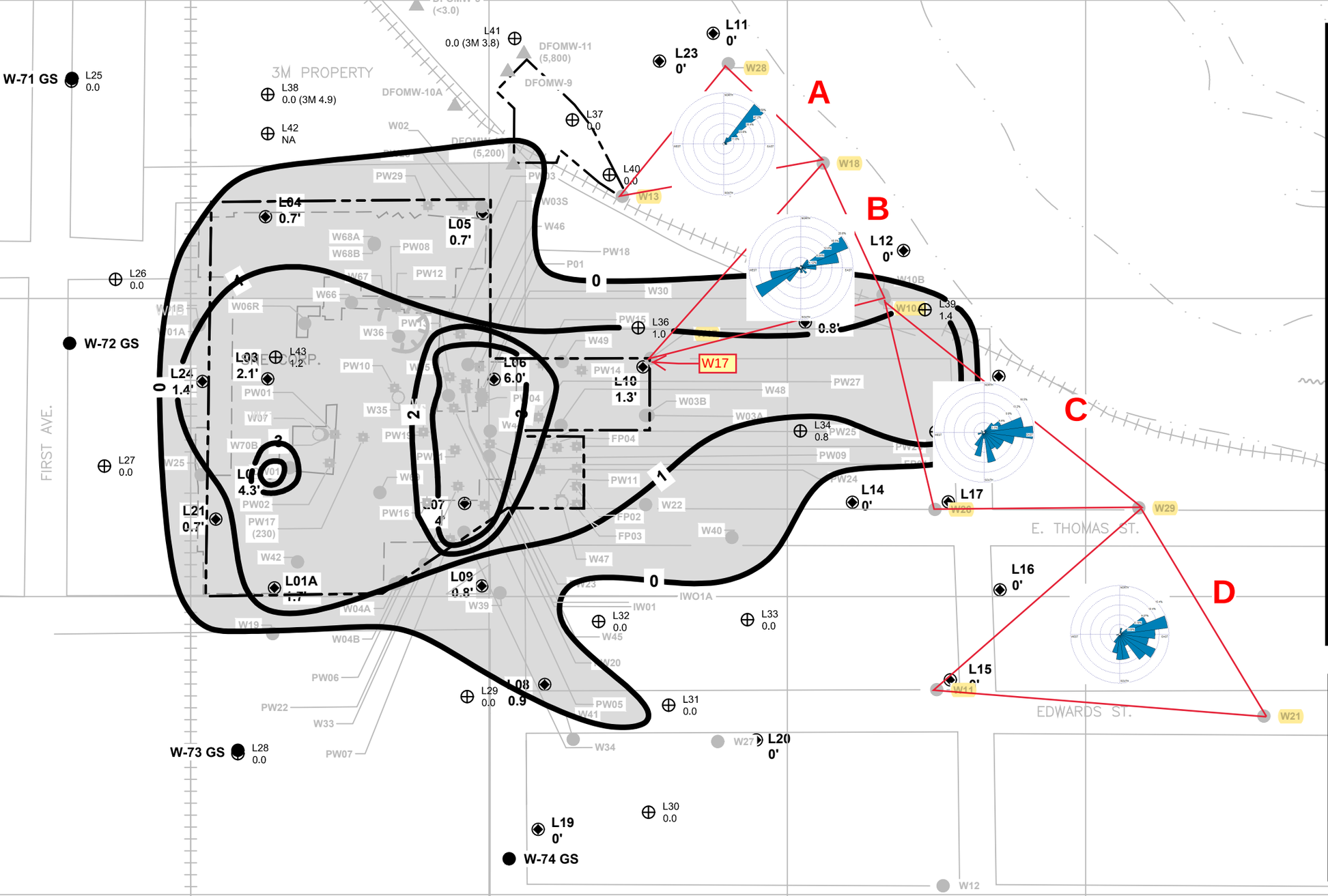

Figure Legend

A

Triangle groups of three wells (indicated by highlighted wells at each vertex of the triangle) used for the calculation of local hydraulic gradients

Rose diagrams show relative frequencies of hydraulic gradient directions based on data from 1994-2019.

Figure C-1
Local Groundwater Gradient Directions within Triangle Groups of Wells

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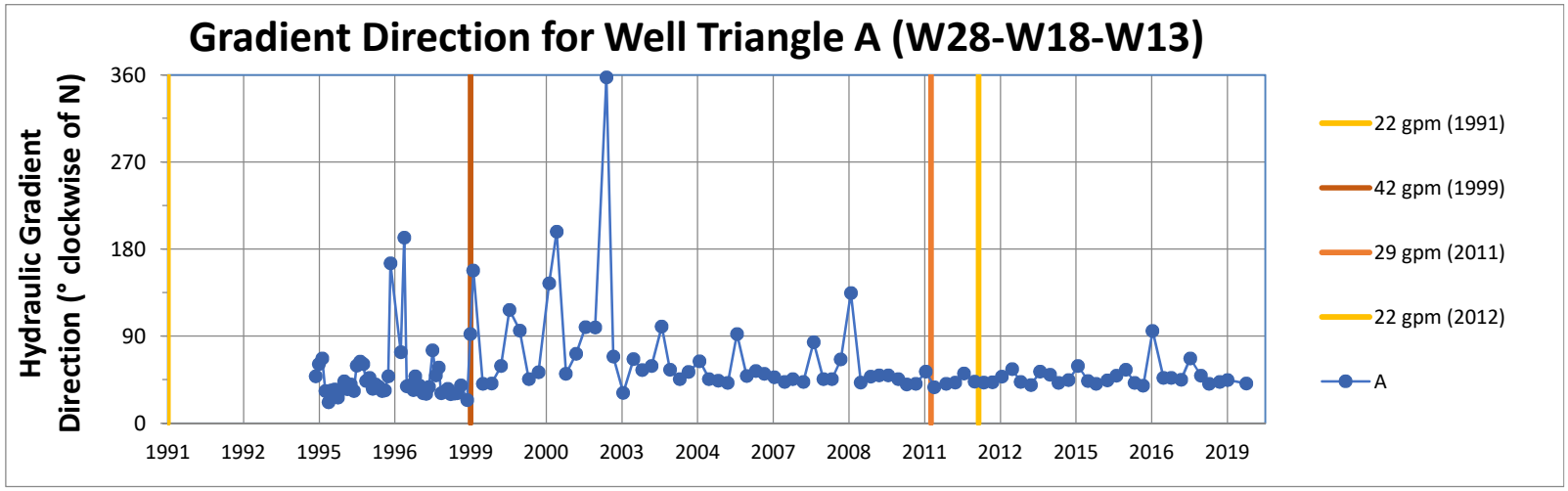
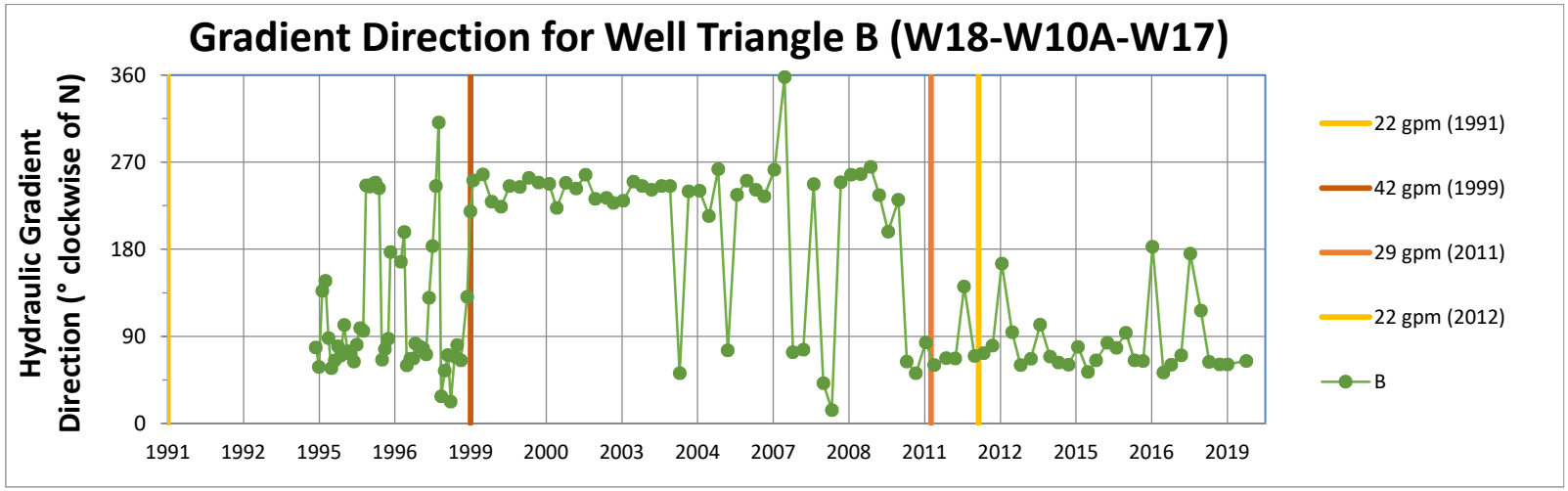
Figure C-2
Gradient Direction Graphs, Triangles A & B





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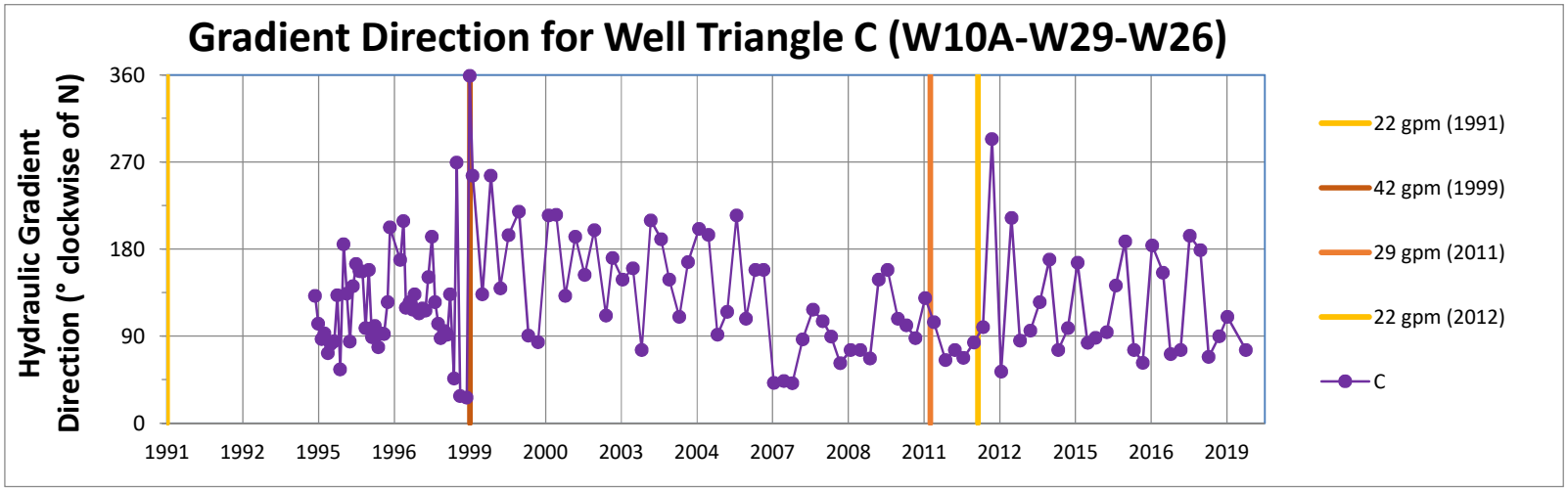
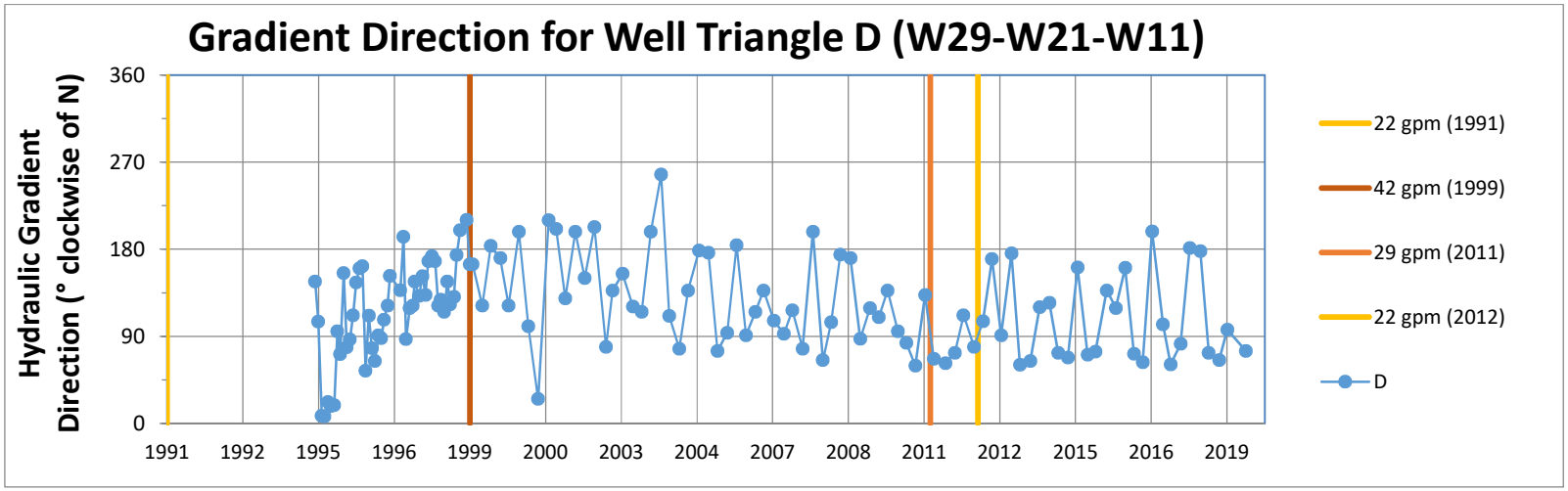
Checked by: L. Hoerning, 1/9/2020
Edited by: K. Quinn
Checked by: L. Auner, 1/14/2020

Figure C-3
Gradient Direction Graphs, Triangles C & D





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Checked by: L. Hoerning, 1/9/2020
Edited by: K. Quinn
Checked by: L. Auner, 1/14/2020

Appendix D
Geochemical Water Quality Data Analysis

Appendix D
Water Chemistry

Groundwater sample analytical results were evaluated to assess oxidation-reduction (redox) conditions in several wells along the profile lines for the concentration-distance graphs and an additional upgradient well. Oxidation-reduction reactions are typically due to biological activity that drive groundwater from oxidative conditions to progressively stronger reducing conditions. This sequence of reducing conditions is:

- **Oxygen Reducing –** Aerobic
- **Nitrate Reducing** - Weakly Anaerobic
- **Manganese Reducing** - Moderately Anaerobic
- **Iron Reducing** - Strongly Anaerobic
- **Sulfate Reducing and Methanogenic** - Very Strongly Anaerobic

To assess redox conditions, results for nitrate-N, nitrate+nitrite-N, dissolved manganese, and dissolved iron were graphed for a subset of the wells in the monitoring program, as shown in Figures D-1 through D-11. Given that nitrite concentrations are typically low or non-detect, the nitrate+nitrite-N analysis is considered equivalent to the nitrate-N analysis. A summary of these groundwater results and interpretation of redox conditions for each well evaluated is provided in Table D-1.

Table D-1
General Water Chemistry Redox Conditions Summary

WELL	NITRATE-N AND NITRATE+NITRITE-N (mg/L)	DISSOLVED MANGANESE (ug/L)	DISSOLVED IRON (ug/L)	REDOX CONDITIONS
Northeast Profile				
W13	<10	<100	<200	Aerobic
Southeast Profile				
W27	Typically <1 NO_3+NO_2 and <0.3 NO_3	>10,000	>3,400	Strongly anaerobic Iron reducing
W11	Typically 0.5 to 3	Increasing trend from ~20 to 1,500	ND	Weakly to moderately anaerobic Nitrate reducing, trending to manganese reducing
W21	Typically 2-4, once 220	--	--	Aerobic to weakly anaerobic Nitrate reducing
Background				
W01A	1-7	--	--	Aerobic
W08	mostly <8, once 220	ND	up to 135, mostly ND	Weakly anaerobic Nitrate reducing
Other Wells				
W10A	<0.5	2,500-3,500	1,000-1,500	Strongly anaerobic Iron reducing
W22	Typically 0.5 or less. Spike of 6 in July 2019	Typically >1,000. Drop to ND in July 2019	Typically ND, except a spike up to 390 in Jan. 2017	Moderately anaerobic Manganese reducing, but aerobic in July 2019
W40	Typically <0.2	>3,000	>2,400 except ND July 2019	Strongly anaerobic Iron reducing, except manganese reducing in July 2019 (W40R)
W26	Typically 1 to 4 since 2010 and typically ND or <1 prior to 2010.	Typically ND, except 4,270 in July 2019	Typically <300, except for 164 in July 2019	Weakly anaerobic Nitrate reducing except for spike of manganese reducing in July 2019
W29	Typically 0-1	up to 100	ND	Weakly to moderately reducing Nitrate reducing or manganese reducing

Redox Conditions Key

Aerobic
Nitrate reducing
Manganese reducing
Iron reducing

Prepared by: L. Auner, 1/15/2020
Checked by: K. Quinn, 1/21/2020

Notes:
ND = Non-detect
-- = Not analyzed

Figure D-1

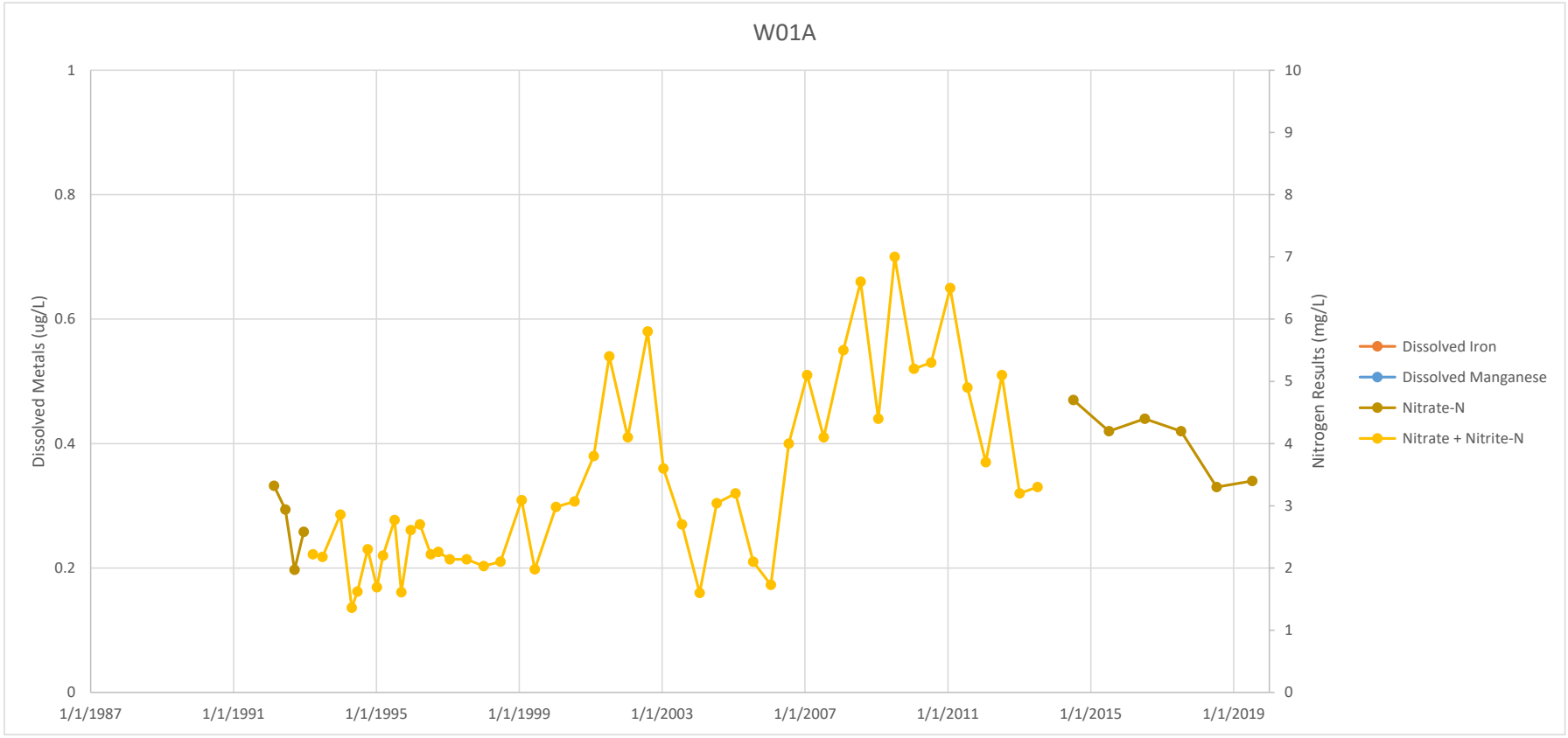

W01A
Dissolved Metals (ug/L)
Nitrogen Results (mg/L)
1
0.8
0.6
0.4
0.2
0
10
9
8
7
6
5
4
3
2
1
0
1/1/1987
1/1/1991
1/1/1995
1/1/1999
1/1/2003
1/1/2007
1/1/2011
1/1/2015
1/1/2019
Dissolved Iron
Dissolved Manganese
Nitrate-N
Nitrate + Nitrite-N

Figure D-2

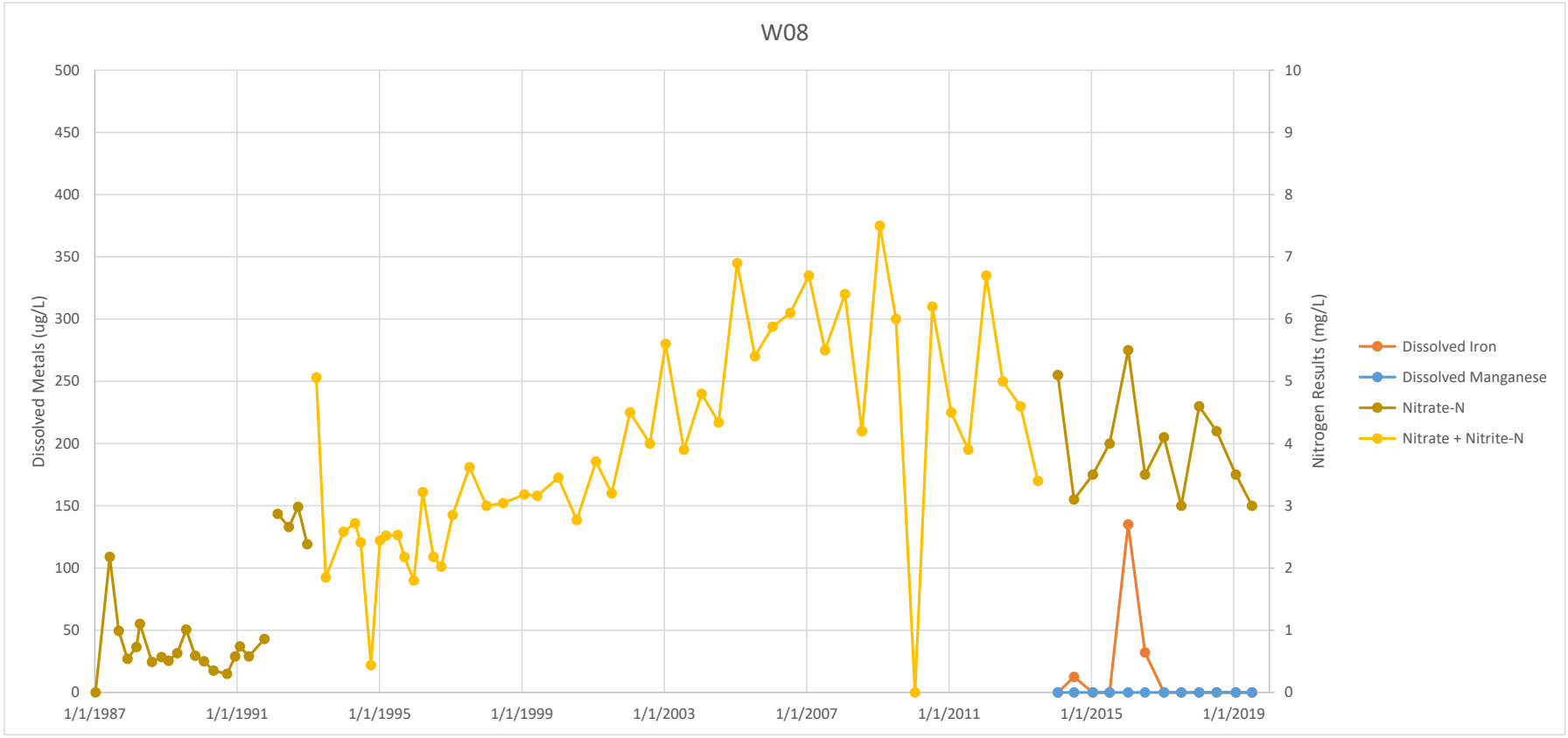

W08
Dissolved Metals (ug/L)
Nitrogen Results (mg/L)
500
450
400
350
300
250
200
150
100
50
0
10
9
8
7
6
5
4
3
2
1
0
1/1/1987
1/1/1991
1/1/1995
1/1/1999
1/1/2003
1/1/2007
1/1/2011
1/1/2015
1/1/2019
Dissolved Iron
Dissolved Manganese
Nitrate-N
Nitrate + Nitrite-N

Figure D-3

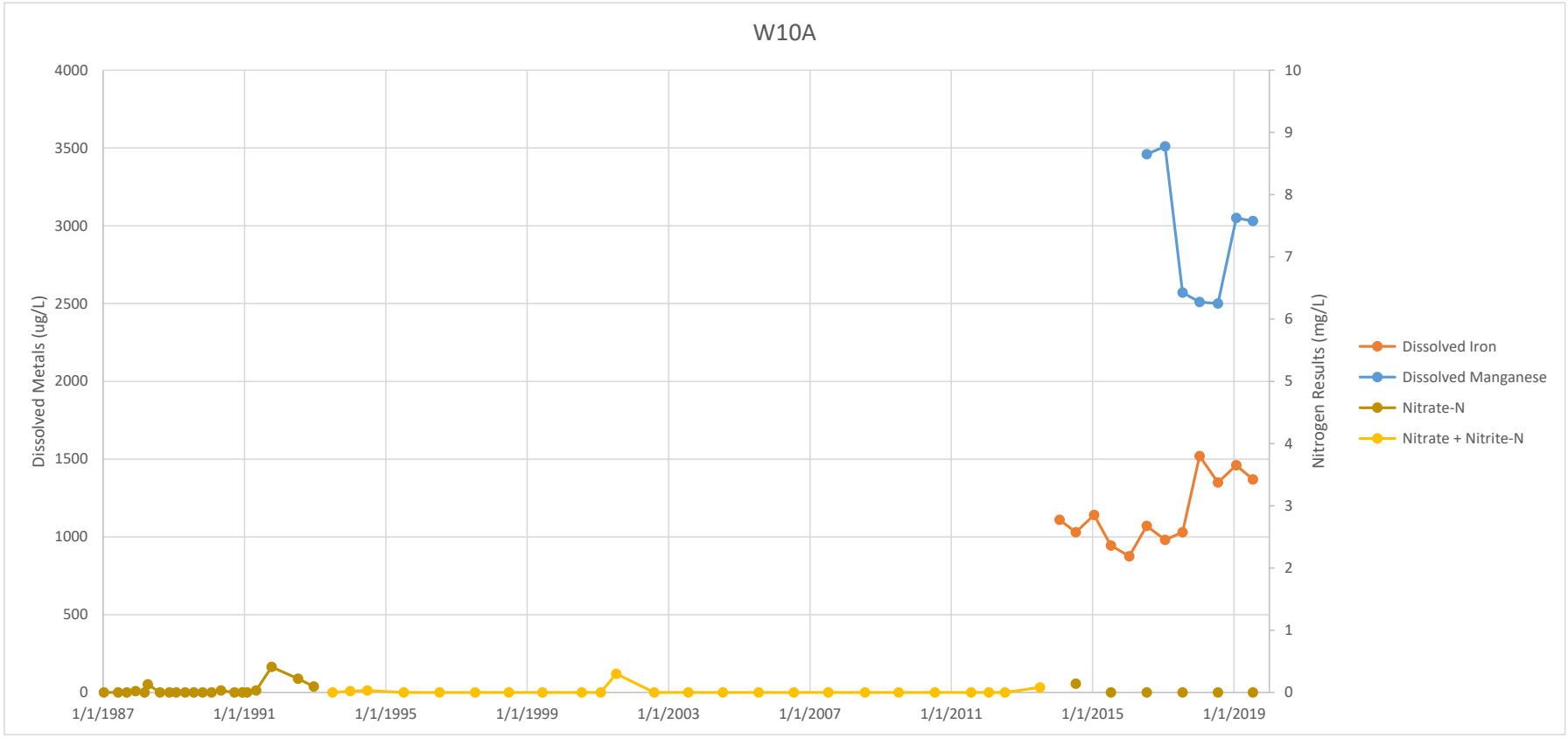

W10A
Dissolved Metals (ug/L)
Nitrogen Results (mg/L)
4000
3500
3000
2500
2000
1500
1000
500
0
10
9
8
7
6
5
4
3
2
1
0
1/1/1987
1/1/1991
1/1/1995
1/1/1999
1/1/2003
1/1/2007
1/1/2011
1/1/2015
1/1/2019
Dissolved Iron
Dissolved Manganese
Nitrate-N
Nitrate + Nitrite-N

Figure D-4

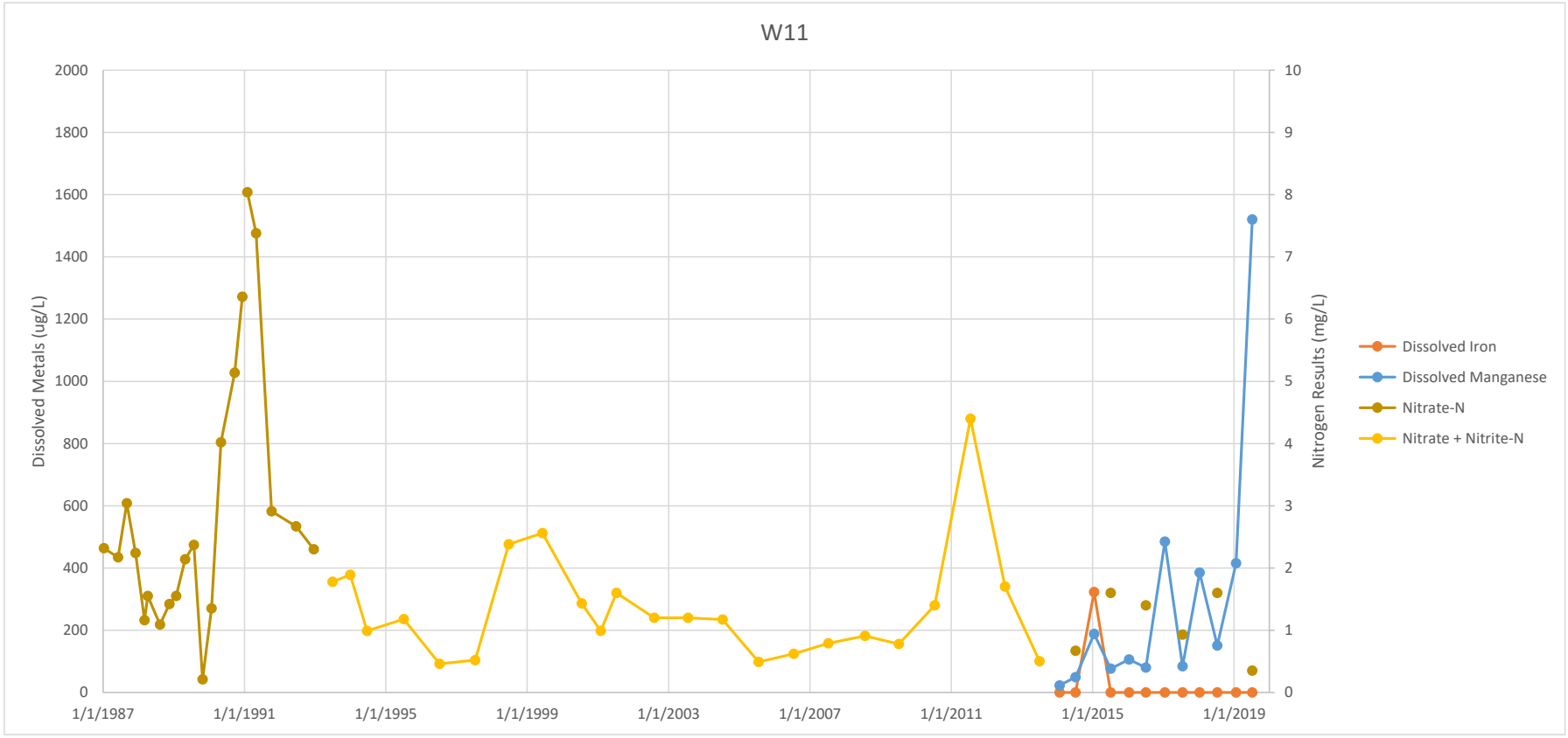

W11
Dissolved Metals (ug/L)
Nitrogen Results (mg/L)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
10
9
8
7
6
5
4
3
2
1
0
1/1/1987
1/1/1991
1/1/1995
1/1/1999
1/1/2003
1/1/2007
1/1/2011
1/1/2015
1/1/2019
Dissolved Iron
Dissolved Manganese
Nitrate-N
Nitrate + Nitrite-N

Figure D-5

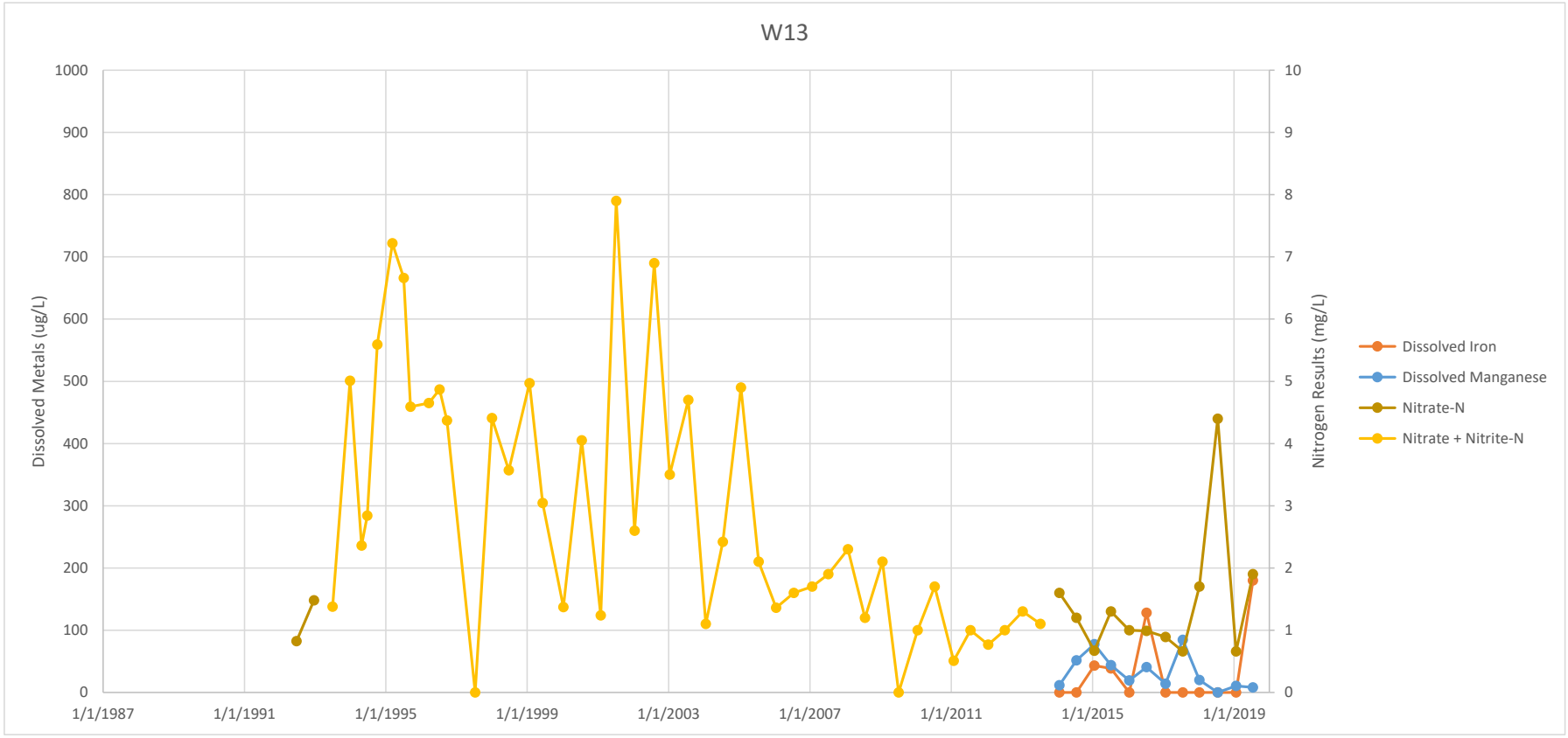

W13
Dissolved Metals (ug/L)
Nitrogen Results (mg/L)
1000
900
800
700
600
500
400
300
200
100
0
10
9
8
7
6
5
4
3
2
1
0
1/1/1987
1/1/1991
1/1/1995
1/1/1999
1/1/2003
1/1/2007
1/1/2011
1/1/2015
1/1/2019
Dissolved Iron
Dissolved Manganese
Nitrate-N
Nitrate + Nitrite-N

Figure D-6

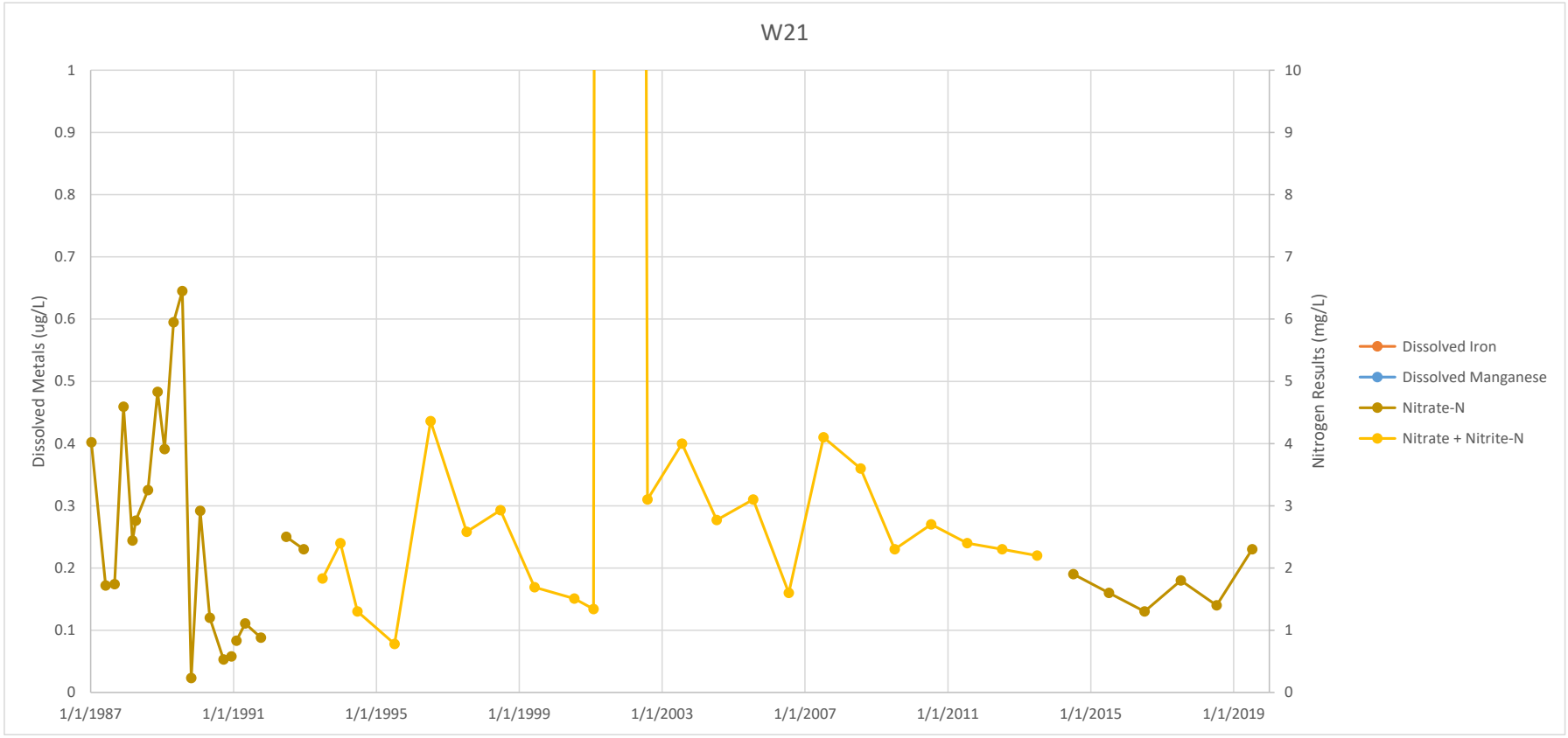

W21
Dissolved Metals (ug/L)
Nitrogen Results (mg/L)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
9
8
7
6
5
4
3
2
1
0
1/1/1987
1/1/1991
1/1/1995
1/1/1999
1/1/2003
1/1/2007
1/1/2011
1/1/2015
1/1/2019
Dissolved Iron
Dissolved Manganese
Nitrate-N
Nitrate + Nitrite-N

Figure D-7

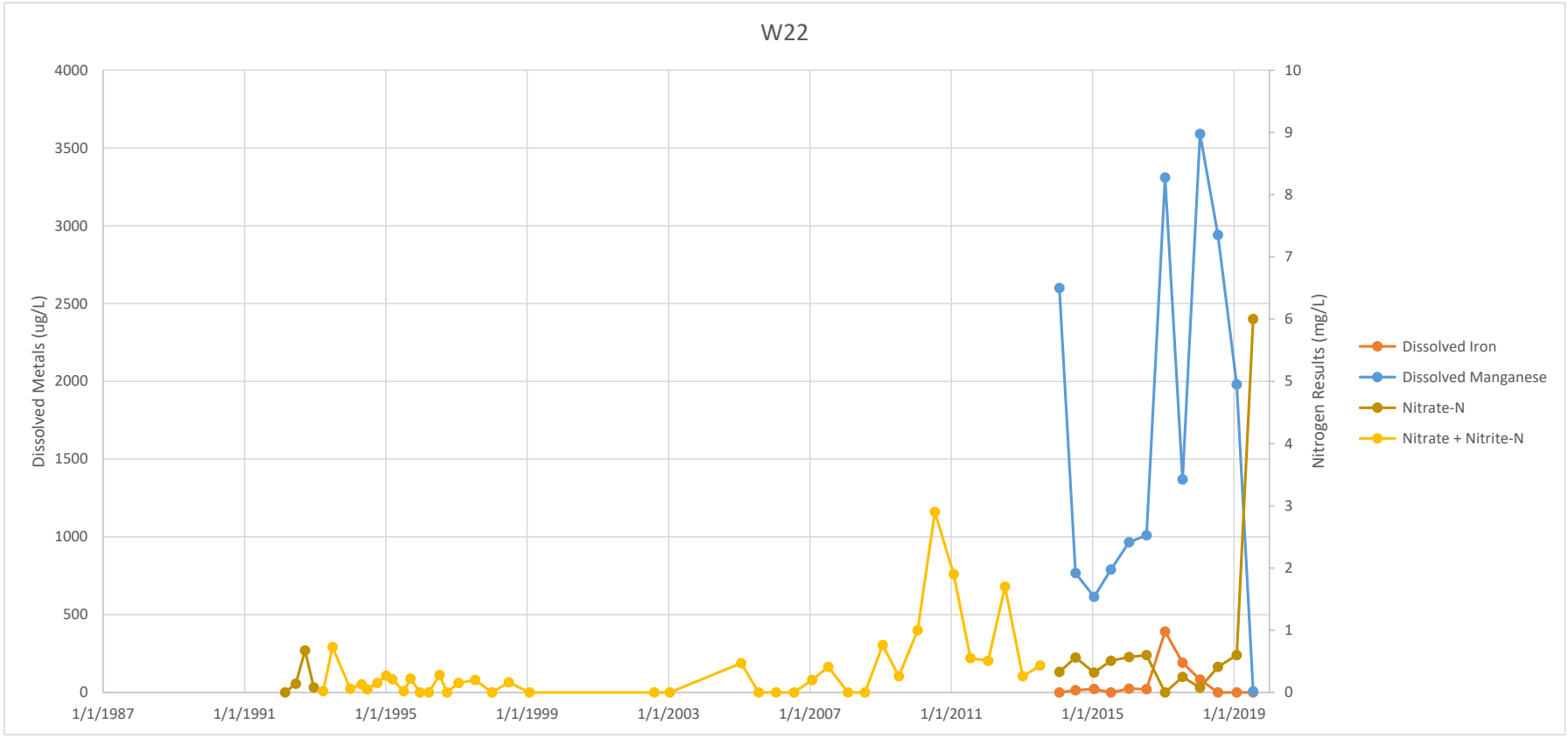

W22
Dissolved Metals (ug/L)
Nitrogen Results (mg/L)
4000
3500
3000
2500
2000
1500
1000
500
0
10
9
8
7
6
5
4
3
2
1
0
1/1/1987
1/1/1991
1/1/1995
1/1/1999
1/1/2003
1/1/2007
1/1/2011
1/1/2015
1/1/2019
Dissolved Iron
Dissolved Manganese
Nitrate-N
Nitrate + Nitrite-N

Figure D-8

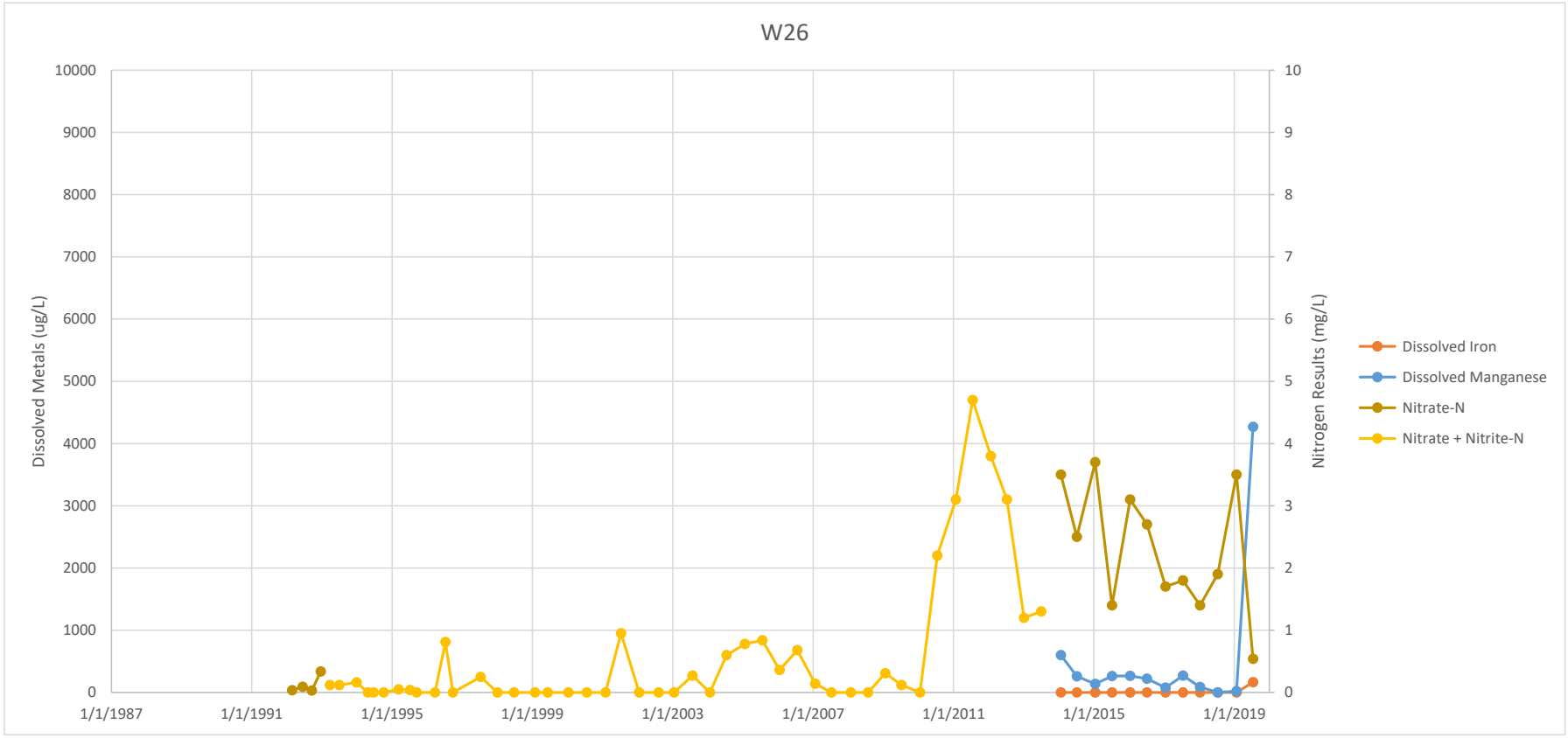

W26
Dissolved Metals (ug/L)
Nitrogen Results (mg/L)
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
10
9
8
7
6
5
4
3
2
1
0
1/1/1987
1/1/1991
1/1/1995
1/1/1999
1/1/2003
1/1/2007
1/1/2011
1/1/2015
1/1/2019
Dissolved Iron
Dissolved Manganese
Nitrate-N
Nitrate + Nitrite-N

Figure D-9

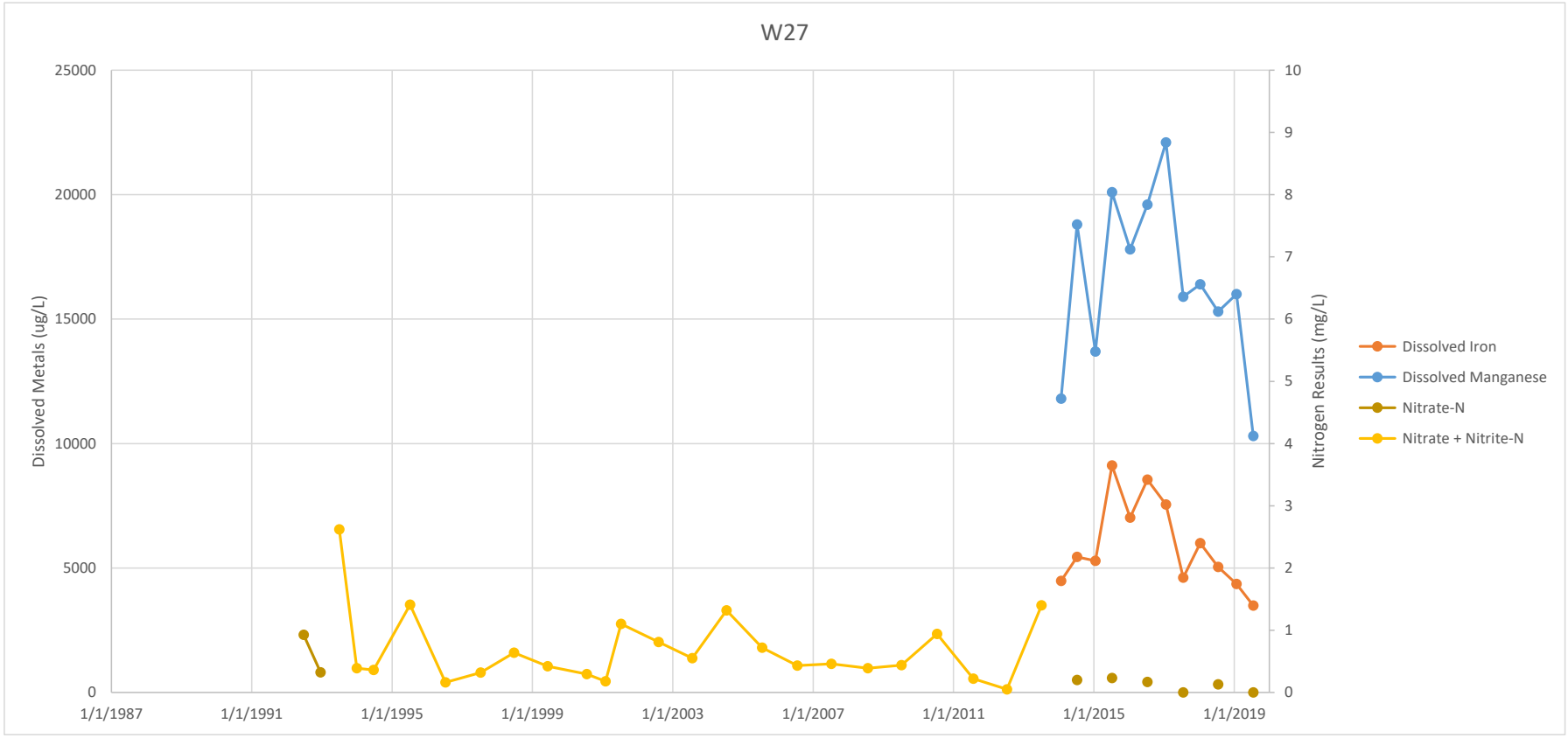

W27
Dissolved Metals (ug/L)
Nitrogen Results (mg/L)
25000
20000
15000
10000
5000
0
10
9
8
7
6
5
4
3
2
1
0
1/1/1987
1/1/1991
1/1/1995
1/1/1999
1/1/2003
1/1/2007
1/1/2011
1/1/2015
1/1/2019
Dissolved Iron
Dissolved Manganese
Nitrate-N
Nitrate + Nitrite-N

Figure D-10

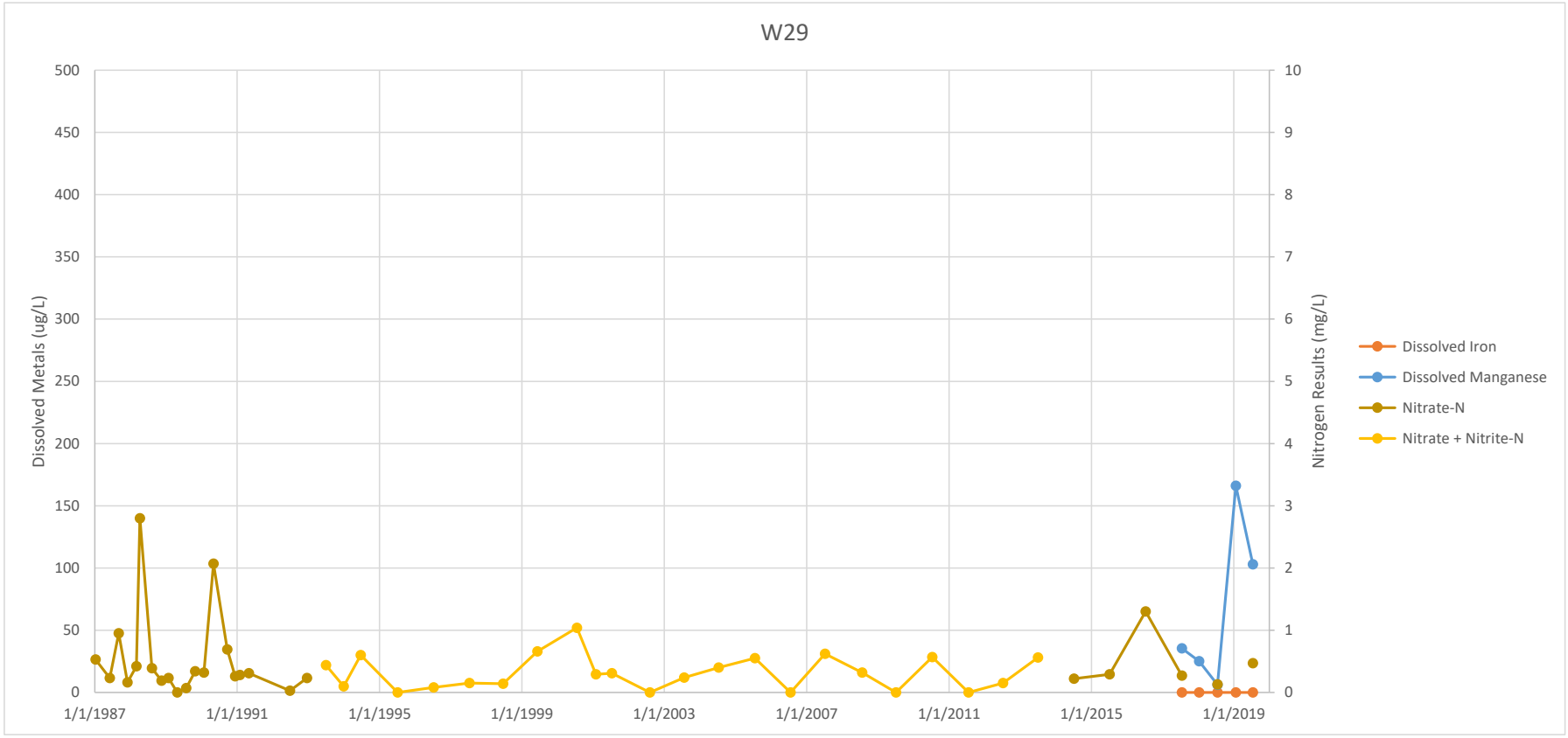
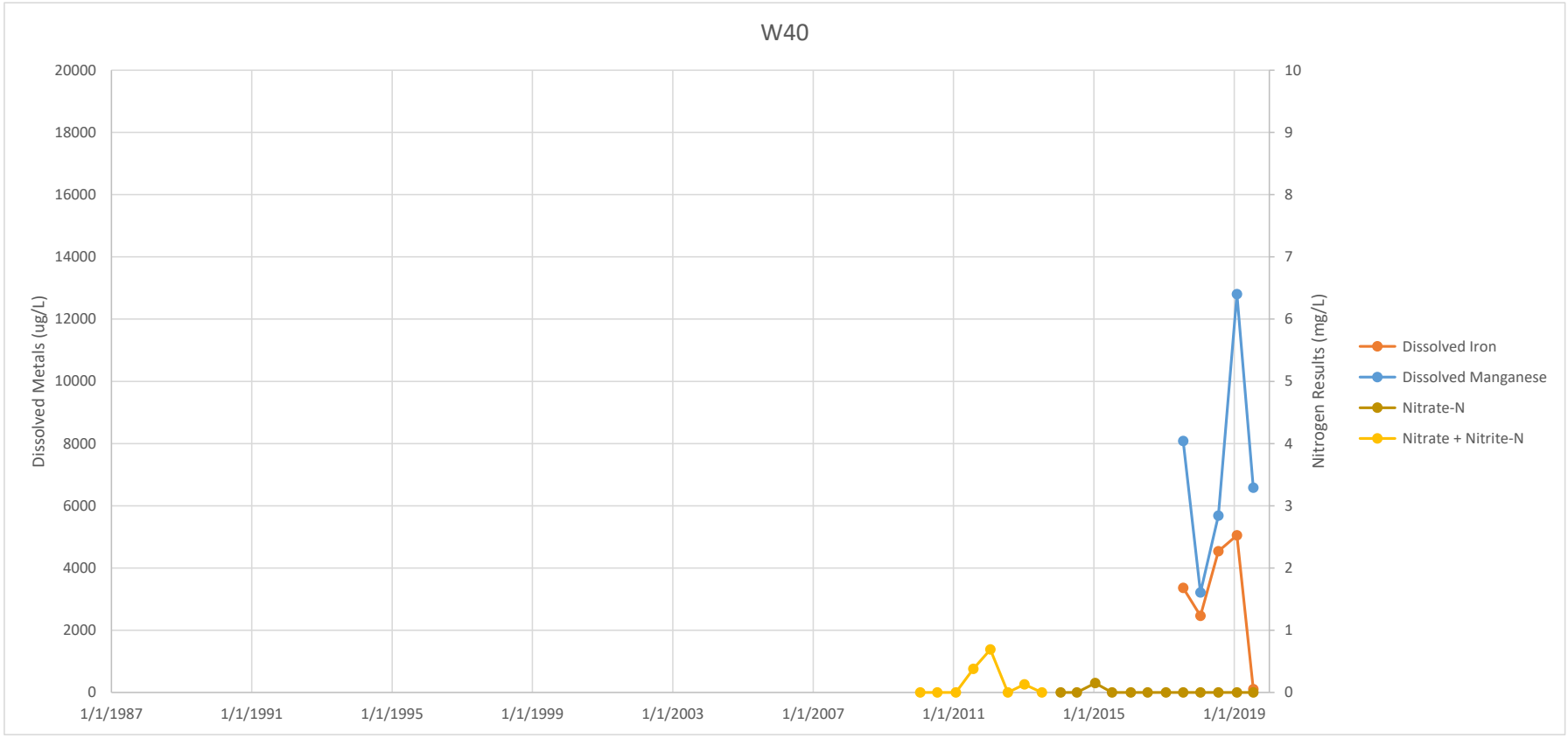
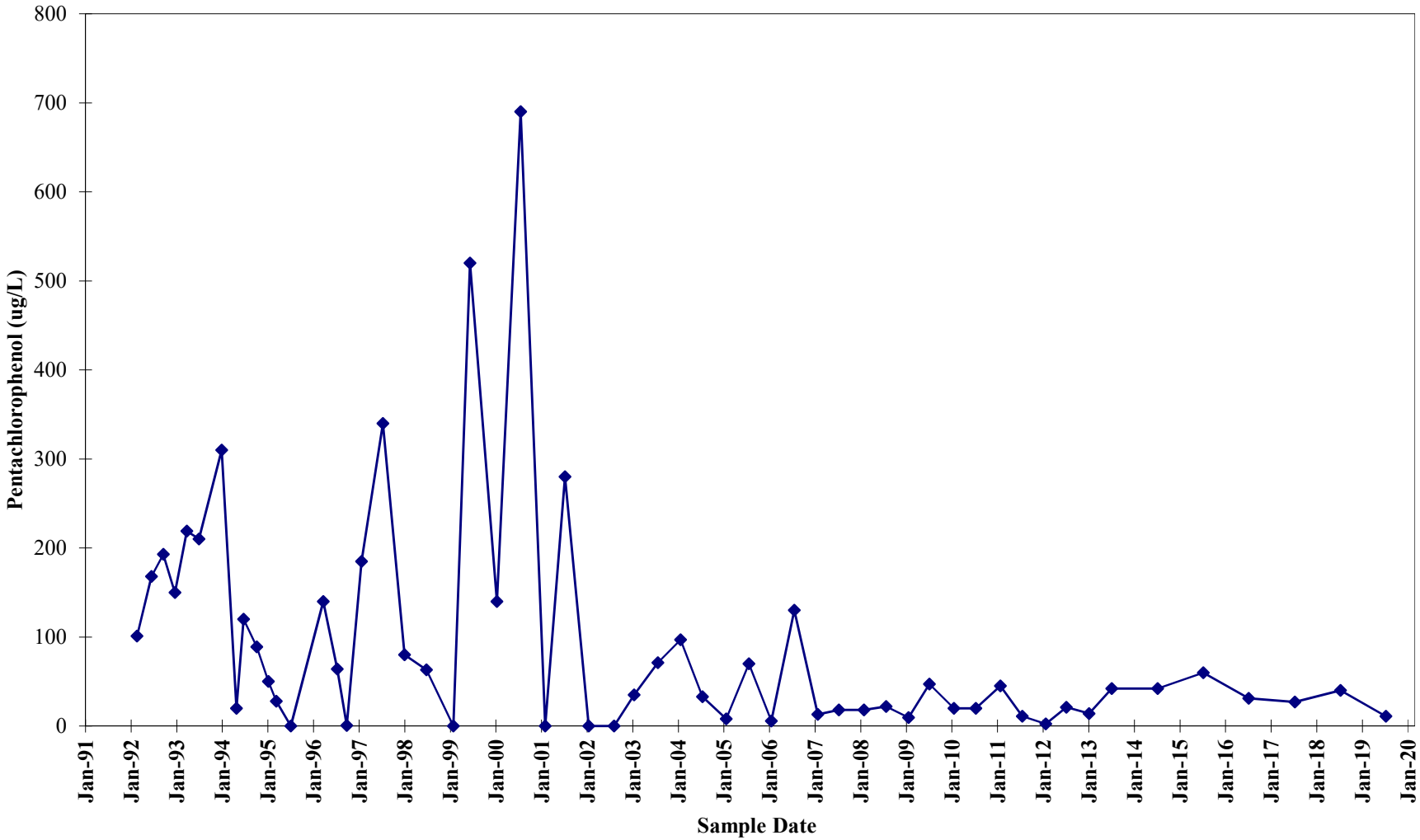

W29
Dissolved Metals (ug/L)
Nitrogen Results (mg/L)
500
450
400
350
300
250
200
150
100
50
0
10
9
8
7
6
5
4
3
2
1
0
1/1/1987
1/1/1991
1/1/1995
1/1/1999
1/1/2003
1/1/2007
1/1/2011
1/1/2015
1/1/2019
Dissolved Iron
Dissolved Manganese
Nitrate-N
Nitrate + Nitrite-N

Figure D-11

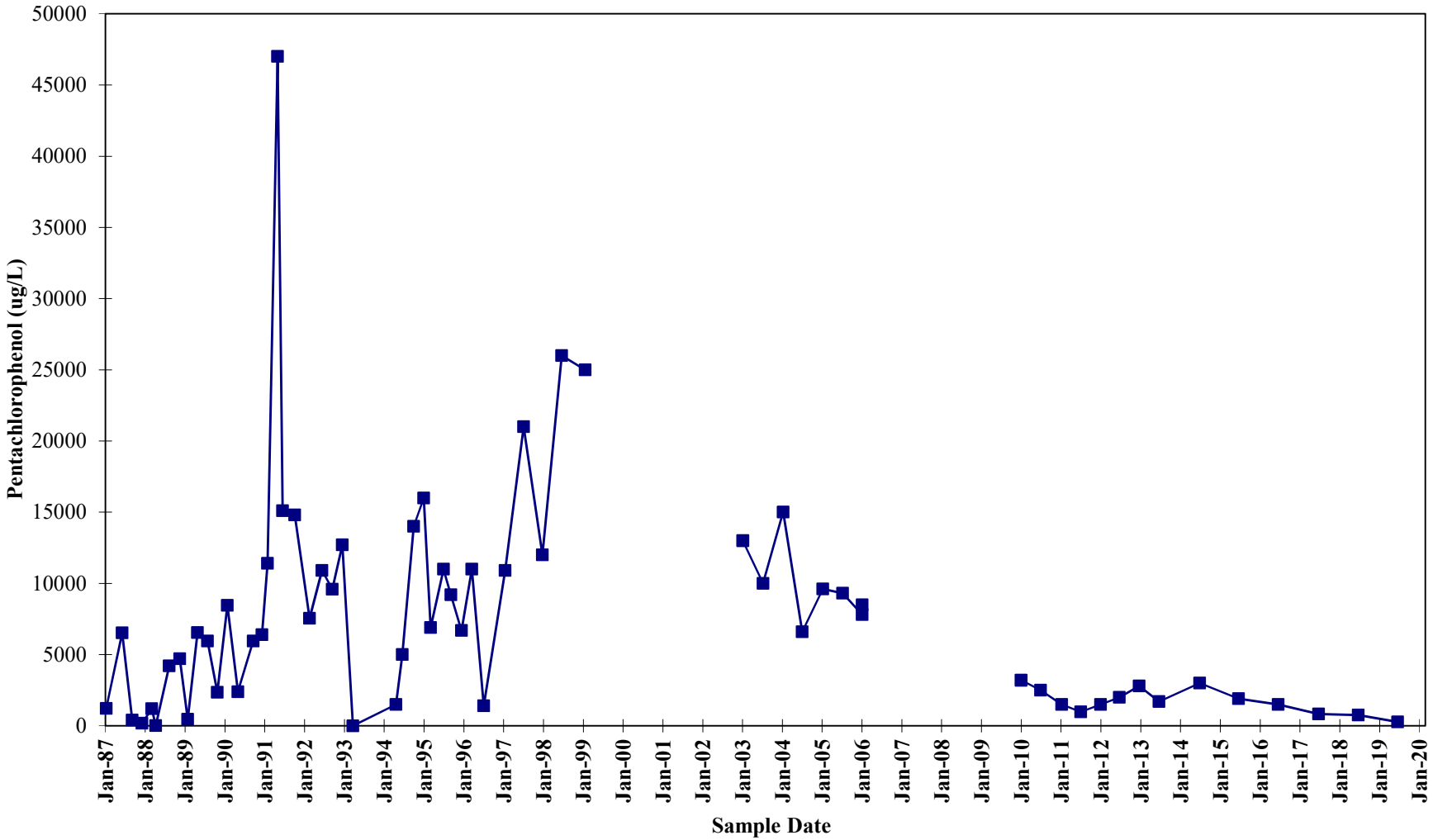

W40
Dissolved Metals (ug/L)
Nitrogen Results (mg/L)
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
10
9
8
7
6
5
4
3
2
1
0
1/1/1987
1/1/1991
1/1/1995
1/1/1999
1/1/2003
1/1/2007
1/1/2011
1/1/2015
1/1/2019
Dissolved Iron
Dissolved Manganese
Nitrate-N
Nitrate + Nitrite-N

Appendix E
Time Concentration Graphs

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W01A

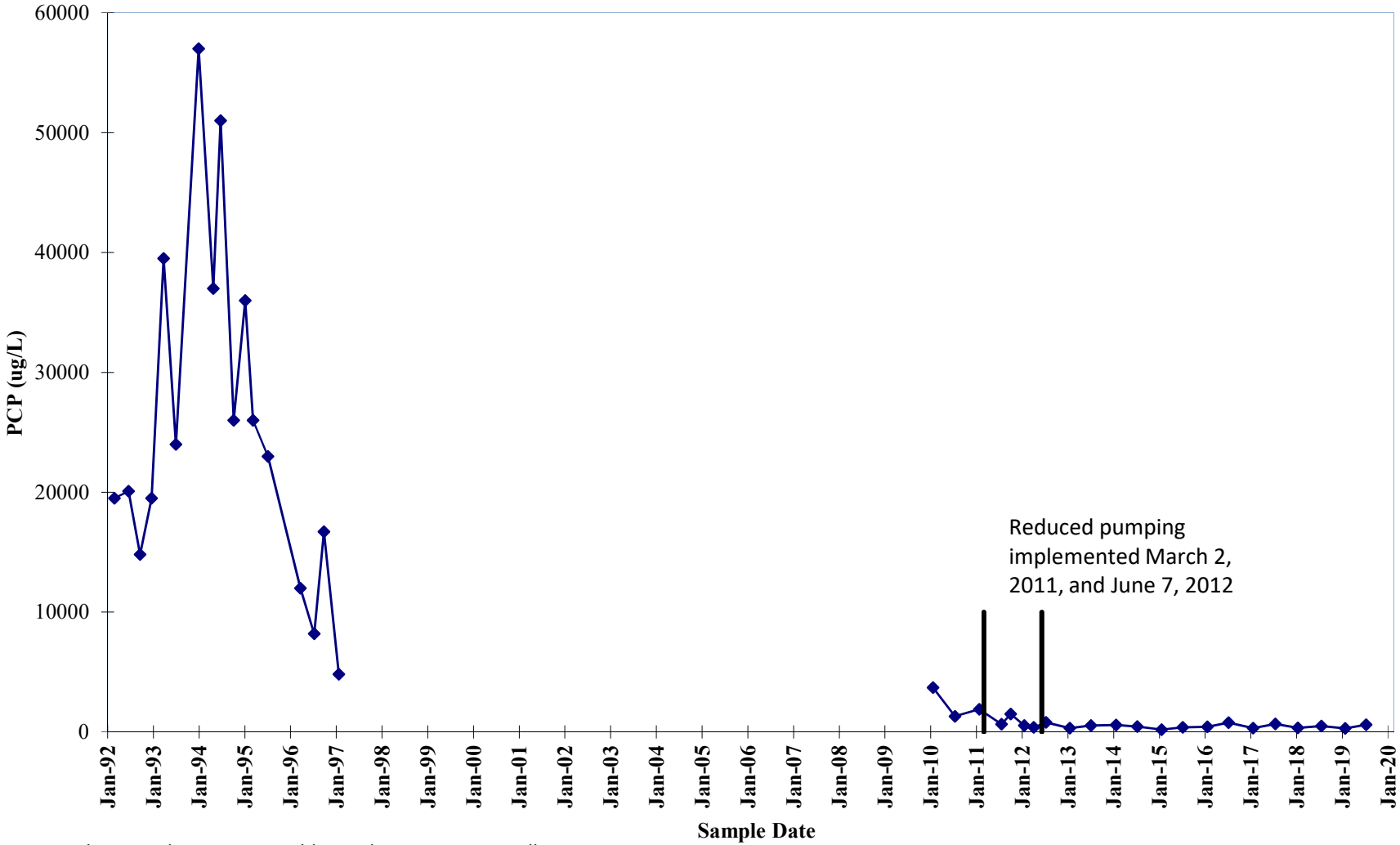


Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W02



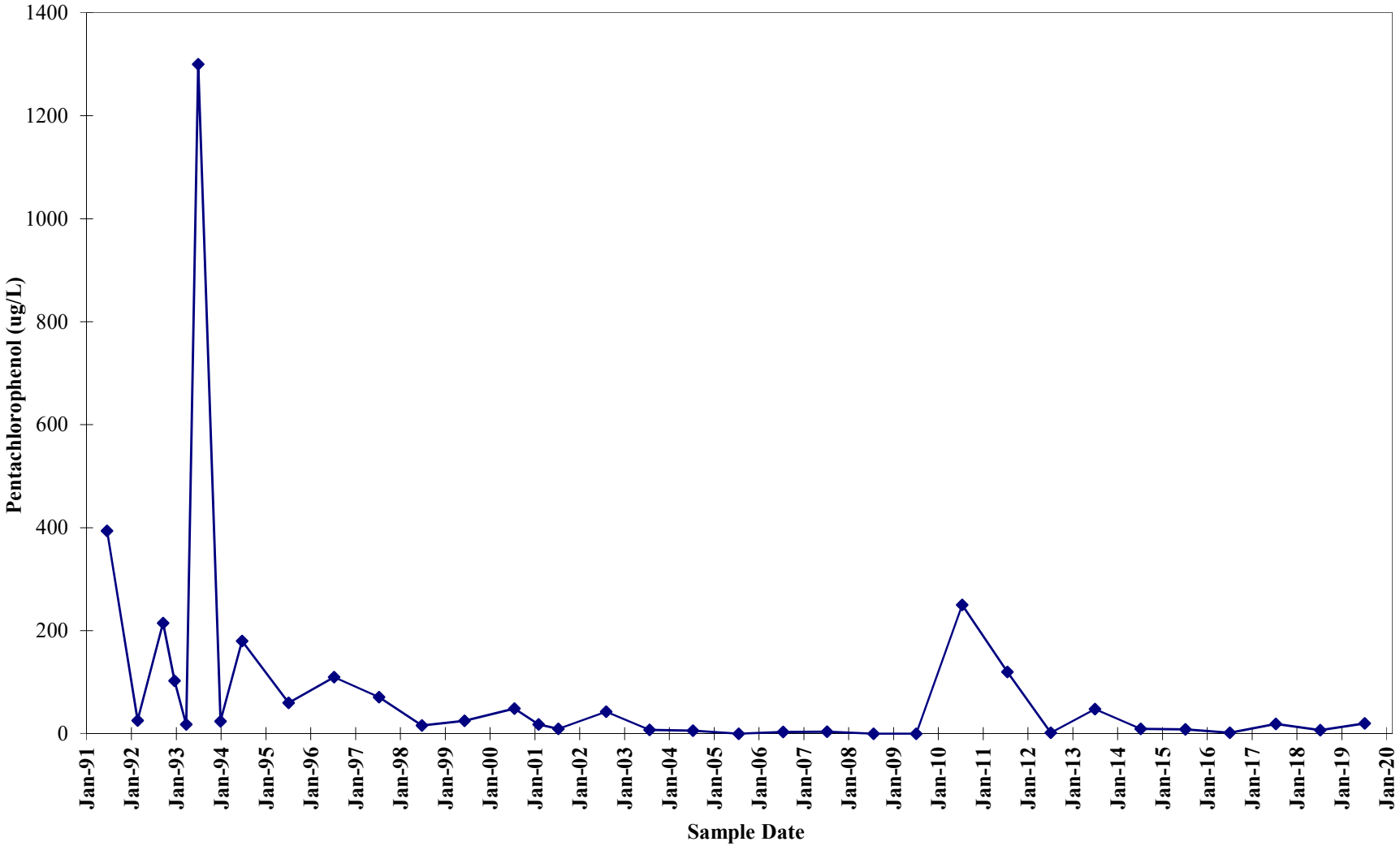
PCP data gap due to measurable product present in well.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W03A

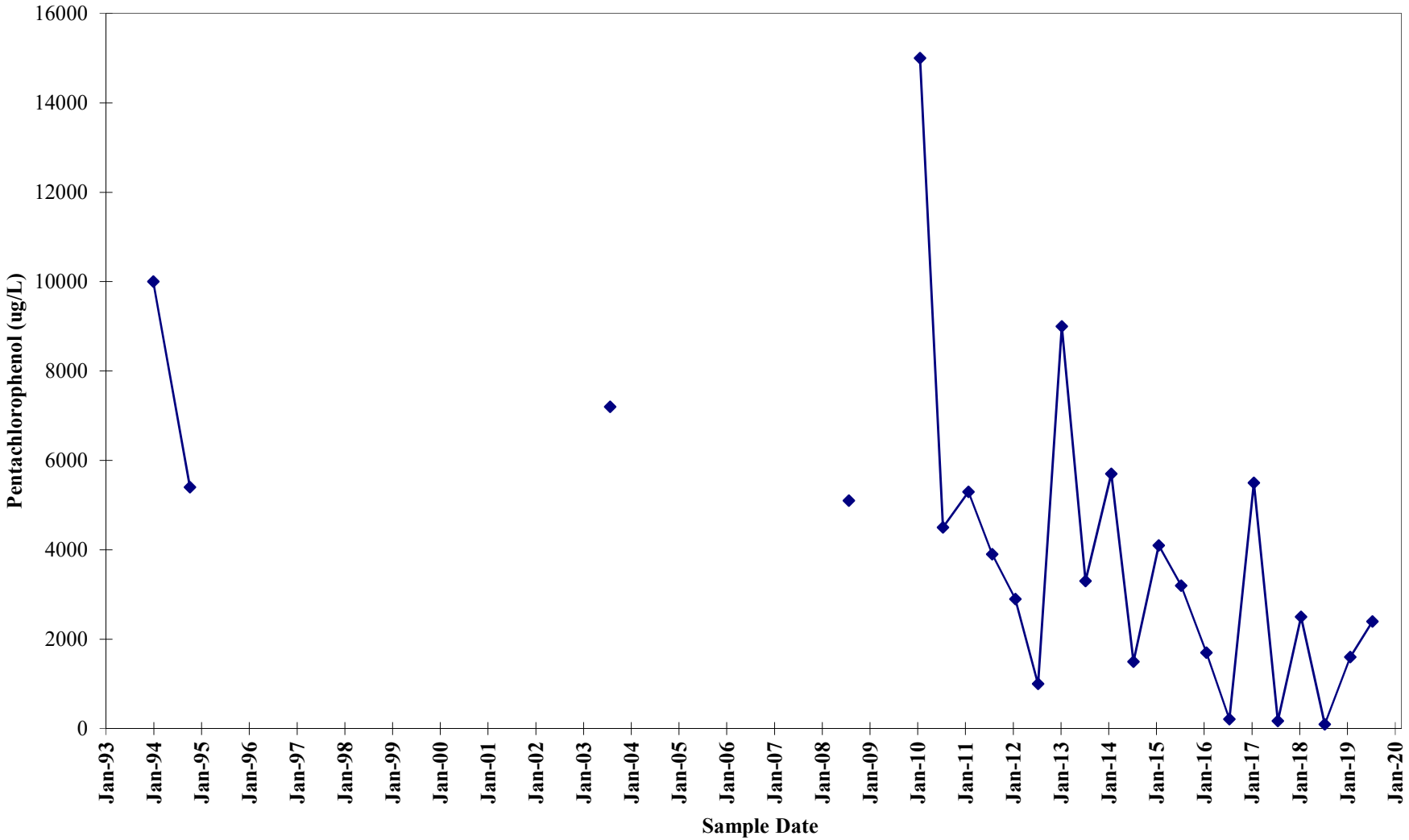


PCP data gap due to measurable product present in well.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W03B

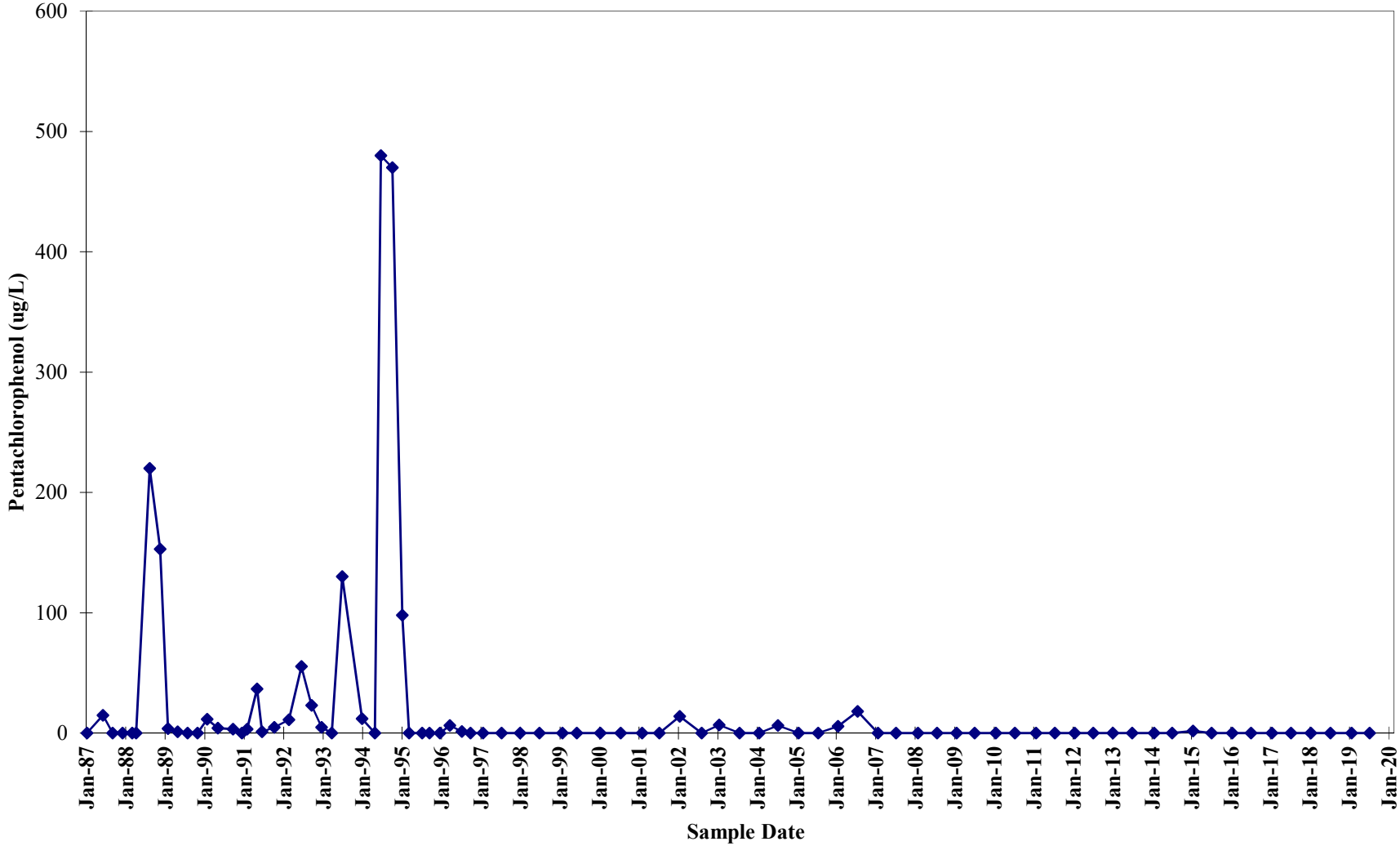


Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W06R

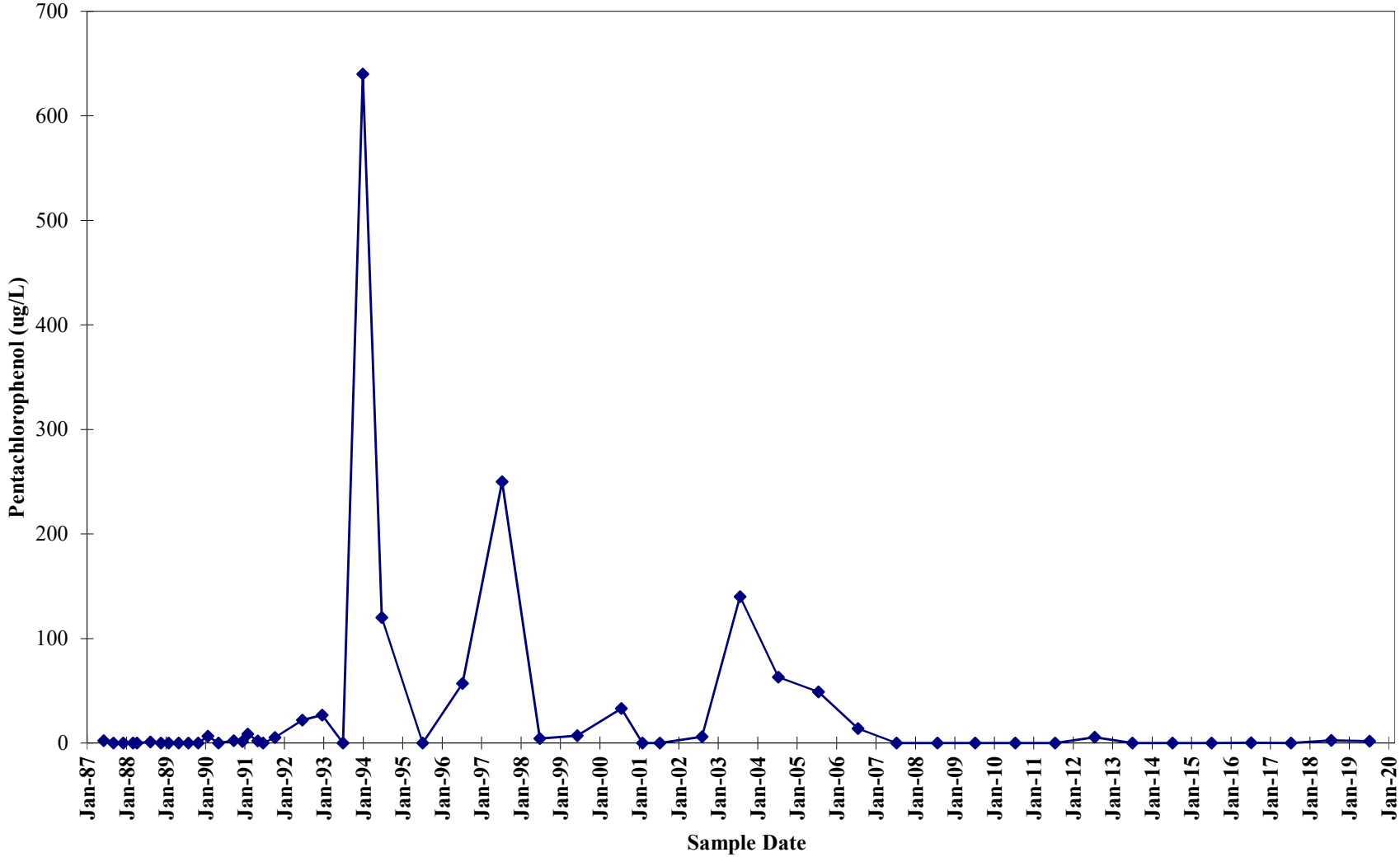


PCP data gap due to measurable product present in well.

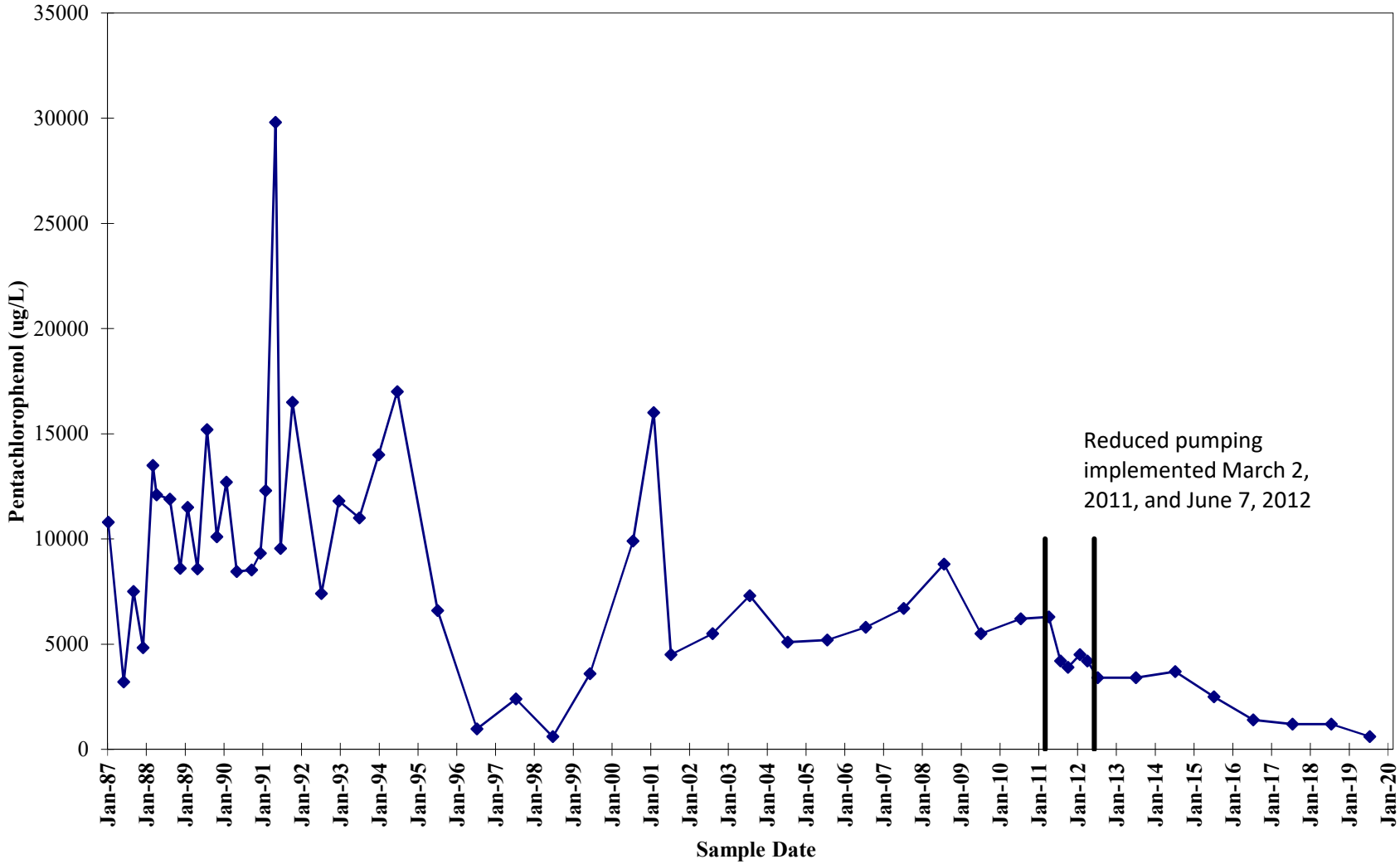
Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W08



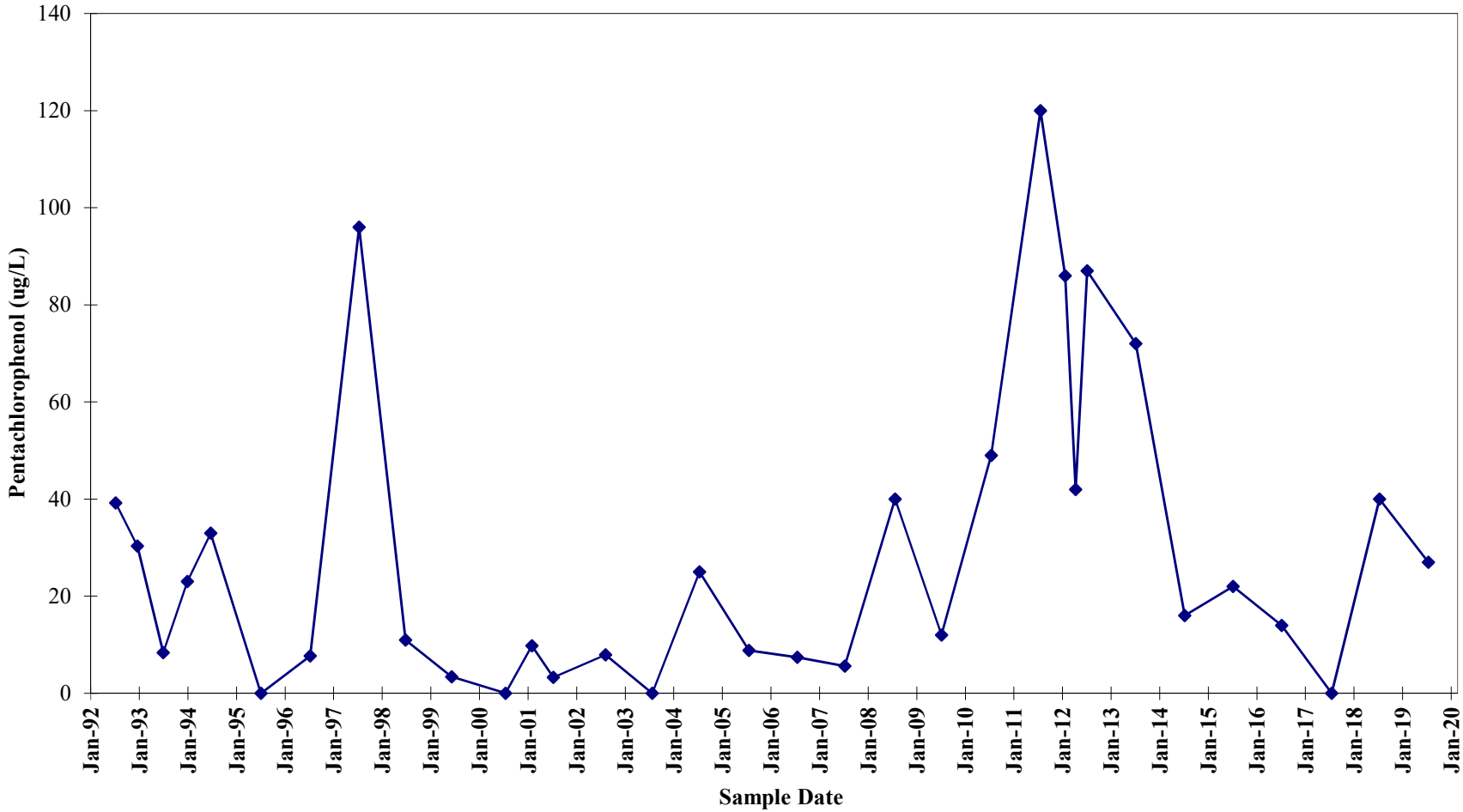
Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W09



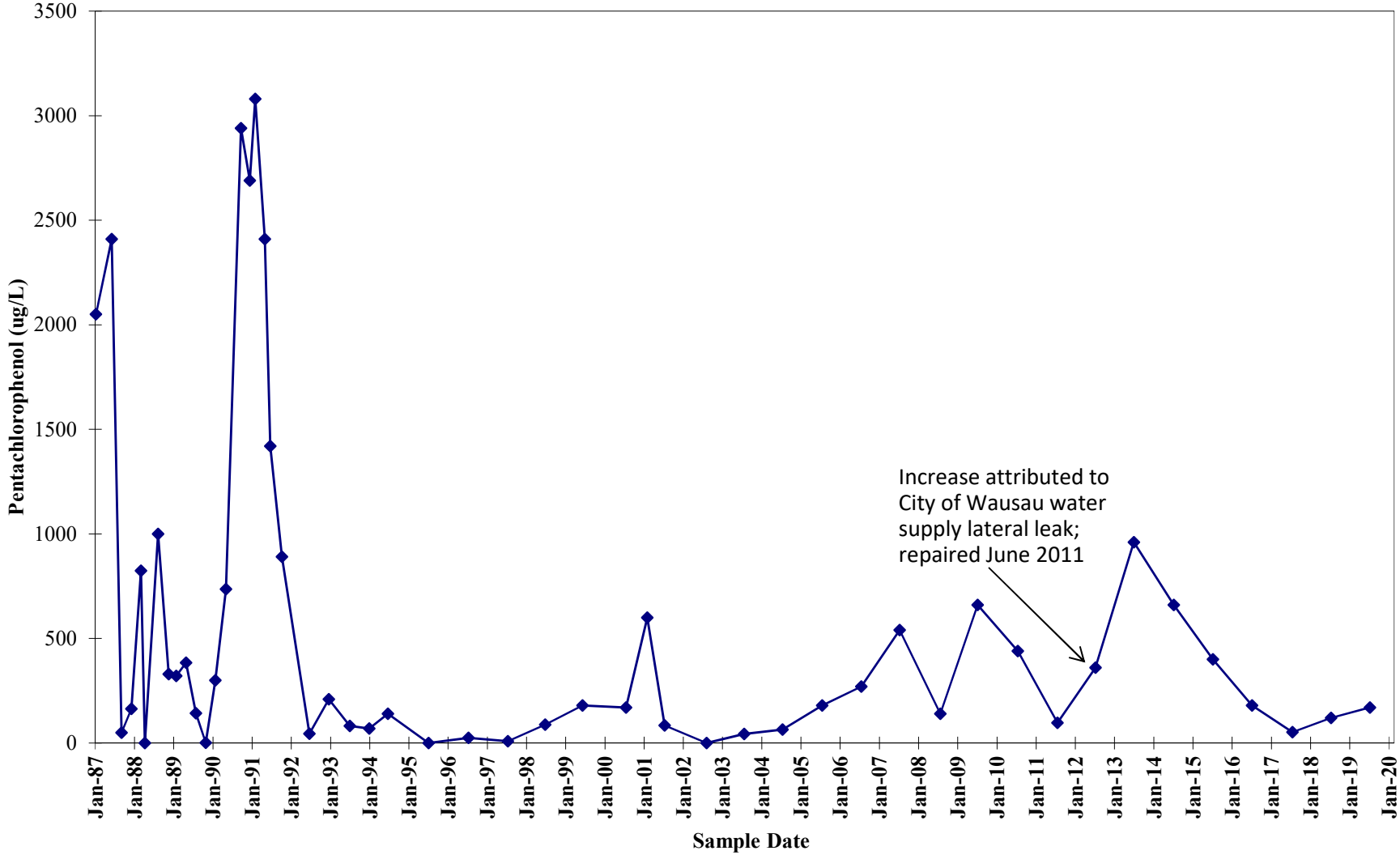
Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W10A



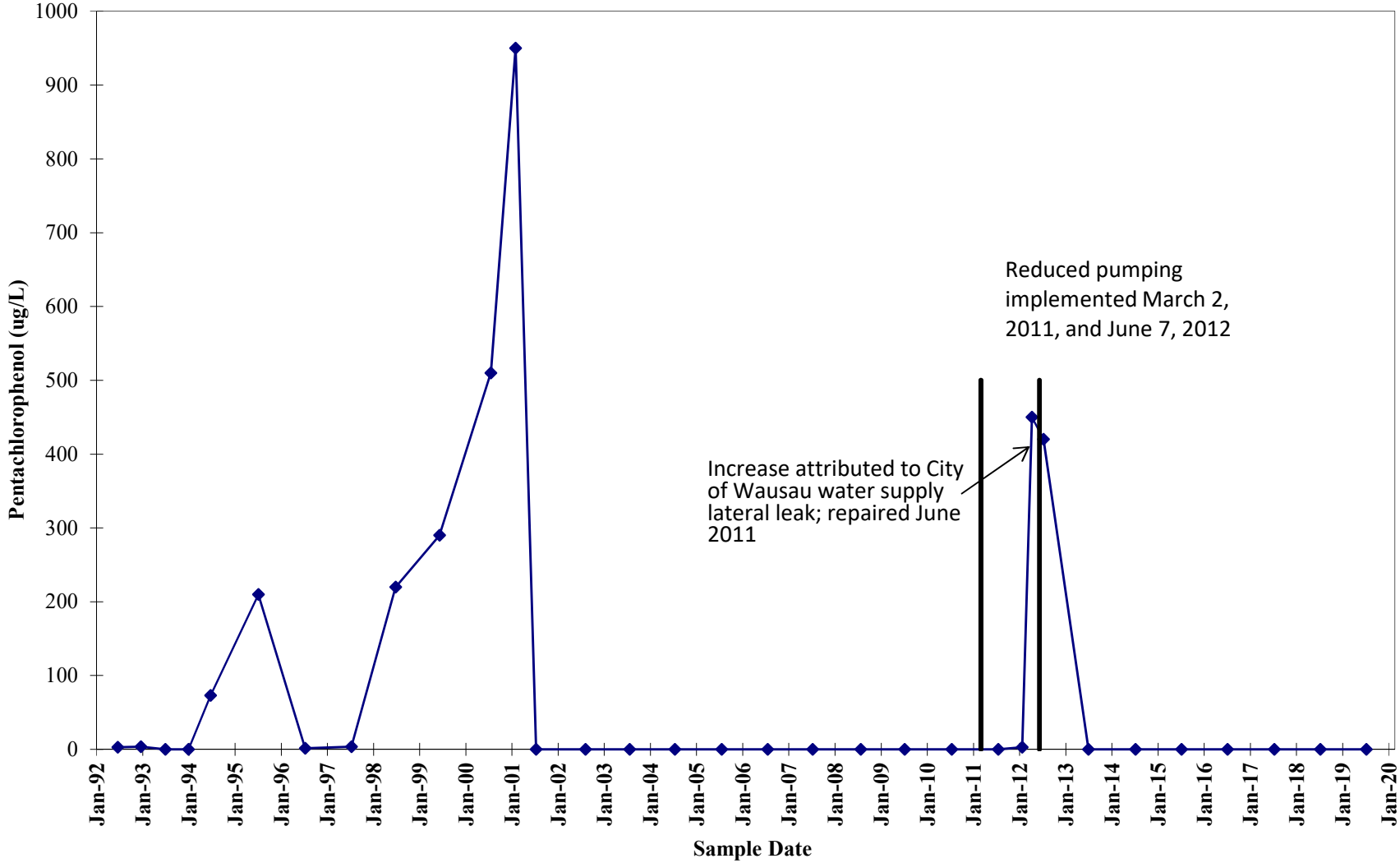
Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W10B



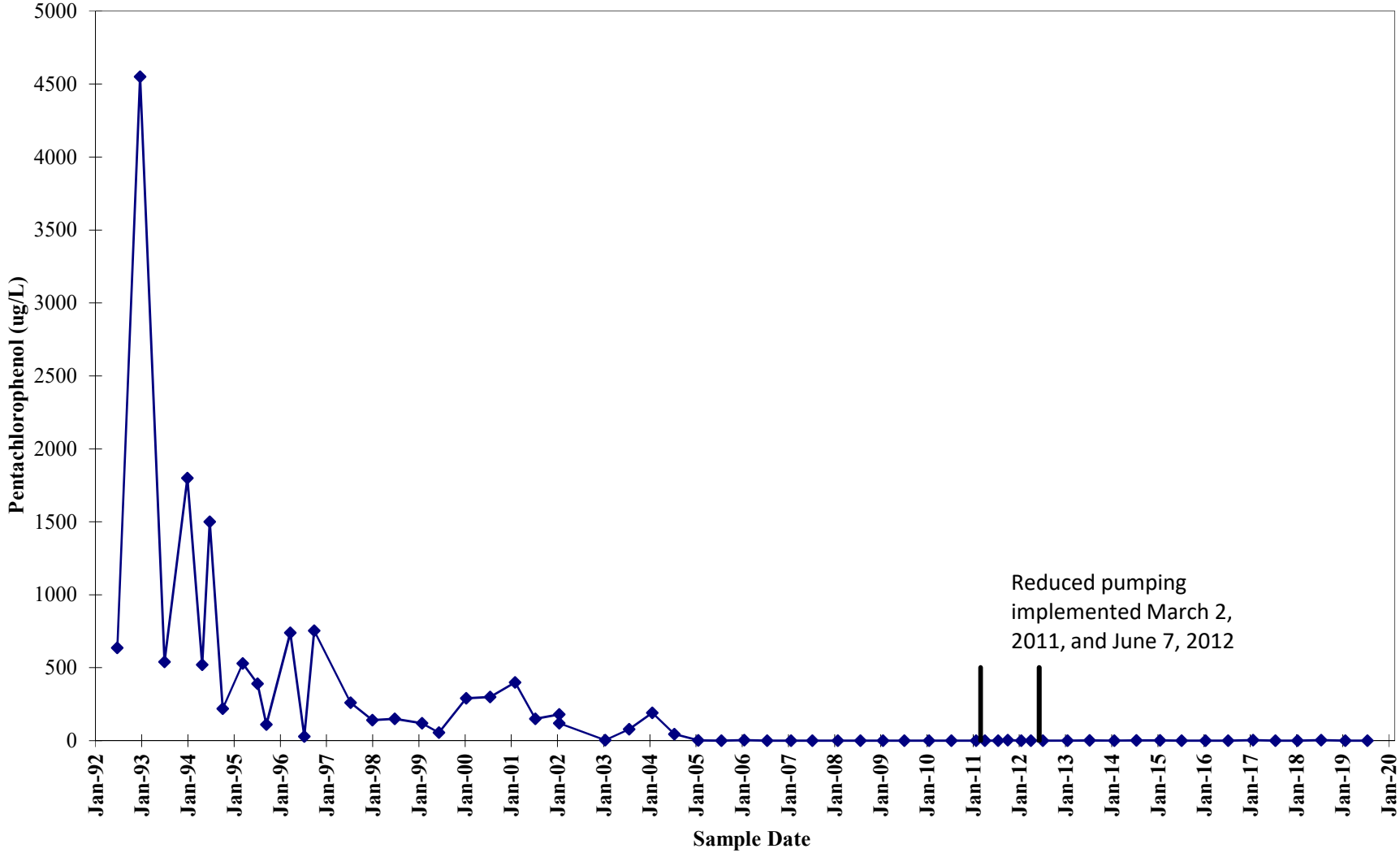
Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W11



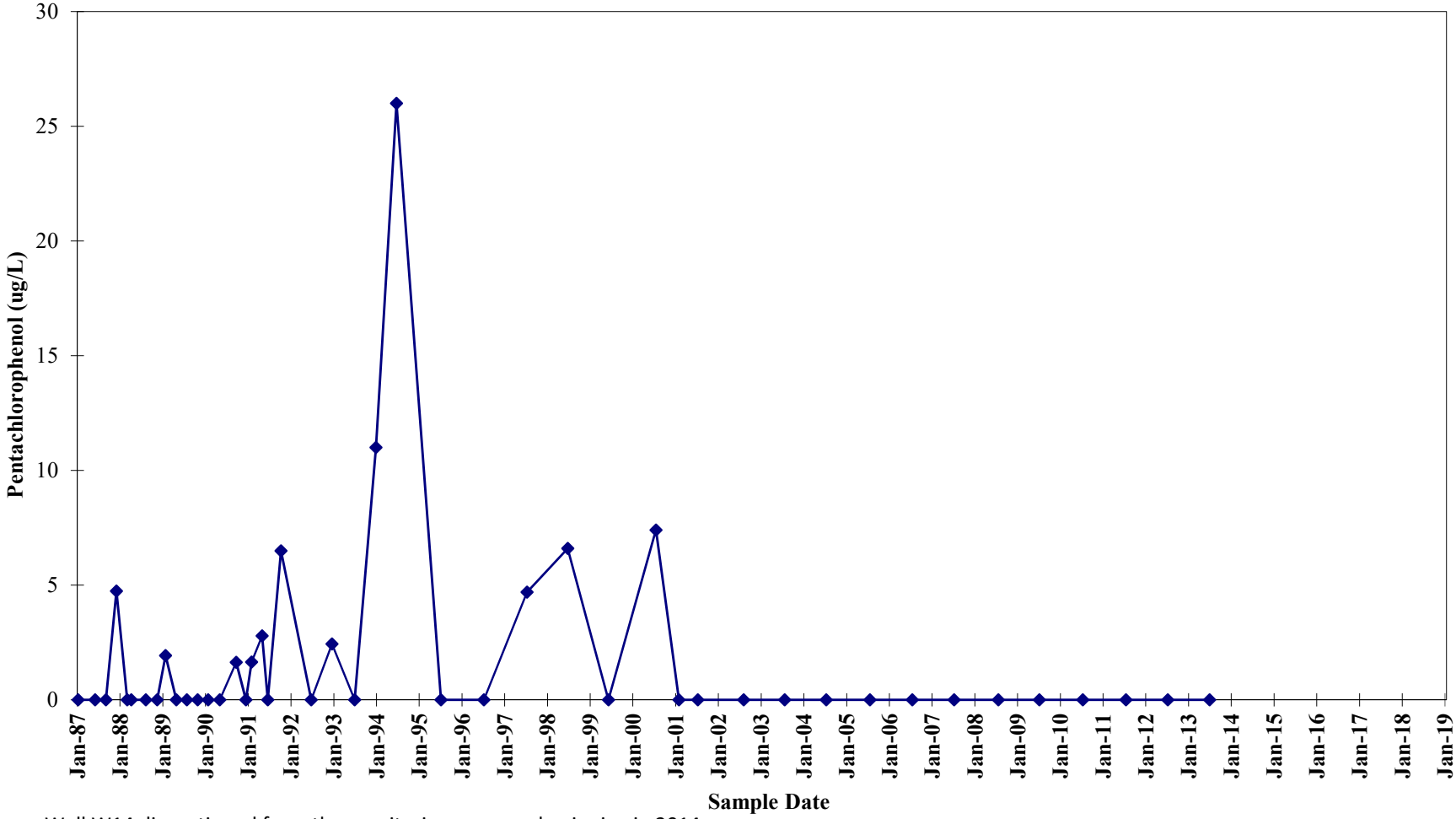
Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W12



Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W13

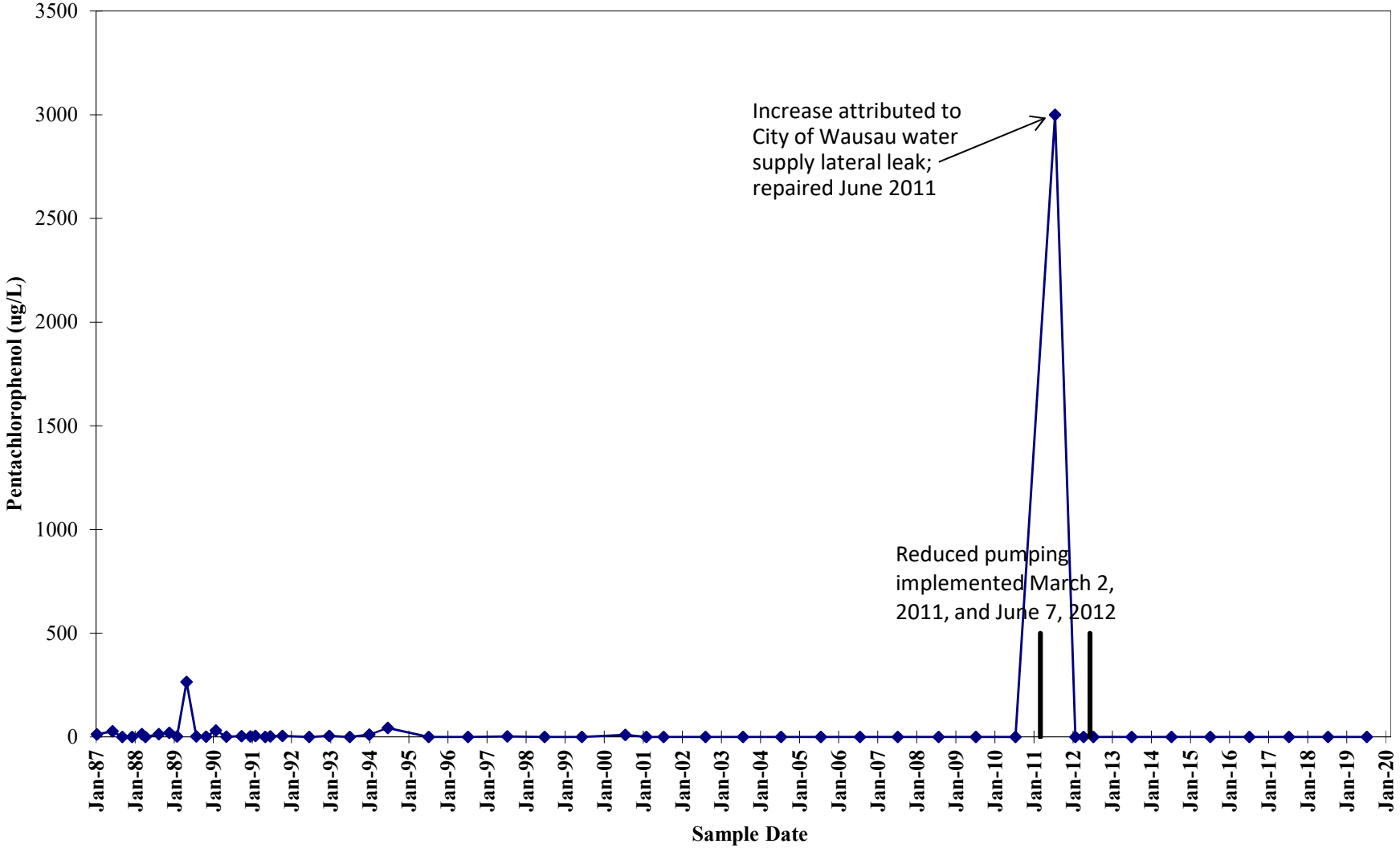


Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W14

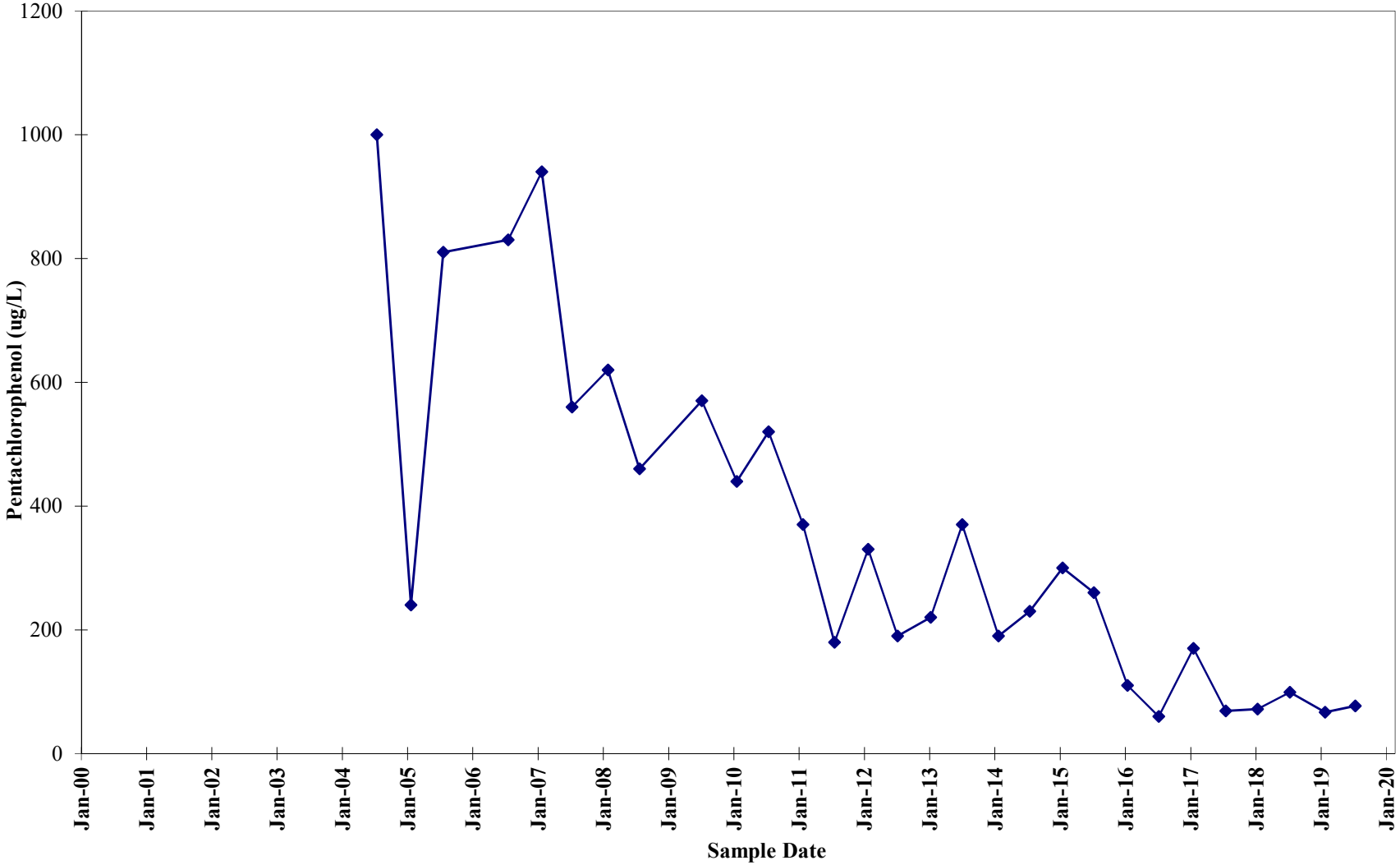


Well W14 discontinued from the monitoring program beginning in 2014.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W16

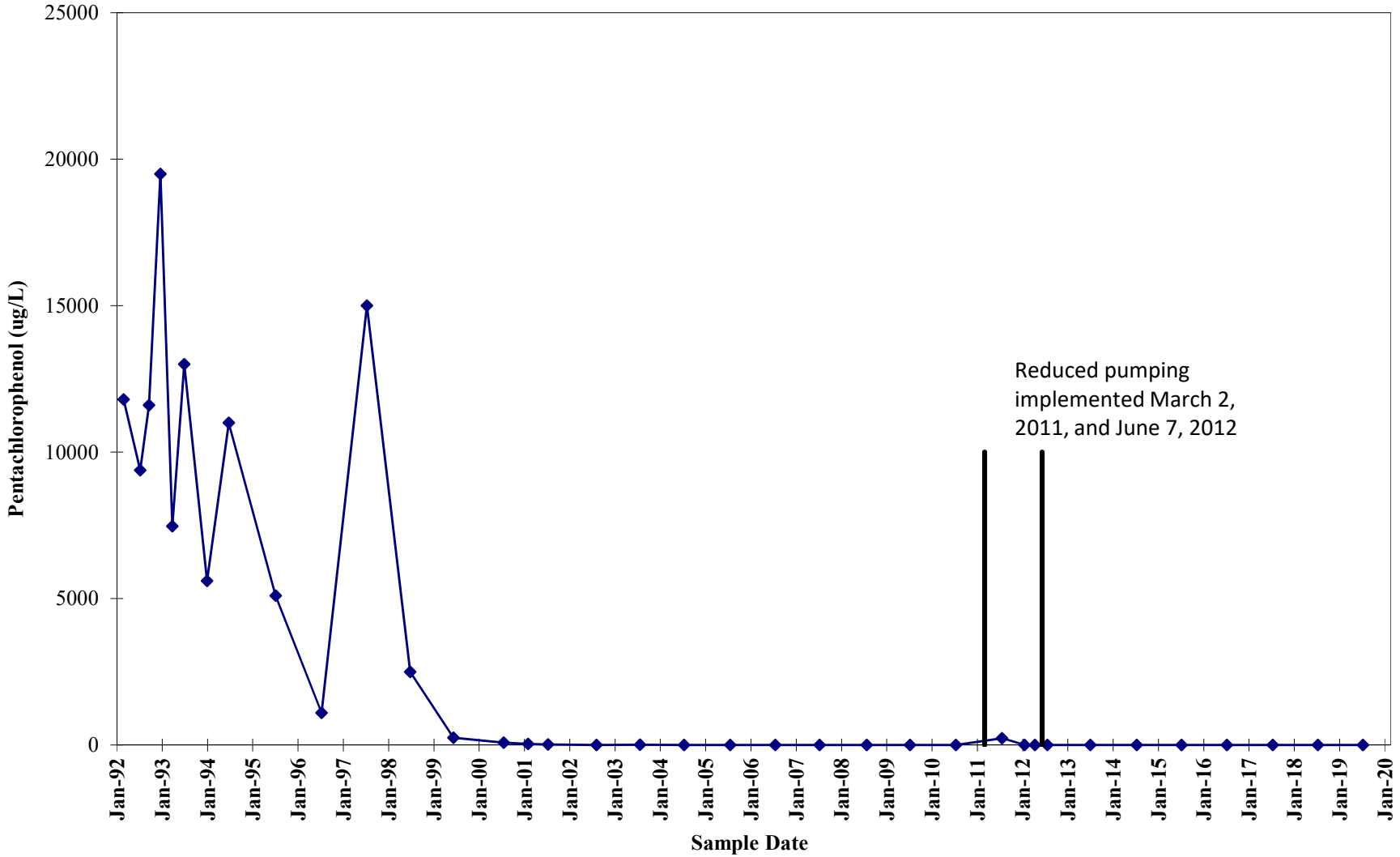


Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W17

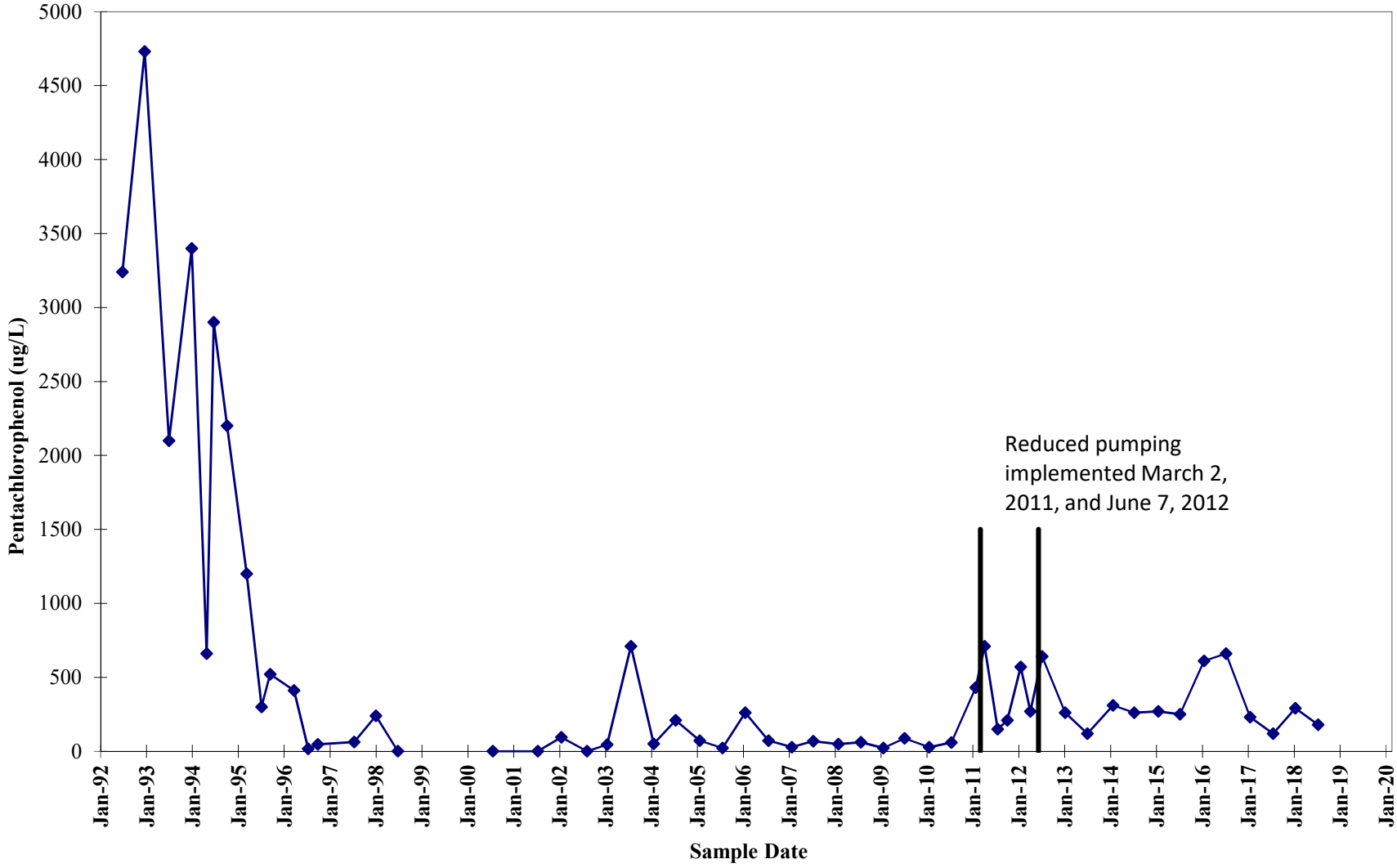


PCP data gap due to measurable product in well.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W18

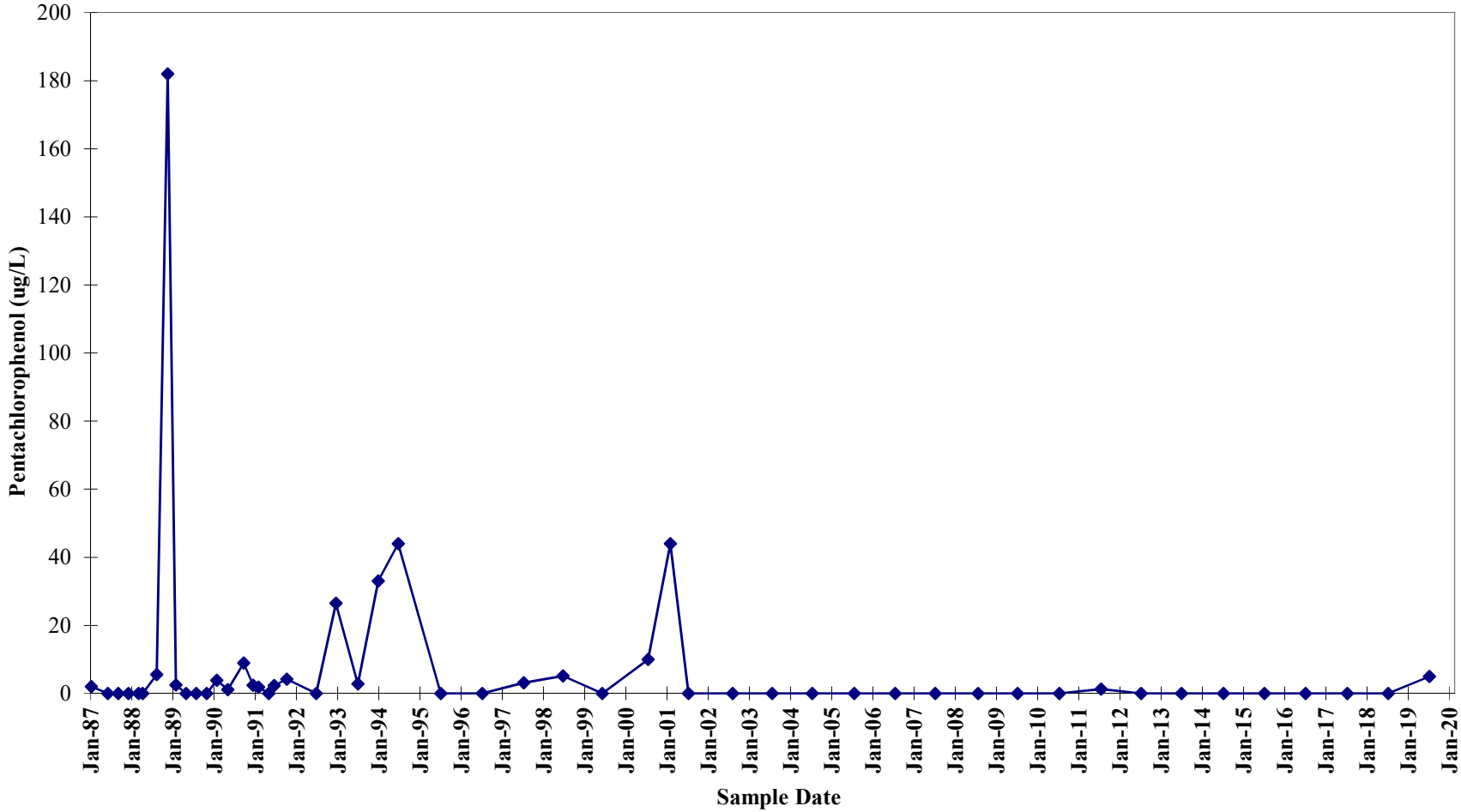


Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W19

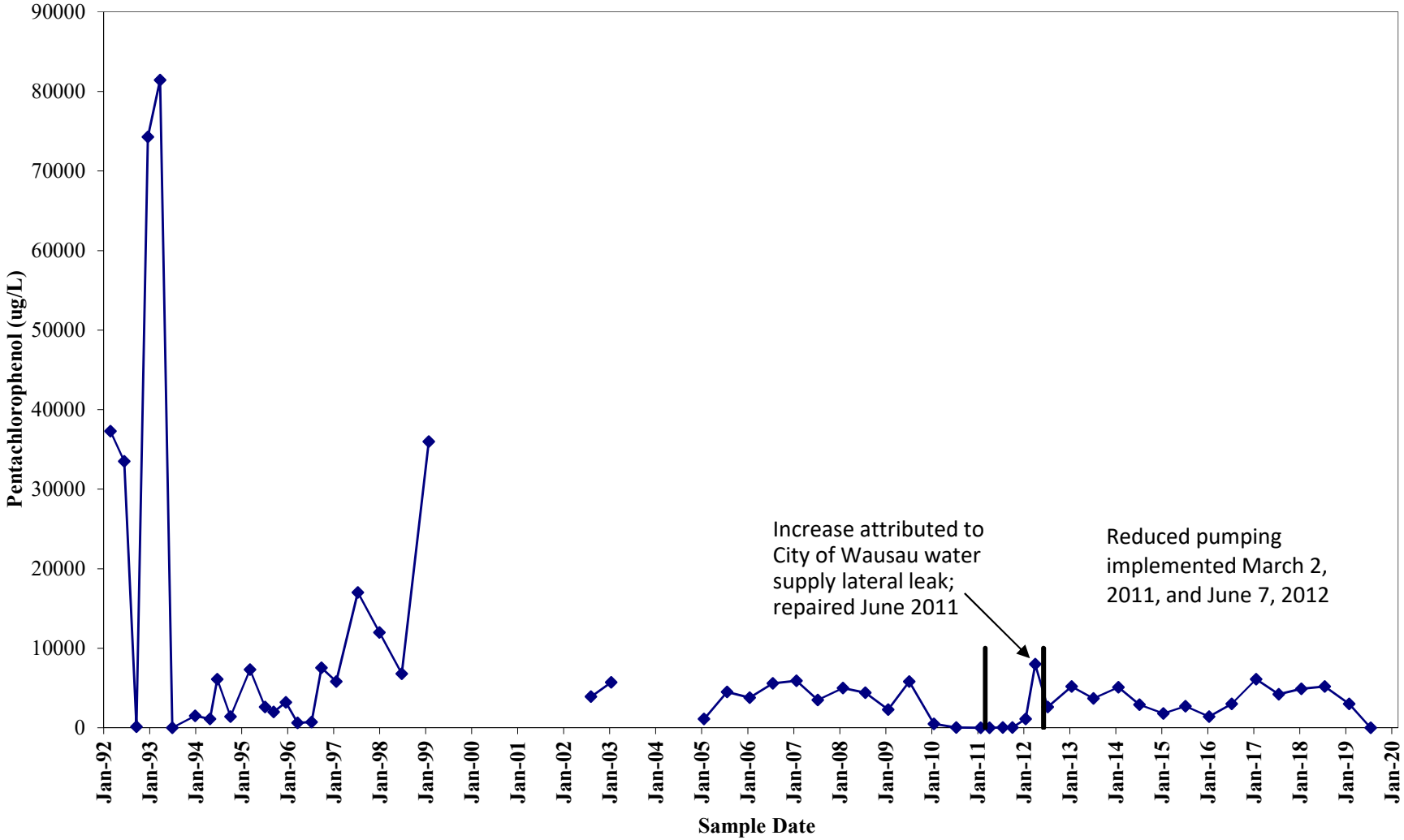


Well W19 was abandoned on March 28, 2019 to facilitate the Thomas Street reconstruction.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W21

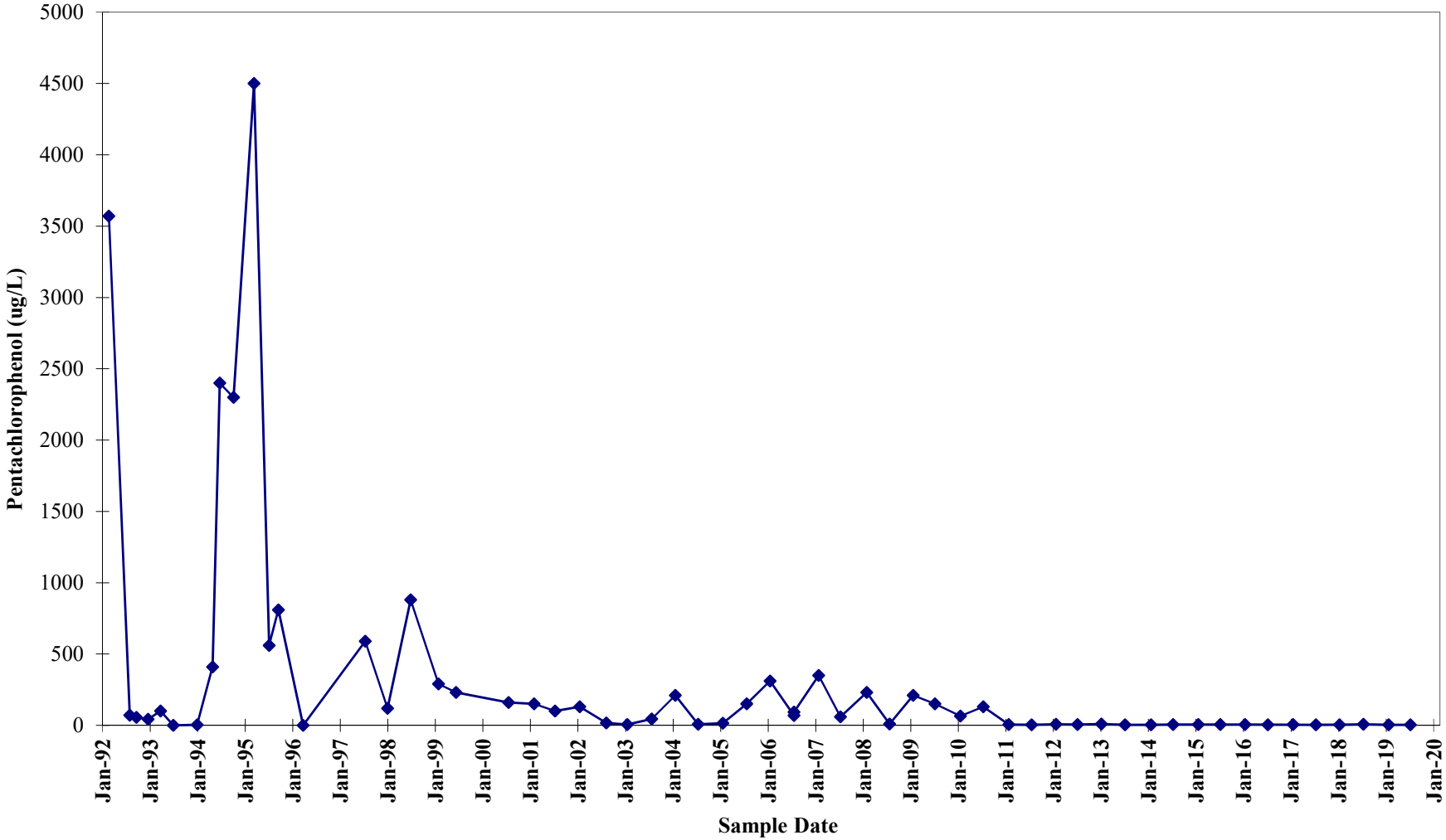


Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W22

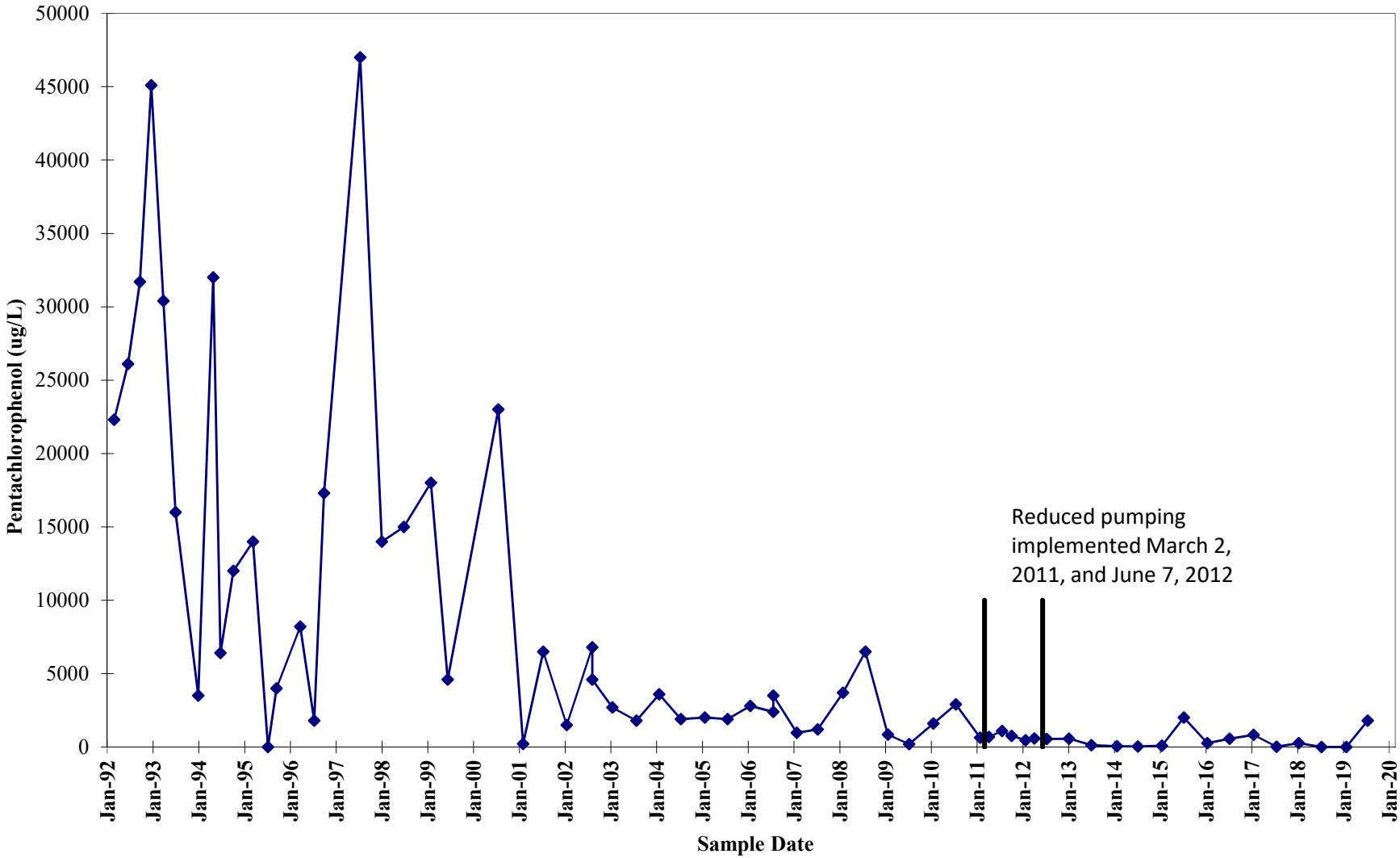


PCP data gap due to measurable product present in well.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W25

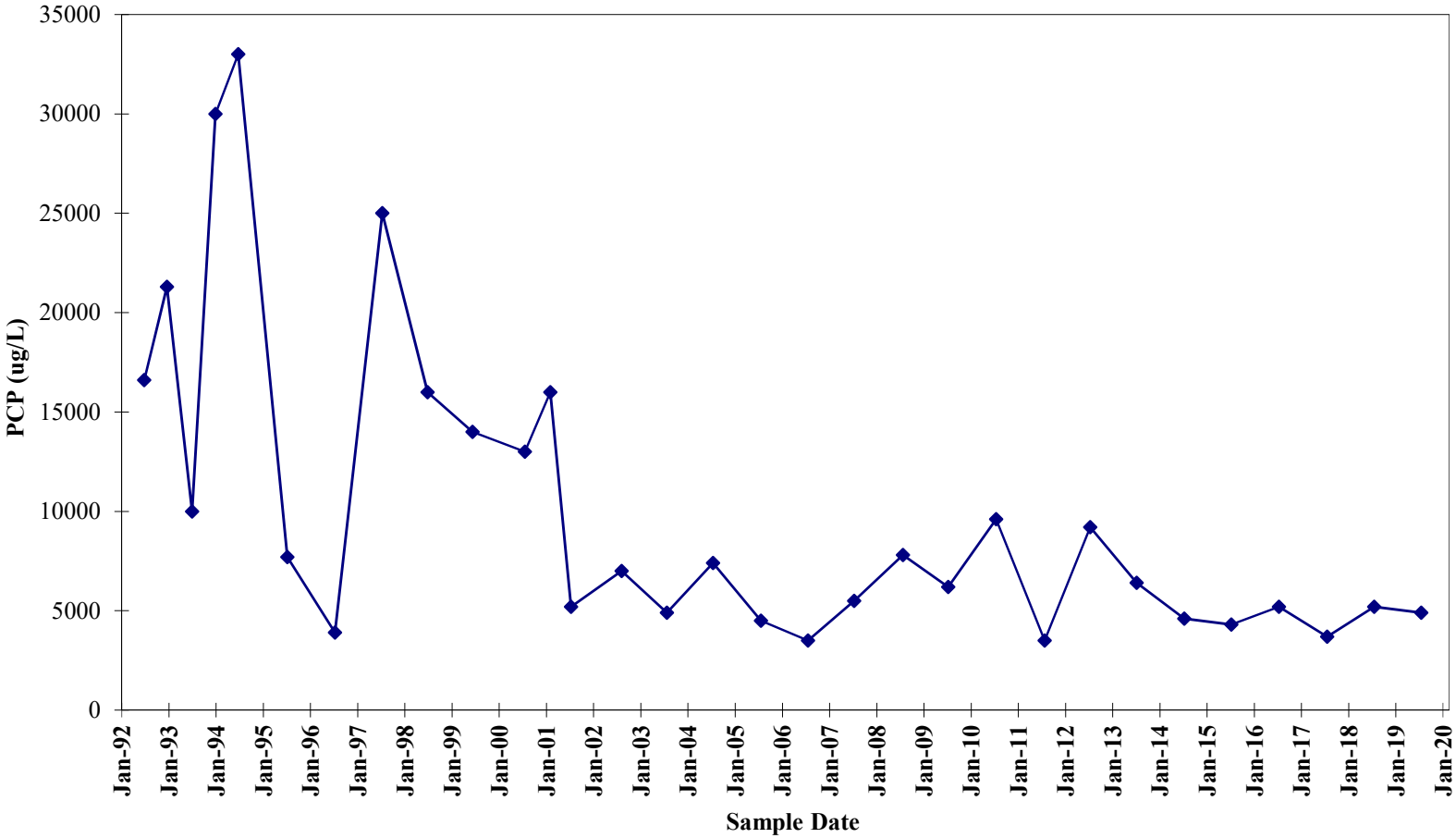


Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W26/W26R

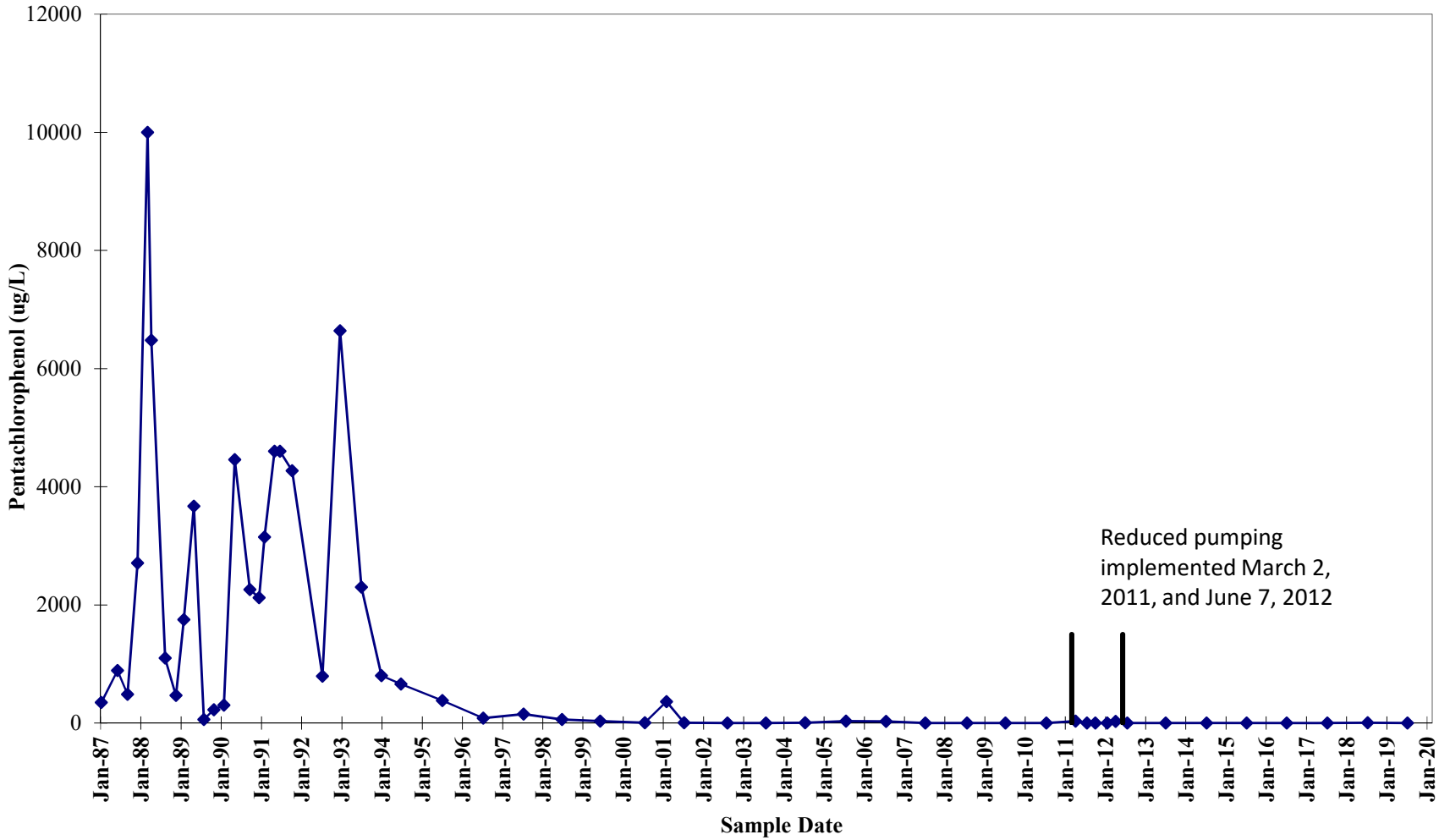


Well W26 was abandoned on March 28, 2019 to facilitate the Thomas Street reconstruction.
Replacement well W26R was installed on June 24, 2019.

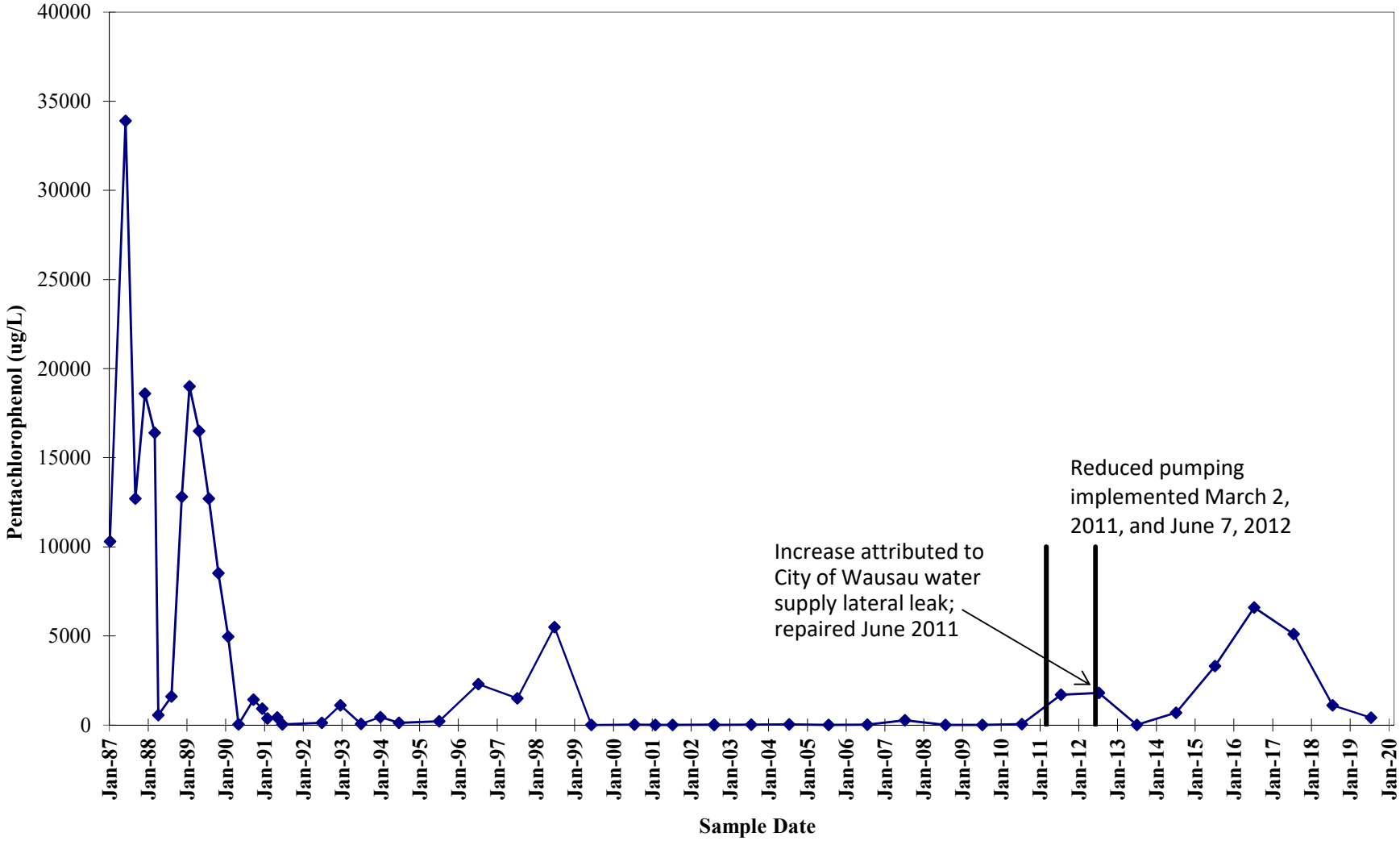
Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W27



Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W28

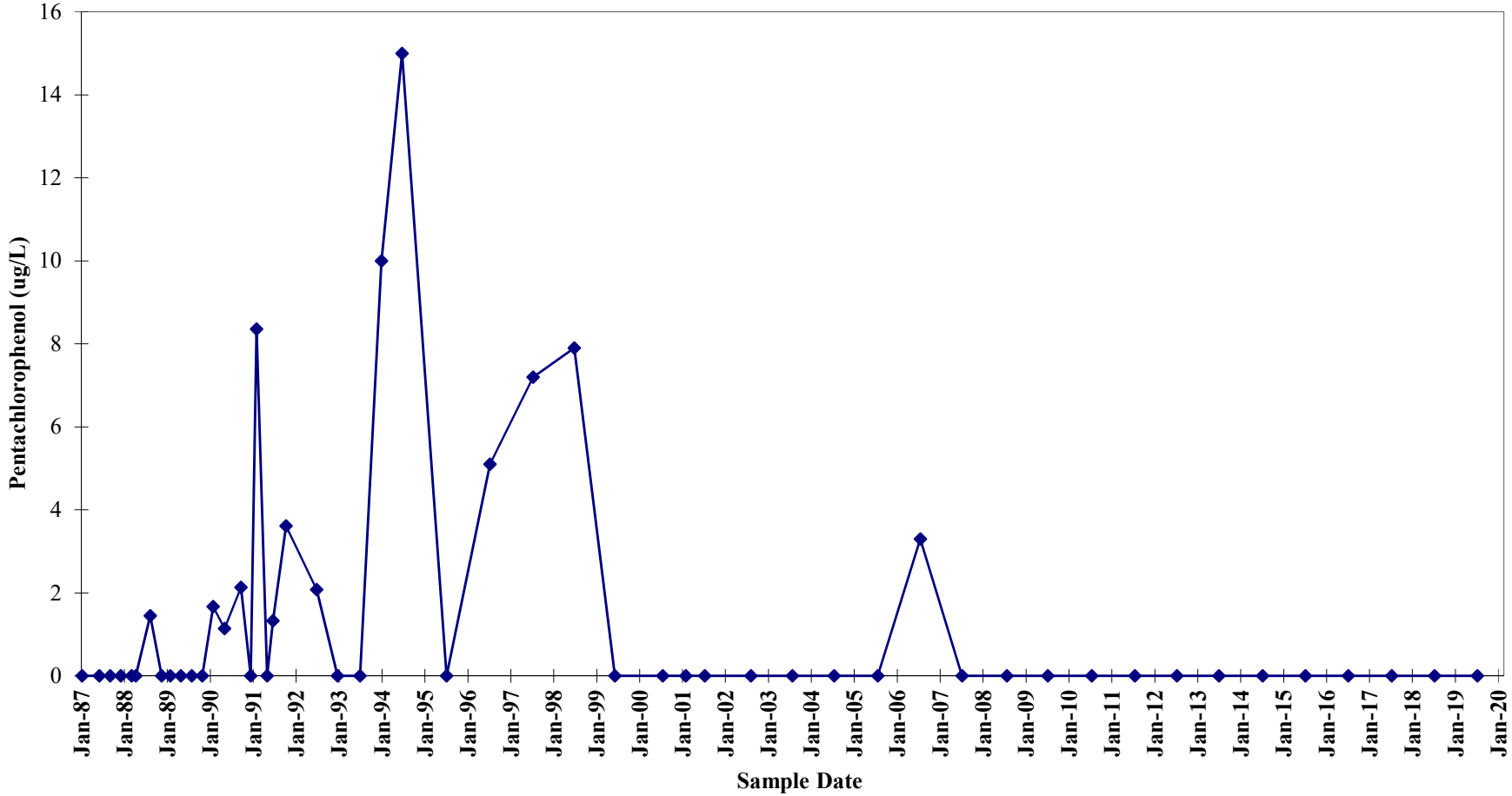


Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W29/W29R

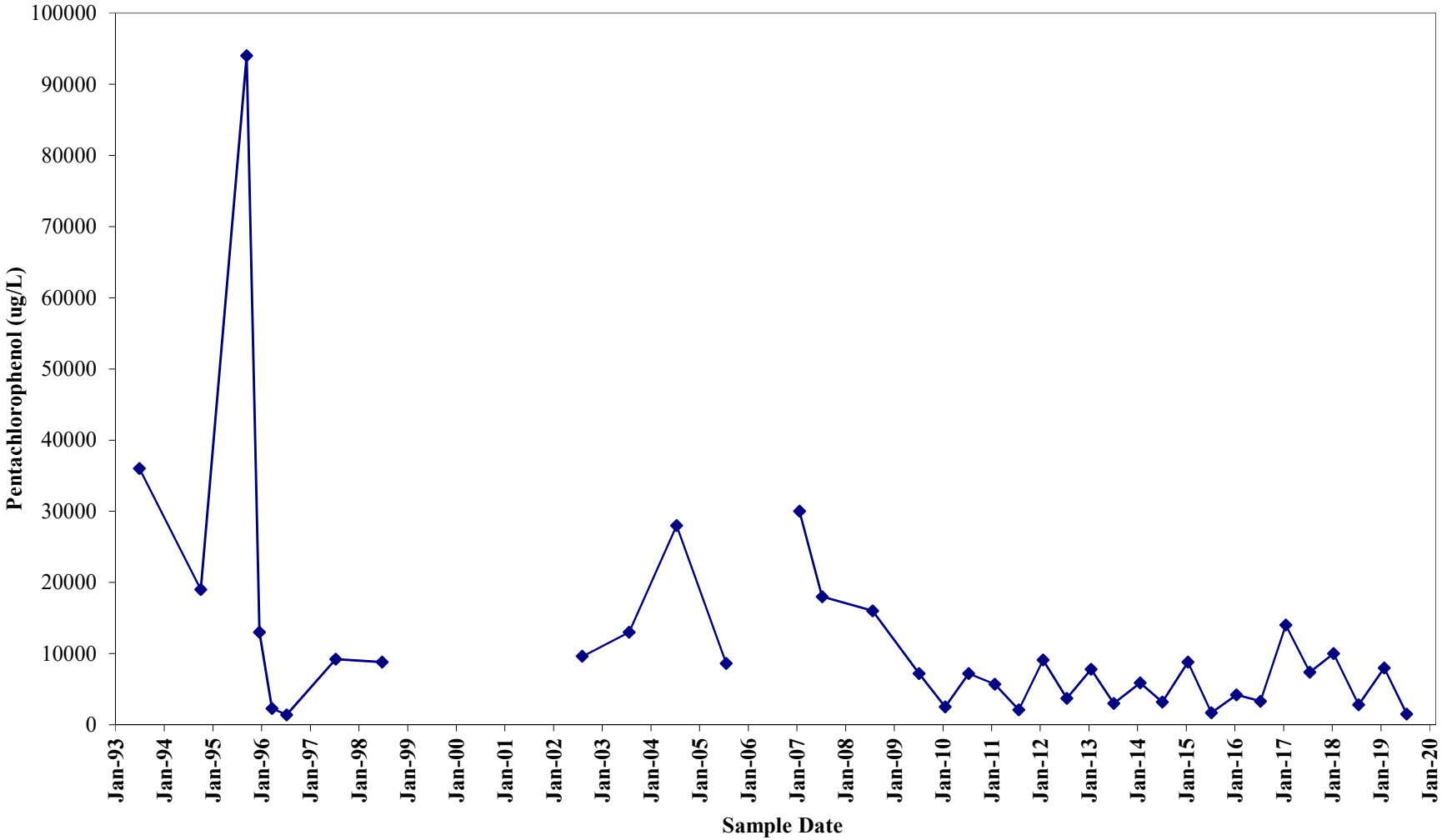


Well W29 was abandoned on March 28, 2019 to facilitate the Thomas Street reconstruction.
Replacement well W29R was installed on June 24, 2019.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W32

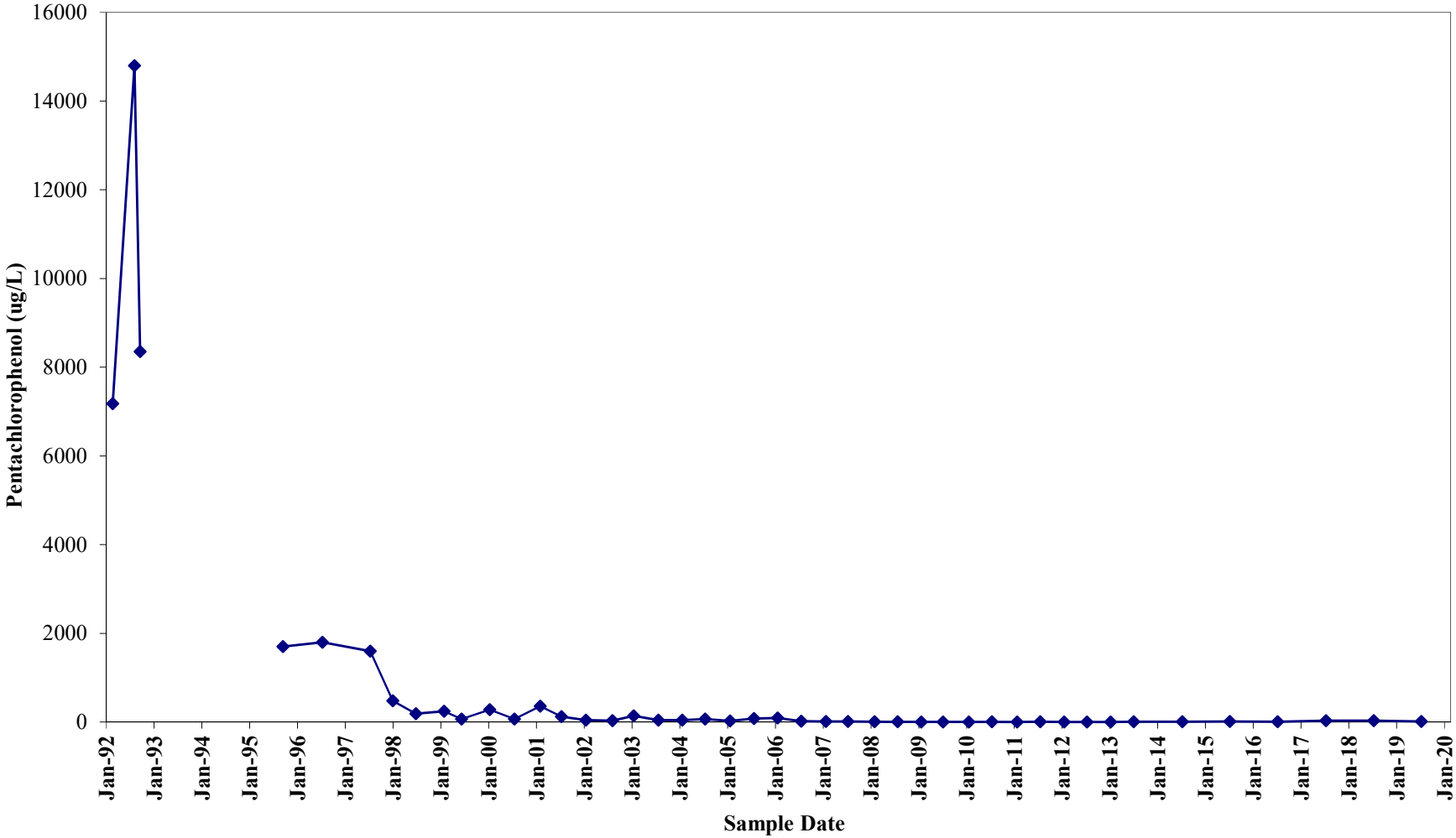


Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W33



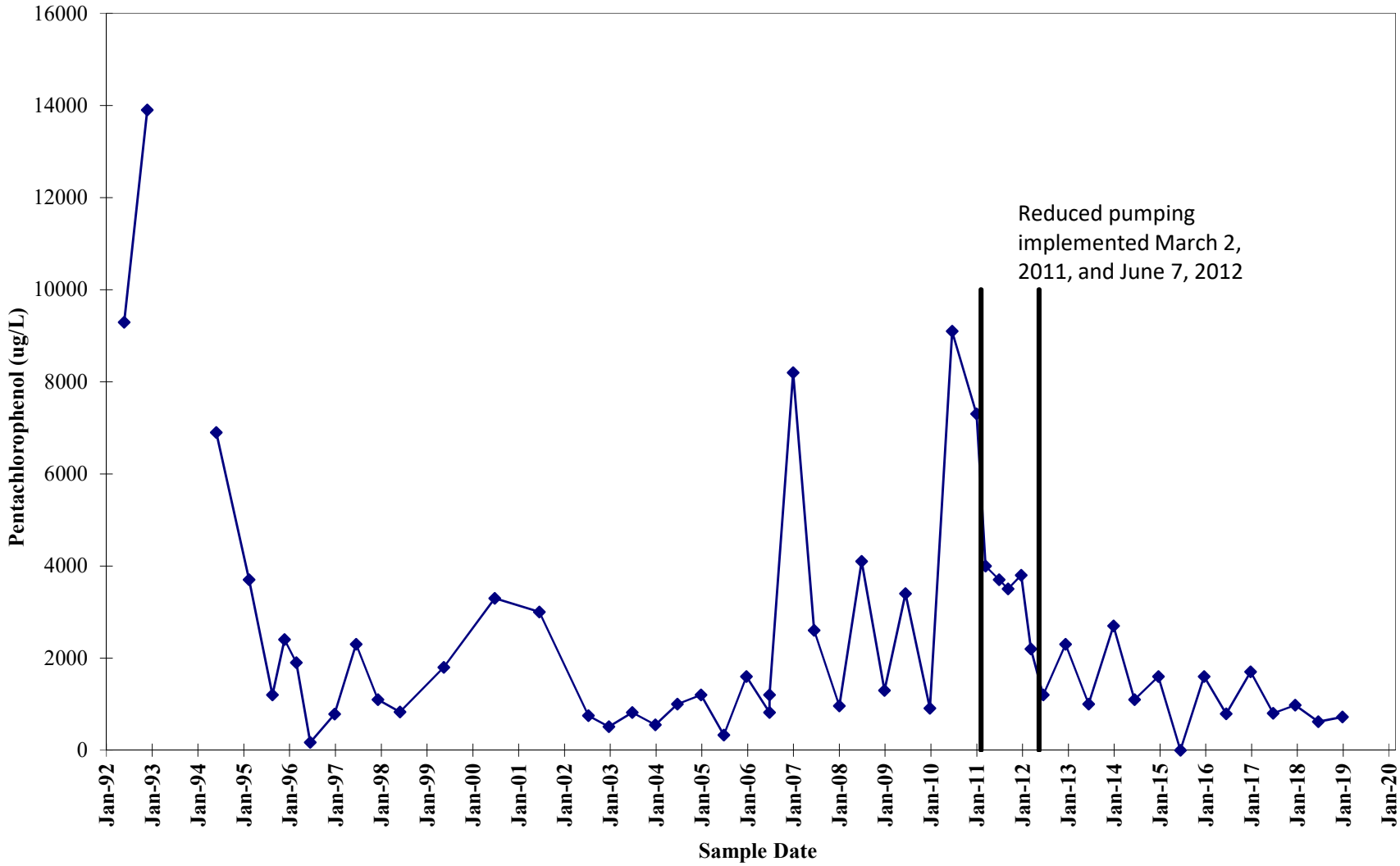
PCP data gap due to measurable product present in well.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W36



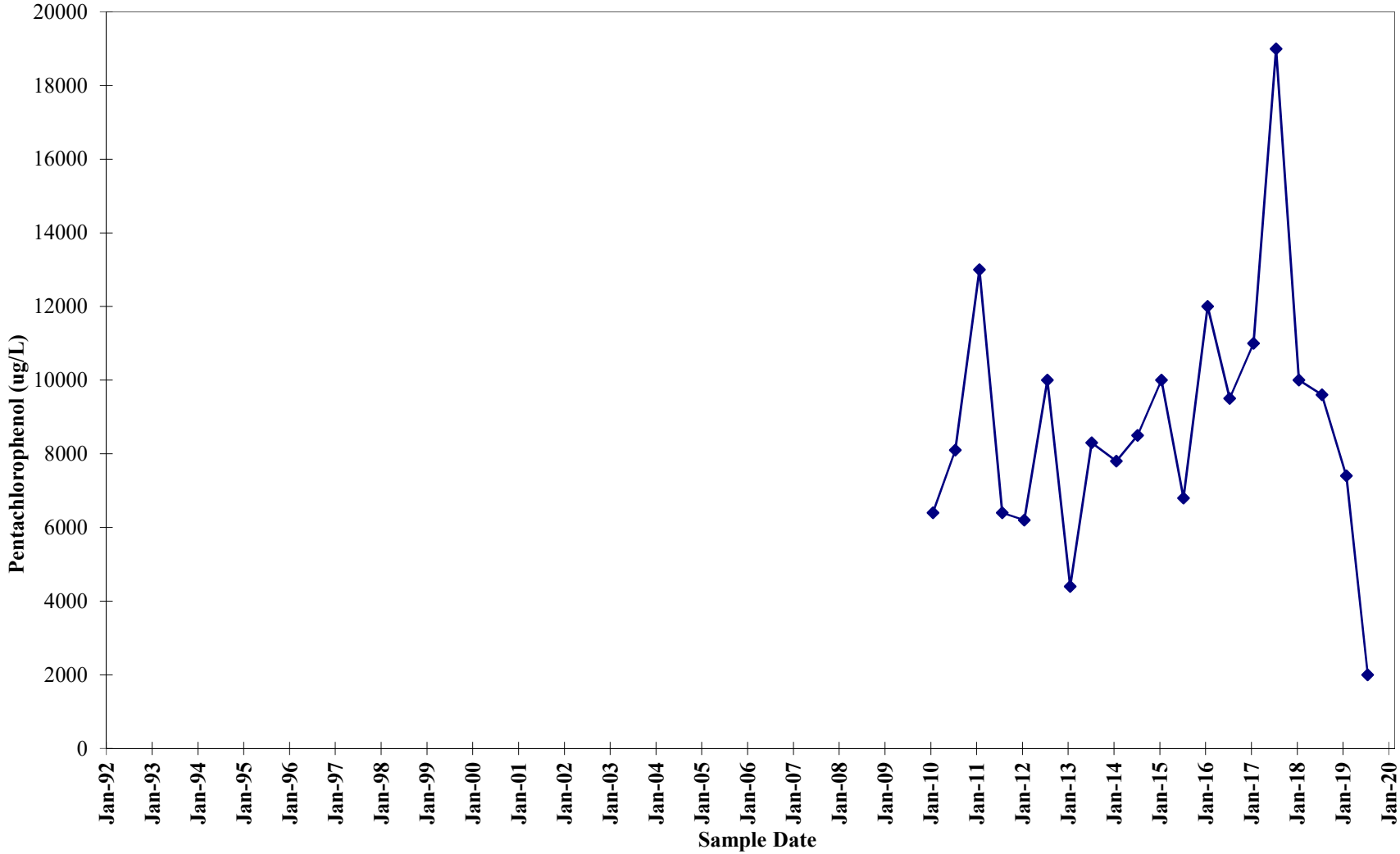
PCP data gap due to measurable product present in well.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W39



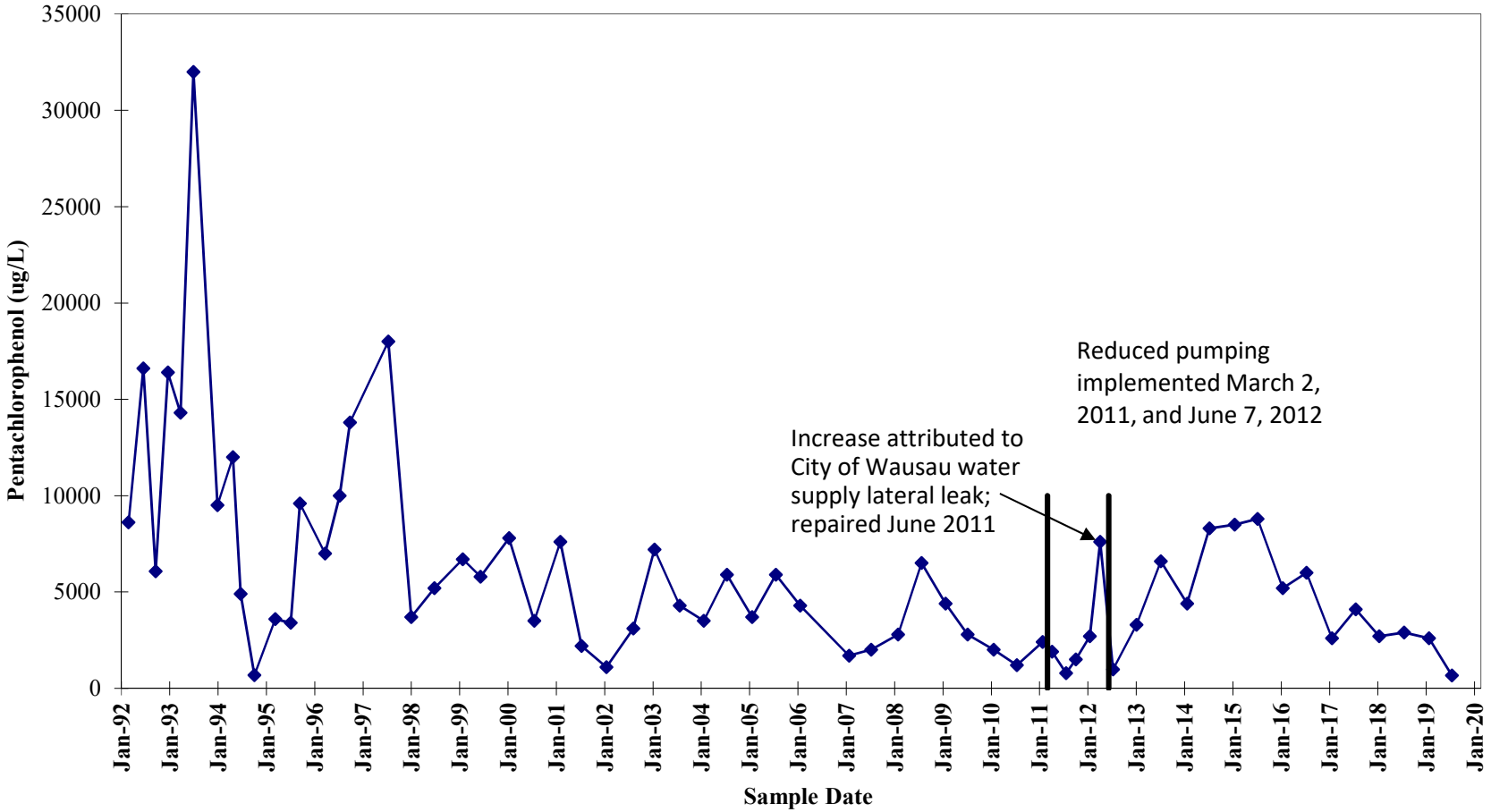
PCP data gap due to measurable product present in well.
Well W39 was abandoned on March 28, 2019 to facilitate the Thomas Street reconstruction.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W40/W40R

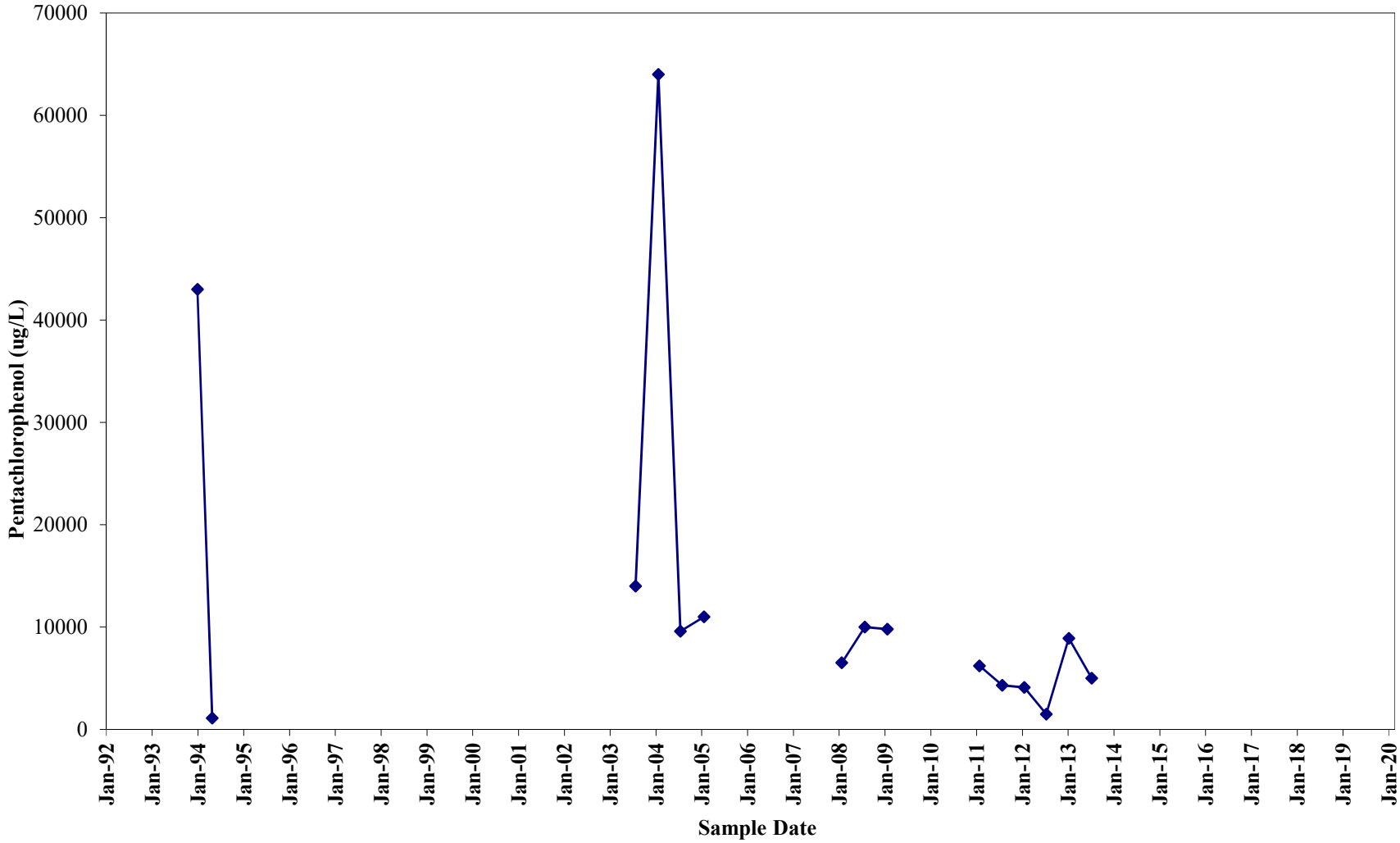


PCP data gap due to measurable product present in well.
Spike in PCP concentration in July 2017 probably due to presence of a small amount of product in water sample.
Well W40 was abandoned on March 28, 2019 to facilitate the Thomas Street reconstruction.
Replacement well W40R was installed on June 24, 2019.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W41

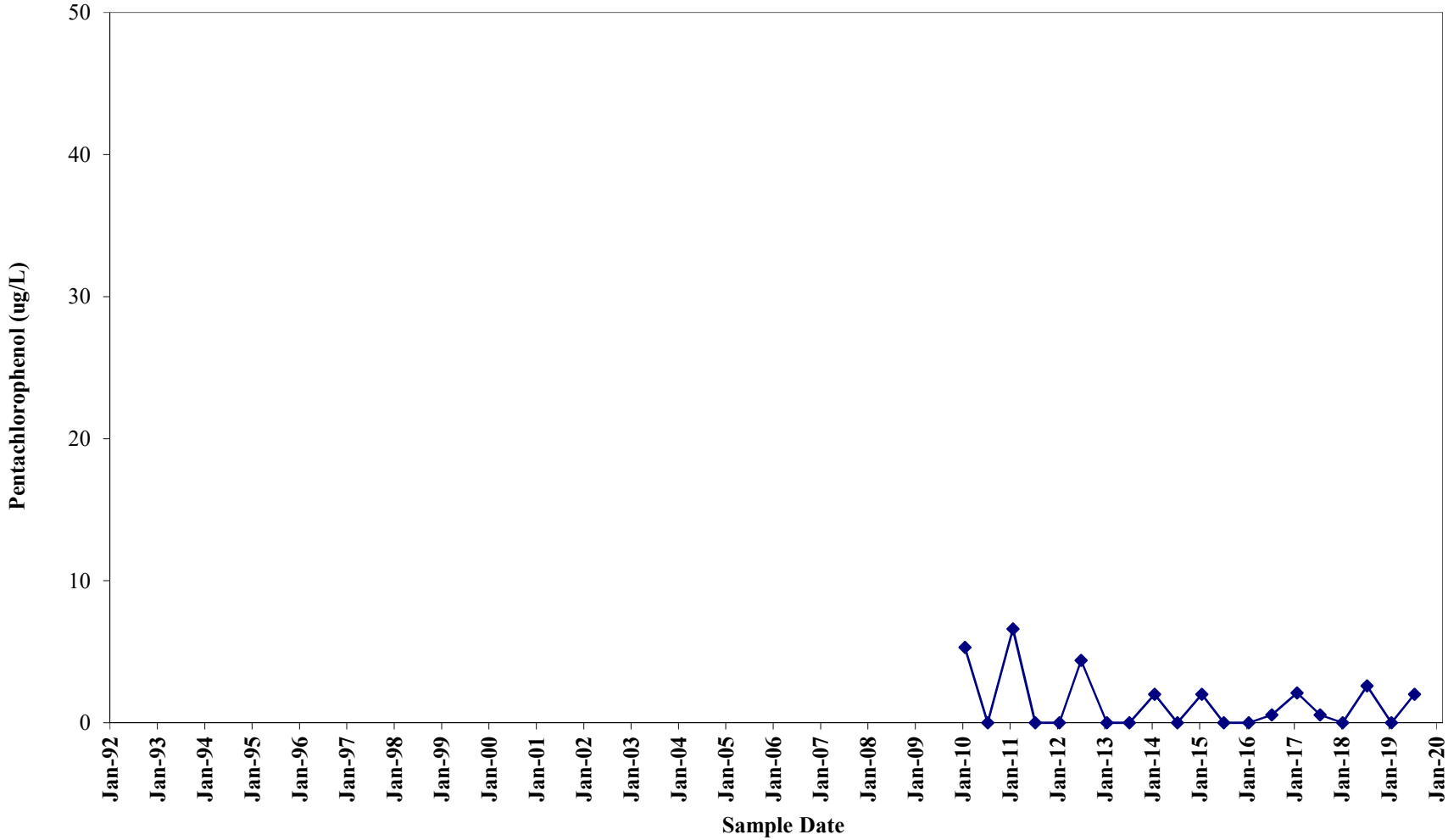


Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W69

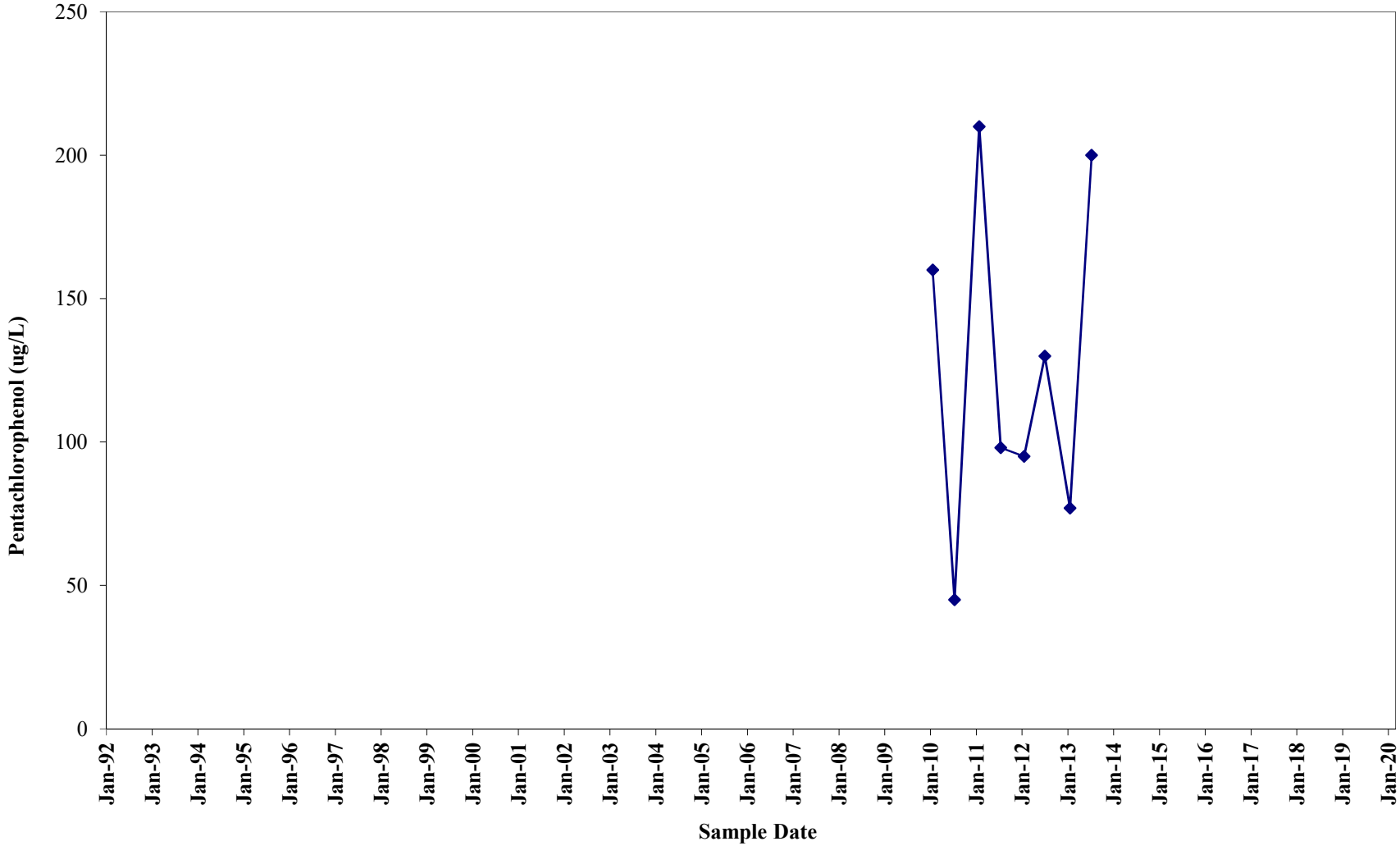


PCP data gap due to measurable product present in well.
Well W69 discontinued from the monitoring program beginning in 2014.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well DFOMW5

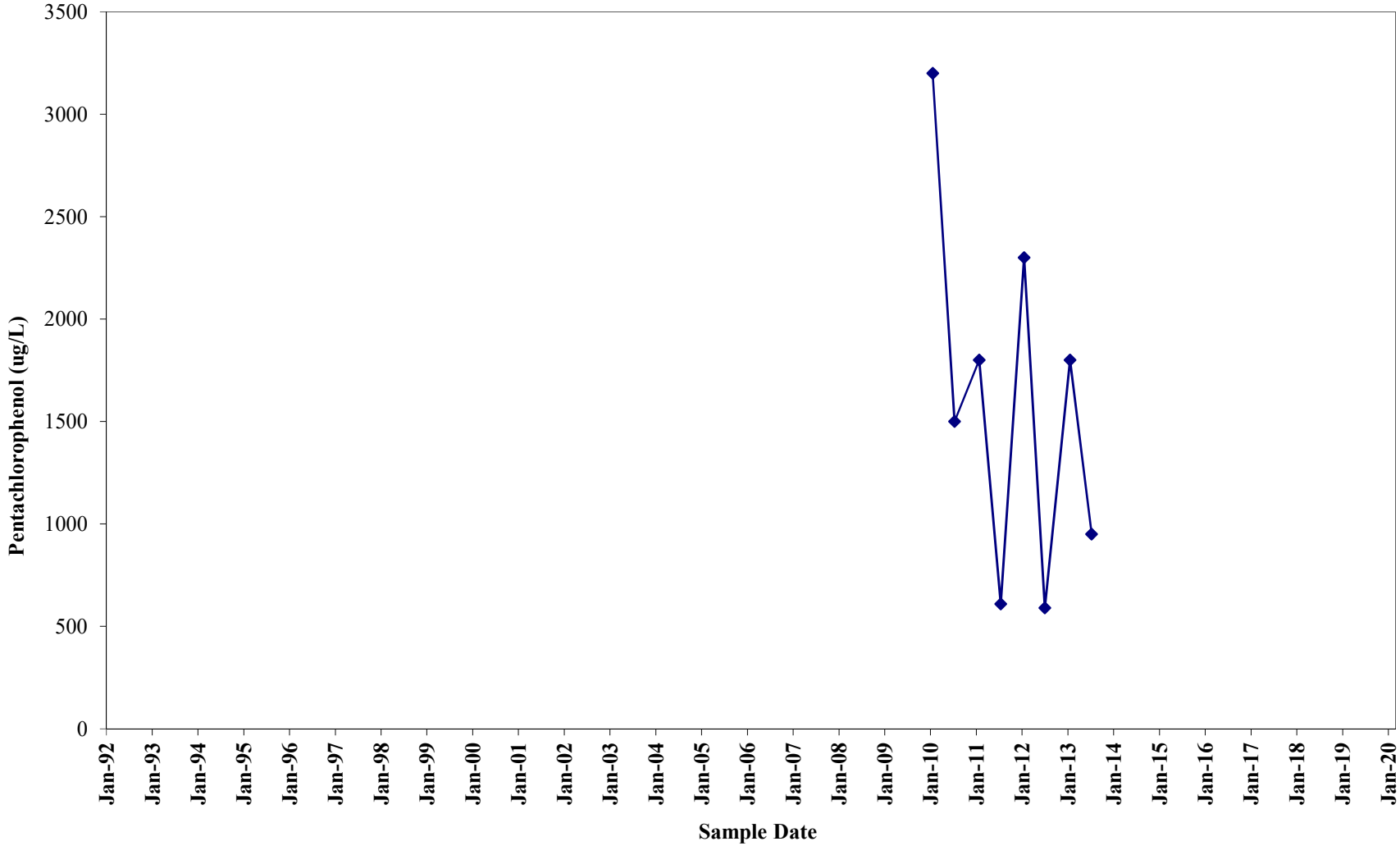


Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well DFOMW9



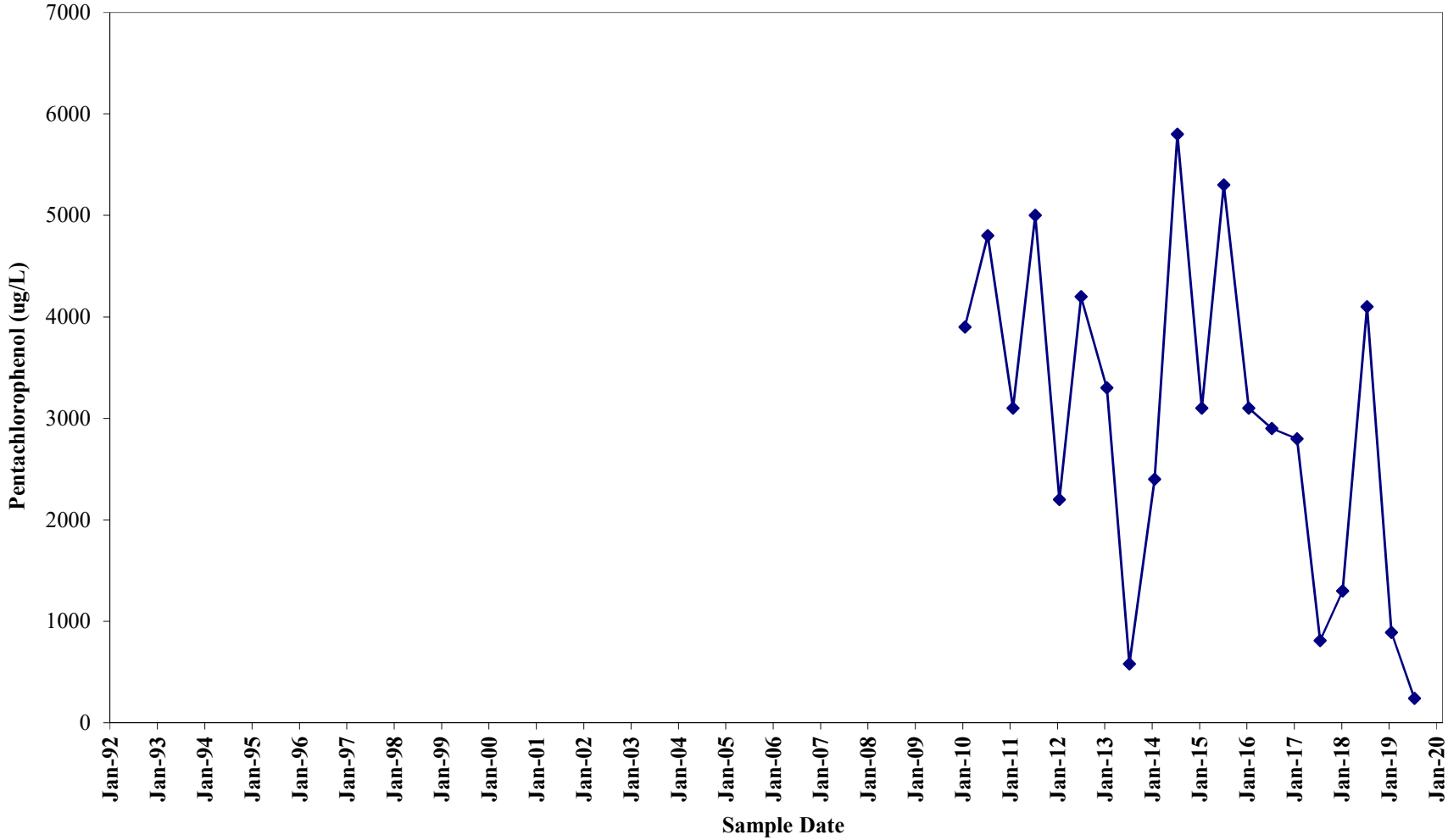
Well DFOMW9 discontinued from monitoring program beginning in 2014.
3M abandoned this well in 2015.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well DFOMW10A

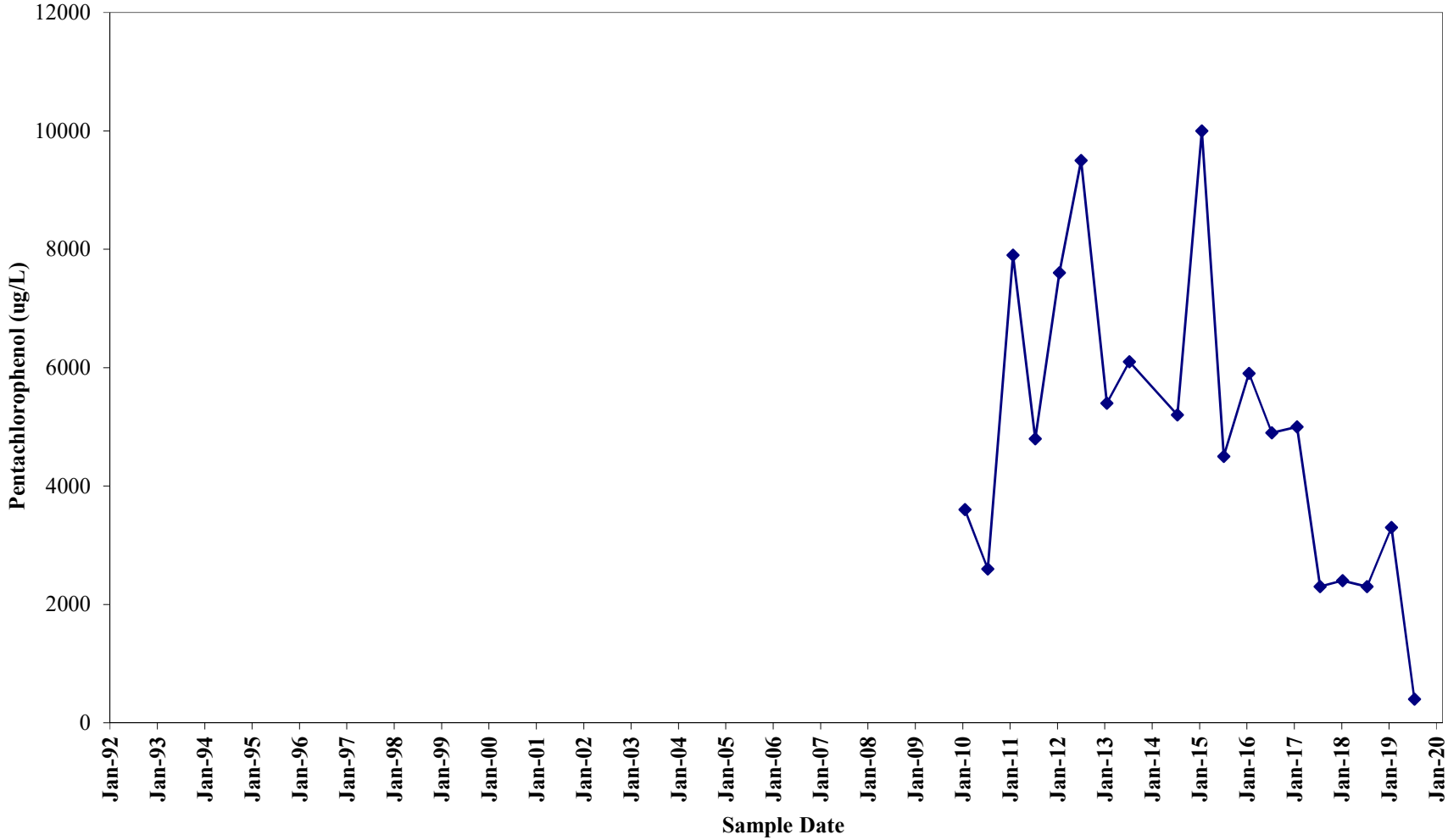


Well DFOMW10A discontinued from monitoring program beginning in 2014.
3M abandoned this well in 2015.

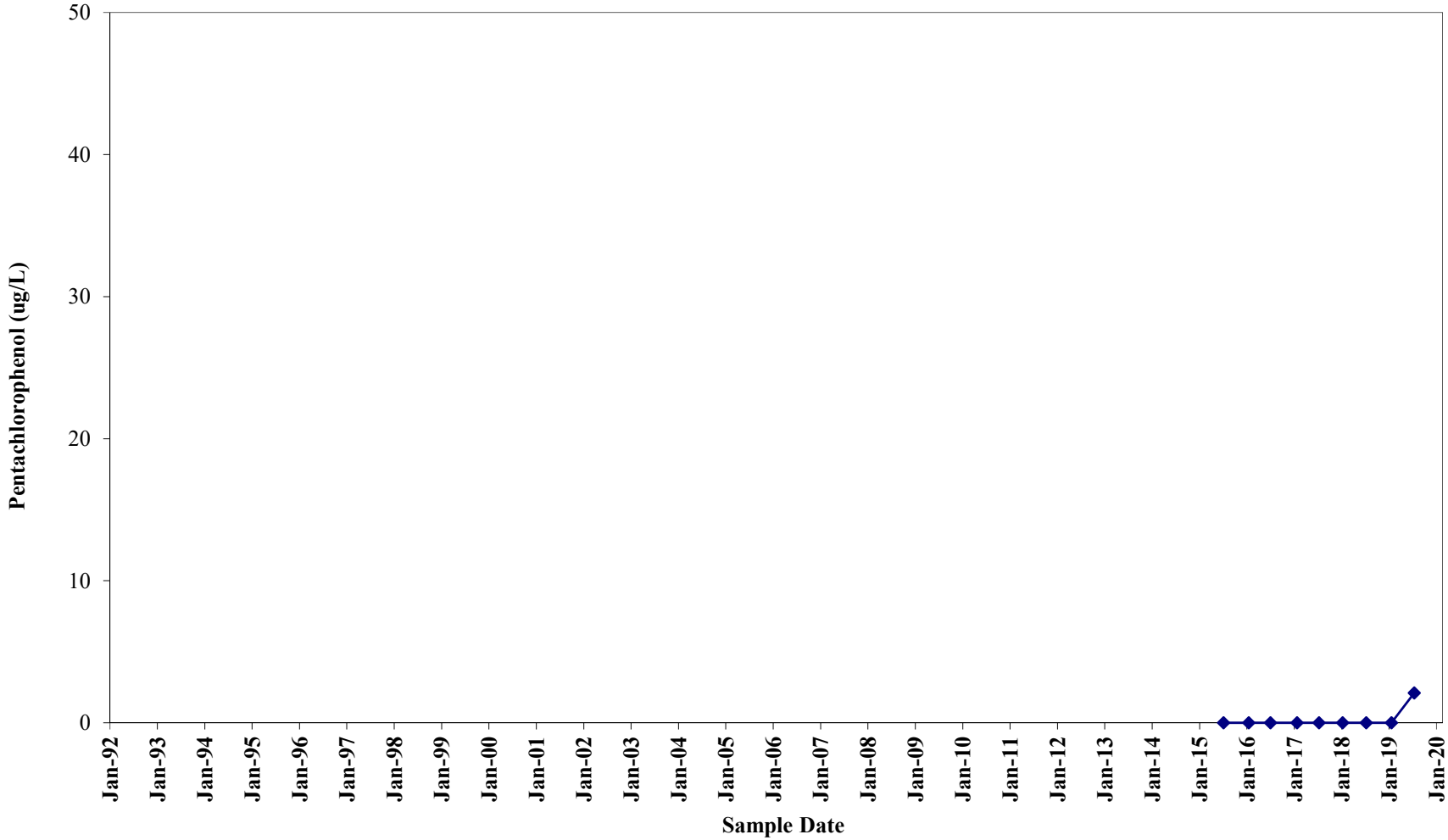
Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well DFOMW11



Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well DFOMW12

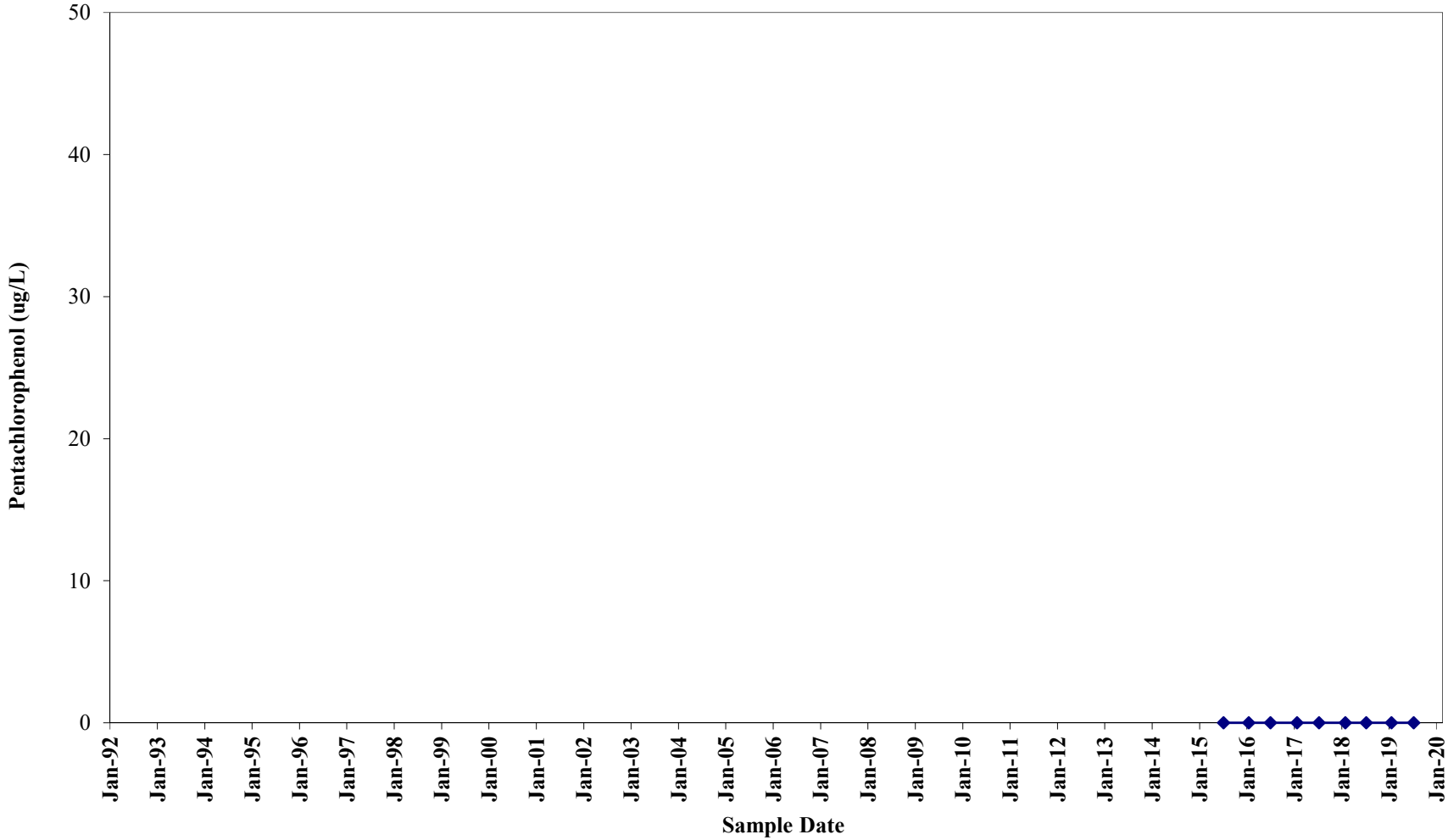


Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W71



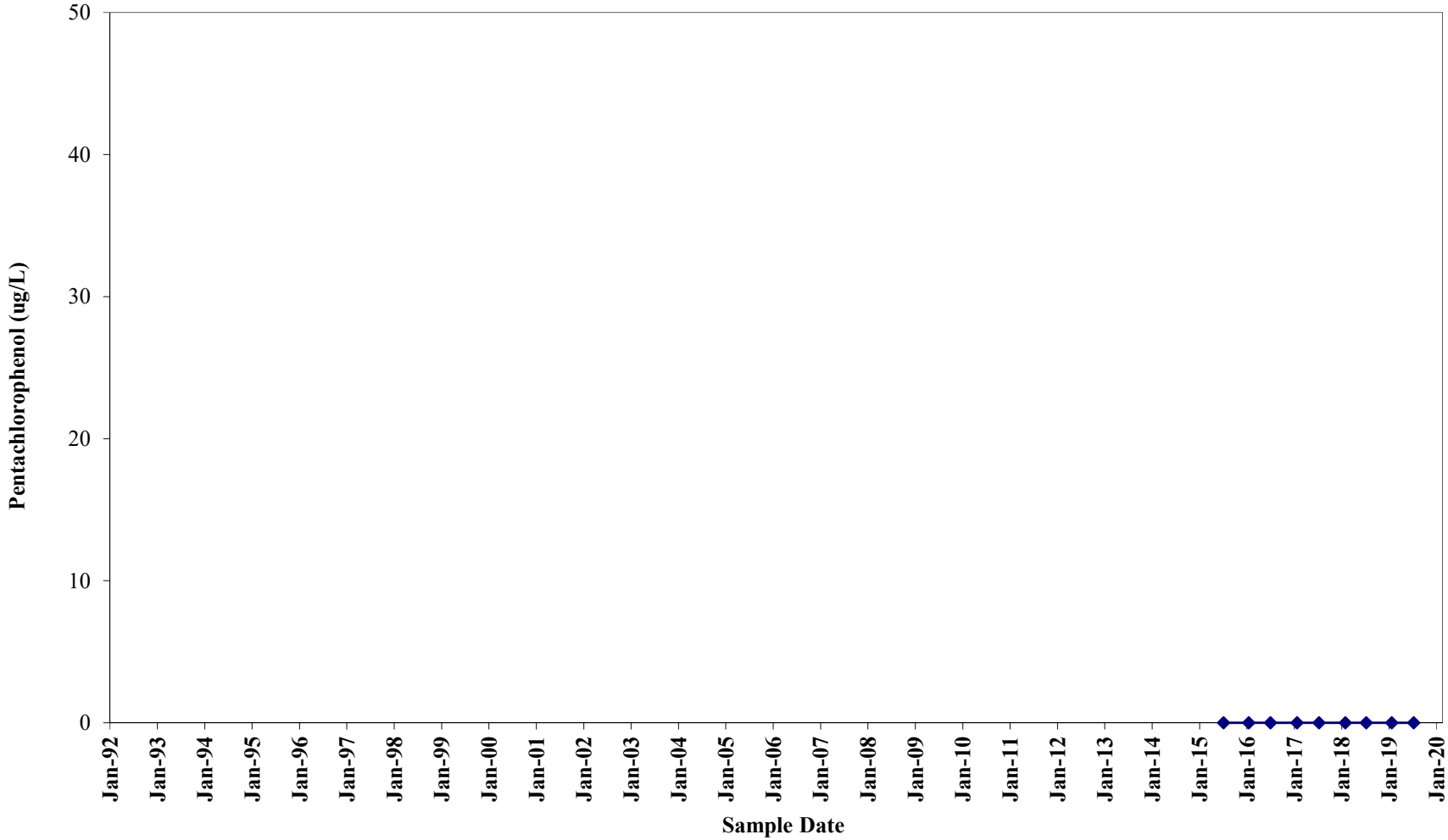
Well W71 installed in June 2015.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W72



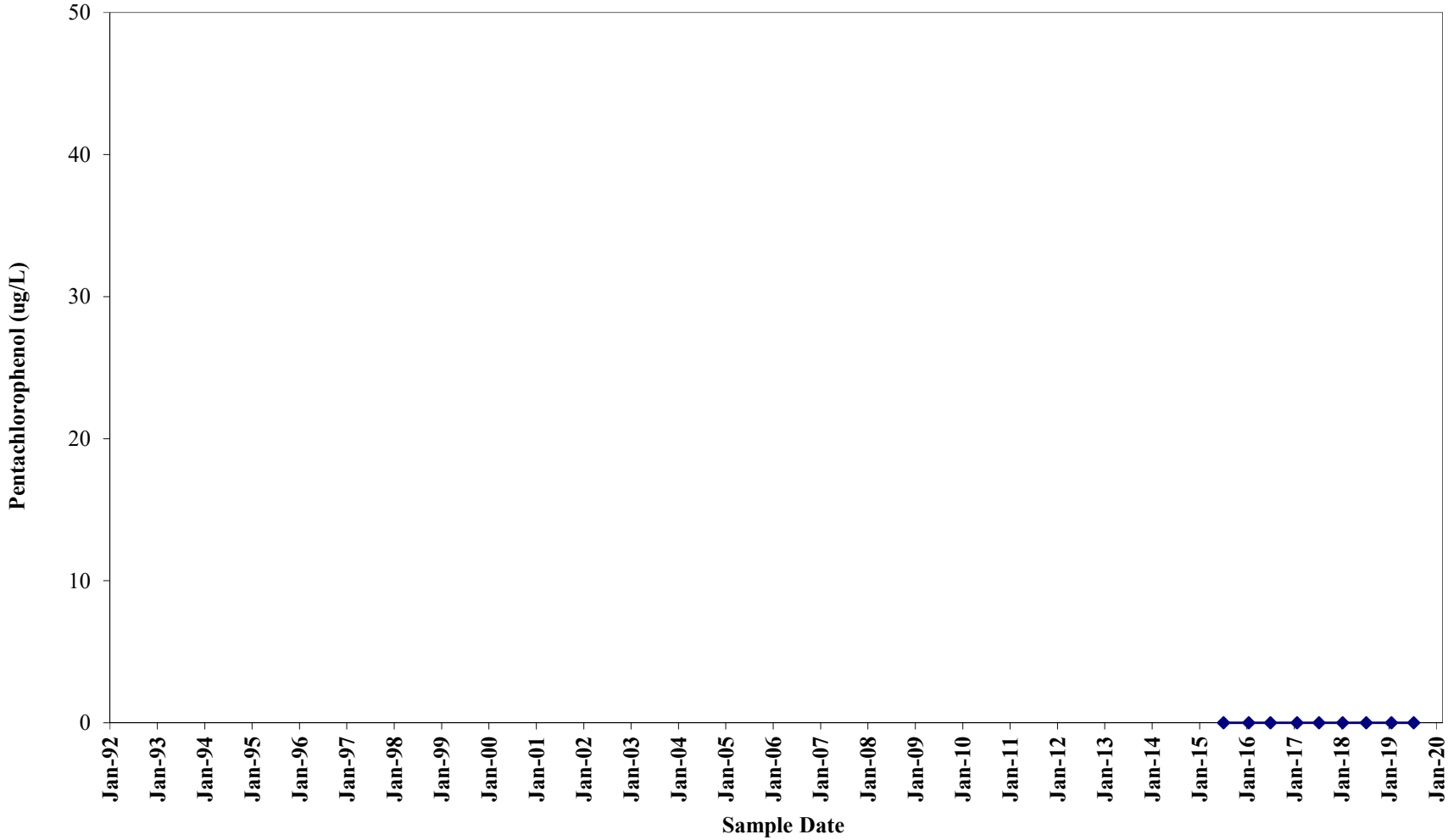
Well W72 installed in June 2015.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W73



Well W73 installed in June 2015.

Pentachlorophenol Concentrations
Historical Groundwater Monitoring
Well W74



Well W74 installed in June 2015.