Thank you for signing in early

The webinar will begin promptly at
12:00 pm ET, 9:00 am PT

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SERDP and ESTCP Webinar Series

The webinar will begin promptly at 12:00 pm ET, 9:00 am PT

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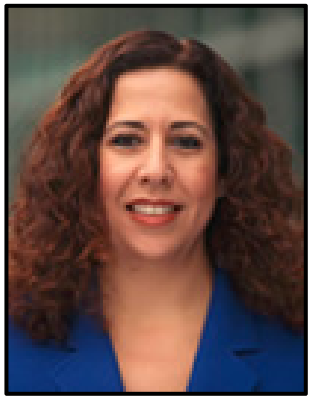
SERDP & ESTCP Webinar Series

Managing Sites Impacted by 1,4-Dioxane: New Developments in Molecular Tools and Conceptual Site Models

June 28, 2018

SERDP DOD • EPA • DOE | ESTCP

Welcome and Introductions



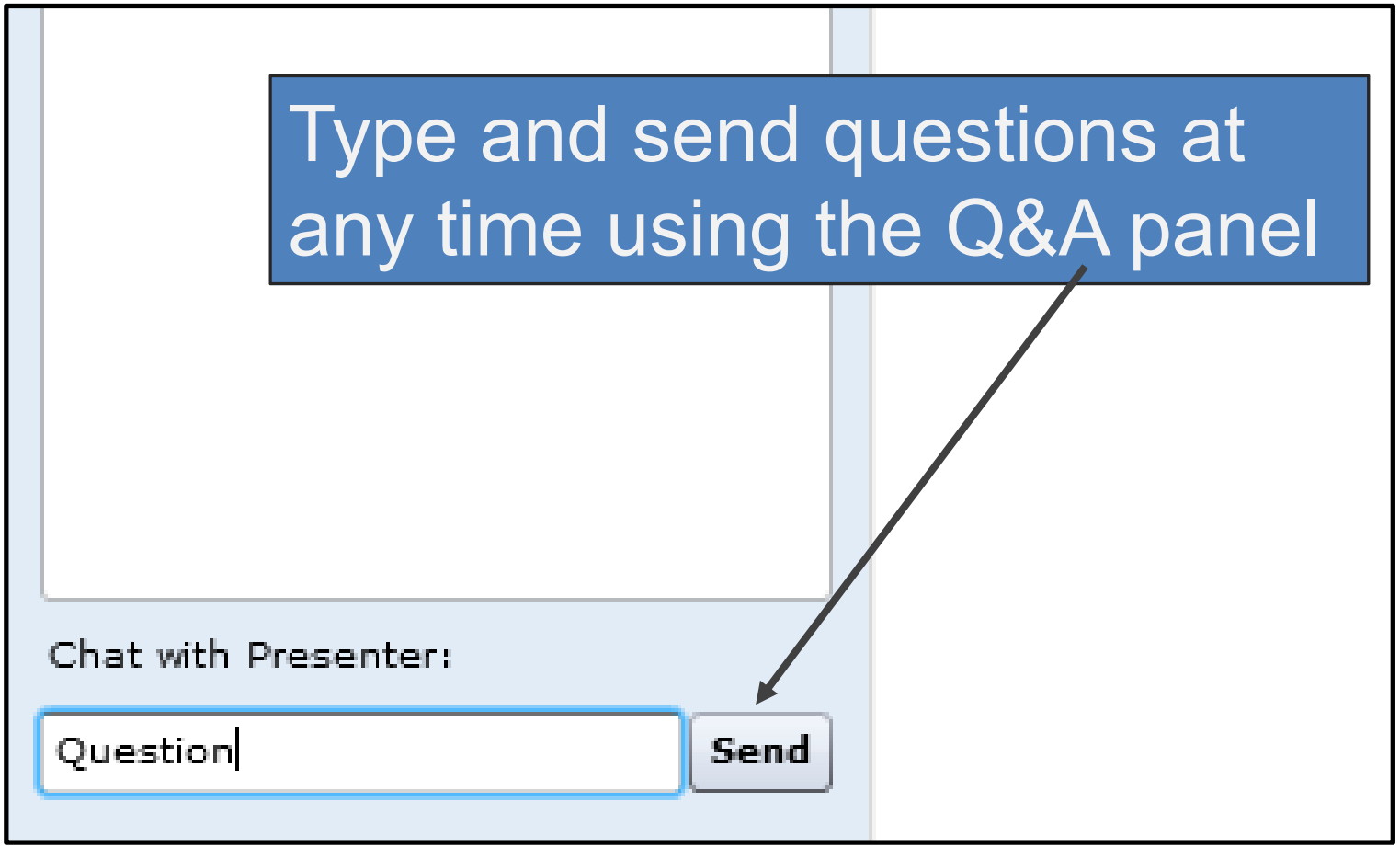
Rula A. Deeb, Ph.D.
Webinar Coordinator

SERDP DOD • EPA • DOE | ESTCP

Webinar Agenda

- **Webinar Logistics** (5 minutes)
  Dr. Rula Deeb, Geosyntec Consultants
- **Overview of SERDP and ESTCP** (5 minutes)
  Dr. Andrea Leeson, SERDP and ESTCP
- **Developing and Field-Testing Genetic Catabolic Probes for Monitored Natural Attenuation of 1,4-Dioxane**
  Dr. Pedro Alvarez, Rice University (25 minutes + Q&A)
- **Improving the Conceptual Site Model for Management of 1,4-Dioxane and Chlorinated Solvent Contaminated Sites**
  Dr. David Adamson, GSI Environmental (25 minutes + Q&A)
- **Final Q&A session**

How to Ask Questions



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- Submit a question using the chat box

SERDP and ESTCP Overview

Andrea Leeson, Ph.D.
SERDP and ESTCP

SERDP DOD • EPA • DOE | ESTCP

SERDP

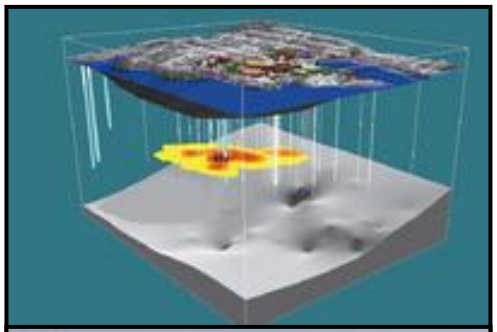
- Strategic Environmental Research and Development Program
- Established by Congress in FY 1991
  - DoD, DOE and EPA partnership
- SERDP is a requirements driven program which identifies high-priority environmental science and technology investment opportunities that address DoD requirements
  - Advanced technology development to address near term needs
  - Fundamental research to impact real world environmental management

ESTCP

- Environmental Security Technology Certification Program
- Demonstrate innovative cost-effective environmental and energy technologies
  - Capitalize on past investments
  - Transition technology out of the lab
- Promote implementation
  - Facilitate regulatory acceptance

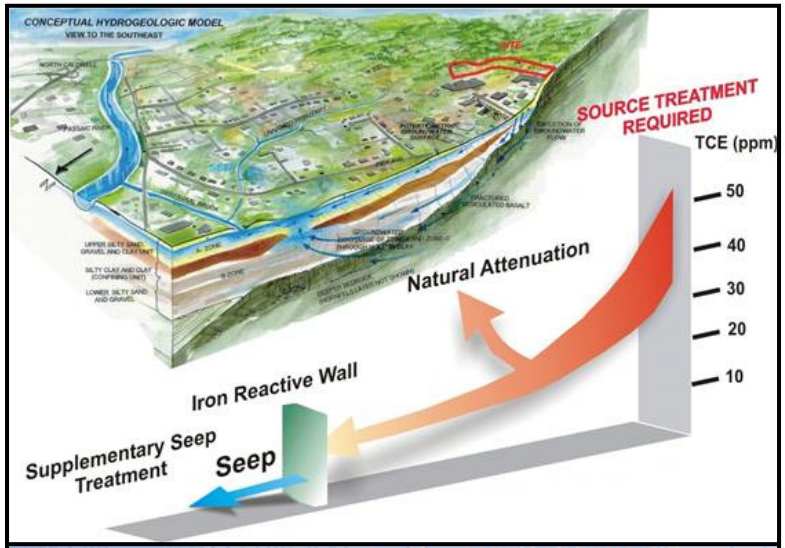
Program Areas

1. Environmental Restoration
2. Installation Energy and Water
3. Munitions Response
4. Resource Conservation and Resiliency
5. Weapons Systems and Platforms



Environmental Restoration

- Major focus areas
  - Contaminated groundwater
  - Contaminants on ranges
  - Contaminated sediments
  - Wastewater treatment
  - Risk assessment



SERDP and ESTCP Webinar Series

Date	Topic
July 12, 2018	Innovative Low Cost Building Automation Sensors and Controls
August 9, 2018	Energy Sustainable Wastewater Treatment Systems for Forward Operating DoD Installations
August 23, 2018	Sediment Volume Search Sonar Development
September 6, 2018	Informing Restoration Programs for Threatened and Endangered Plant Species
September 20, 2018	Plant Diversity and Biological Nitrogen Fixation in Longleaf Pine Ecosystems at Military Installations
October 4, 2018	SERDP & ESTCP Chlorinated Solvents Workshop Overview and Feature Project

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Save the Date!

SERDP • ESTCP
SYMPOSIUM
2018 | Enhancing DoD's Mission Effectiveness

A three-day symposium showcasing the latest technologies that enhance DoD's mission through improved environmental and energy performance

November 27-29, 2018

Washington Hilton Hotel

Registration is open

Developing and Field-Testing Genetic Catabolic Probes for Monitored Natural Attenuation of 1,4-Dioxane

Dr. Pedro Alvarez
Rice University

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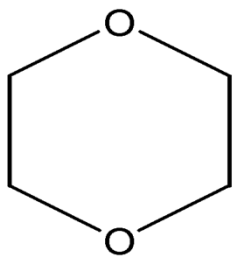
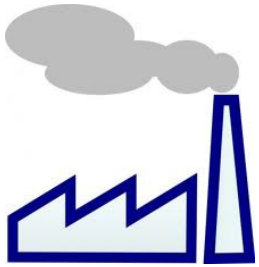
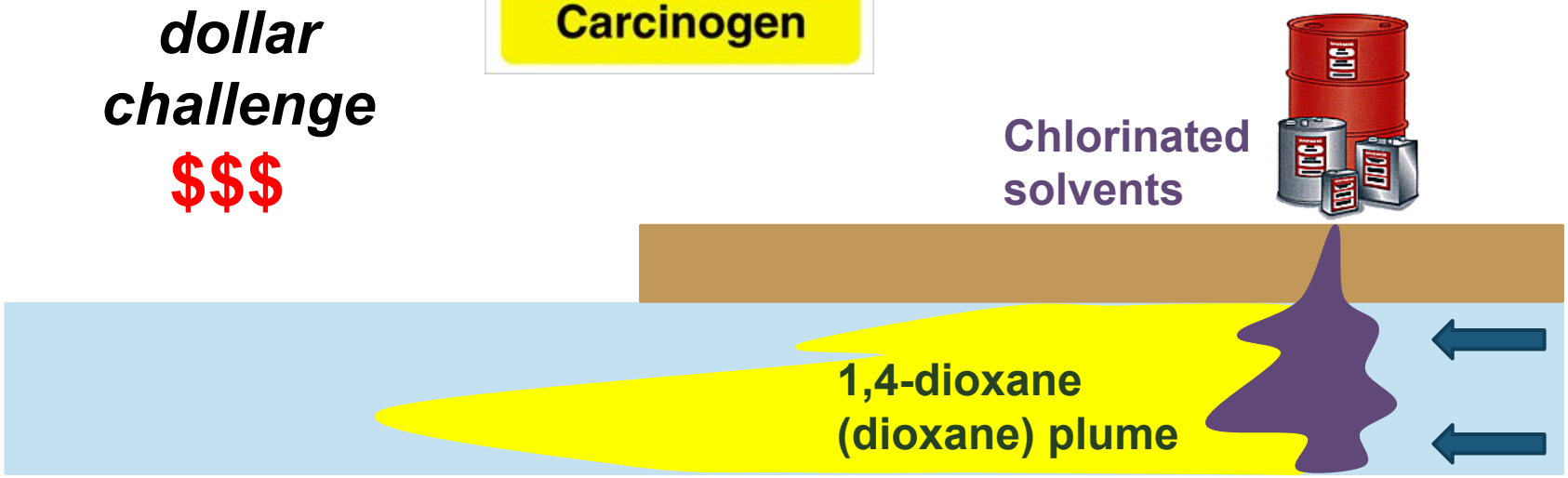
Background on 1,4-Dioxane



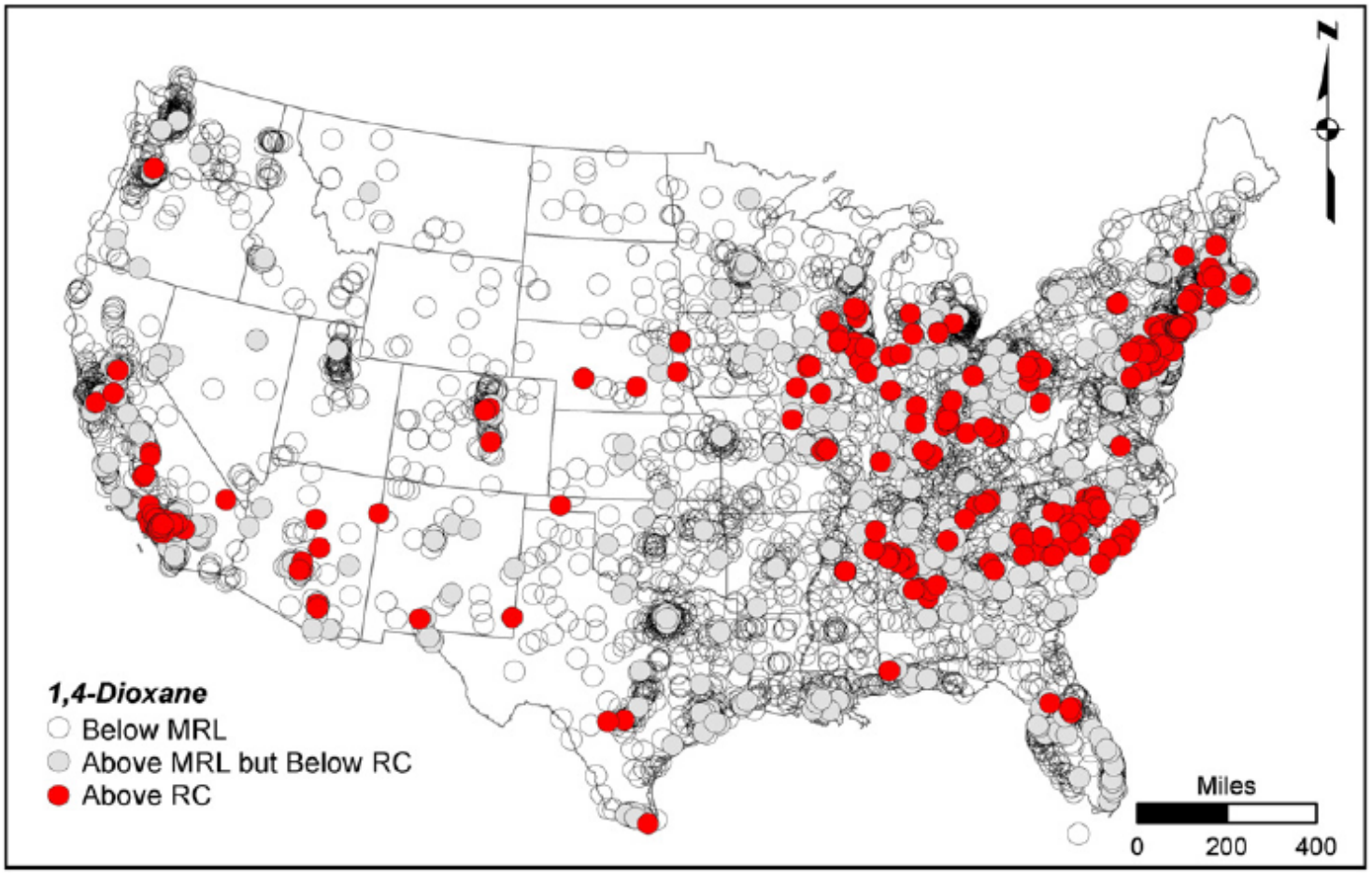


Multi-billion dollar challenge

$$$

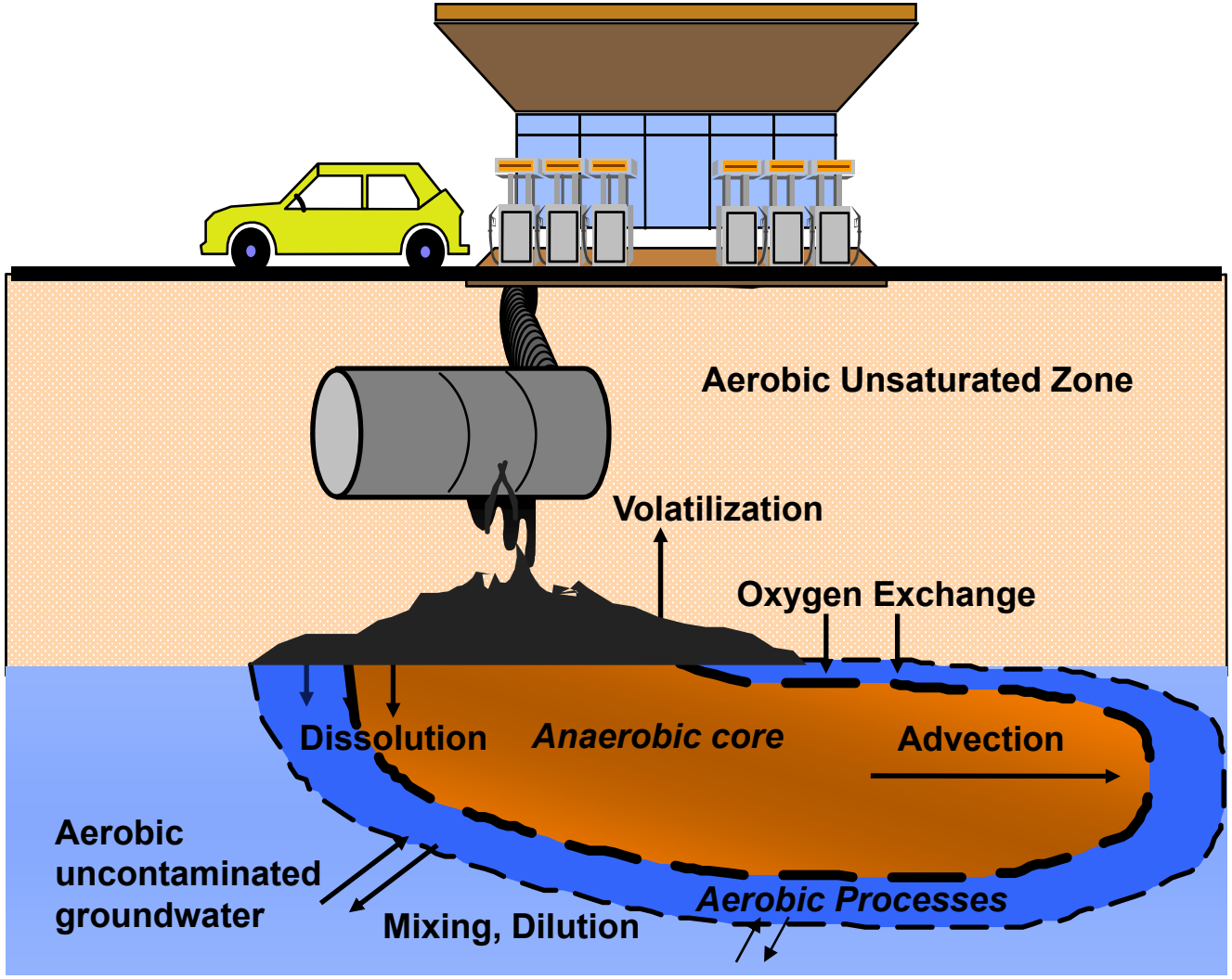


Widespread 1,4-Dioxane Contamination



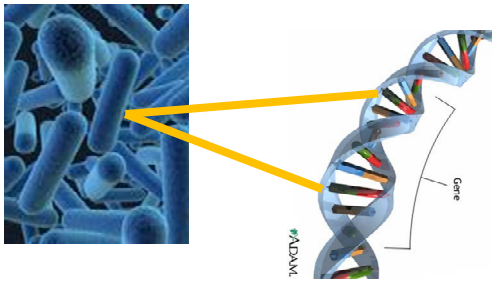
Adamson et al., 2017 Sci. Total. Environ. 596: 236-245

Natural Attenuation



Biomarkers

Question	Target	Information Gained
Who is there?	Ribosomal (r)RNA	Phylogenetic identity
	Genes for rRNA on DNA	
What can they degrade?	Catabolic genes on DNA	Phenotypic potential
What genes are being expressed?	Messenger (m)RNA	Phenotypic activity
Who is active and what are they doing?	Produced protein	Phenotypic activity Phylogenetic identity

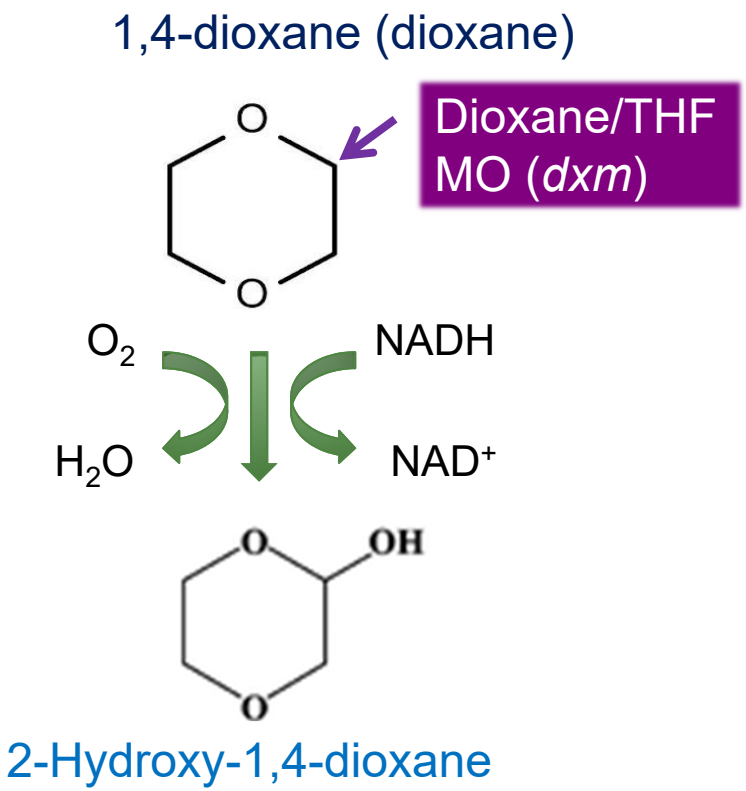


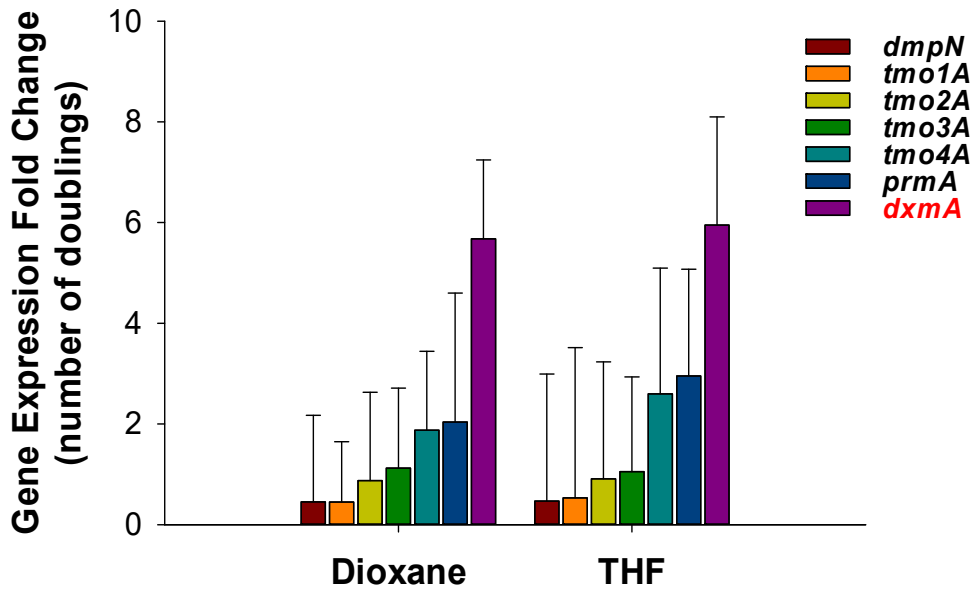
Alvarez and Illman, 2006. Bioremediation technologies

Objectives

- Develop functional gene probe(s)
  - Quantify dioxane biodegradation
  - Support monitored natural attenuation (MNA) decisions and performance assessment
- Specific objectives
  1. Assess reliability of biomarker
  2. Discern site-specific factors influencing biomarker abundance/activity
  3. Isolate or identify dioxane degraders
  4. Characterize novel catabolic gene sequences

Development of Probe





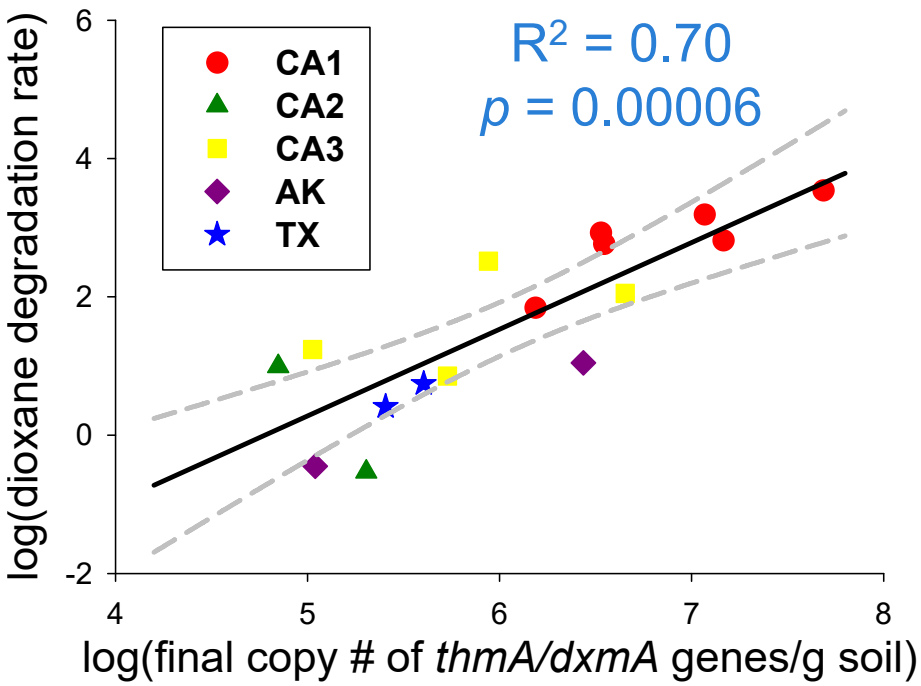
***thm*A/*dxm*A Probe: 5′-ACG-CCT-ATT-ACA-TCC-AGC-AGC-TCG-A-3′**

Li et al., 2013. Environ. Sci. Technol. Lett.1(1): 122-127

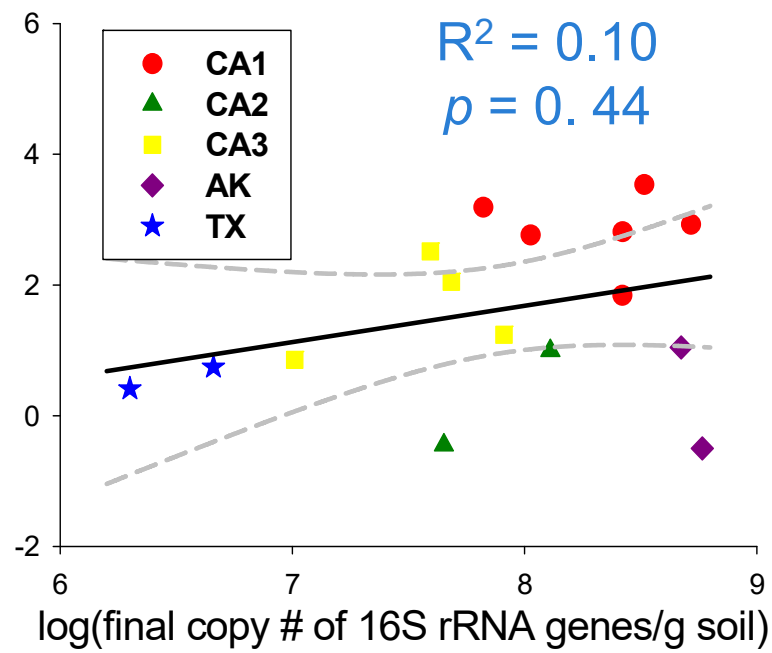
1. Probe Reliability

Degradation activity correlated with dxmA, not 16s

thmA/dxmA



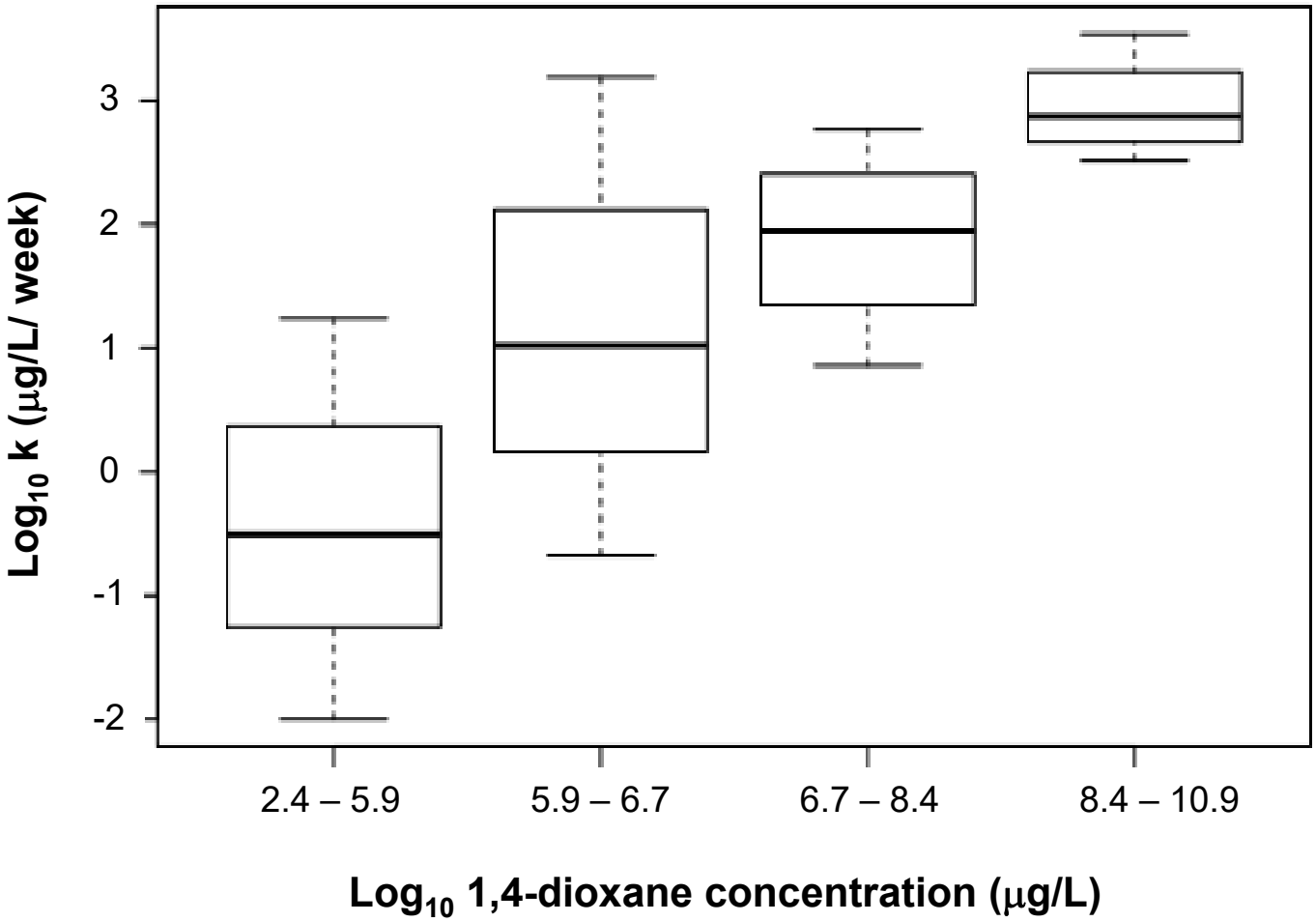
16s rRNA



Li et al., 2013. Environ. Sci. Technol. Lett.1(1): 122-127

2. Factors Affecting Biomarker Abundance

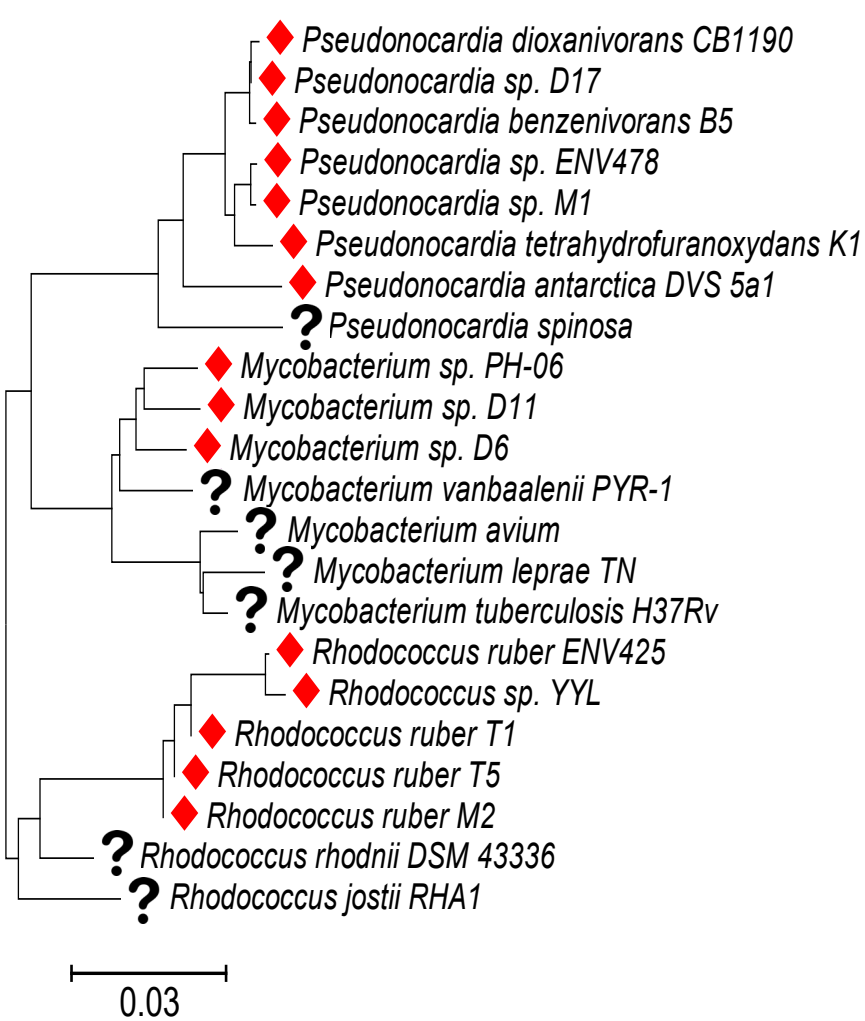
Aerobic sites with more dioxane better suited for MNA



Da Silva et al., 2018. J. Environ. Mngmt, 206:60-64

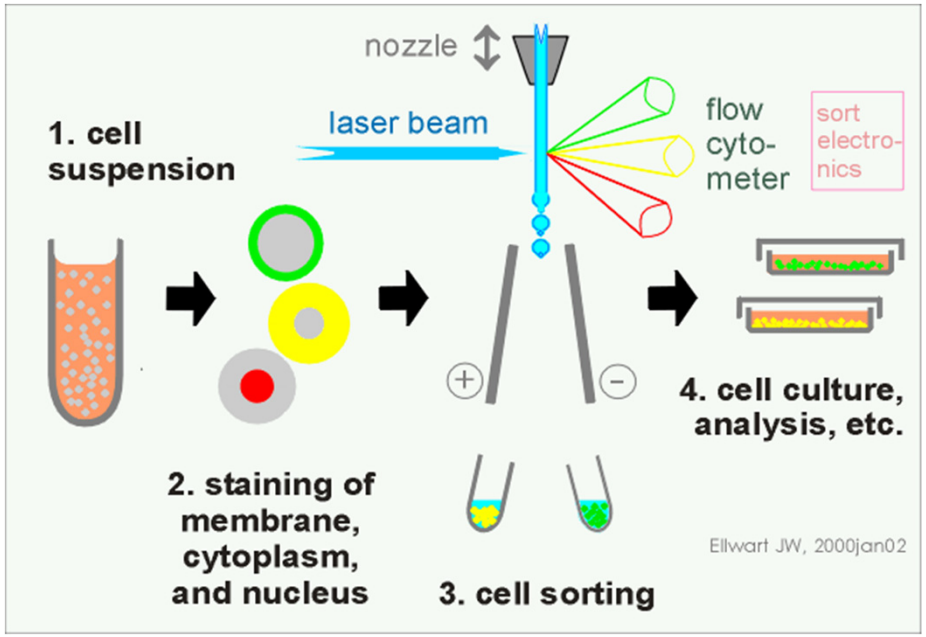
3. Identify Dioxane Degraders

Phylogenetic probe Pse631 for cell sorting



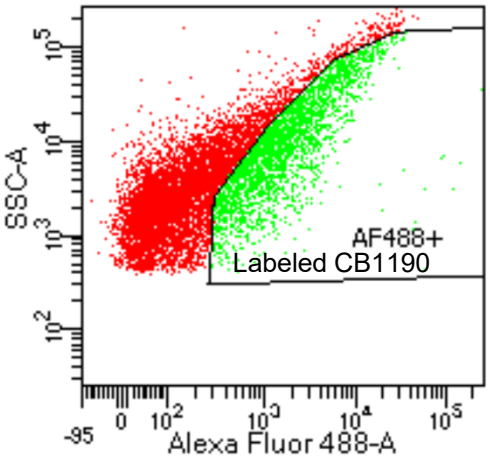
Pse631

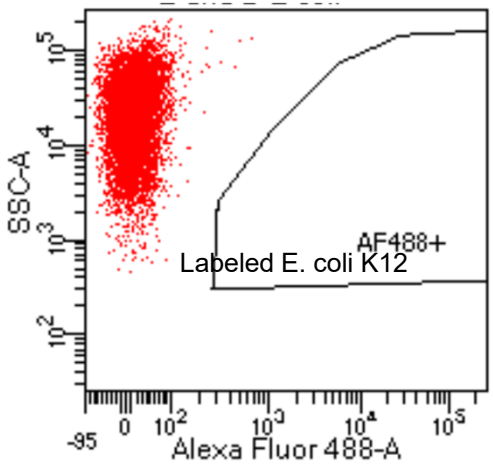
18 out of 34 known species that degrade dioxane are *Pseudonocardia*

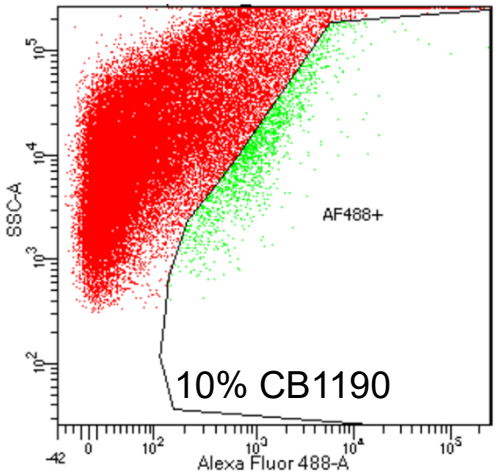


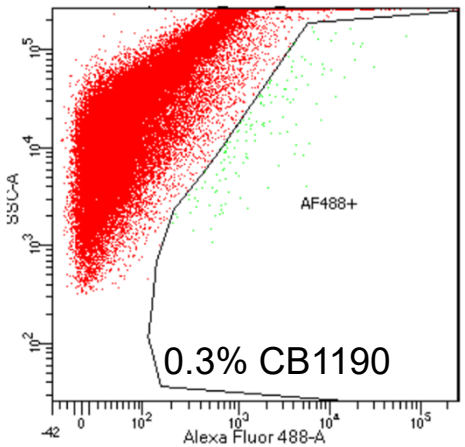
Identify Dioxane Degraders

Probe Pse631 is specific and reliably sensitive









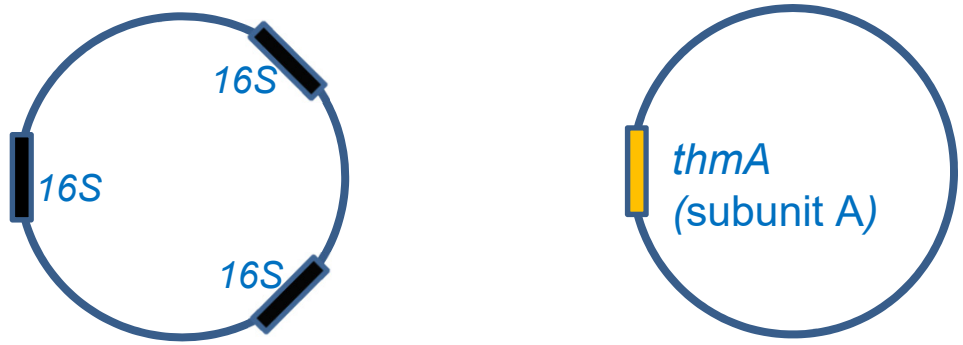
Bacteria	Hybridization efficiency %
CB1190	35.6 ± 4.2
Unlabeled CB1190	0.2 ± 0.1
B. Subtilis	0.0 ± 0.1
E. coli	0.0 ± 0.1
PH-06	0.1 ± 0.1

Sample	AF488+ (positively labeled)	Detection efficiency (%)
CH2M control	0.1±0.0%	-
10% CB1190	3.7±0.4%	36.7
3% CB1190	1.4±0.1%	47.8
1% CB1190	0.7±0.1%	67.4
0.3% CB1190	0.3±0.1%	89.1
0.1% CB1190	0.1±0.0%	100

Li et al., 2018. Appl. Microbiol. Biotechnol. 1-12

Identify Dioxane Degraders

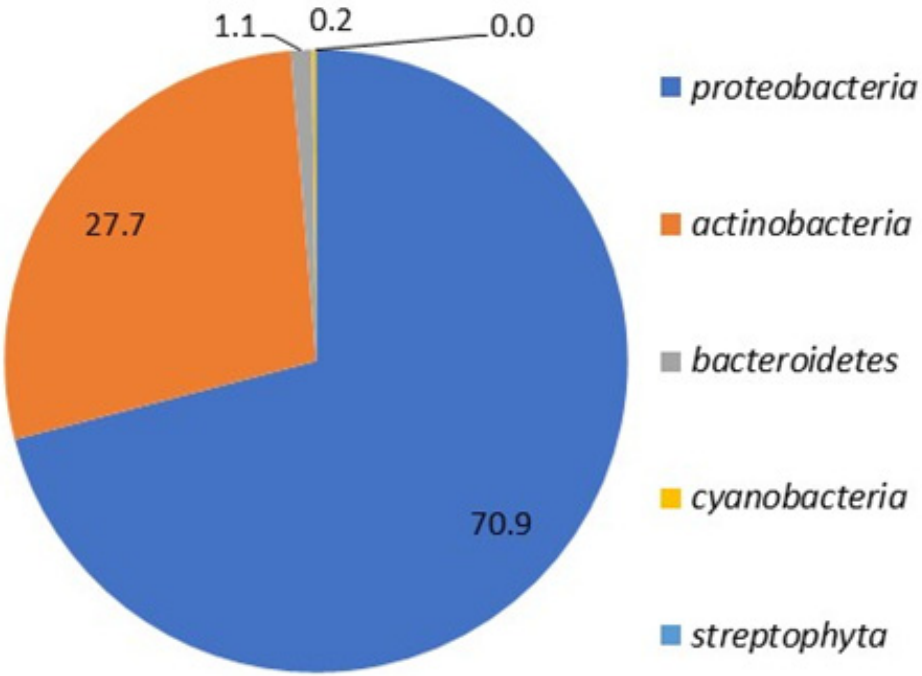
Strategy to amplify signal of thmA/dxmA probe



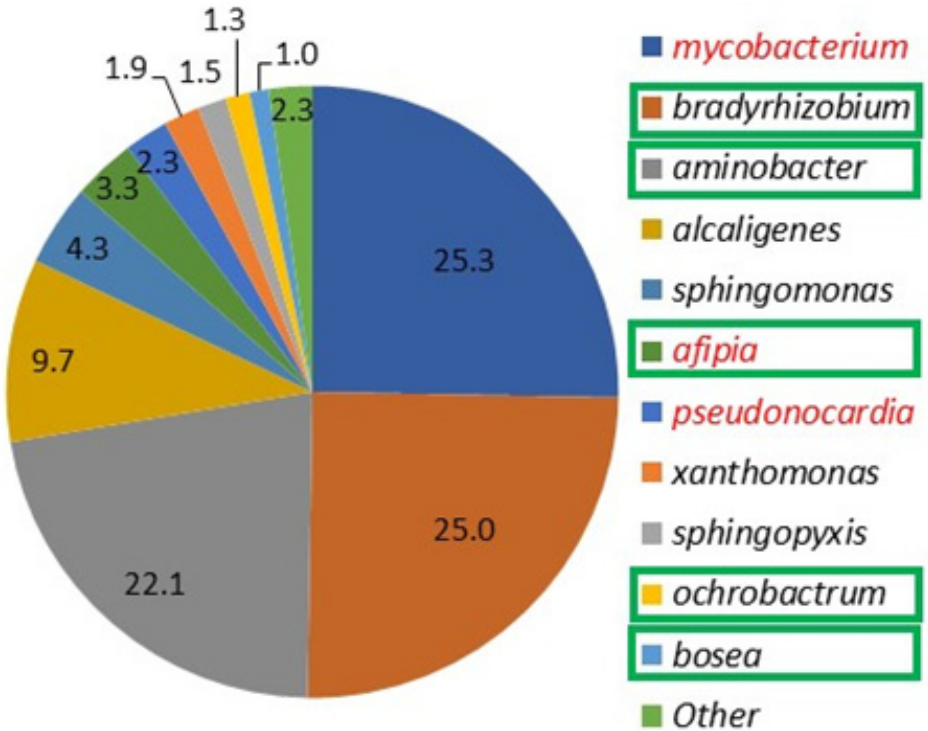
Strategy	Regular probe (multiple copies)	*thmA* probe (single copy)
Longer probe	22 base pairs	304-317 base pairs
Multiple probes covering different locations		
Multiple fluorophores on one probe	Alexa488	Alexa488

Identify Dioxane Degraders

Cell sorting to find novel dioxane degraders with thmA



Phylum

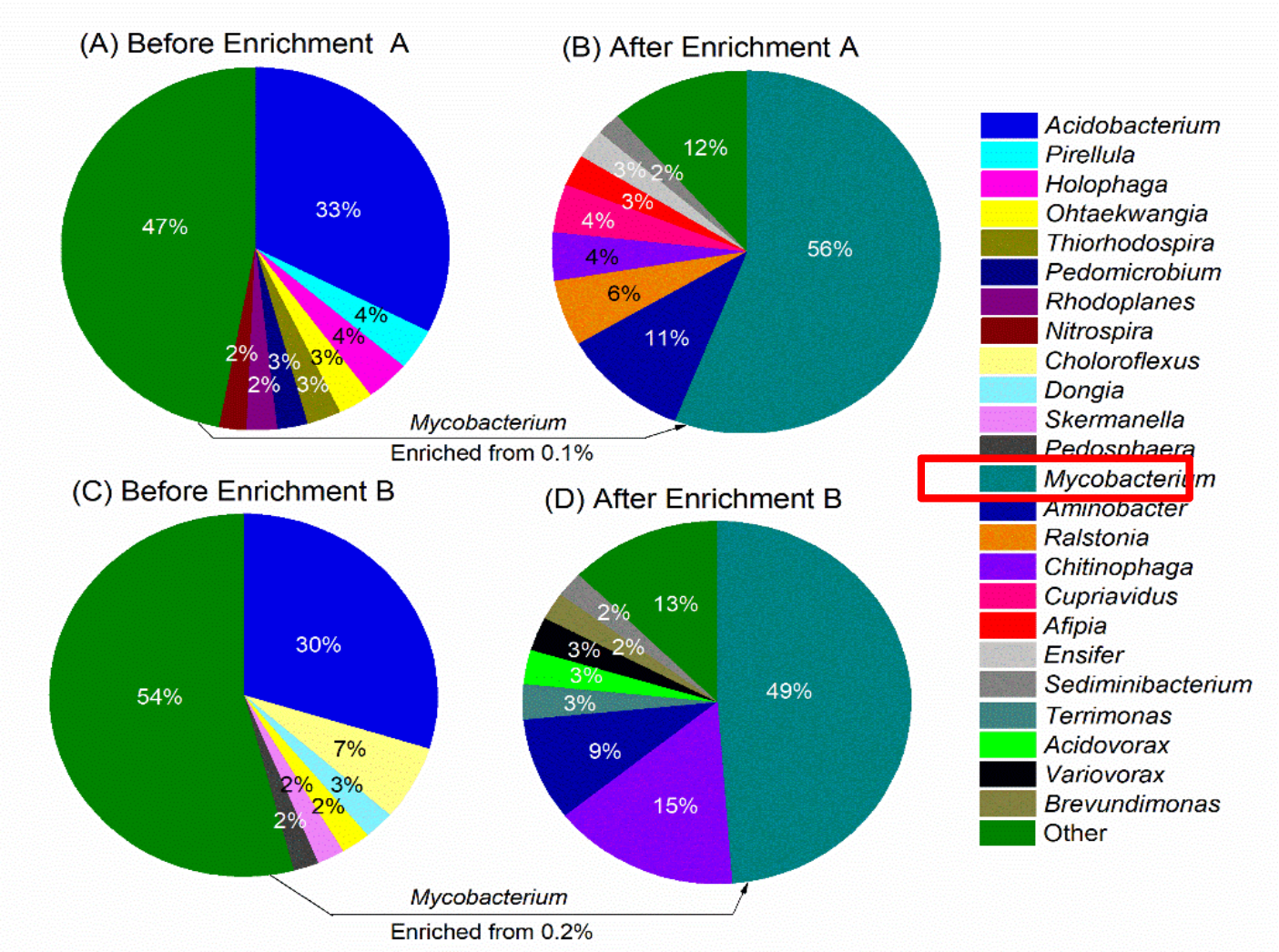


Genus

Observed five *Rhizobiales*

4. Characterize Novel Gene Sequences

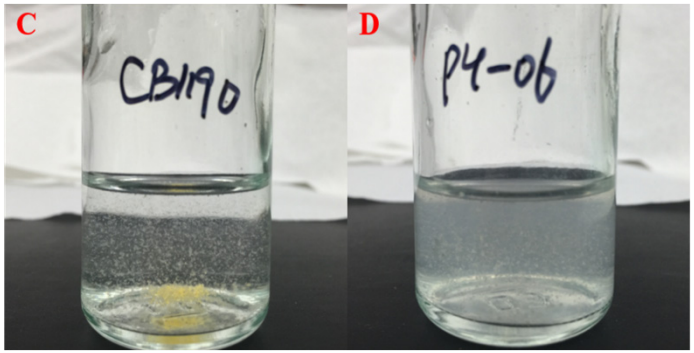
thmA absent in garden soil dioxane degraders

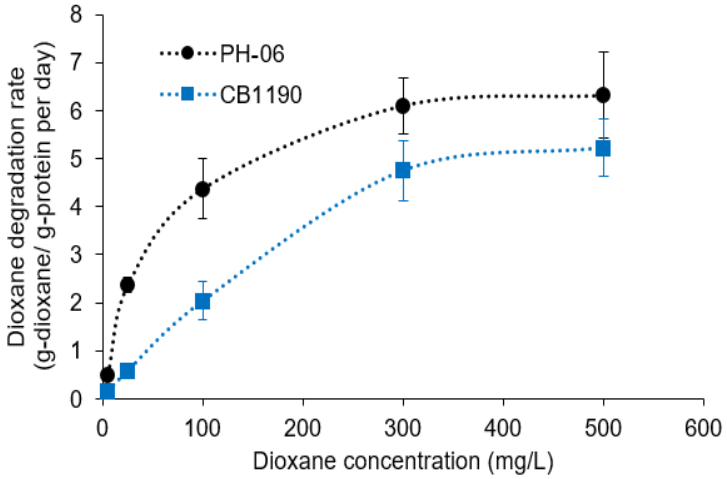


He et al., 2018. Microbiol Biotechnol. 11(1):189-198

Characterize Novel Gene Sequences

- *Mycobacterium dioxanotrophicus* PH-06
- Greater potential for bioaugmentation (compared with CB1190)
  - Higher affinity for dioxane
  - Higher cell yield
  - Less clumping

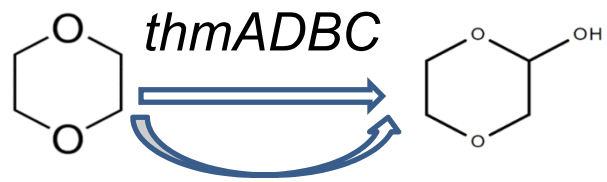




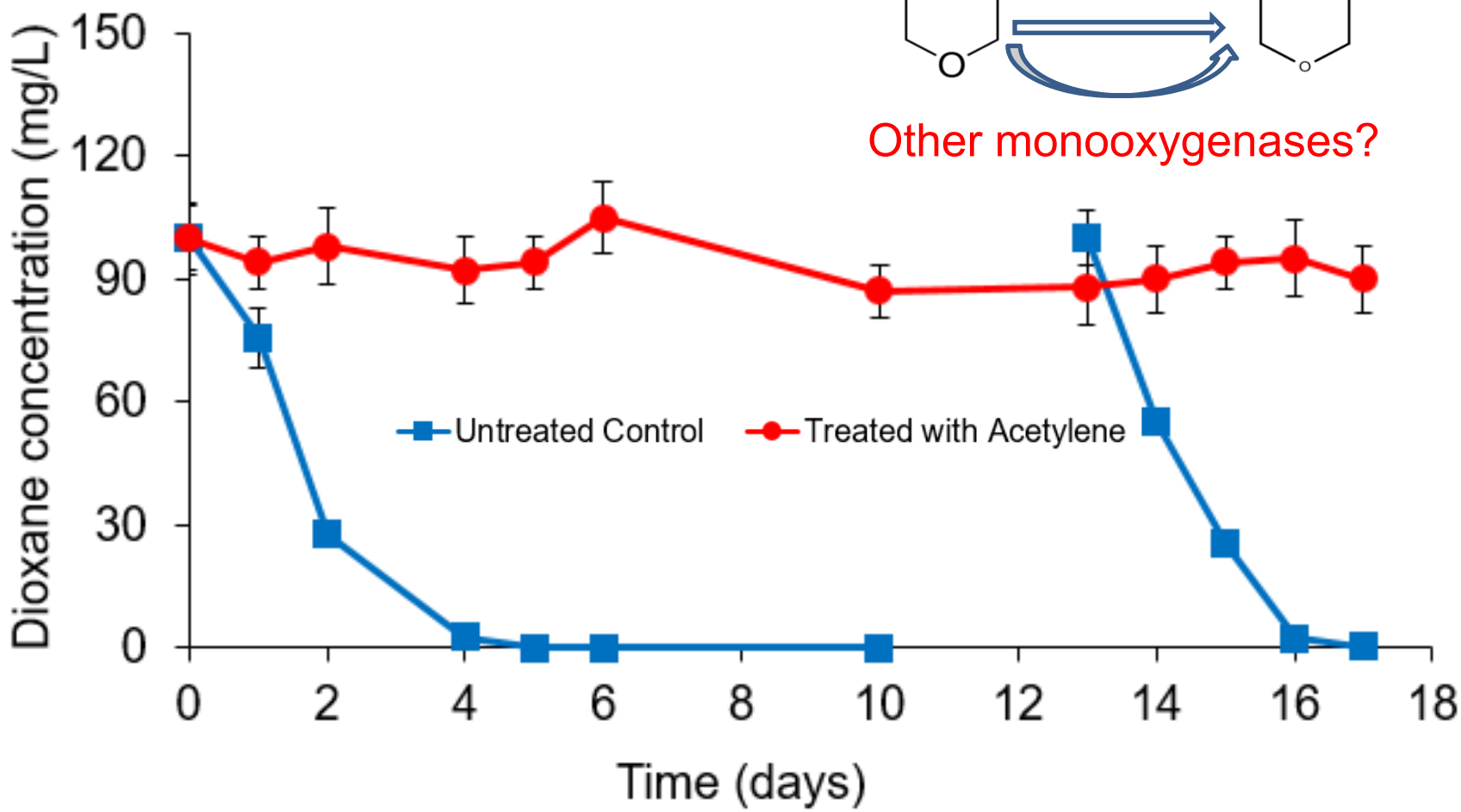
He et al., 2017. Environ. Sci. Technol. Lett. 4(11):494-499

Characterize Novel Gene Sequences

Acetylene inhibitor showed monooxygenases are key

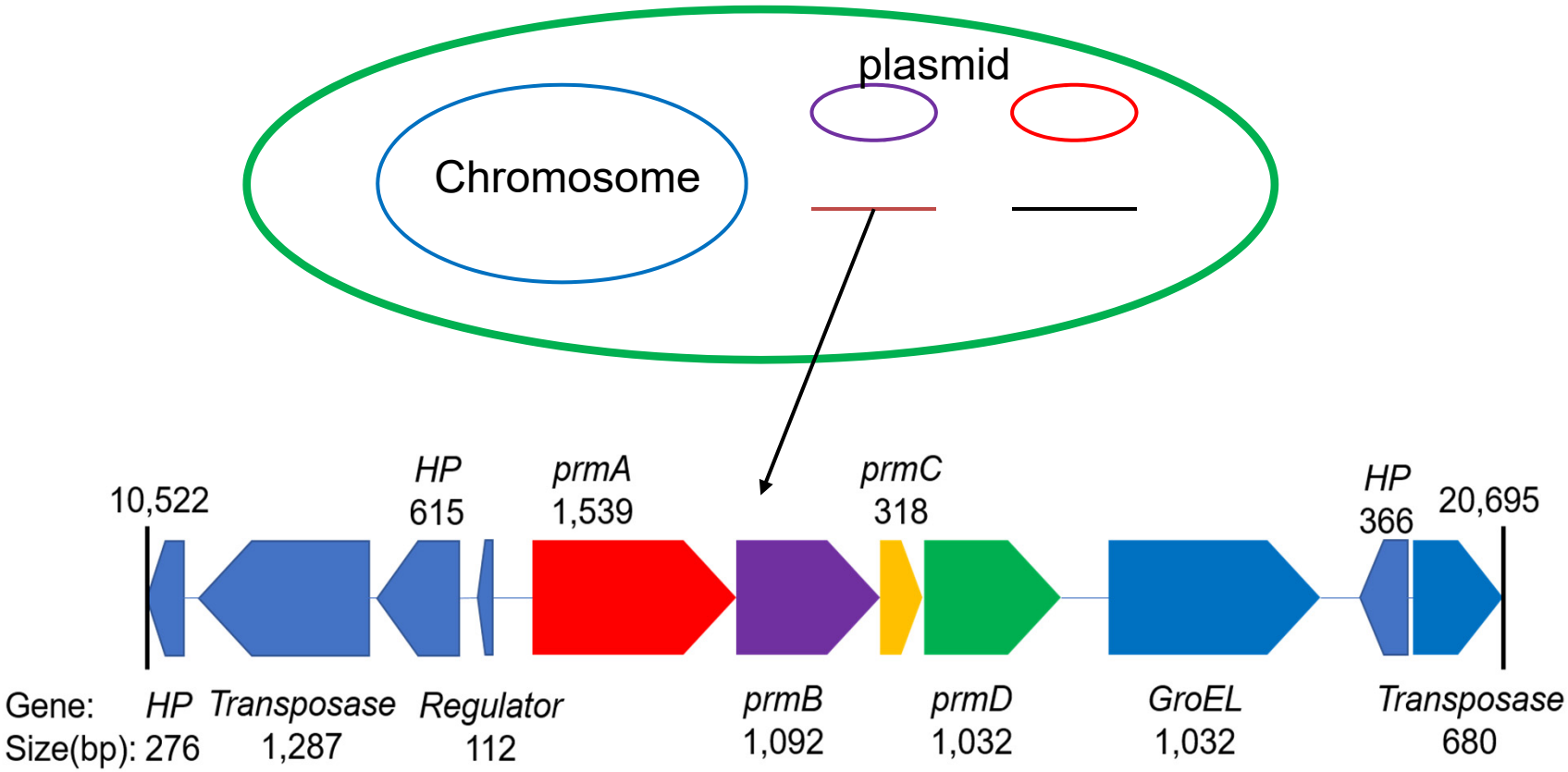


Other monooxygenases?



Characterize Novel Gene Sequences

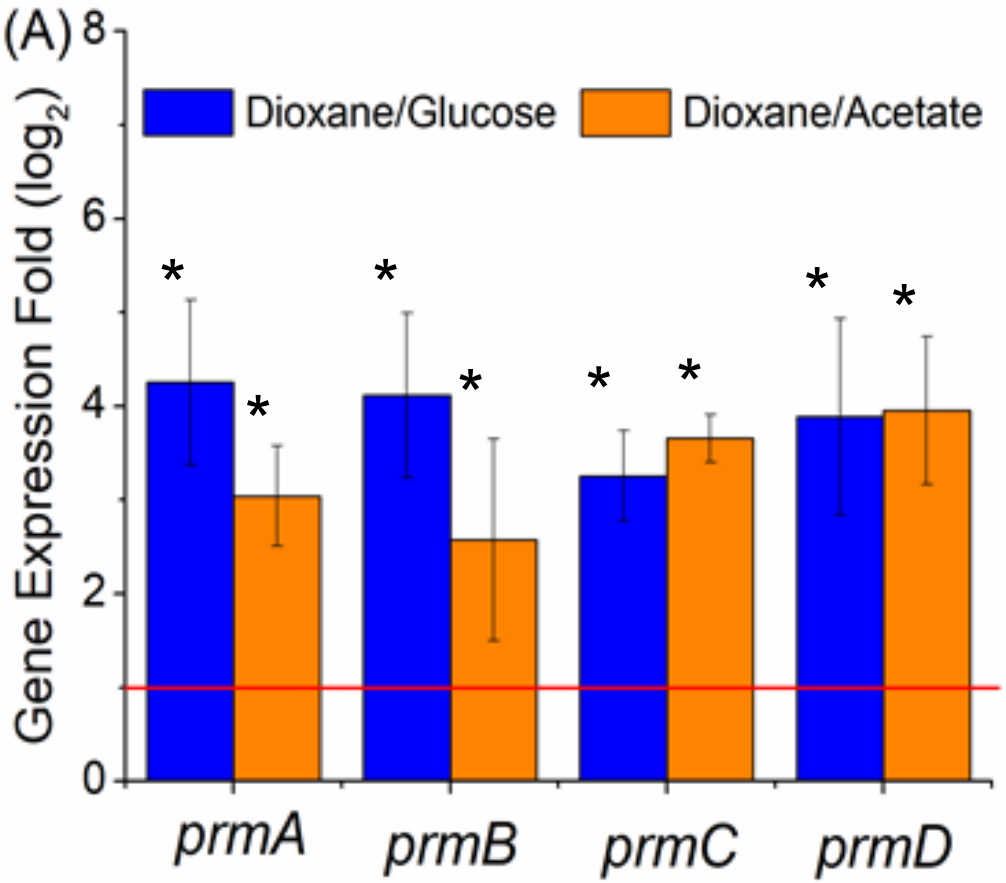
Whole genome sequencing of PH-06 identified only one SDIMO gene cluster: prmABCD



He et al., 2017. Genome Announc. 5(35): e00625-17
He et al., 2017. Environ. Sci. Technol. Lett. 4(11):494-499

Characterize Novel Gene Sequences

RNA-seq and RT-qPCR confirmed the upregulation of prmABCD by dioxane



He et al., 2017. Environ. Sci. Technol. Lett. 4(11):494-499

Characterize Novel Gene Sequences

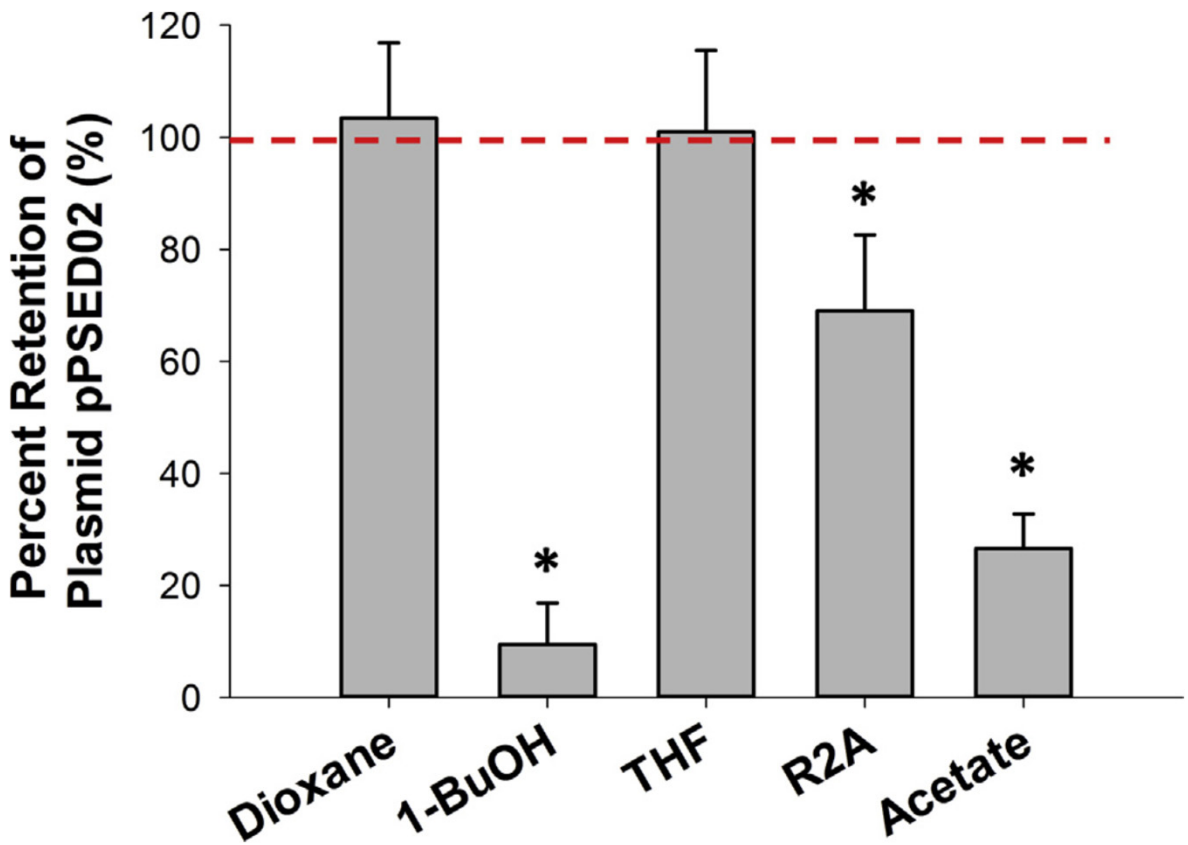
Designed biomarker prmA to target prmABCD

- Two criteria
  - Avoid di-iron centers (DE*RH motif): active site
  - Target the surrounding hydrophobic residues: substrate recognition and binding
- Biomarker is selective (no false positives)
- Similar to *thmA*, dioxane degradation rates were significantly correlated with *prmA* (not 16S rRNA) concentrations

He et al., 2017. Environ. Sci. Technol. Lett. 4(11):494-499

Characterize Novel Gene Sequences

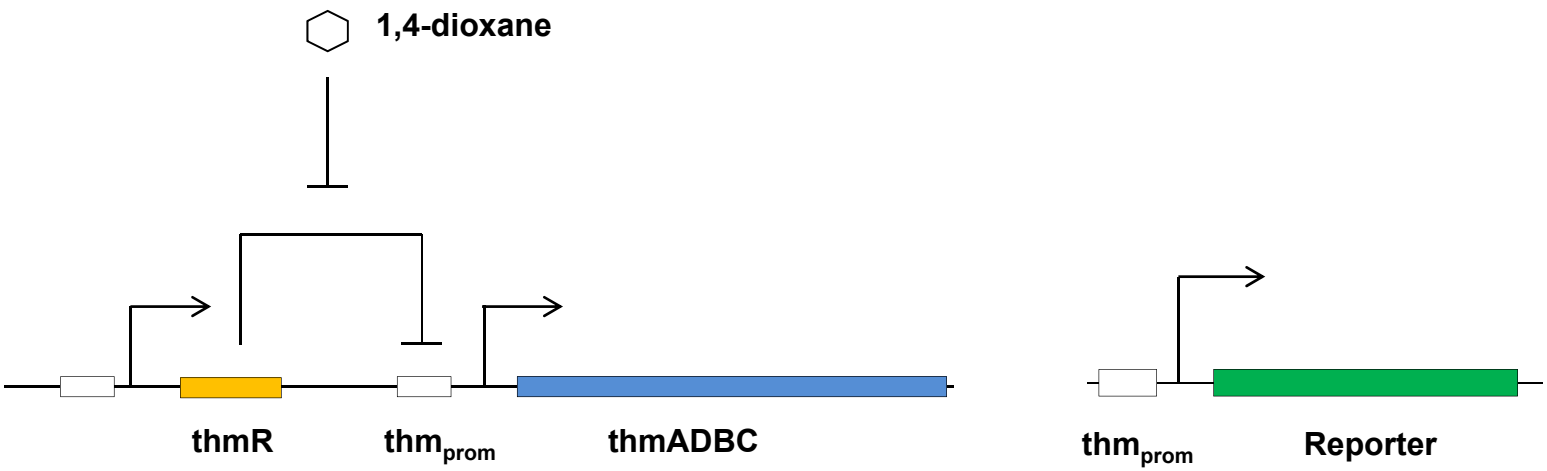
Plasmid-coded degradation capacity is lost when auxiliary substrates are added



Li et al., 2017. Water Res. 112: 217-225

Next Steps

- Use reporter protein to monitor dioxane degradation activities and choose appropriate biostimulants
  - Insert reporter into chromosome or plasmid using transposon-directed mutagenesis
  - Use reporter expression dynamics to screen for novel inducers or monitor plasmid loss under non-inducing growth conditions



Conclusions

- *dxmA* conservatively indicates dioxane degradation capacity
  - Not a single false positive yet!
  - Does not detect all degraders (false negatives are possible)
- Dioxane degradation is more likely at higher concentrations, provided that O_2 is available
- Fluorescence activated cell sorting (FACS) with our catabolic gene probes highlight the ability to separate genomes of unknown potential dioxane degraders
- Dioxane degradation was observed in uncontaminated soil,
- *Mycobacterium* spp. dominated in our enriched consortia
- Discovered new catabolic gene (*prmABCD*) in *Mycobacterium dioxanotrophicus* PH-06, and designed a selective probe

DoD Benefits

- Many dioxane plumes could be effectively managed by MNA at a small fraction of alternative treatment costs
- This project contributes a simple forensic tool to enable site-specific decisions to select or reject MNA, and to assess intrinsic bioremediation performance

Acknowledgements

- Dr. Jacques Mathieu
  - Research Scientist at Rice University
  - Provides expertise in molecular biotechnology
- Dr. Yu Yang
  - Postdoctoral Associate at Rice University
  - DNA probes for flow cytometry and FACS
- Ya He
  - Graduate student at Rice University
  - Isolates novel degraders, transcriptomic studies with pure cultures
- Ruonan Sun
  - Graduate student at Rice University
  - Monitors degradation of 1,4-dioxane in microcosms

For additional information, please visit

https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Emerging-Issues/ER-2301

Speaker Contact Information

alvarez@rice.edu; 713-348-5903

SERDP DOD • EPA • DOE | ESTCP

Q&A Session 1

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SERDP & ESTCP Webinar Series

Improving the Conceptual Site Model for Management of 1,4-Dioxane and Chlorinated Solvent Contaminated Sites

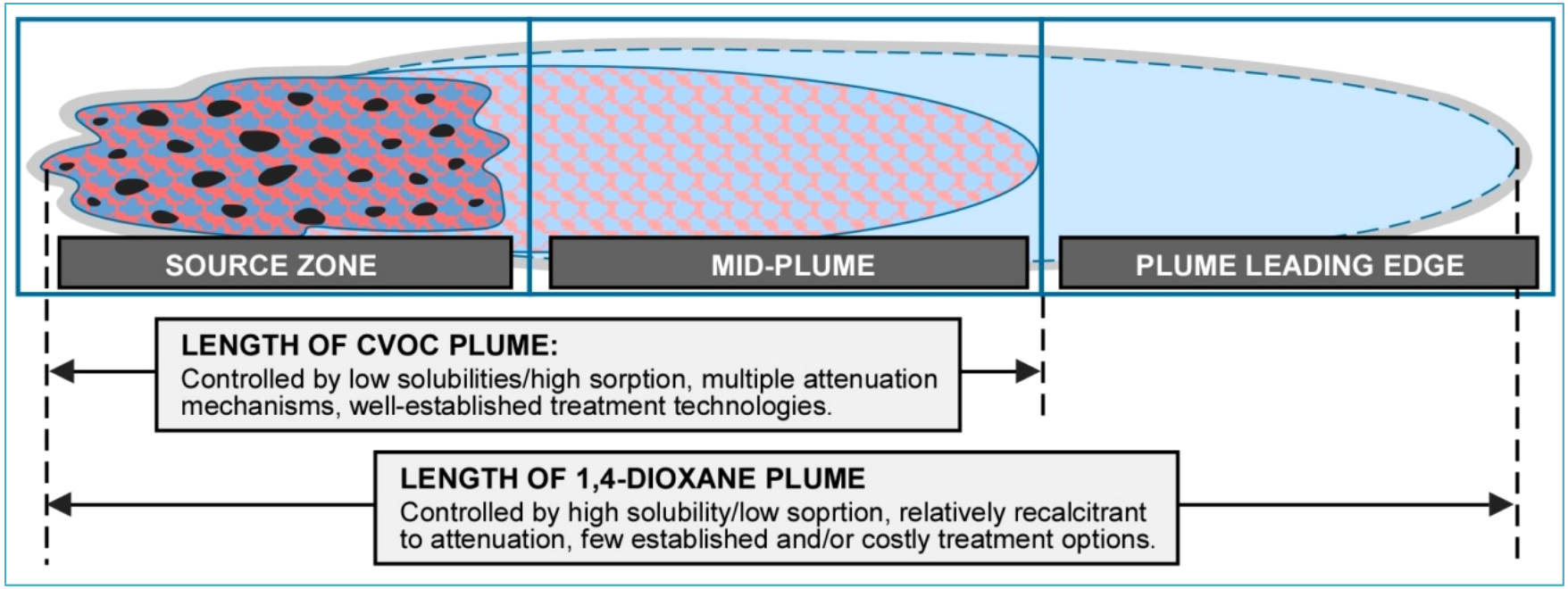
Dr. David Adamson
GSI Environmental Inc.

SERDP DOD • EPA • DOE | ESTCP

Agenda

- Objectives
- Dioxane conceptual model
  - Plume length
  - Delineation
  - Attenuation
  - Treatment trains
  - Matrix diffusion
- Implications for site management

Traditional Conceptual Model for Dioxane and CVOC* Sites



Model is based on perception that was never technically validated

*CVOC – Chlorinated volatile organic compounds

Overall Objective

- Improve the technical basis for conceptual site model development and management strategies for dioxane and CVOCs
- Methods
  - “Big data” studies
  - Proof-of-concept modeling
  - Focused field studies
  - Bench-scale treatability testing

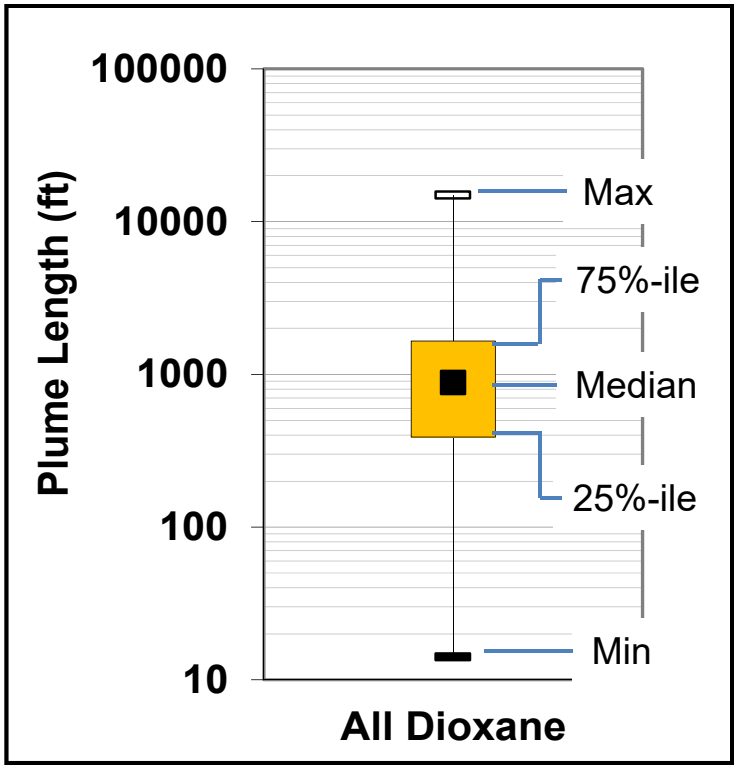
Dioxane Conceptual Model

Hypothesis testing

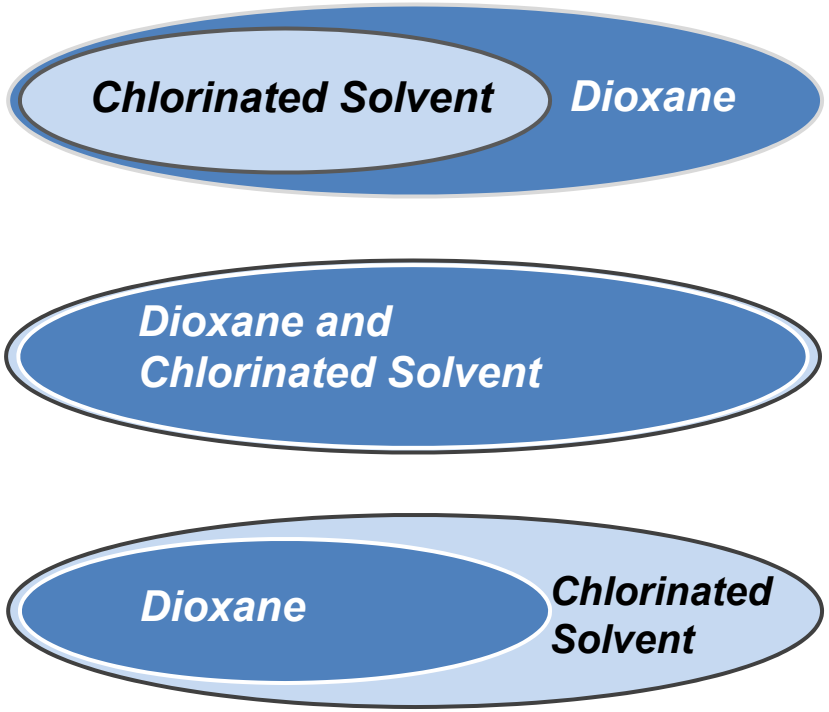
Hypothesis	Do Data Support Hypothesis?
Dioxane usually forms “large, dilute” plumes	
Dioxane plumes are longer than chlorinated solvent plumes	
Dioxane plumes are poorly delineated	
Dioxane is not attenuating	
Geochemical conditions and co-occurring contaminants influence dioxane attenuation	
Treatment trains can be effective remediation strategy for dioxane	
Matrix diffusion is not an important process for dioxane	

What is a “Typical” Dioxane Plume?

	Dioxane
Median length	880 ft
Median maximum concentration	370 µg/L
% Co-occurrence with chlorinated solvents	95%
% with some form of remediation	> 90% n = 109 sites



Are Dioxane Plumes Longer Than Chlorinated Solvent Plumes?



Dioxane plume is longest = 21%

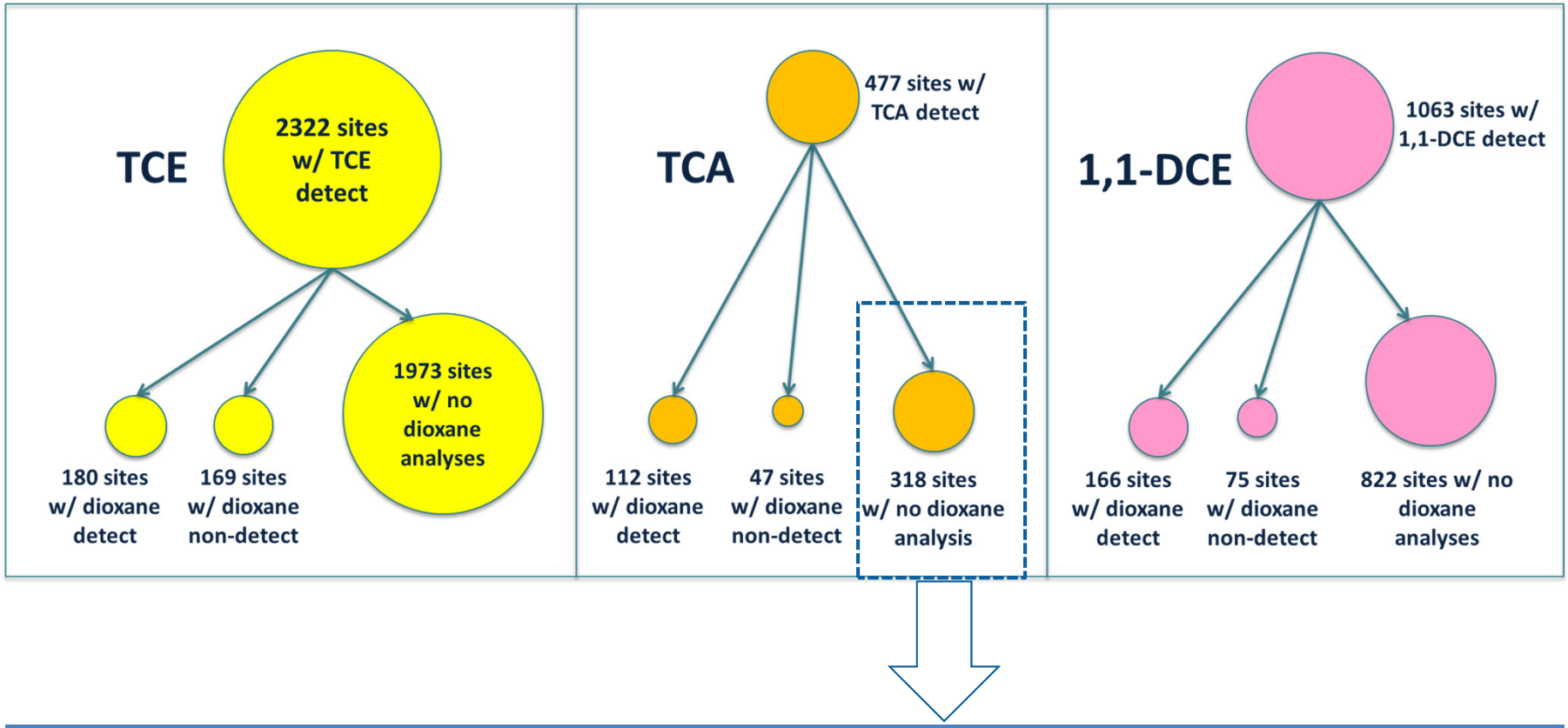
Plumes are same length = 23%

Chlorinated solvent plume is longest = 56%

n = 105 sites where dioxane and chlorinated solvents co-occur

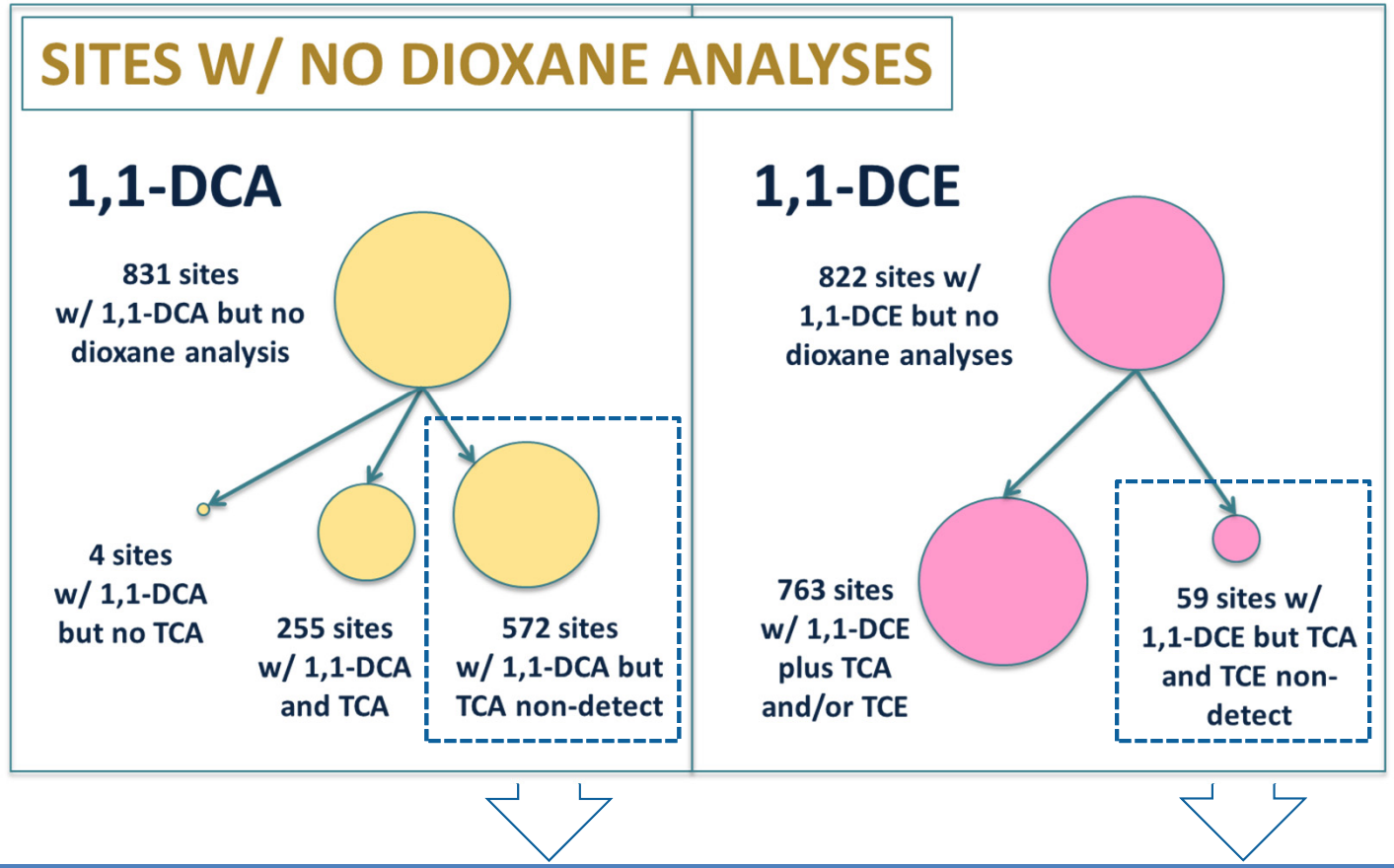
Adamson et al., 2014, ES&T Letters 1: 254-258

Are Dioxane Sites Poorly Delineated?



Evidence for large number of "unidentified" dioxane sites:
(318 sites)*(75% of TCA sites w/ dioxane) = 239 "unidentified" dioxane sites

Are Dioxane Sites Poorly Delineated?



Evidence for large number of "unidentified" dioxane sites:
400 to 500 additional "unidentified" dioxane sites

Are Dioxane Sites Attenuating?

Median order of magnitude (OoM) reduction

	Median OoM Reduction at Dioxane Sites [a] (# of sites)	Comparison to Dioxane [b] (p-value [c])
Dioxane	0.59 (n=106)	--
TCA	0.94 (n=90)	Larger than dioxane (p=0.01)
TCE	0.45 (n=129)	Not different than dioxane (p=0.14)
1,1-DCE	0.57 (n=121)	Not different than dioxane (p=0.77)
1,1-DCA	0.43 (n=124)	Not different than dioxane (p=0.10)

[a] OoM reduction in well with historic maximum sitewide concentration

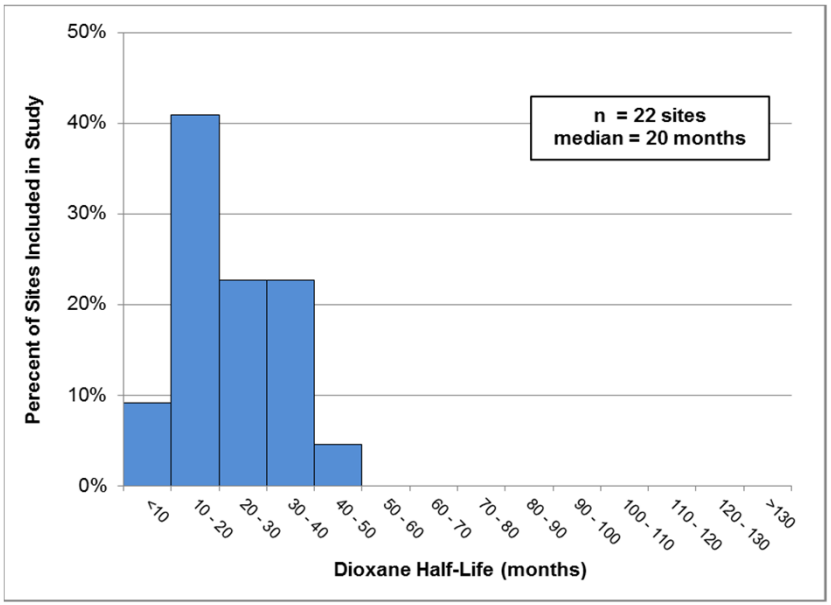
[b] Same pattern is observed if all chlorinated solvent sites are included (i.e., sites with no detectable dioxane or no dioxane analyses) or only 2013 data are used for current max concentration

[c] Wilcoxson Signed Rank test (non-parametric, two-tailed; $p<0.05$)

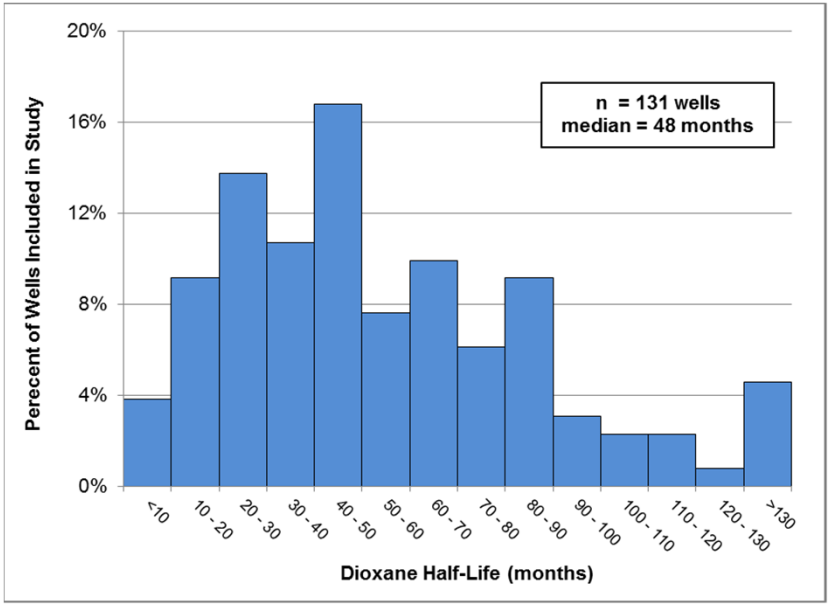
Are Dioxane Sites Attenuating?

Source attenuation rate coefficients

Site-wide values
(Geotracker data)



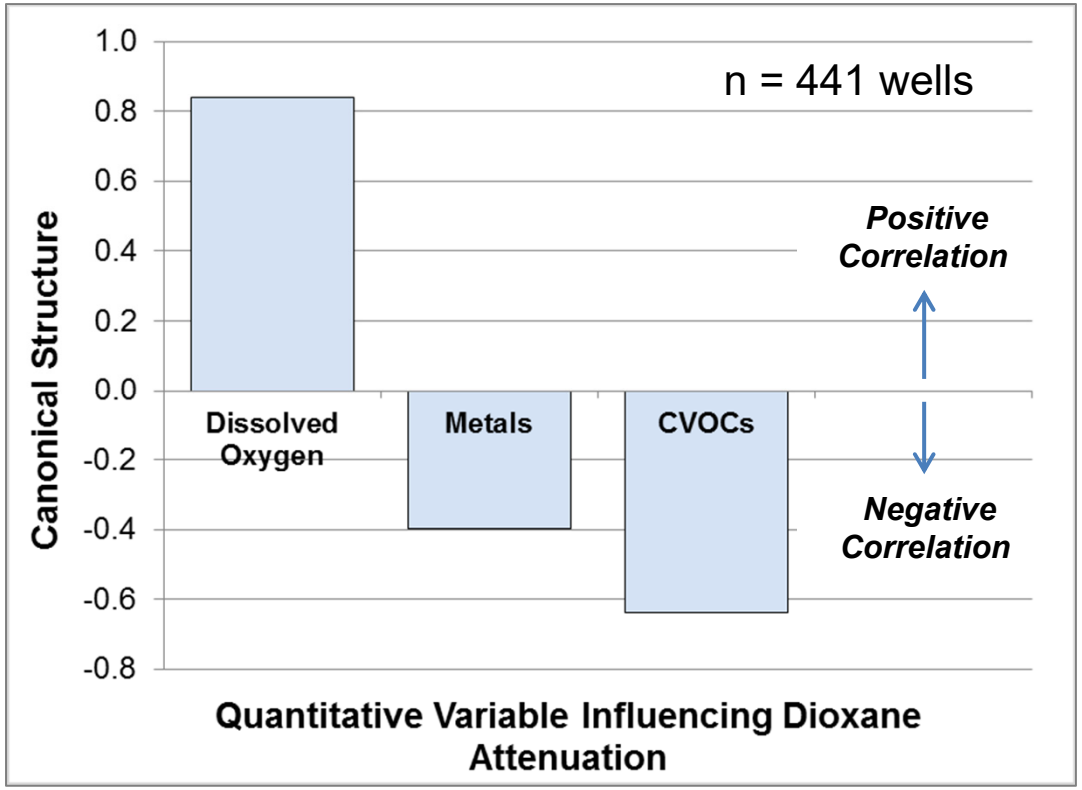
Well-specific values
(Air Force data)



Adamson et al., 2015, ES&T 49: 6510-6518

Are Dioxane Sites Attenuating?

Linear discriminant analysis of Air Force data

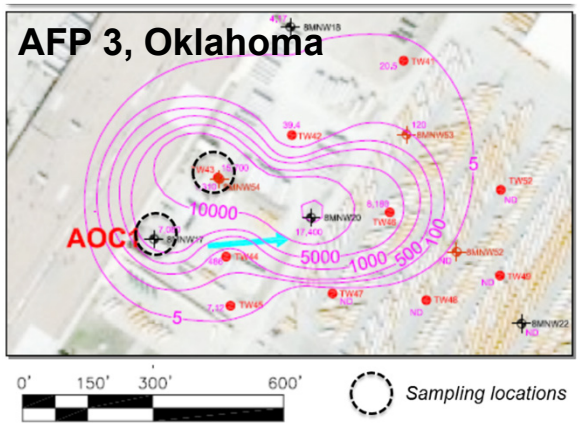


Influences of key variables on field rates are consistent with laboratory study observations of dioxane biodegradation

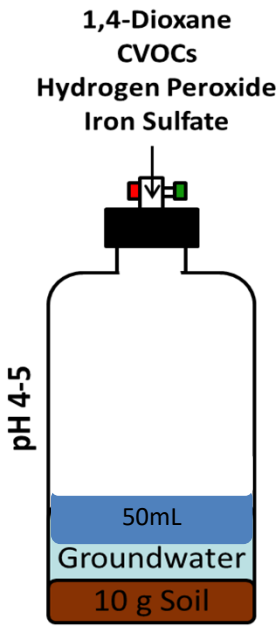
Adamson et al., 2015, ES&T 49: 6510-6518

Can Treatment Trains be an Effective Strategy for Dioxane Sites?

- Oxidation + biodegradation
- Oxidation + catalysis
- Catalysis + biodegradation
- Oxidation + catalysis + biodegradation



Monitor contaminant removal and shifts in microbial community over time

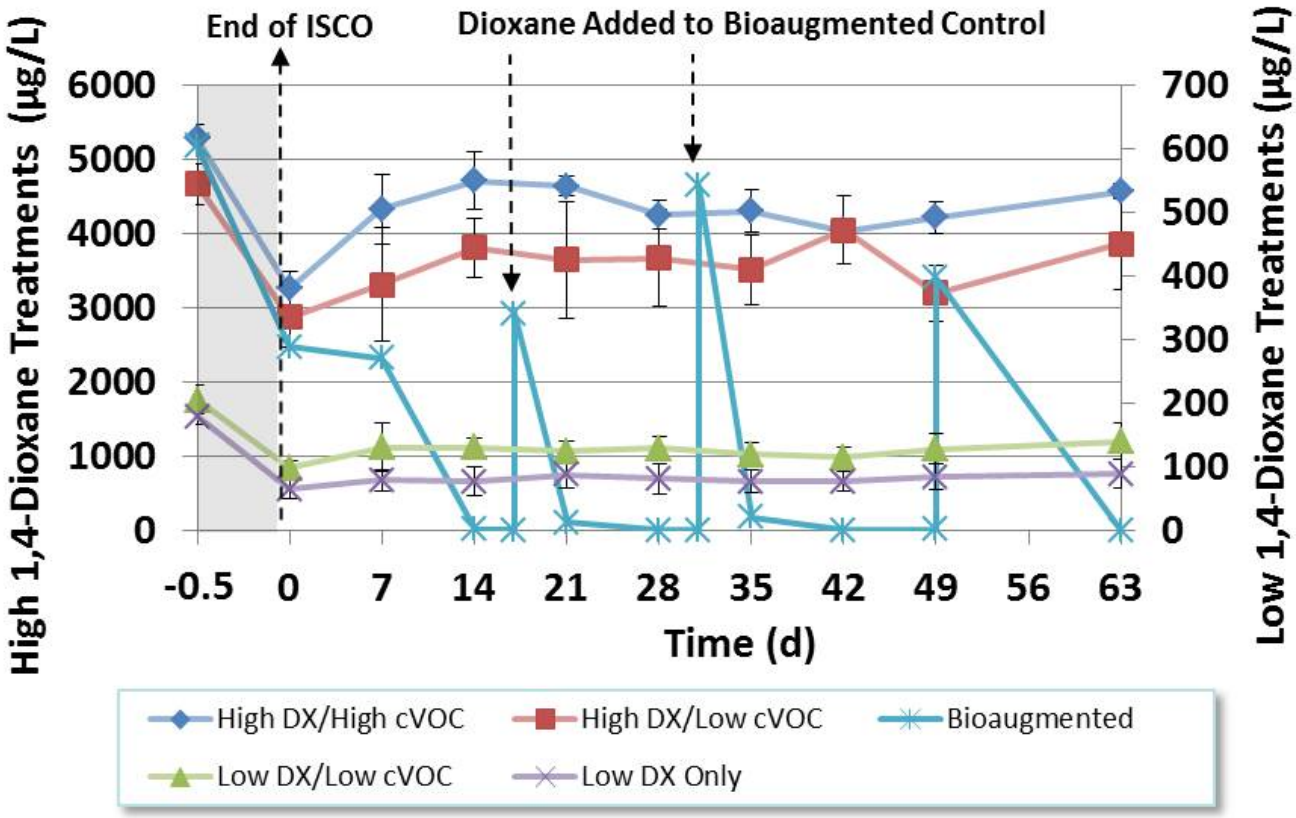


Can Treatment Trains be an Effective Strategy for Dioxane Sites?

- *Example: Oxidation + biodegradation*
  - Oxidation to remove CVOCs (inhibitory to biodegradation)
  - Bioaugment to overcome lack of native dioxane degraders
  - Community shifts to oxidant tolerant species with long-term recovery

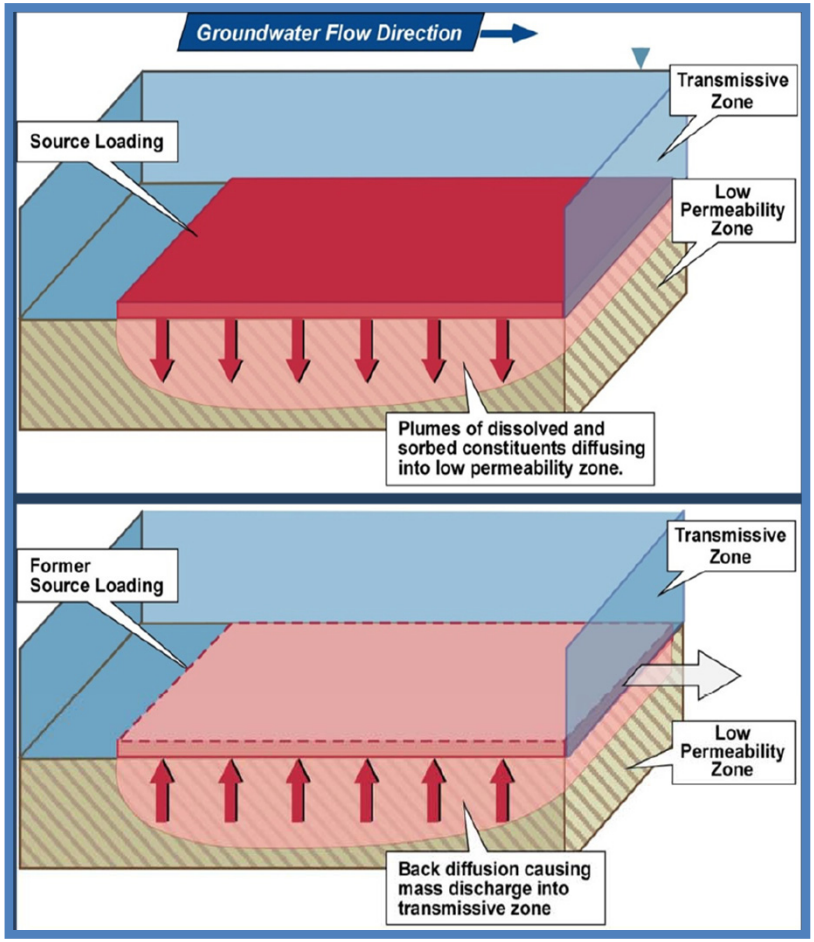
Can Treatment Trains be an Effective Strategy for Dioxane Sites?

- *Example: Oxidation + biodegradation*



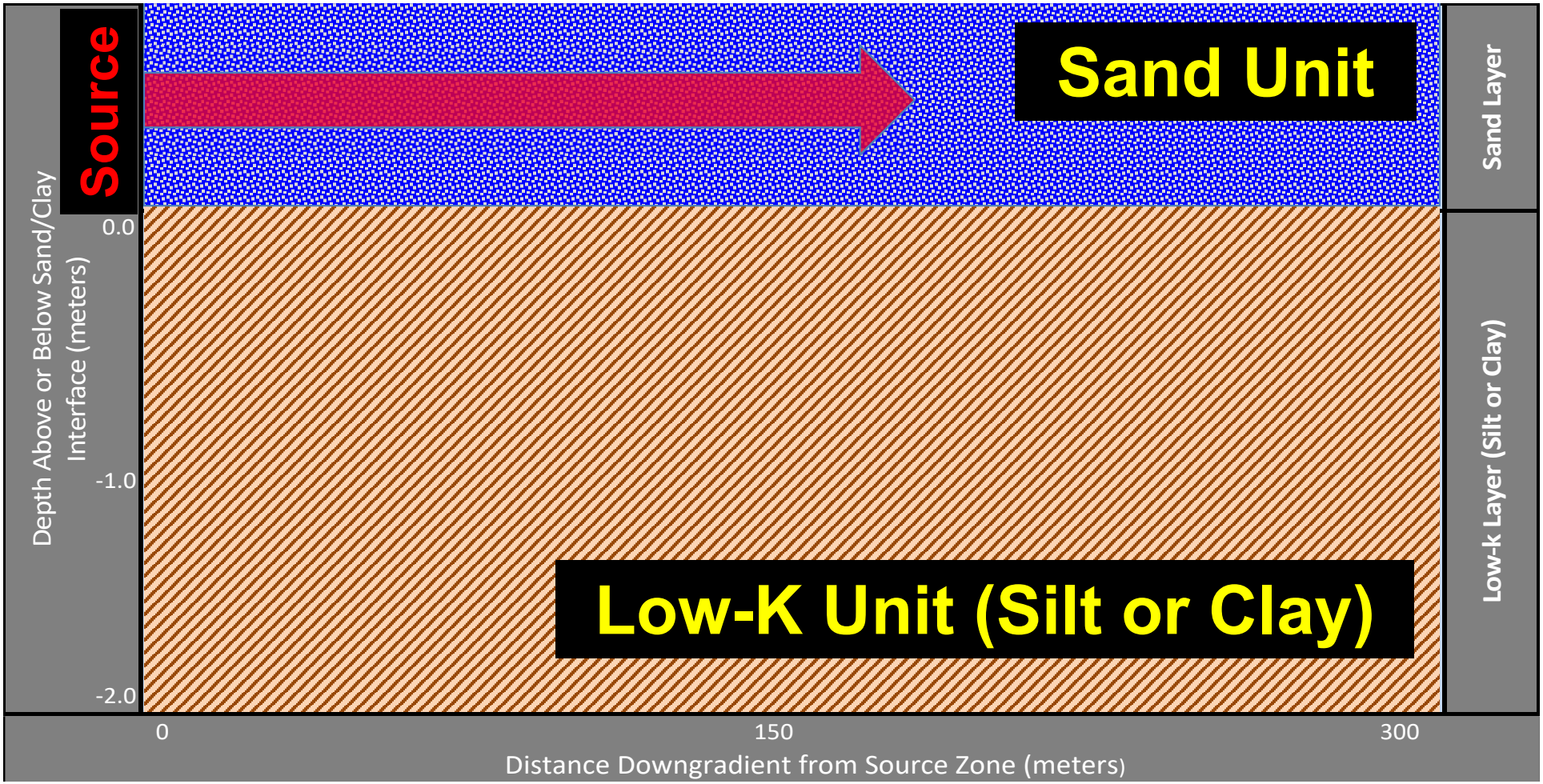
Miao et al., 2018, in preparation for submittal to Water Research

Is Matrix Diffusion Important for Dioxane Sites?

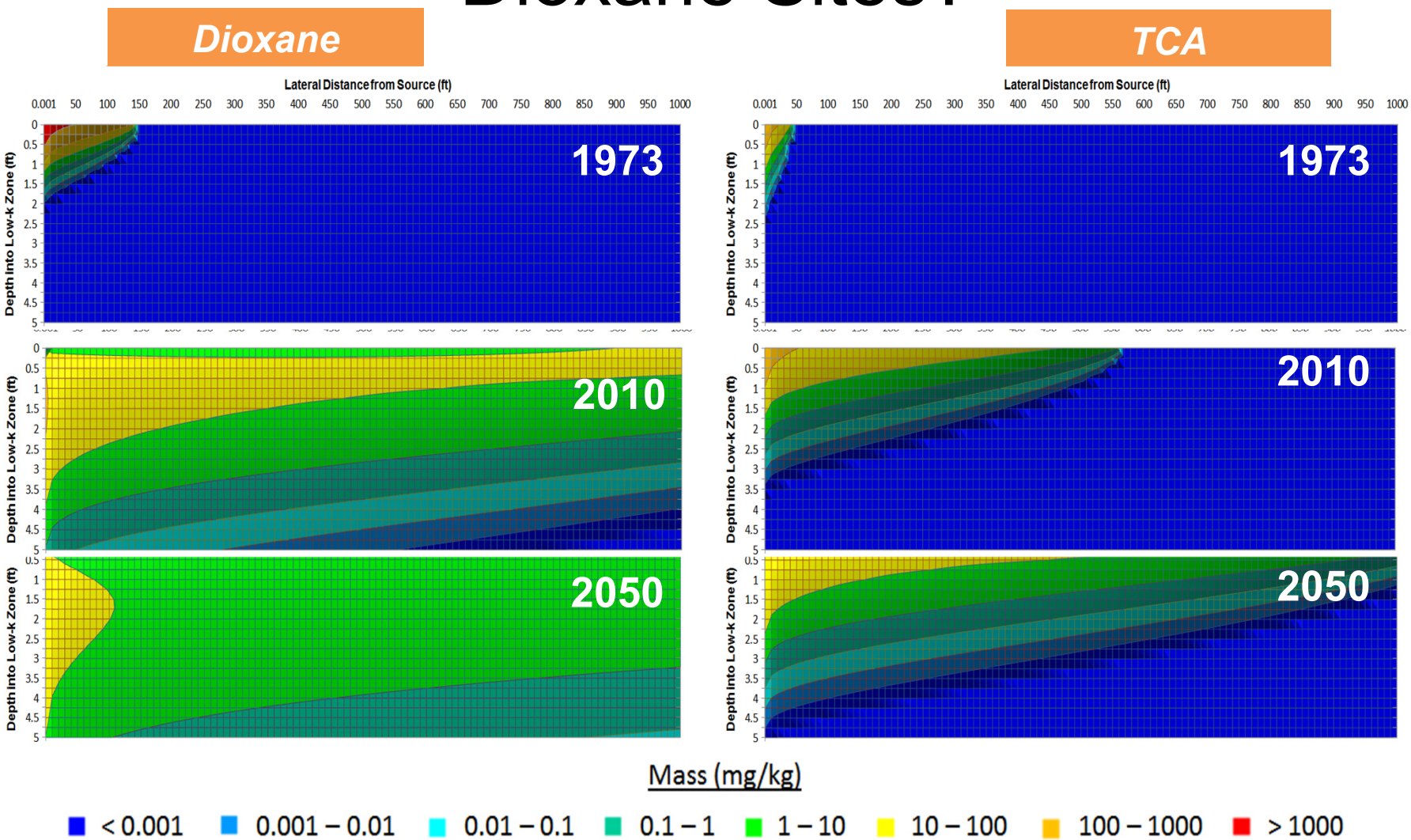


- Recognize importance at chlorinated solvents
- Contributes to long-term persistence of contaminants stored in these lower-k zones
- Exacerbated by high concentration gradient and mobility
- Mitigated by short “loading period” and lack of sorption

Matrix Diffusion Modeling: Co-Release of Dioxane and TCA in 1970



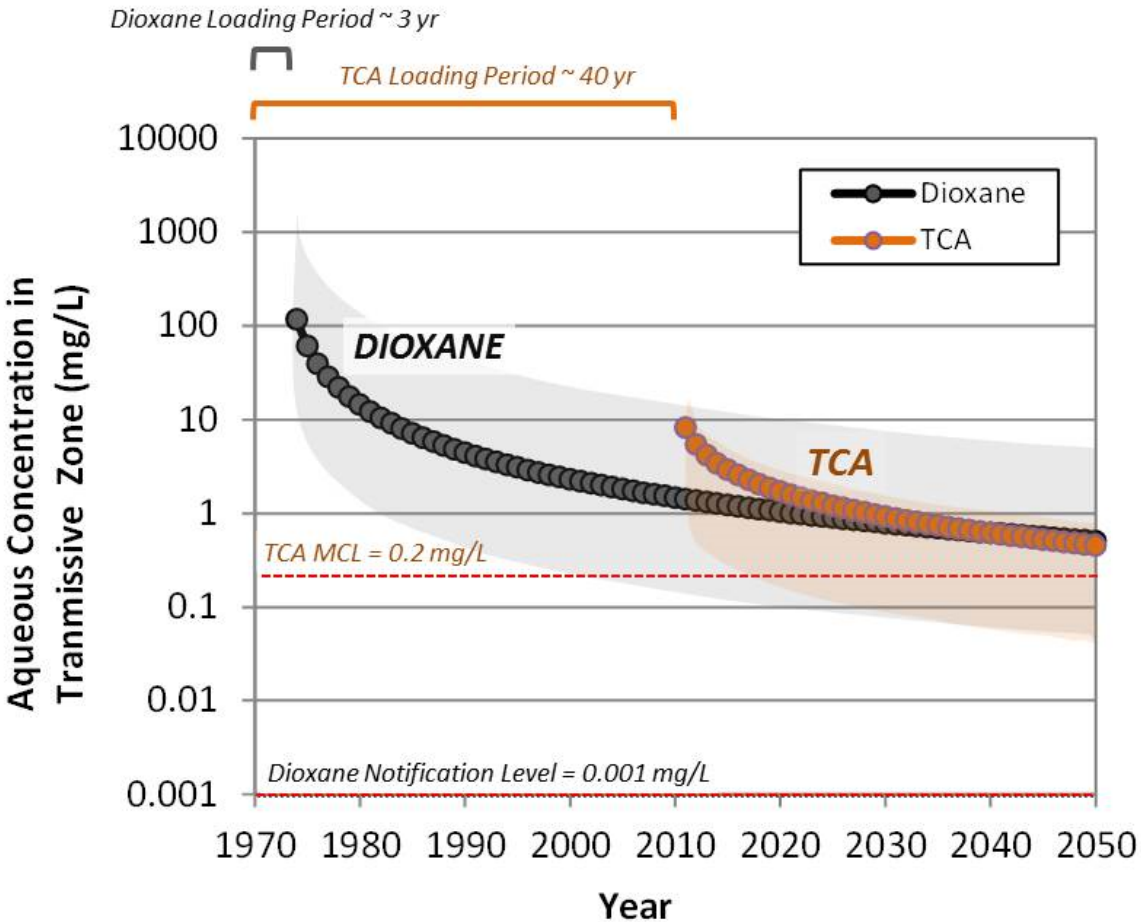
Is Matrix Diffusion Important for Dioxane Sites?



Adamson et al., 2016, Sci. Total Environ. 562: 98-107

Is Matrix Diffusion Important for Dioxane Sites?

Source zone modeling results



Adamson et al., 2016, Sci. Total Environ. 562: 98-107

Dioxane Conceptual Model

Hypothesis test results, 1 of 2

Hypothesis	Do Data Support Hypothesis?
Dioxane usually forms “large, dilute” plumes	**NO**, similar to chlorinated solvents
Dioxane plumes are longer than chlorinated solvent plumes	**YES**, if co-occurrence ignored **NO**, for co-occurring plumes
Dioxane plumes are poorly delineated	**NO**, but data suggest many *unidentified* plumes
Dioxane is not attenuating	**NO**, evidence for dioxane attenuation at some sites; similar to 1,1-DCE, less than TCA

Dioxane Conceptual Model

Hypothesis test results, 2 of 2

Hypothesis	Do Data Support Hypothesis?
Geochemical conditions and co-occurring contaminants influence dioxane attenuation	**YES**, but many relationships not strongly predictive; attenuation correlated with co-occurring contaminants and geochemical conditions
Treatment trains can be effective remediation strategy for dioxane	**YES**, critical to remove co-occurring contaminants
Matrix diffusion is not an important process for dioxane	**NO**, can contribute significantly to long-term persistence

DoD Benefits

- Basis for monitored natural attenuation (MNA) as a remedy for 1,4-dioxane is much stronger
  - Plumes are generally dilute and often similar in size to co-occurring chlorinated solvents
  - Data suggests many plumes are attenuating
  - Lots of new ways to collect lines of evidence for MNA (biomarkers, isotopes)

Conclusions

- Dioxane source zones are not like other source zones
- Persistence may be a result of matrix diffusion processes (secondary source)
  - Matches expectations from multi-site survey of concentrations (most sites are “dilute”)
  - Starting to see evidence of this at field sites
  - Important to evaluate as part of site-specific conceptual site model
- Traditional source treatment approaches not very effective at these types of sites

Conclusions

- Site management should focus on longer-term management or targeted treatment
- Evidence that MNA is a viable option
- If treatment is required and/or there is a identifiable source to target, then consider treatment trains
  - Provides a way to remove inhibitory co-occurring CVOCs

For additional information, please visit

https://www.serdp-estcp.org/Program-Areas/Environmental-Restoration/Contaminated-Groundwater/Emerging-Issues/ER-2307

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Q&A Session 2

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