



Comparison of Enzyme Activity Probe Response with TCE Degradation Rates at Five Contaminated Sites in the US

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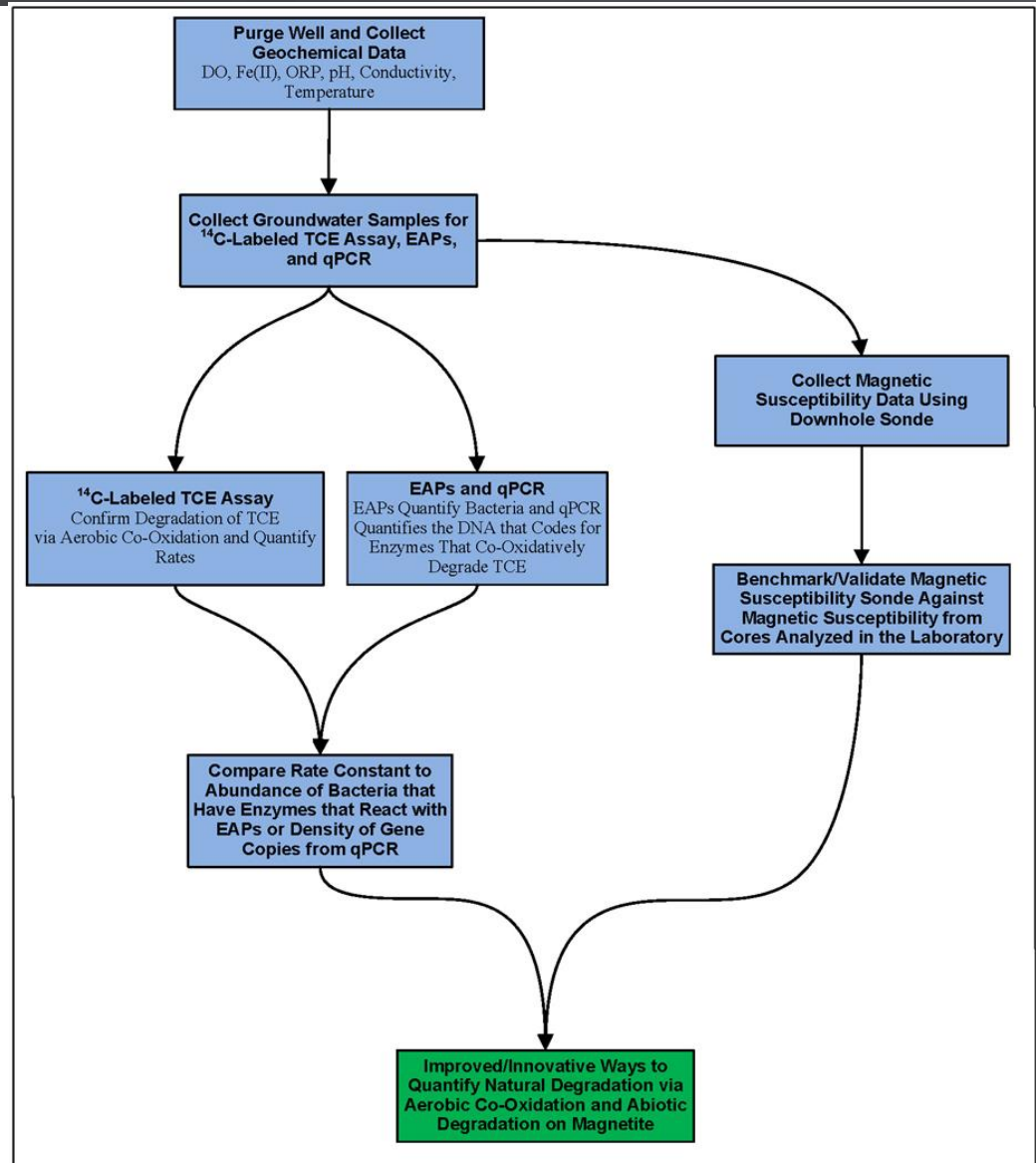
Presentation Outline

- Introduction
- EAP Background
- Overall Analysis Flow Diagram
- Contaminated Sites/Data
 - TCAAP
 - Plattsburgh
 - Hopewell
 - Hill AFB
 - Tooele Army Depot
- EAP/TCE Degradation Rates
- Conclusions
- Acknowledgements

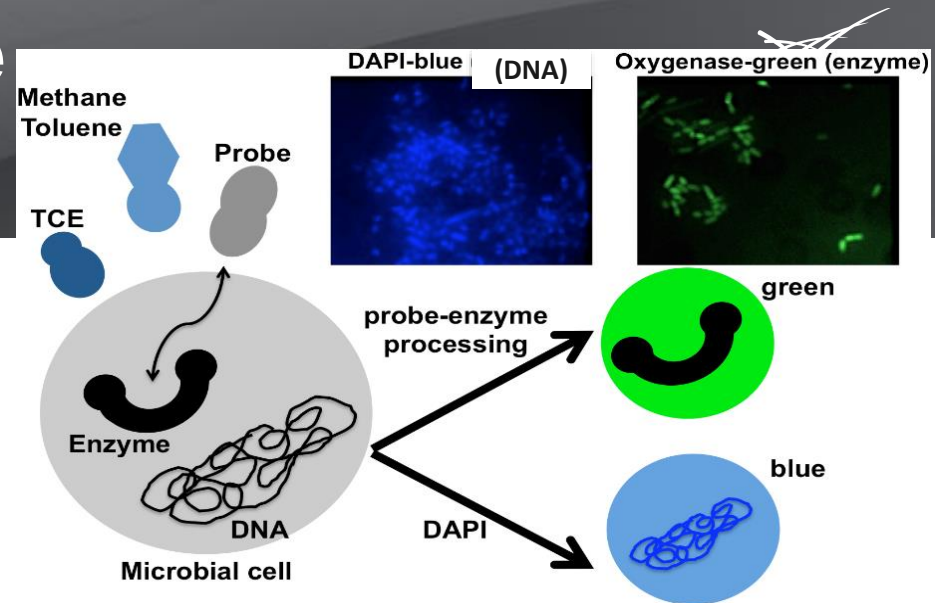


Technical Objectives

- Provide method to readily and inexpensively acquire data required to evaluate monitored natural attenuation at chlorinated solvent contaminated sites
 - Presence of enzymes (qPCR)
 - Activity of Enzymes (EAP)
- Compare results to TCE Co-oxidation rate using ^{14}C -labeled TCE assays

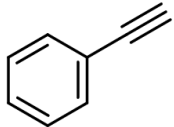
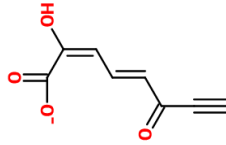
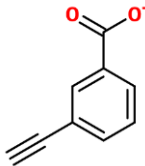
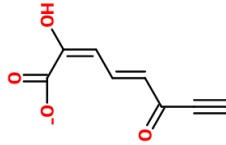
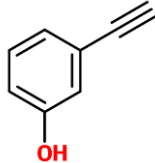
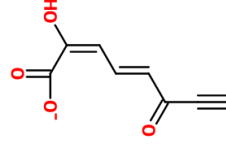
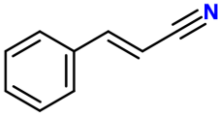
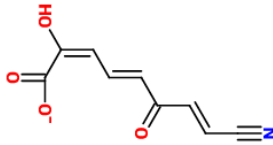
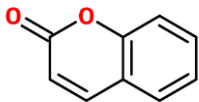
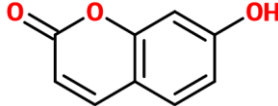


Enzyme Activity Probe Background



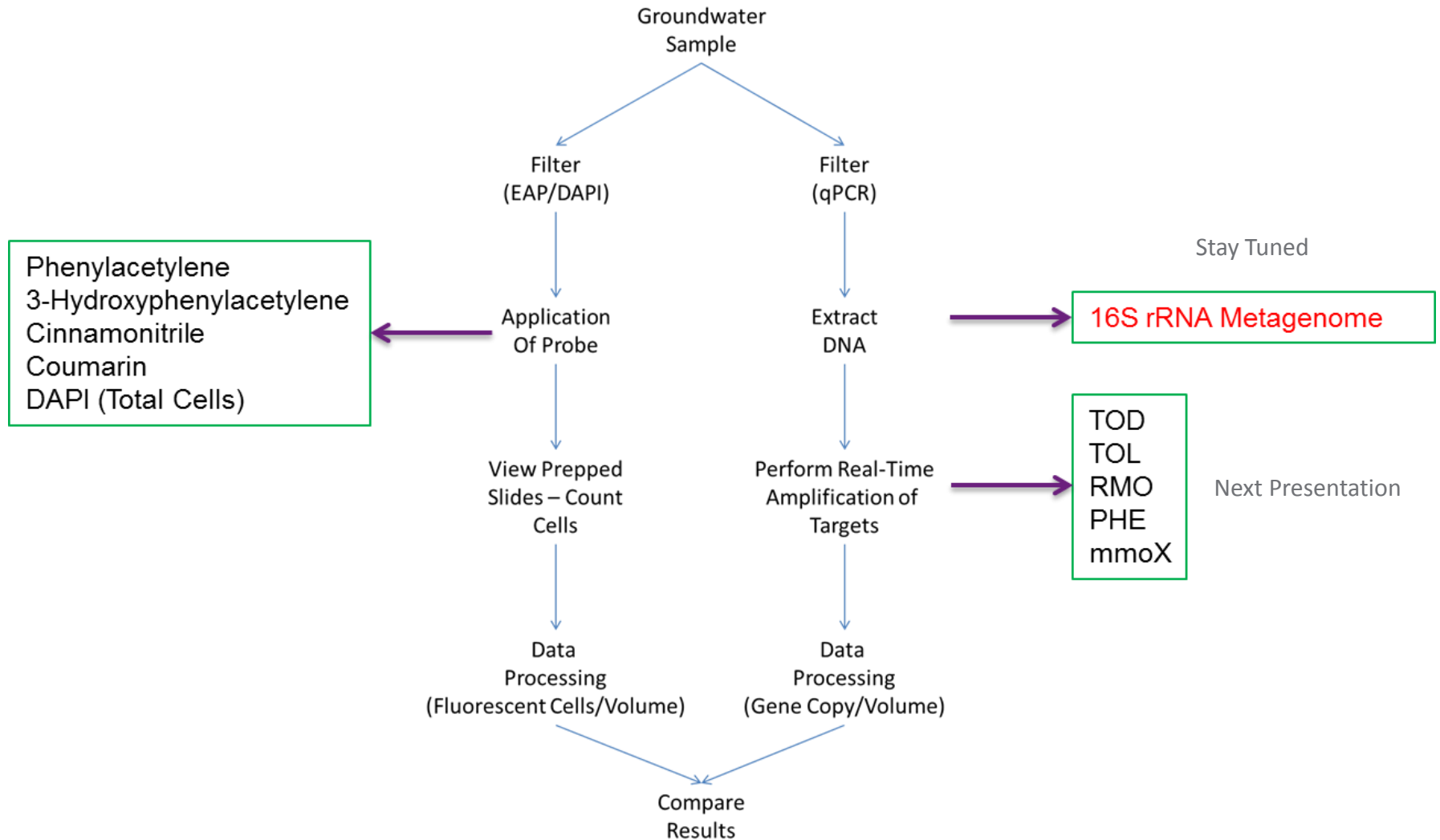
Probe	Pathway
3-Hydroxyphenylacetylene	Toluene-2-monooxygenase Toluene-3-monooxygenase Toluene-2,3-dioxygenase
Phenylacetylene	Toluene-2,3-dioxygenase Toluene-3-monooxygenase Toluene-2-monooxygenase Toluene-side-chain-monooxygenase
<i>trans</i> -cinnamionitrile	Toluene-2,3-dioxygenase
Coumarin	Soluble methane monooxygenase

Probe Sets and Products

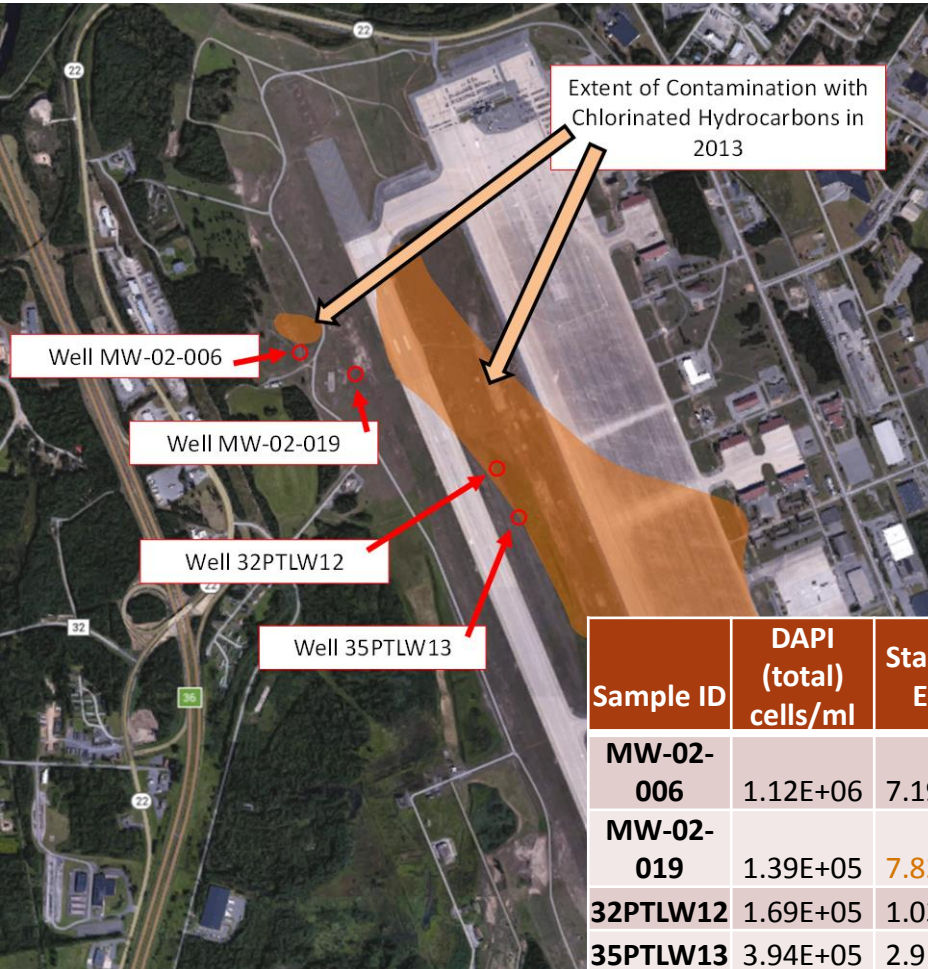
Probe	Structure	Enzyme Pathway(s)	Fluorescent product	Fluorescent structure
Phenylacetylene		<ul style="list-style-type: none"> •Toluene-2,3-dioxygenase 	2-hydroxy-6-oxo-7-octyn-2,4-dienoate	
3-ethynylbenzoate		<ul style="list-style-type: none"> •Side-Chain-monoxygenase 	2-hydroxy-6-oxo-7-octyn-2,4-dienoate	
3-hydroxyphenylacetylene		<ul style="list-style-type: none"> •Toluene-2,3-dioxygenase •Toluene-2-monoxygenase •Toluene-3-monoxygenase 	2-hydroxy-6-oxo-7-octyn-2,4-dienoate	
Cinnamonitrile		<ul style="list-style-type: none"> •Toluene-2,3-dioxygenase 	2-hydroxy-6-oxo-8-cyano-octa-2,4,7-trienoate	
Coumarin		<ul style="list-style-type: none"> •Soluble methane monoxygenase 	7-hydroxycoumarin	



Overall Test Design



Former Plattsburgh AFB, NY



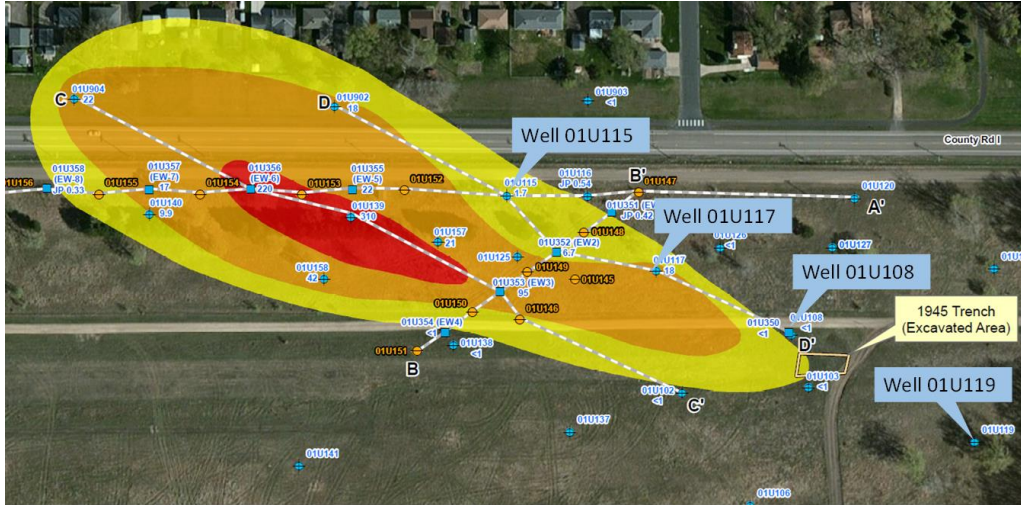
sMMO

Sample ID	Coumarin
MW-02-006	+
MW-02-019	+
32PTLW12	-
35PTLW13	-

Aromatic Oxygenases

Sample ID	DAPI (total) cells/ml	Standard Error	PA (T2-mono) cells/ml	Standard Error	Cinn (T23-di) Cells/ml	Standard Error	3HPA (T3-mono) cells/ml	Standard Error
MW-02-006	1.12E+06	7.19E+04	4.01E+04	8.49E+03	1.43E+04	4.53E+03	2.47E+04	6.28E+03
MW-02-019	1.39E+05	7.82E+03	1.50E+03	8.72E+02	7.13E+02	4.71E+02	2.02E+03	9.37E+02
32PTLW12	1.69E+05	1.03E+04	2.27E+03	1.06E+03	1.76E+03	1.04E+03	1.46E+03	8.88E+02
35PTLW13	3.94E+05	2.91E+04	8.39E+02	6.81E+02	9.25E+02	7.88E+02	9.84E+02	6.40E+02

Twin Cities Army Ammunition Plant (TCAAP)



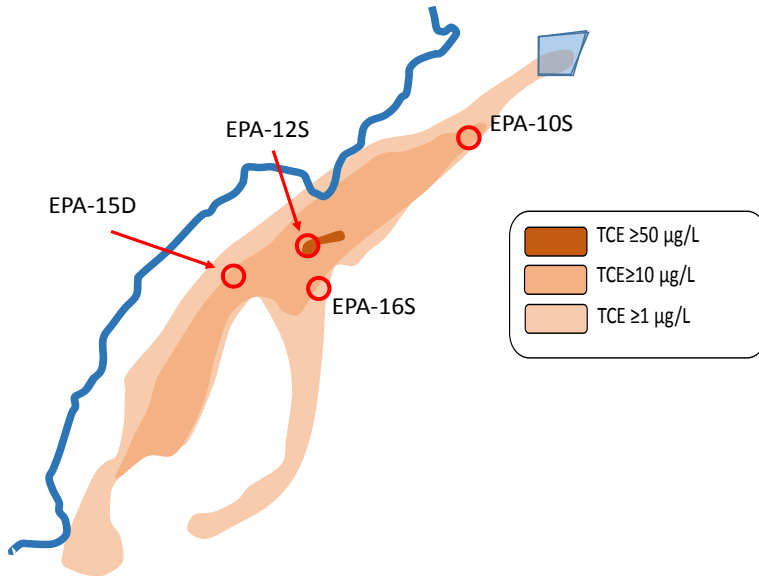
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Sample ID	Coumarin
01U119	-
01U108	-
01U117	-
01U115	-

Aromatic Oxygenases

Sample ID	DAPI (total) cells/ml	Standard Error	PA (T2-mono) cells/ml	Standard Error	Cinn (T23-di) Cells/ml	Standard Error	3HPA (T3-mono) cells/ml	Standard Error
01U119	7.11E+05	4.29E+04	1.38E+03	8.37E+02	1.55E+03	1.06E+03	5.31E+03	2.80E+03
01U108	6.91E+05	4.64E+04	2.03E+03	1.21E+03	5.46E+02	4.89E+02	2.36E+03	1.67E+03
01U117	4.07E+05	2.36E+04	4.06E+02	4.06E+02	4.55E+02	3.42E+02	5.91E+02	4.85E+02
01U115	5.85E+05	2.90E+04	1.41E+05	1.49E+04	1.52E+05	1.66E+04	1.91E+05	1.51E+04

Hopewell Junction



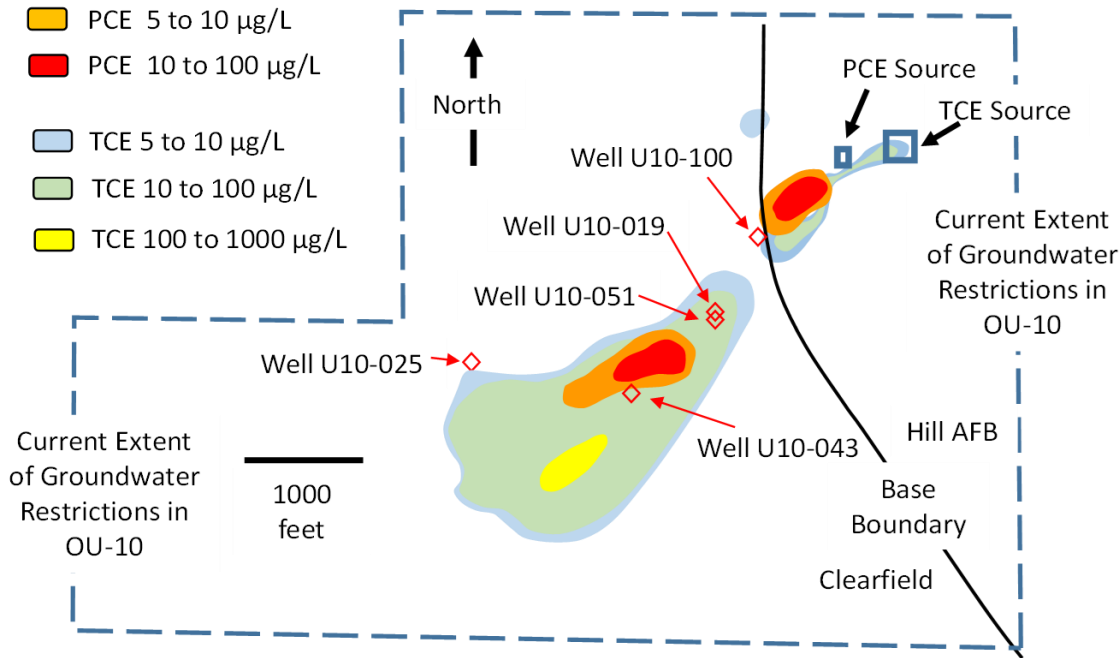
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Sample ID	Coumarin
EPA-10S	-
EPA-12S	-
EPA-15D	-
EPA-16S	-

Aromatic Oxygenases

Sample ID	DAPI (total) cells/ml	Standard Error	PA (T2-mono) cells/ml	Standard Error	Cinn (T23-di) Cells/ml	Standard Error	3HPA (T3-mono) cells/ml	Standard Error
EPA-10S	1.15E+05	5.67E+03	6.82E+02	5.20E+02	2.91E+02	2.62E+02	1.11E+03	6.33E+02
EPA-12S	1.25E+05	6.35E+03	3.05E+03	1.12E+03	1.74E+03	8.30E+02	2.29E+03	8.97E+02
EPA-15D	3.22E+05	1.95E+04	9.68E+03	2.85E+03	5.51E+03	2.12E+03	1.00E+04	3.01E+03
EPA-16S	1.81E+05	9.16E+03	8.63E+03	2.58E+03	7.09E+03	2.16E+03	1.31E+04	2.83E+03

Hill Air Force Base OU10



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Sample ID	Coumarin
U10-043	-
U10-025	-
U10-019	-

Aromatic Oxygenases

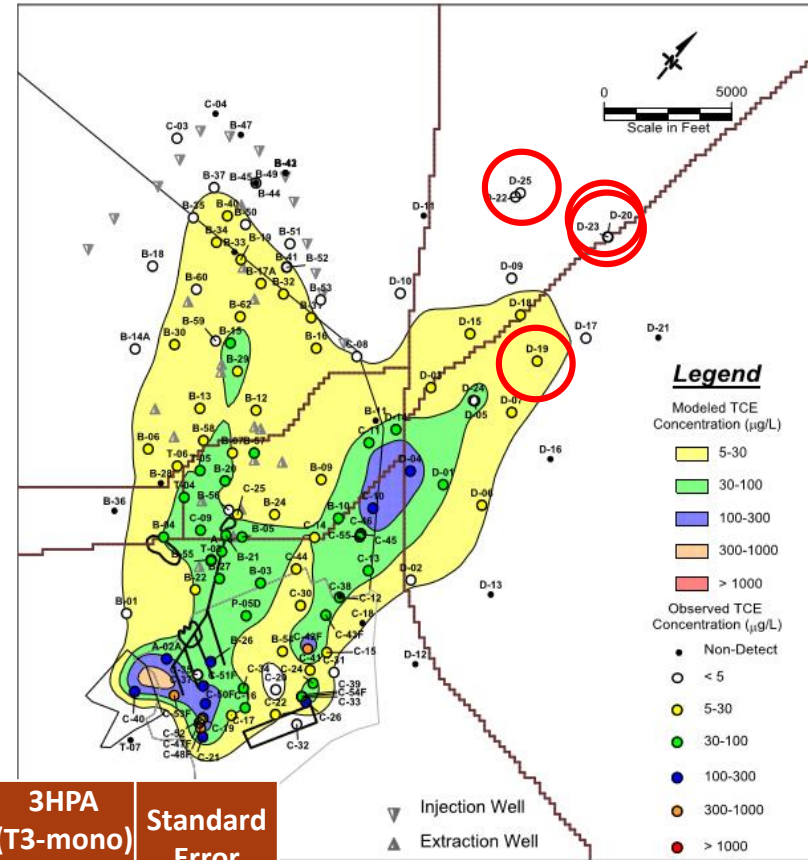
Sample ID	DAPI (total) cells/ml	Standard Error	PA (T2-mono) cells/ml	Standard Error	Cinn (T23-di) Cells/ml	Standard Error	3HPA (T3-mono) cells/ml	Standard Error
U10-043	1.97E+05	1.18E+04	8.80E+02	6.25E+02	3.03E+02	2.28E+02	7.87E+02	4.11E+02
U10-025	2.66E+05	1.15E+04	3.79E+02	3.19E+02	7.58E+01	7.58E+01	6.05E+02	4.35E+02
U10-019	1.21E+06	5.60E+04	5.73E+03	2.67E+03	5.40E+03	2.84E+03	3.12E+03	2.04E+03

sMMO

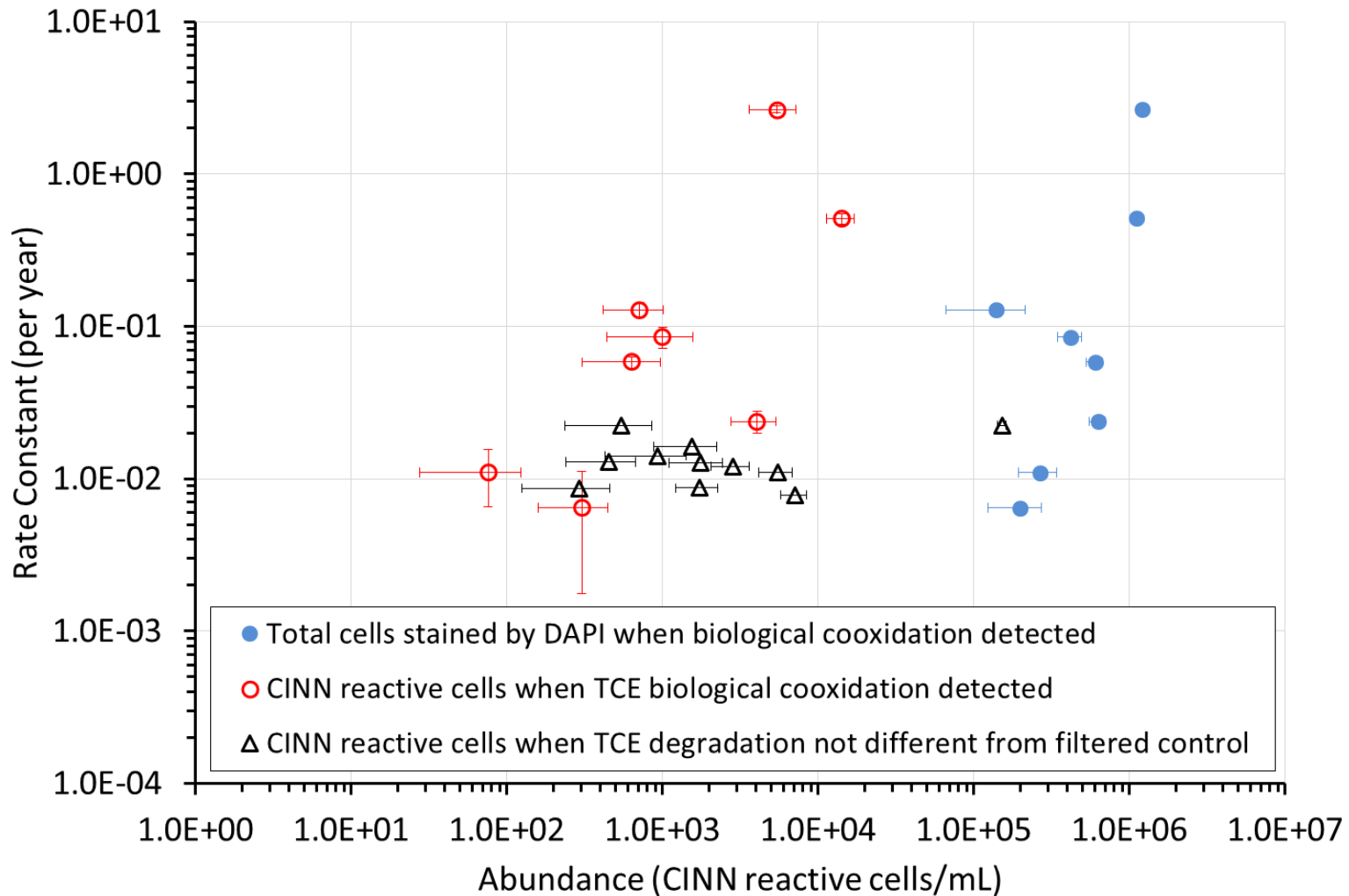
Sample ID	Coumarin
D-20	-
D-23	-
D-25	-
D-19	-

Aromatic Oxygenases

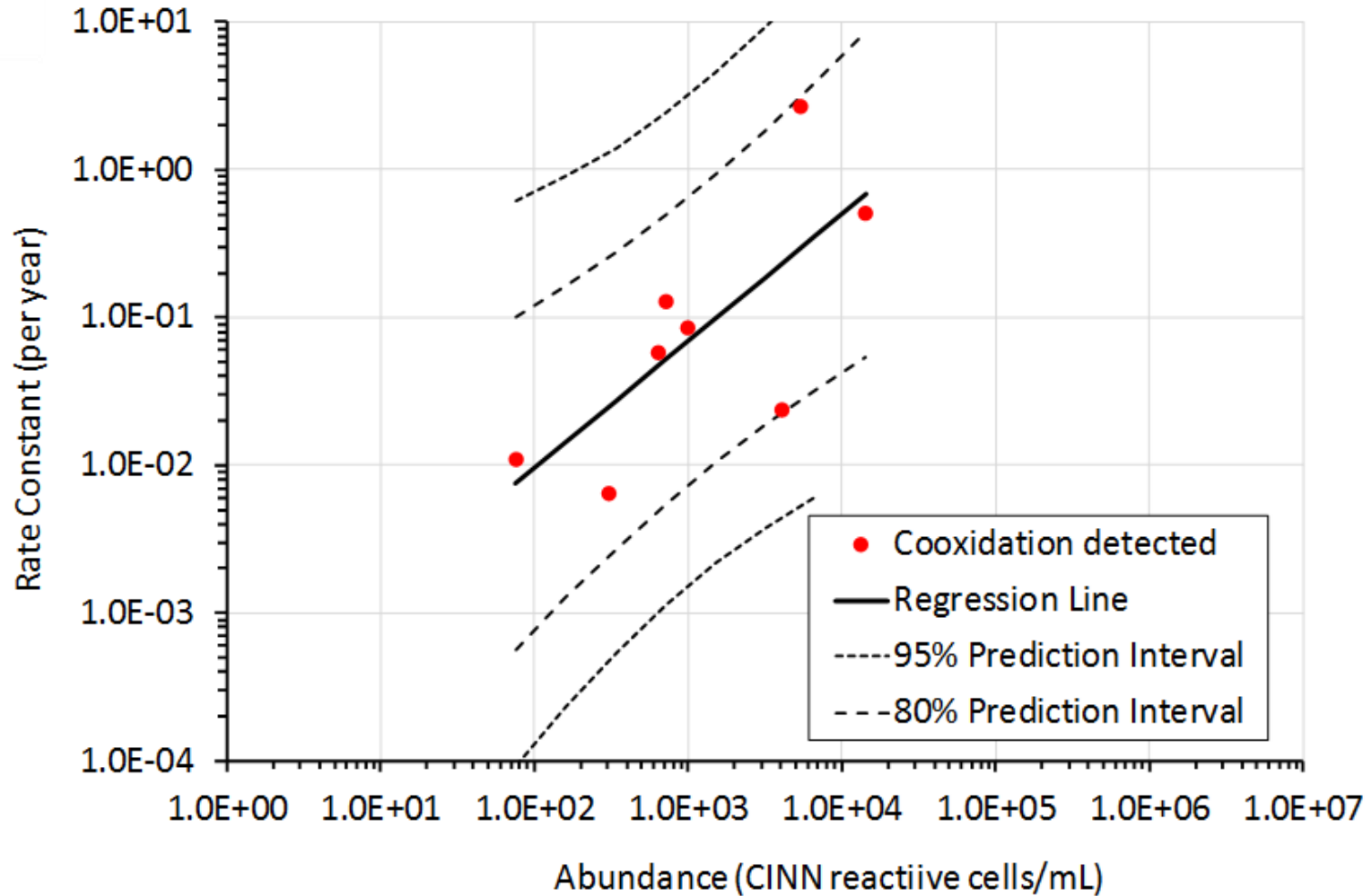
Sample ID	DAPI (total) cells/ml	Standard Error	PA (T2-mono) cells/ml	Standard Error	Cinn (T23-di) Cells/ml	Standard Error	3HPA (T3-mono) cells/ml	Standard Error
D-20	4.18E+05	2.50E+04	2.84E+03	1.57E+03	1.00E+03	8.88E+02	2.62E+03	1.11E+03
D-23	6.28E+05	4.50E+04	6.61E+03	2.91E+03	4.05E+03	2.01E+03	5.58E+03	2.29E+03
D-25	6.01E+05	4.20E+04	1.46E+03	1.12E+03	6.37E+02	5.24E+02	2.53E+03	1.35E+03
D-19	6.46E+05	3.80E+04	3.17E+03	1.71E+03	2.82E+03	1.20E+03	1.60E+03	1.14E+03



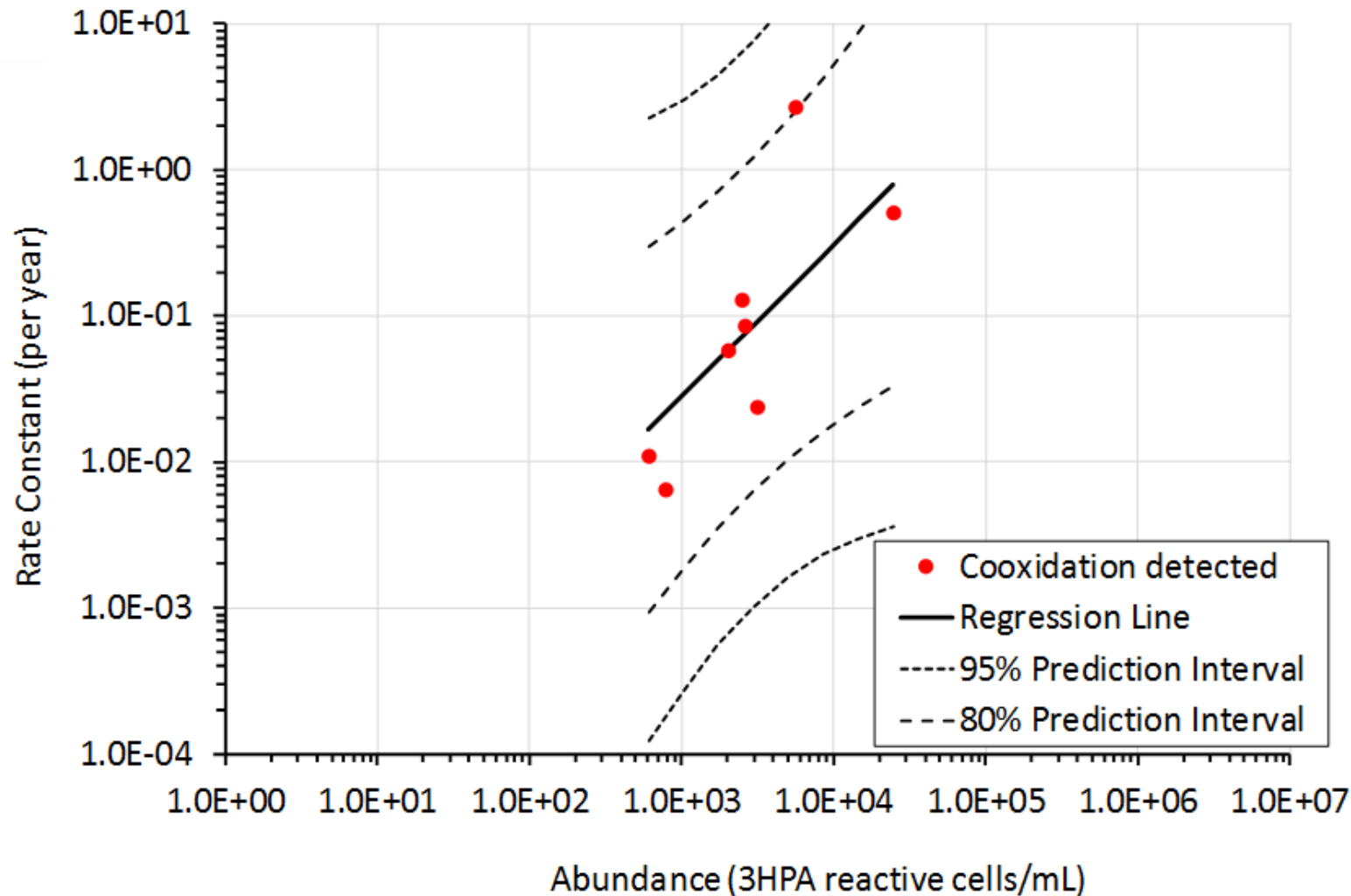
TCE Co-oxidation Rates Associated with Abundance of Toluene-2,3-dioxygenase



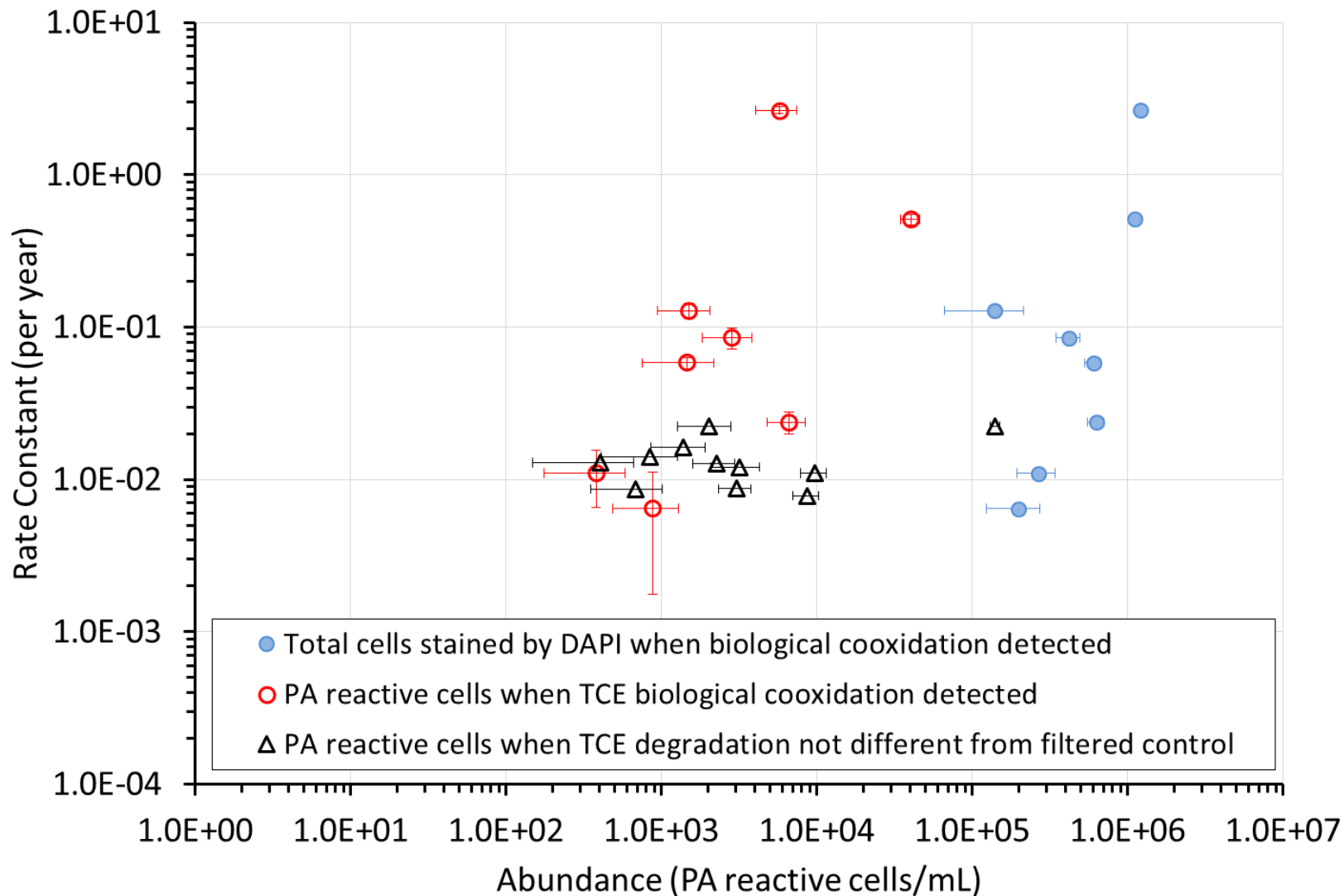
Regression of TCE Co-oxidation Rate Constant on Abundance of Reactive Cells



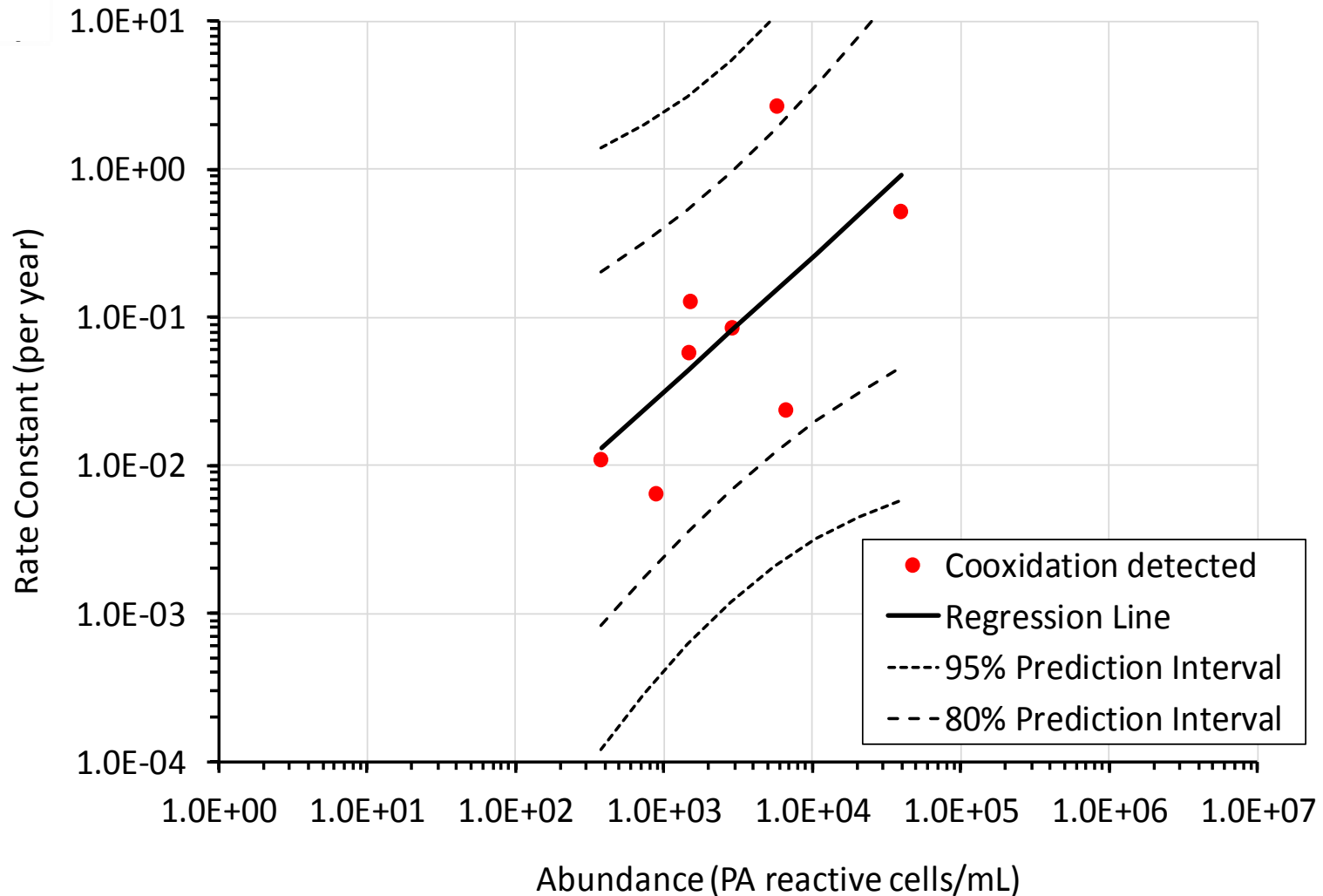
Regression of TCE Co-oxidation Rate Constant on Abundance of Reactive Cells



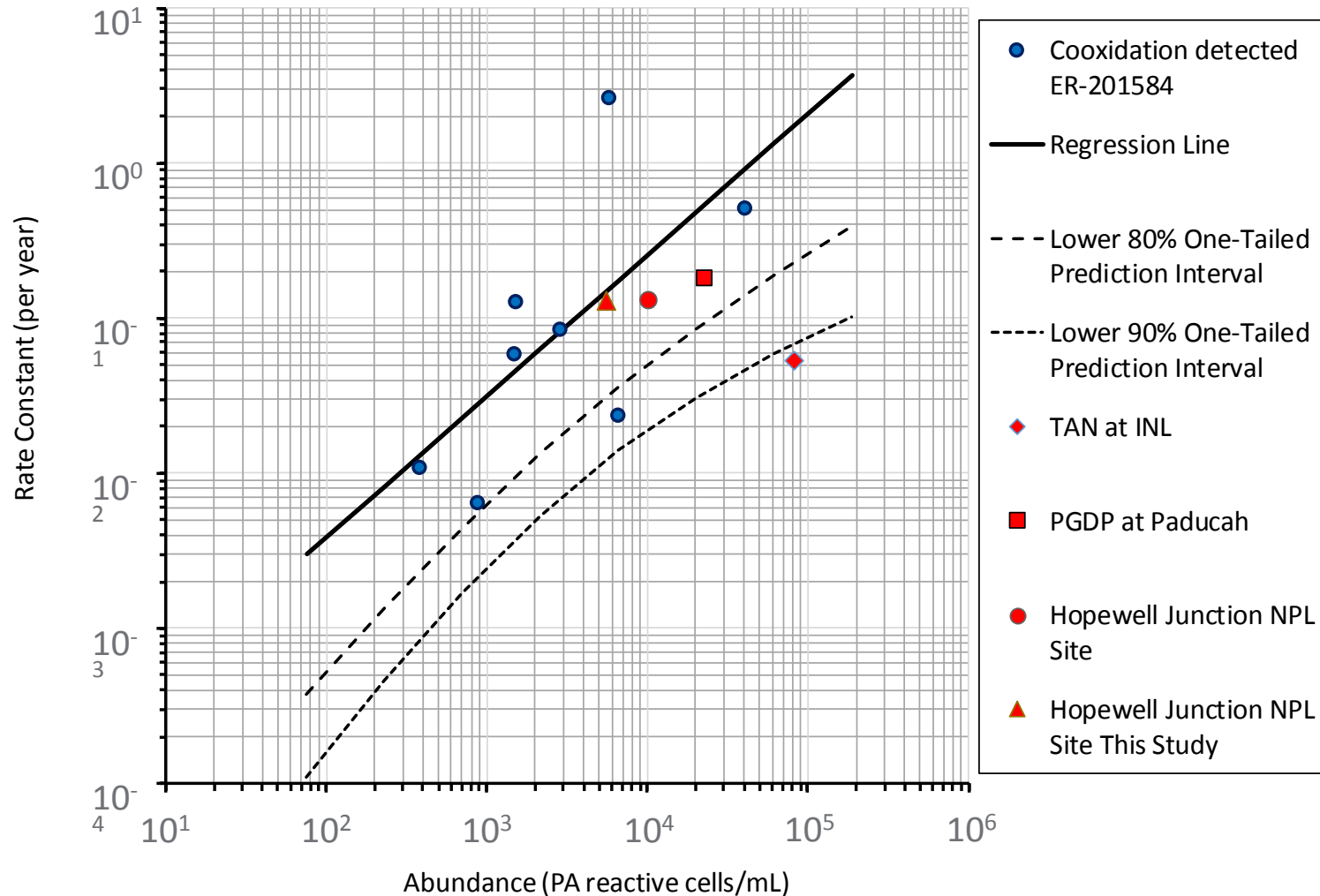
TCE Co-oxidation Rates Associated with Abundance of Toluene-2-monooxygenase



Regression of TCE Co-oxidation Rate Constant on Abundance of Reactive Cells



Extrapolating to Other Sites Where Co-oxidation Rates Have Been Measured



- Number of samples showing activity based on probing with three different aromatic oxygenase probes was low
- Many samples showing presence of active enzymes did not show comparable TCE co-oxidation rate
- Probes can be used along with qPCR as a second line of evidence at sites where TCE co-oxidation rates have been demonstrated
- EAP technology could be applied as initial screen for active enzymes followed by determination of rate constants.

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Thank You for Your Attention

Questions?

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