TCE Co-oxidation Rates and Quantification of Oxygenase Gene Abundances & Expression

ESTCP Project ER-201584

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ESTCP Project ER-201584

- Follow-up to ER-201129
  - Highlights the importance of all degradation mechanisms
  - Furthers management expectation tool (BioPIC)
- MNA Support w/ Quantitative Lines of Evidence for
  - Abiotic Degradation
  - Cometabolic Oxidation

WIEDEMEIER & ASSOCIATES

CLEMSON UNIVERSITY

Pacific Northwest NATIONAL LABORATORY

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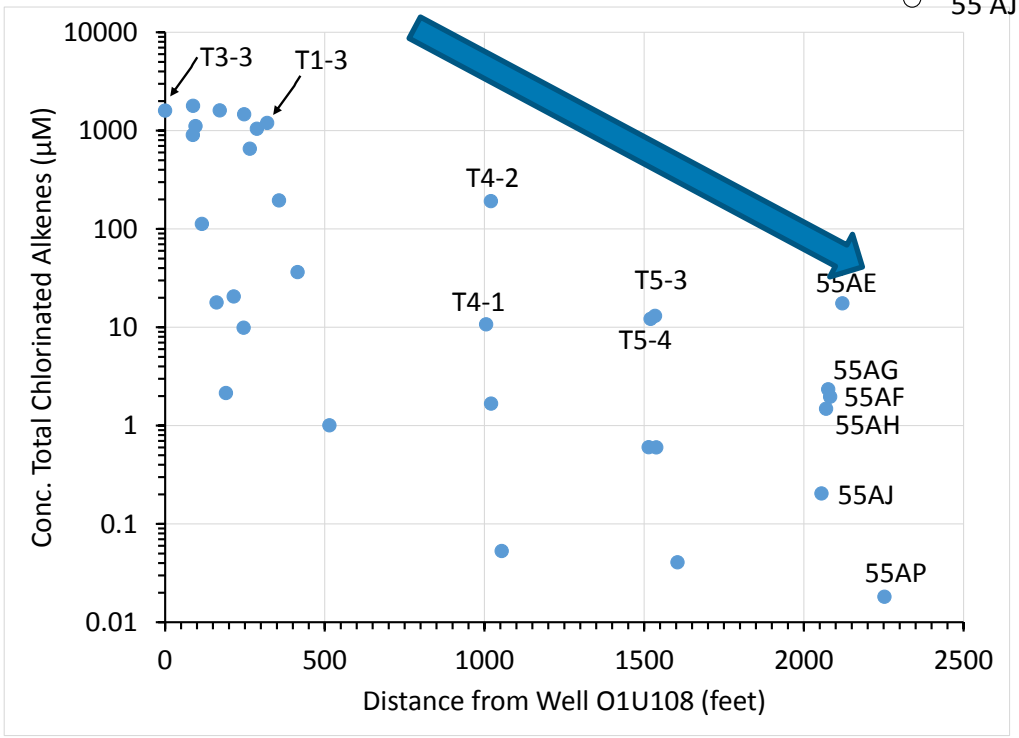
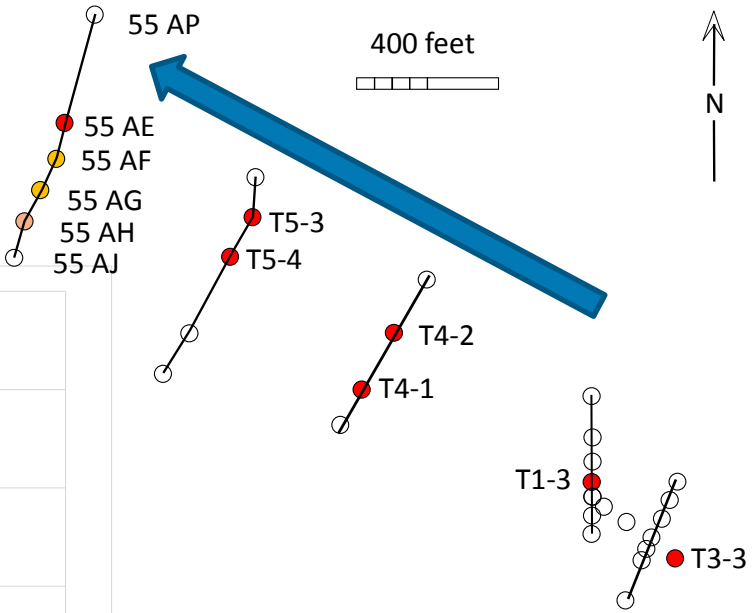
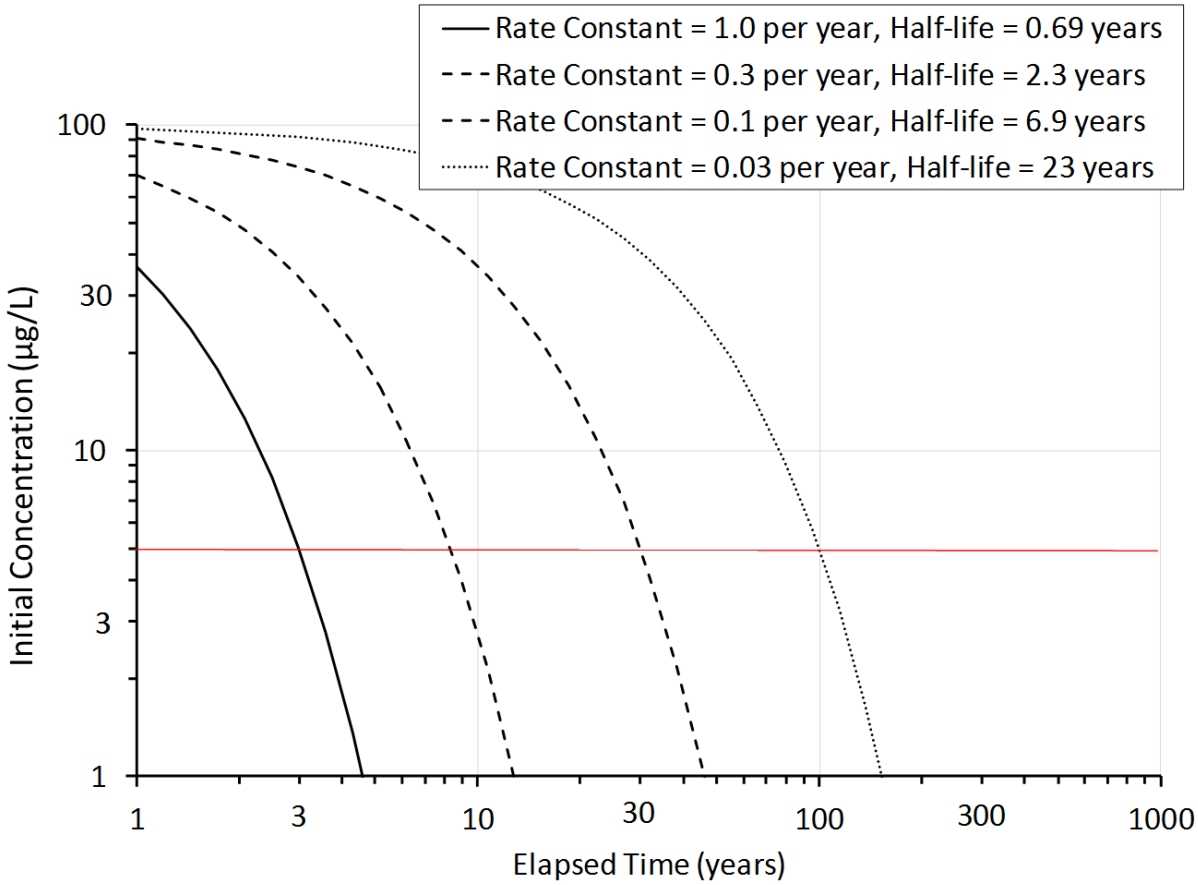
Plume Behavior





Impact of Degradation Rate Constant



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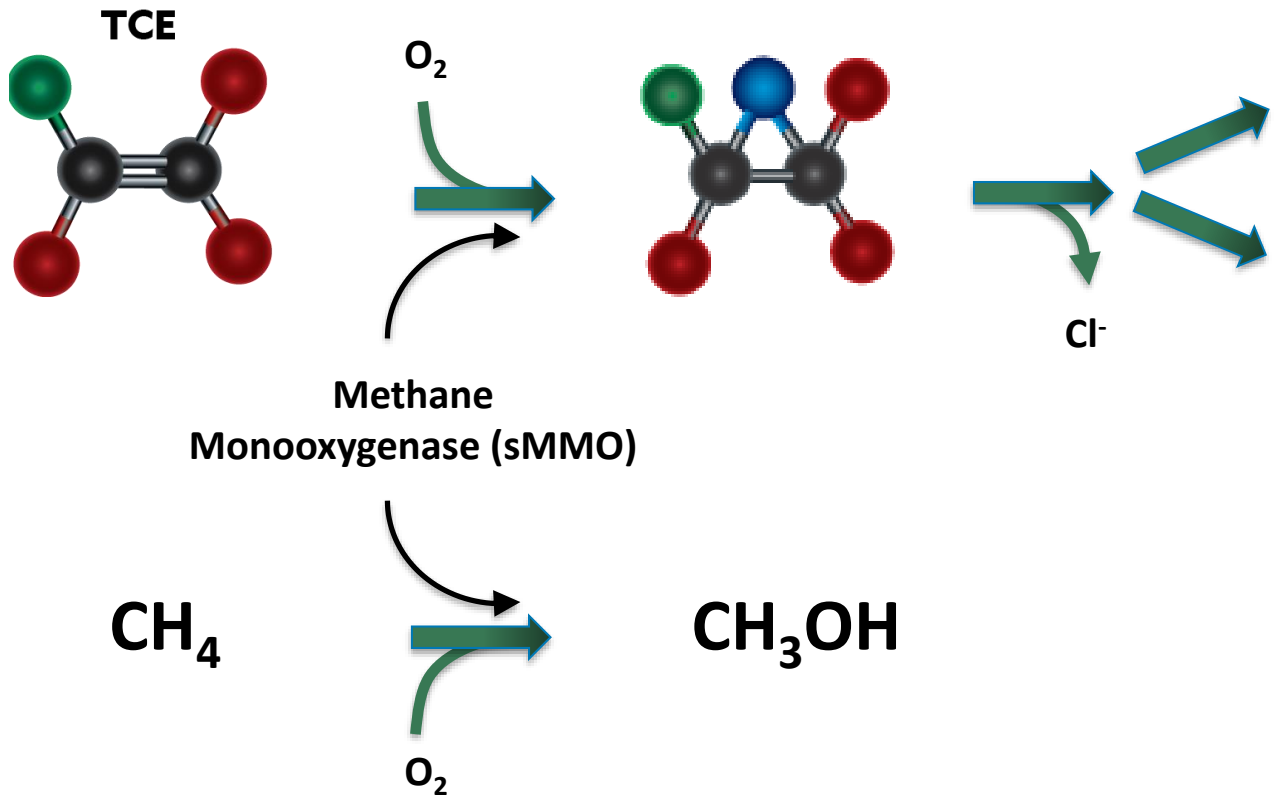
Aerobic Cometabolism of Chlorinated Ethenes



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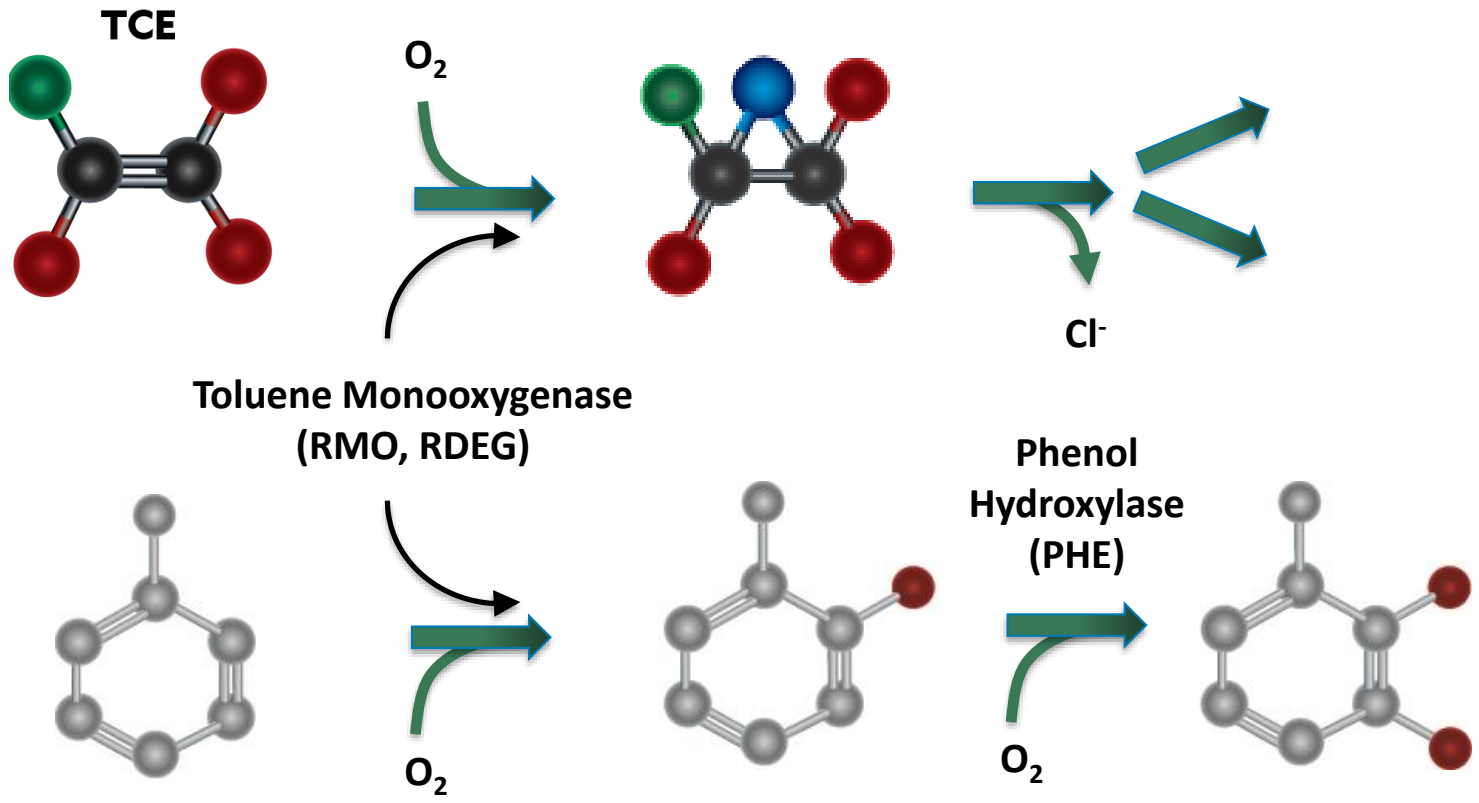

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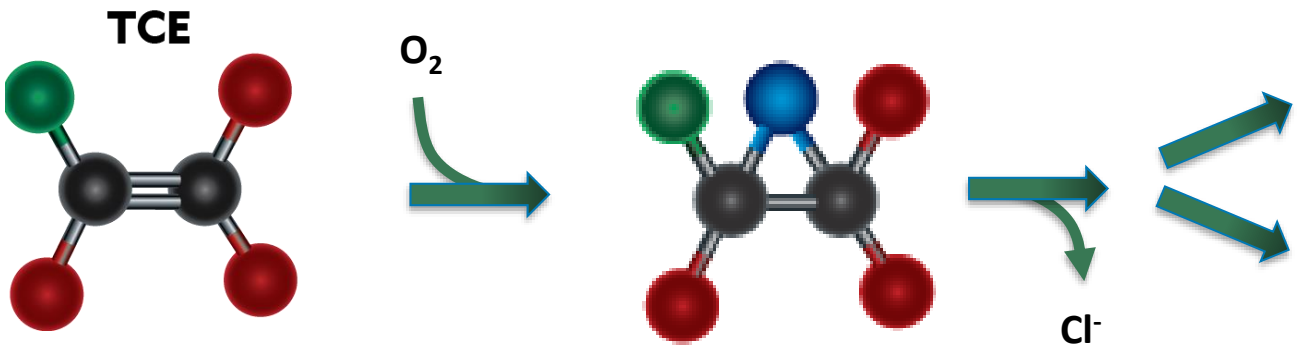

Methane Monooxygenases (sMMO)

Toluene Monooxygenases (RMO & RDEG)

Phenol Hydroxylase (PHE)

Toluene Dioxygenase (TOD)

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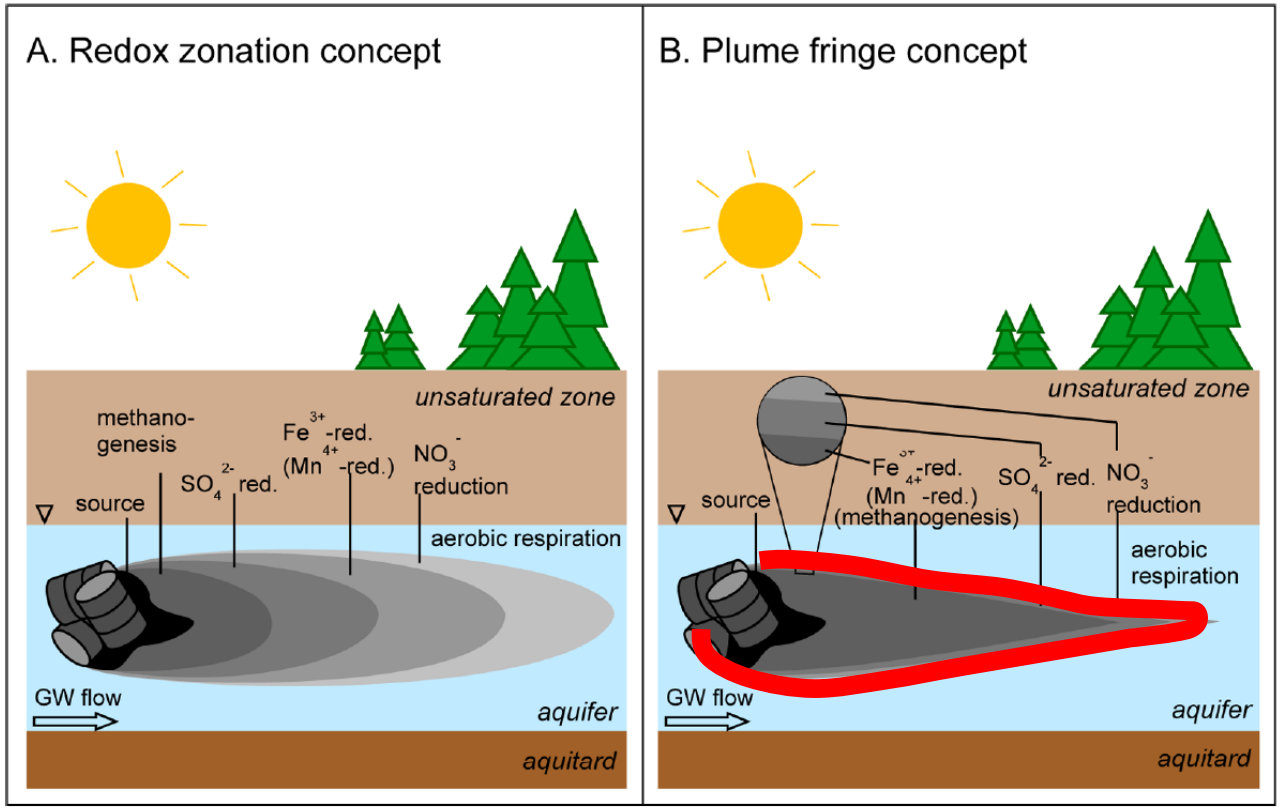
Aerobic Cometabolism

- Primary substrate and oxygen
  - Supports growth
  - Induce oxygenase gene expression
- For MNA…
  - Primary substrate as co-contaminant
  - Primary substrate present at plume fringe
  - Naturally occurring substrate
  - TCE induces oxygenase expression during growth on alternative substrate

Primary Substrate - Plume Fringe



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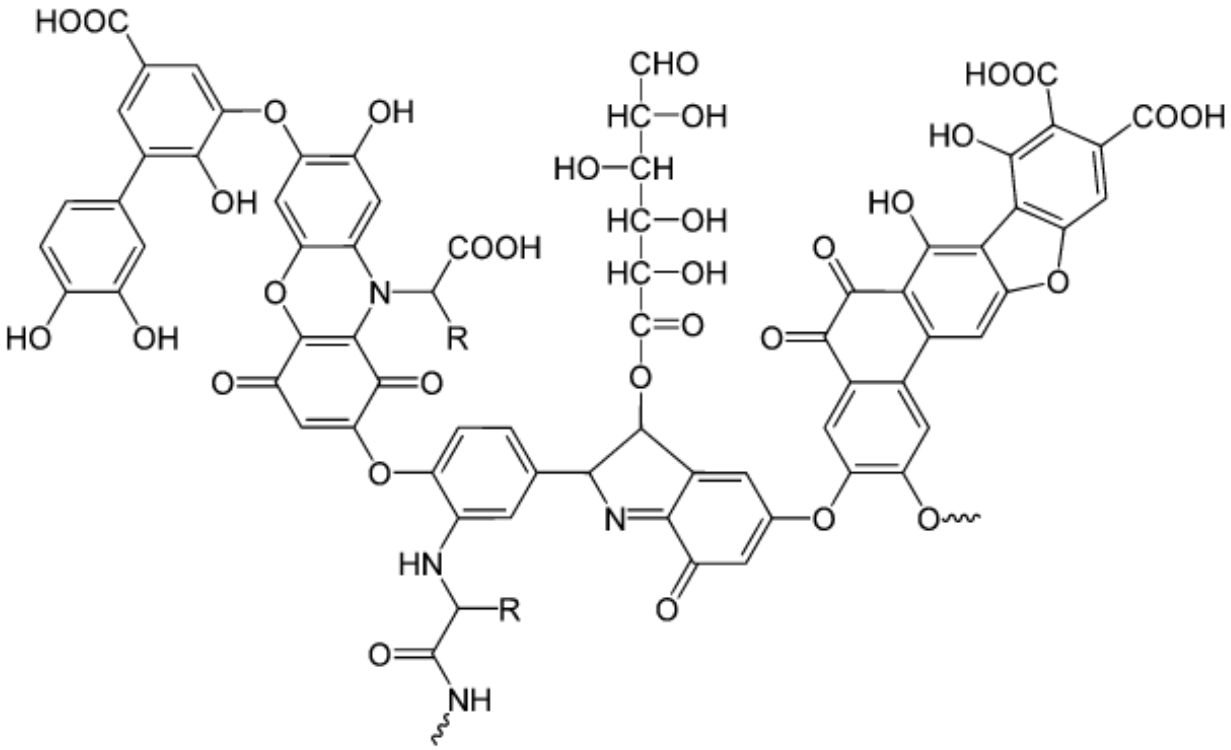
Humic Acid - Naturally Occurring Substrates



en.wikipedia.org/wiki/Humic_acid

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Experimental Plan

Water samples from 5 unique sites
(T.H. Wiedemeier & Associates, Scissortail Environmental)

^{14}TCE Co-oxidation Rate Studies
(Clemson)

EAPs
(PNNL)

qPCR
(MI and PNNL)

RT-qPCR
(MI)

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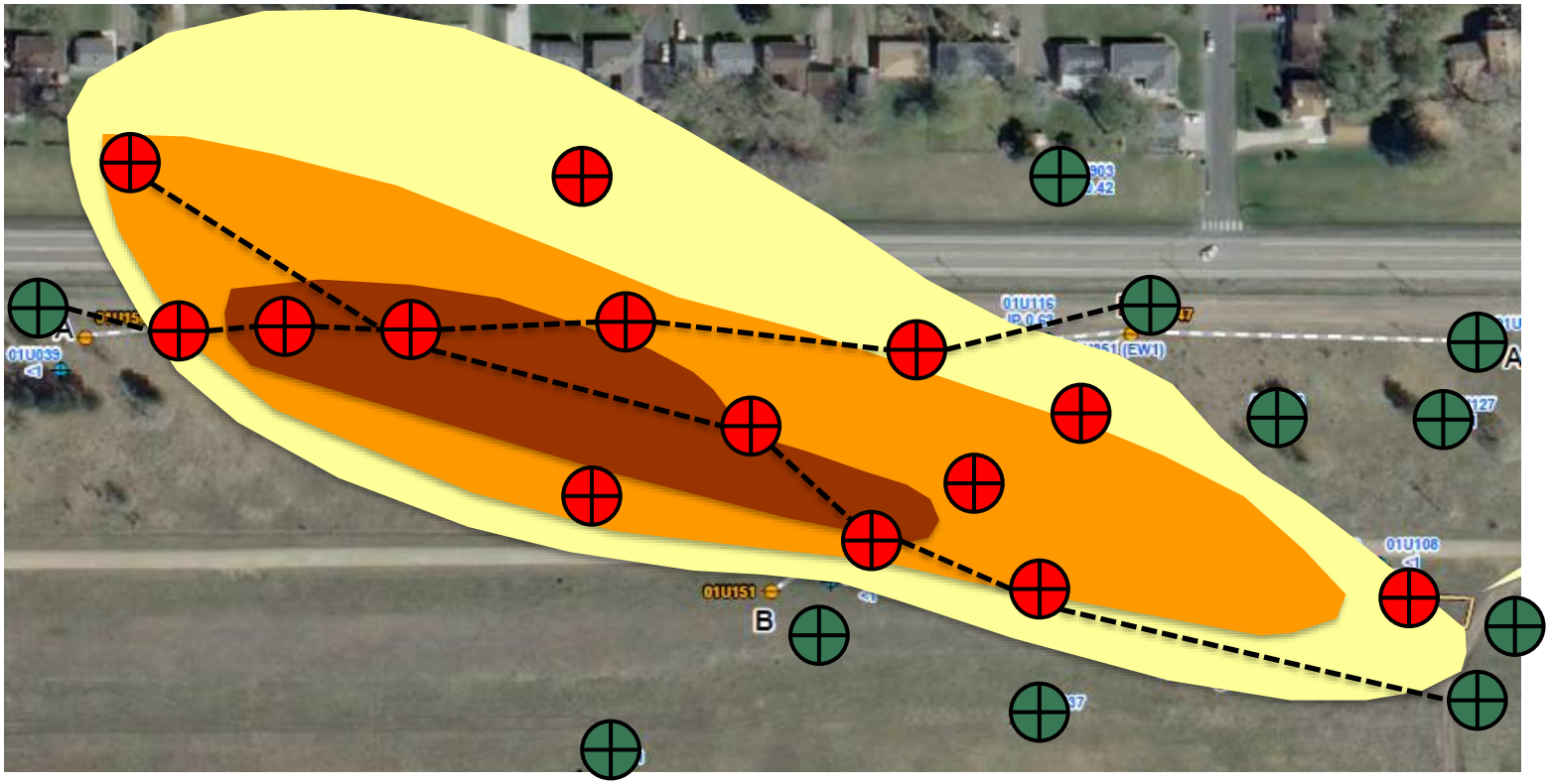
The Sites



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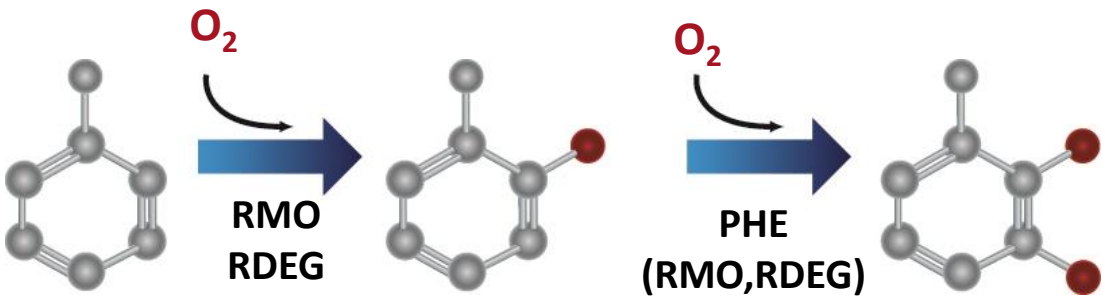
qPCR Targets

Name	Target Genes	Natural Substrate	Induction by TCE ?
RMO	Toluene-3-monooxygenase Toluene-4-monooxygenase	BTEX	Yes
RDEG	Toluene-2-monooxygenase	BTEX	Yes
PHE	Phenol hydroxylase	Phenol	
TOD	Toluene dioxygenase	BT	Yes
sMMO	Soluble methane monooxygenase	Methane	No



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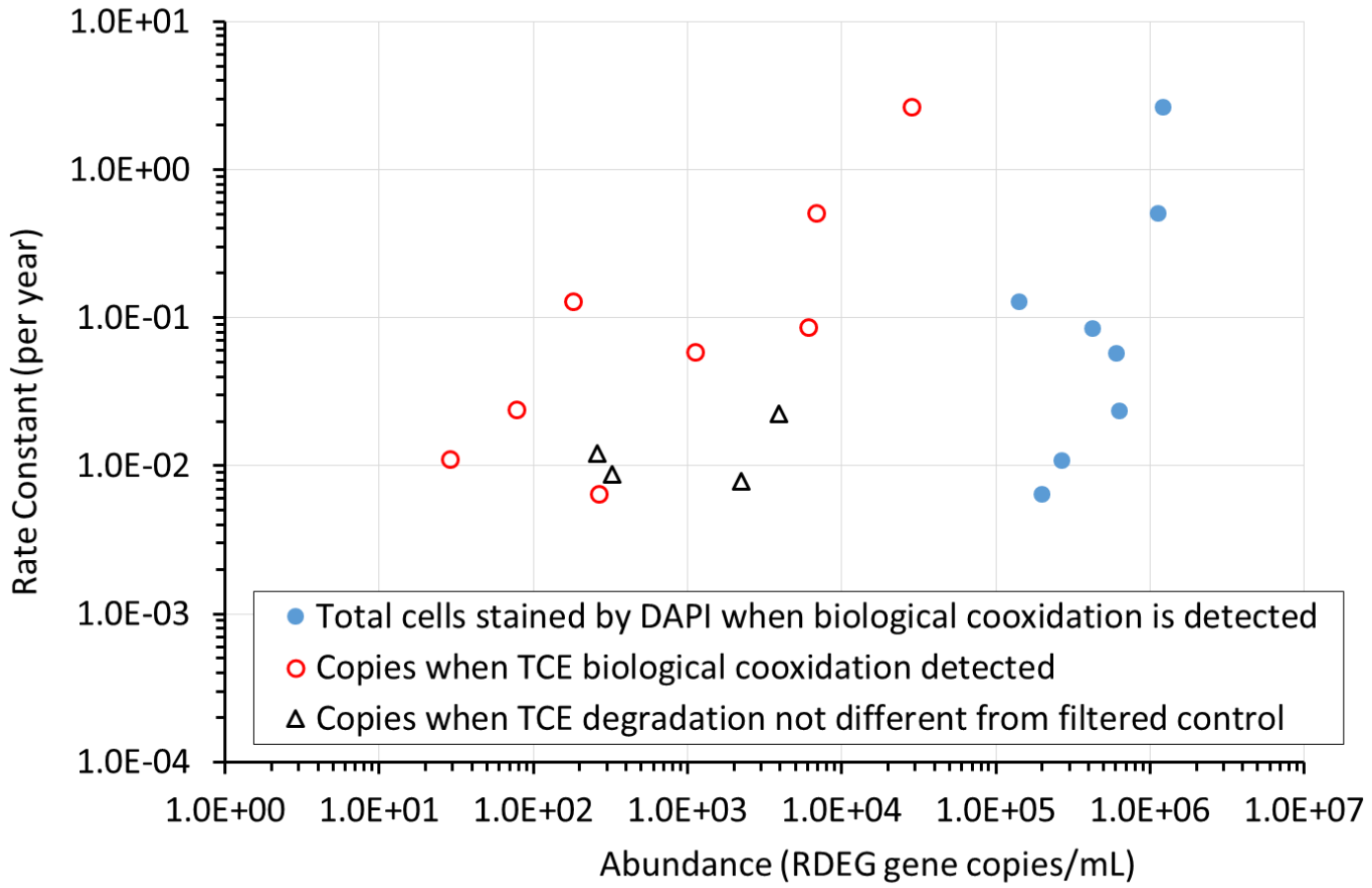
RDEG Concentration & TCE Co-oxidation Rate



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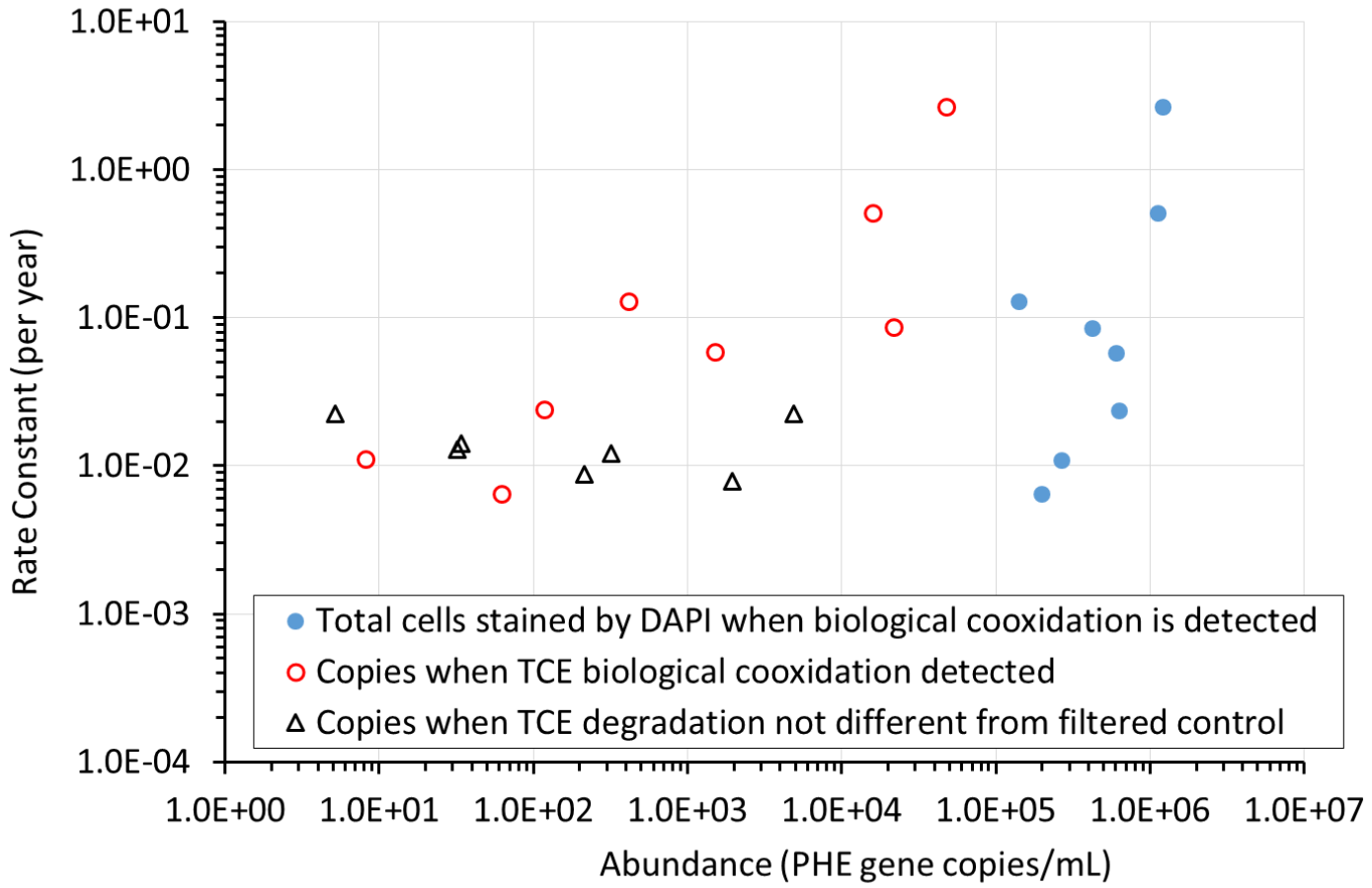
PHE Concentration & TCE Co-oxidation Rate



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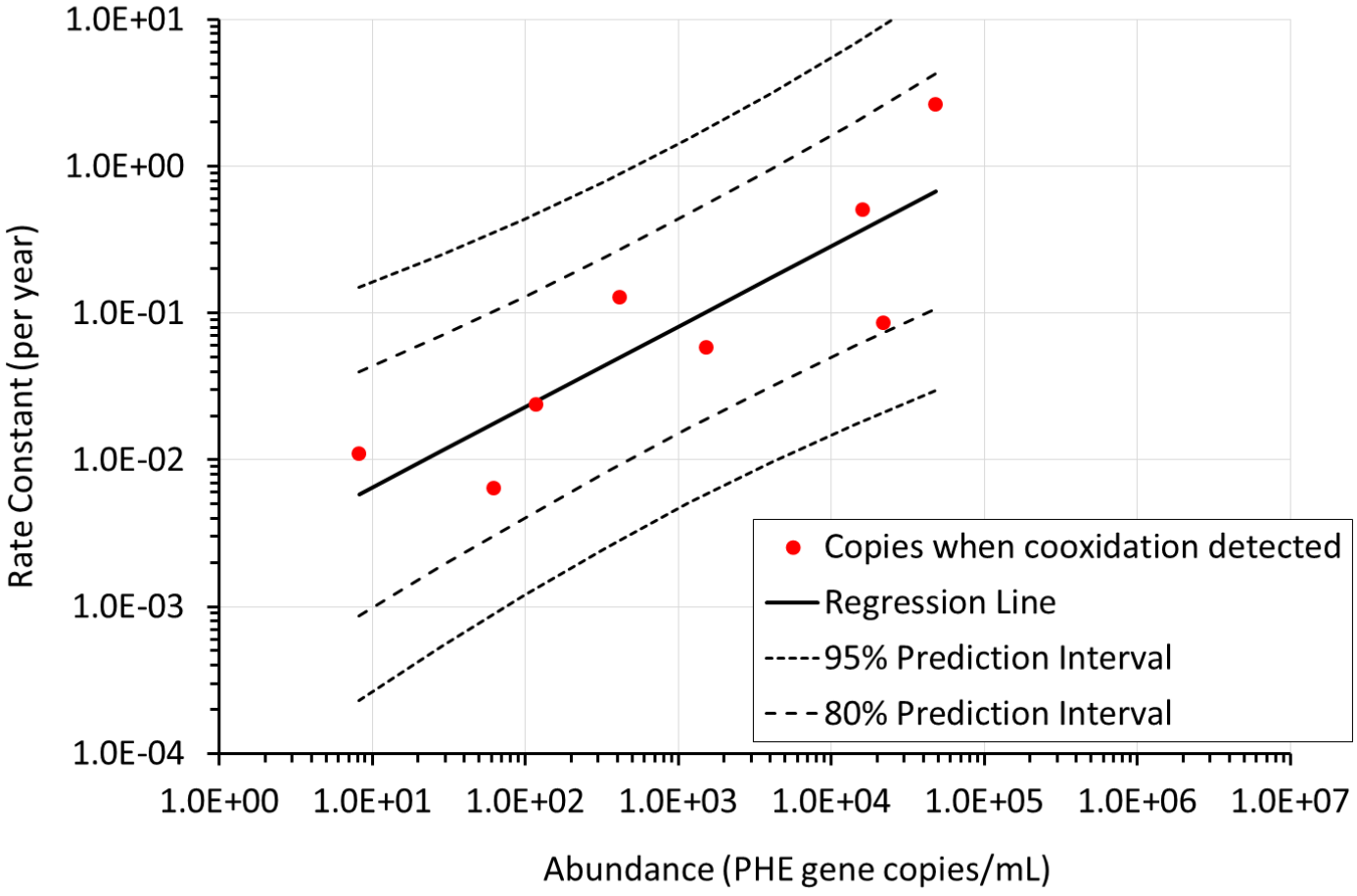
PHE Concentration & TCE Co-oxidation Rate



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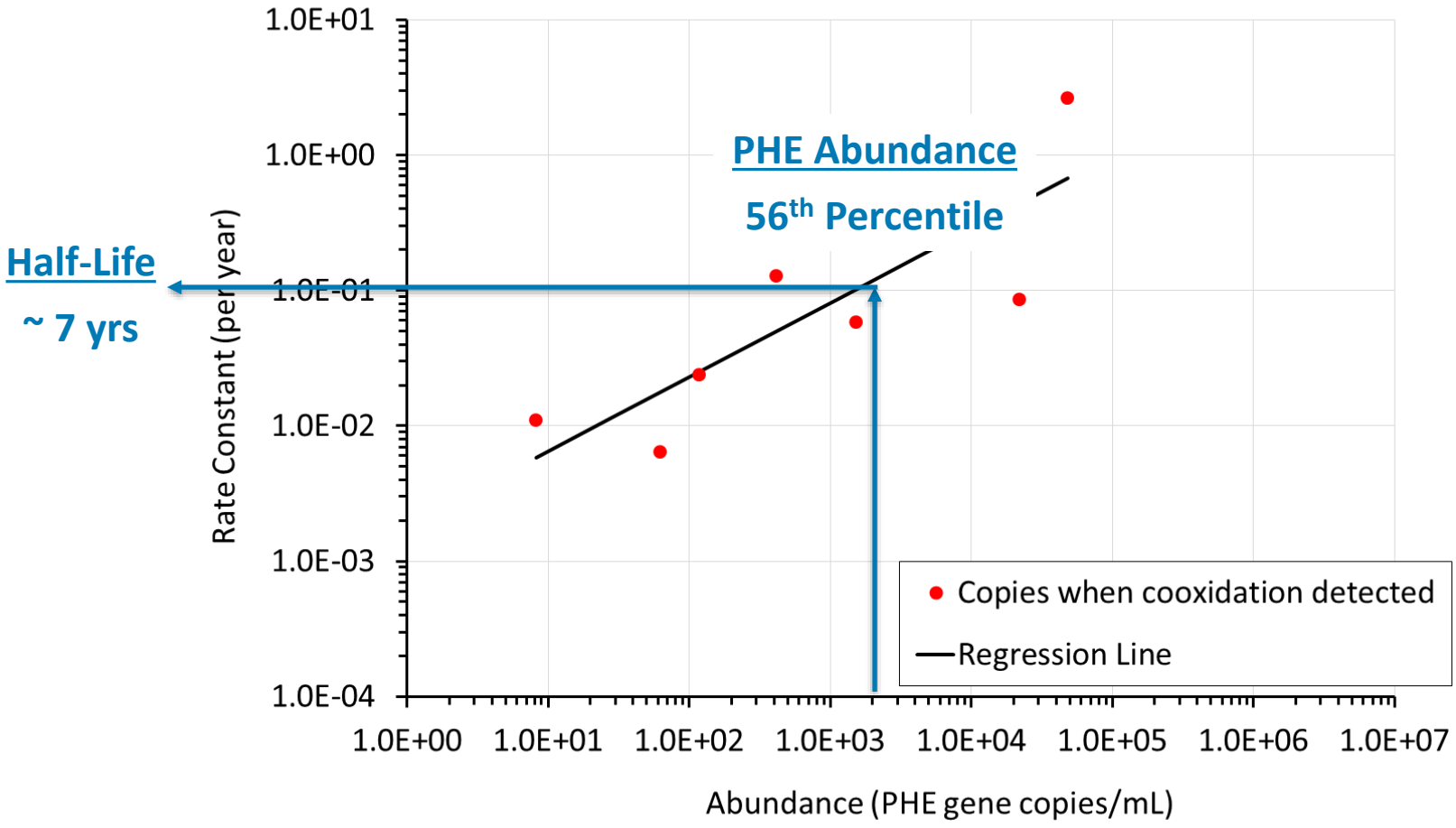

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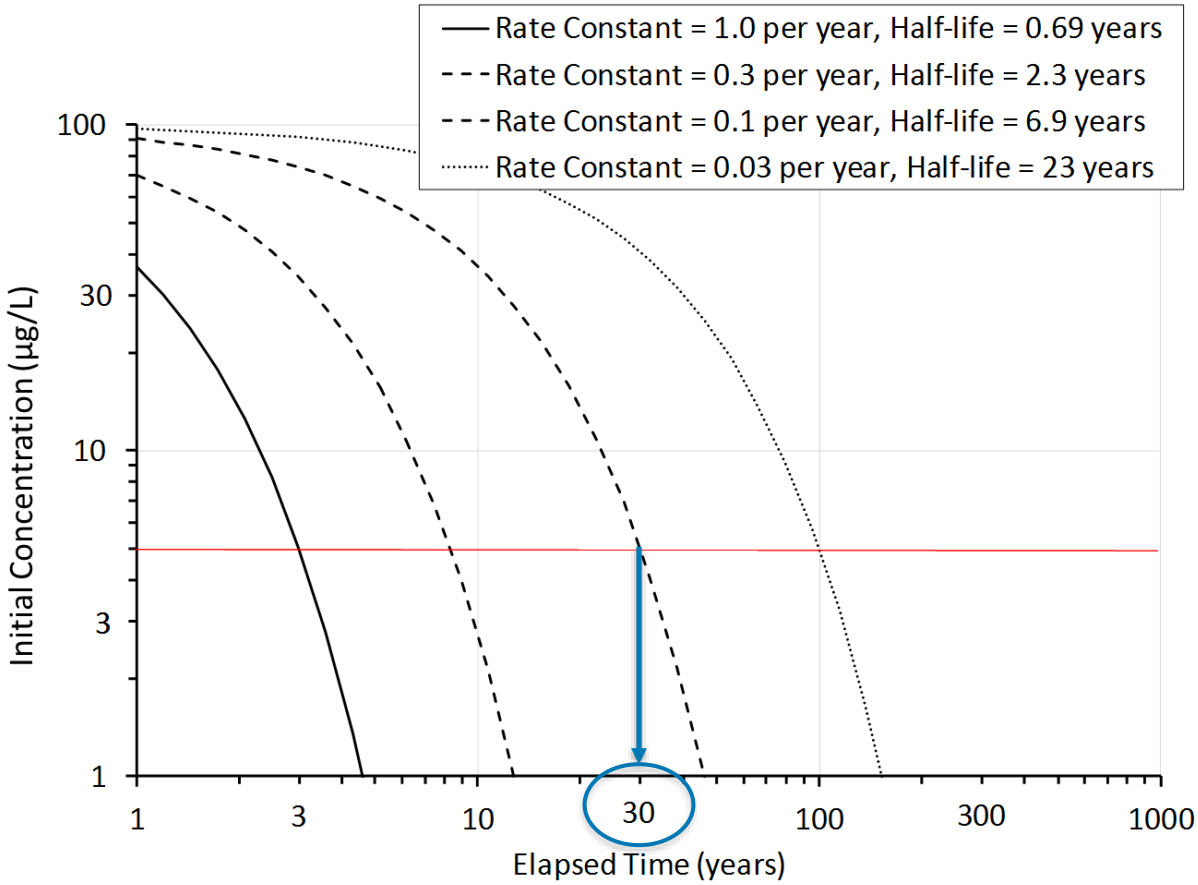
Impact of Degradation Rate Constant



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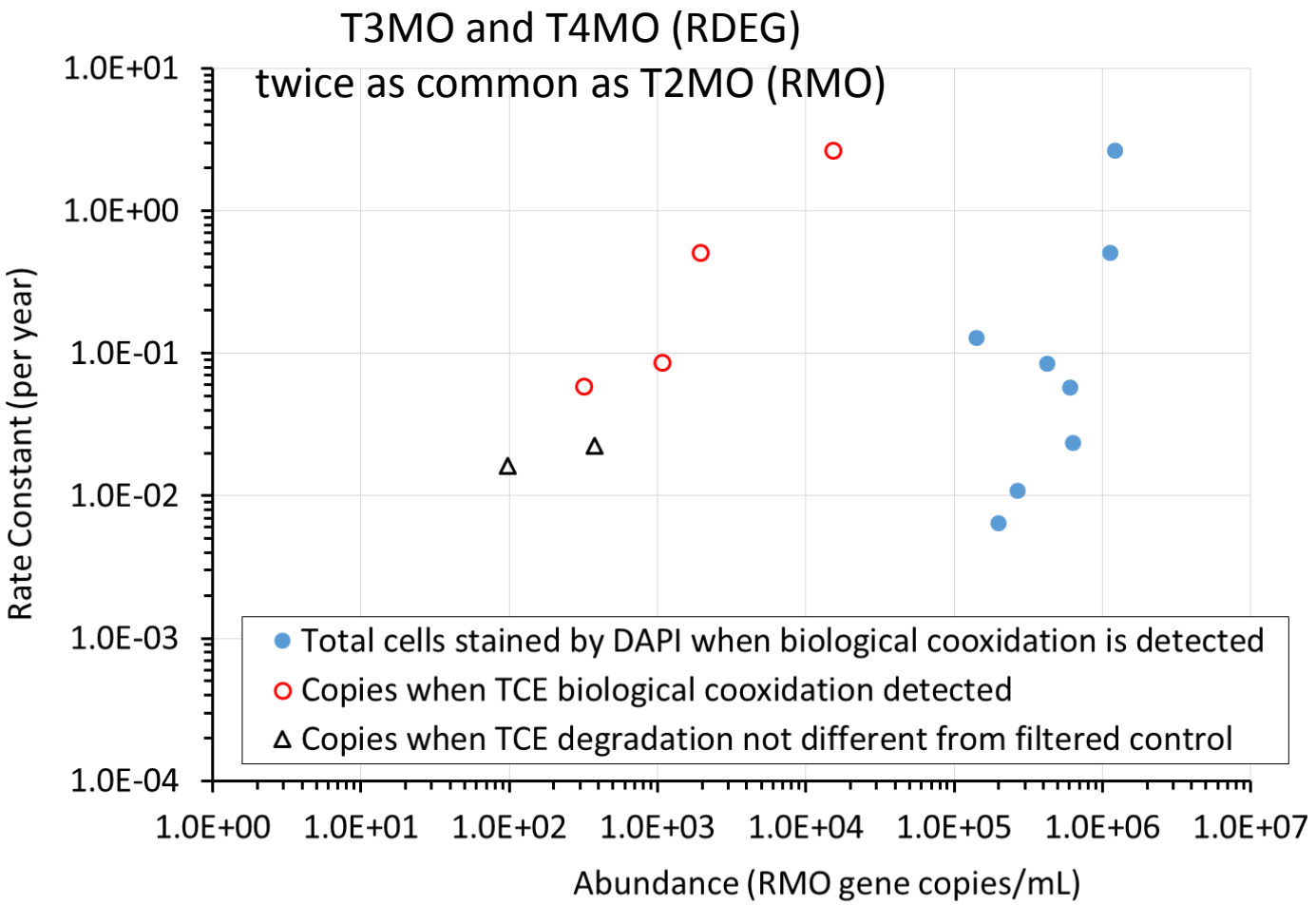
RMO Concentration & TCE Co-oxidation Rate



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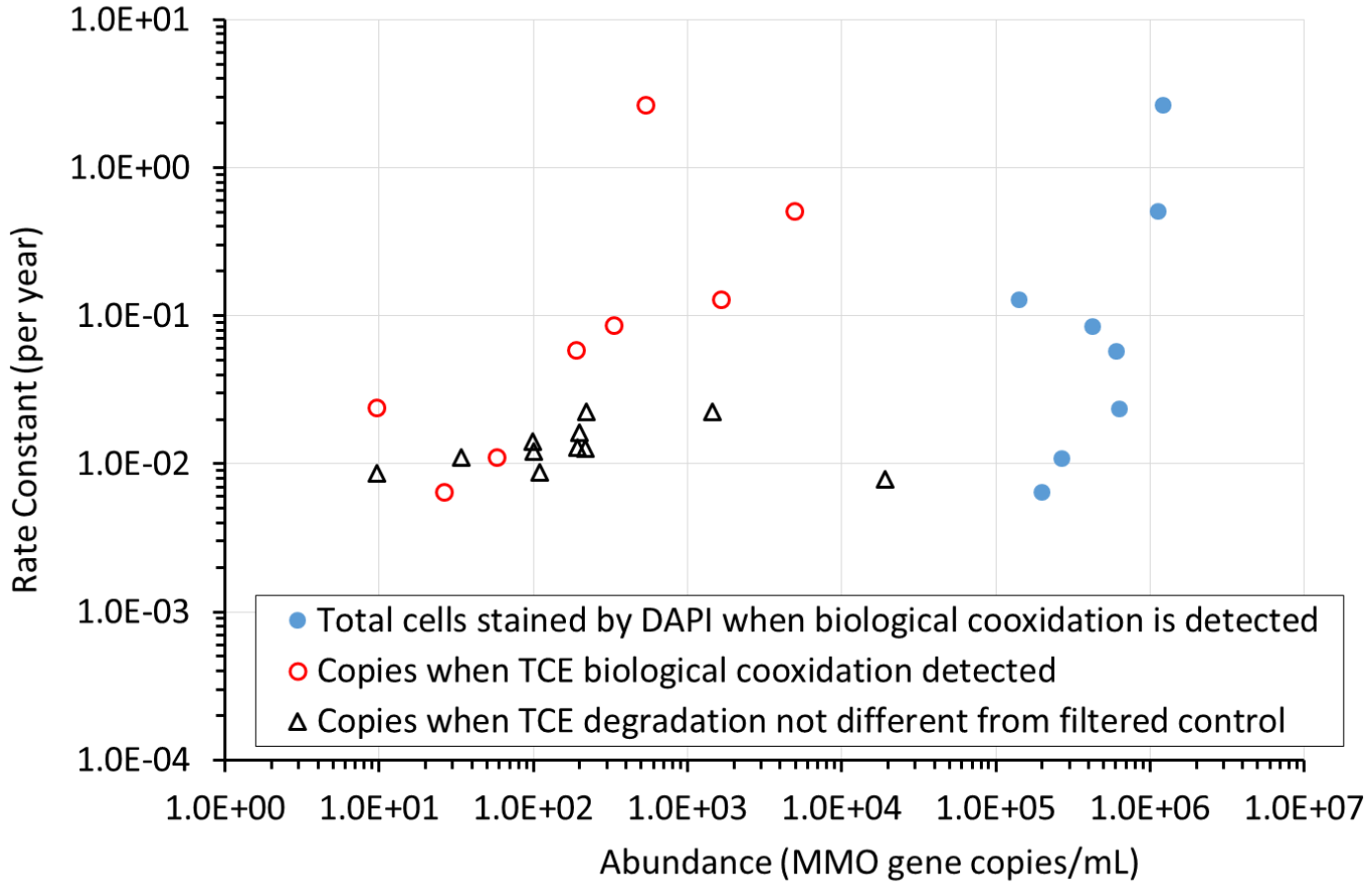
sMMO Concentration & TCE Co-oxidation Rate



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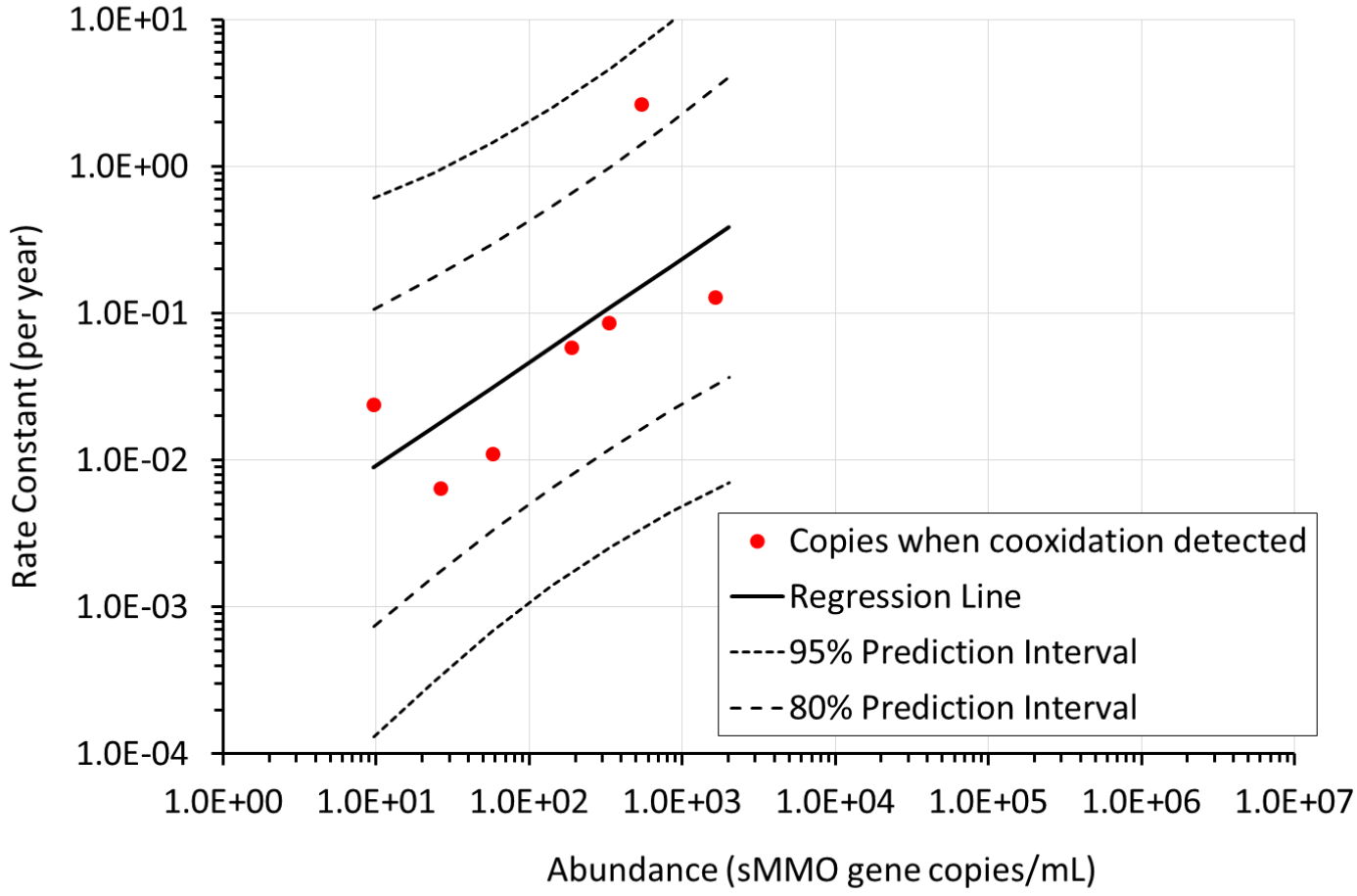
sMMO Concentration & TCE Co-oxidation Rate



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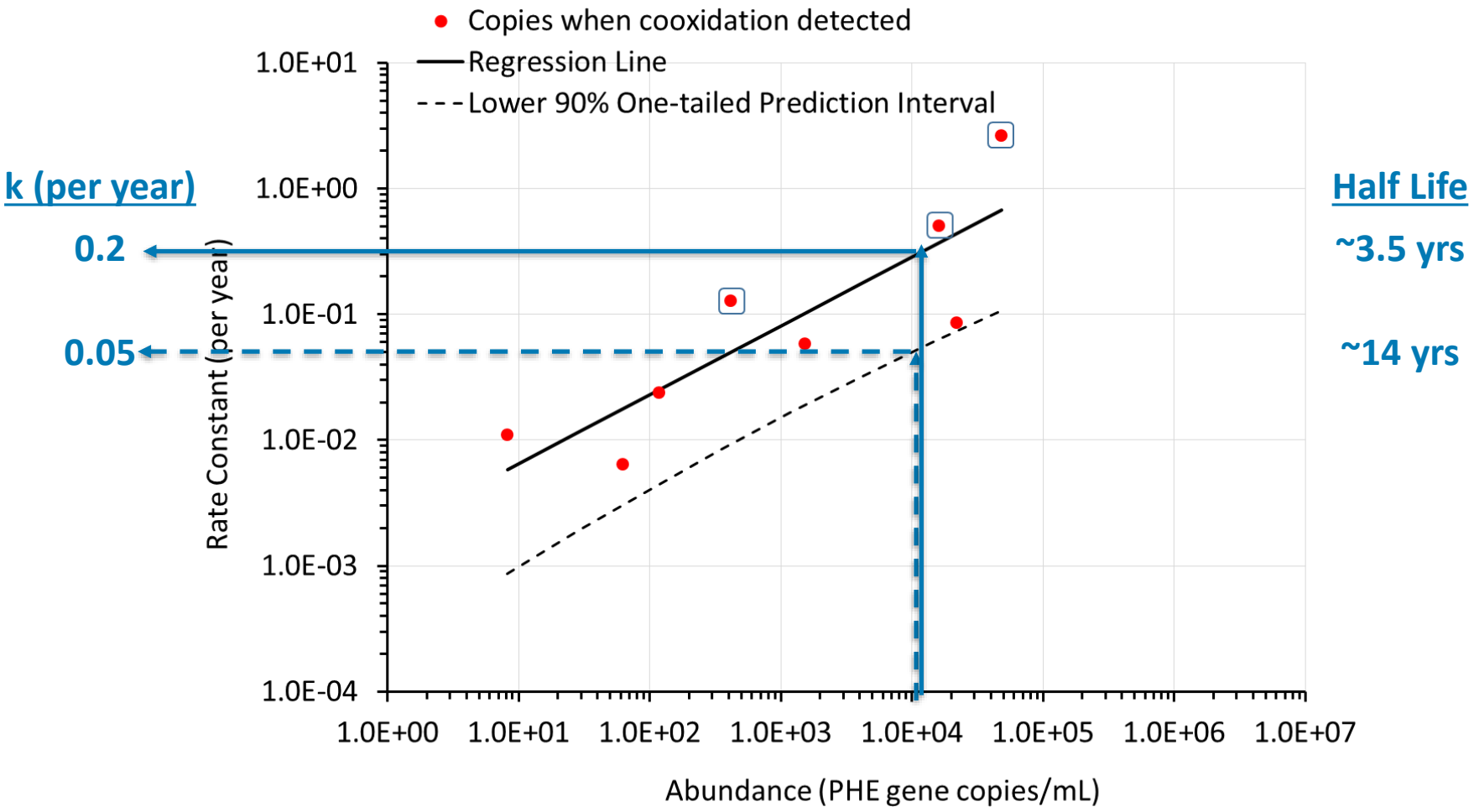
Using Prediction Intervals



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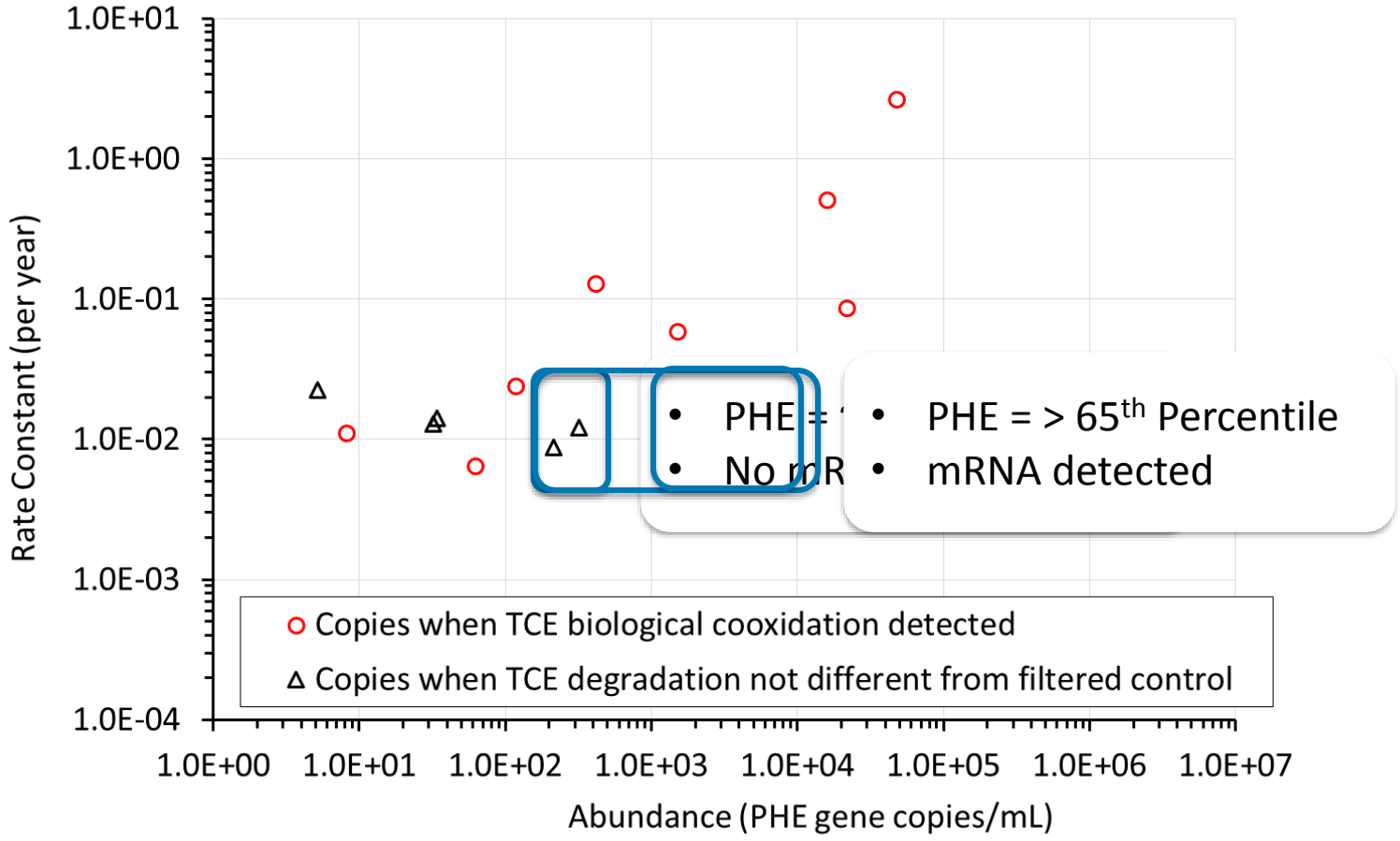
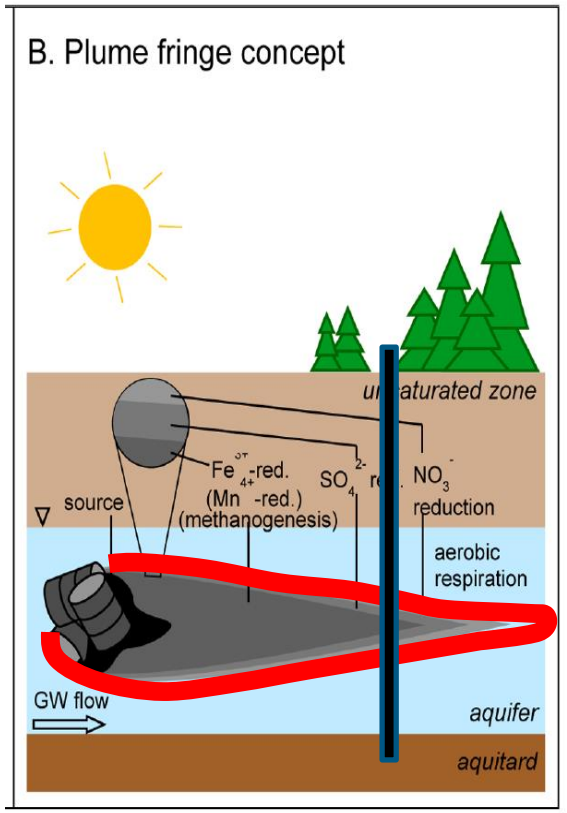
Exceptions – PHE but no significant degradation



Caveats

- Limiting factors
  - Carbon catabolite repression
  - Competitive inhibition
  - Oxygenase inactivation
  - Cellular toxicity
  - Reductant availability (NADH)
- Co-oxidation rates
  - Detection of iron

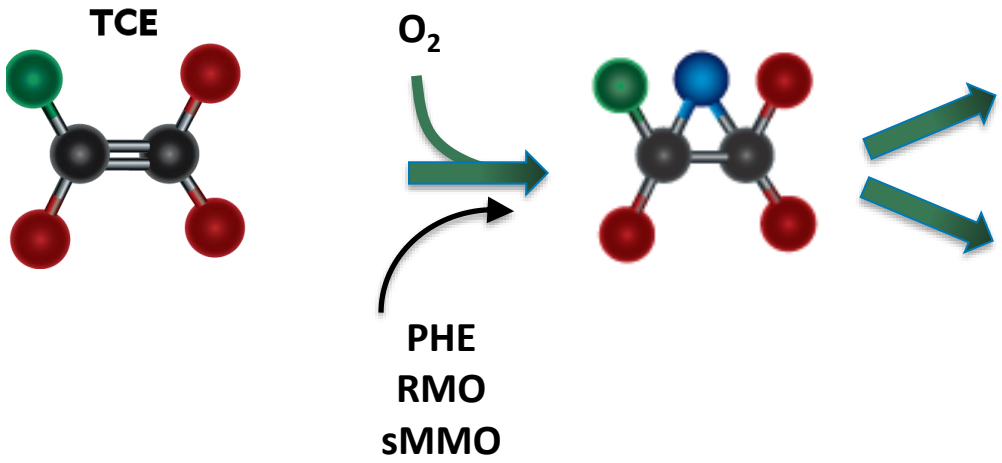


Conclusions

- qPCR is a good second line of evidence
  - Screening co-oxidation as a possible mechanism
  - Compare to rate constants determined from other information (e.g. computer models)
  - RT-qPCR (mRNA) did not provide better correlations

Conclusions

- Recommended Assays
  - PHE (phenol hydroxylase)
  - RMO (ring hydroxylating toluene monooxygenase)
  - sMMO (soluble methane monooxygenase)



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Acknowledgements

- Environmental Security Technology Certification Program (ESTCP)
- Dr. John Wilson

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Questions???

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