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

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Background/Objectives. Enzyme activity probes (EAP) have been applied at numerous DOE, DoD and industrial sites to determine the potential use of monitored natural attenuation (MNA) for remediation of chlorinated solvents, a common groundwater contaminant. As a molecular biological tool, these non-fluorescent probes are transformed by oxygenase enzymes into a quantifiable fluorescent signal, thus providing direct evidence of aerobic cometabolic enzyme activity for subsurface populations. Application of EAPs at contaminated sites can provide valuable information regarding the presence and activity of in situ microbial enzyme systems important for aerobic cometabolism for plume-wide assessment of intrinsic degradation. While EAP provides a quantitative estimate of bacteria active at a contaminated site, there is currently no direct correlation to trichloroethene (TCE) transformation rates. The current study is a first attempt at directly linking EAP results to TCE degradation rates.

Approach/Activities. Groundwater samples from 19 monitoring wells at five chlorinated-solvent-contaminated sites were assayed using EAP. Sites tested included: 1) Former Plattsburgh Airforce Base (New York); 2) New Brighton/Arden Hills Superfund Site (Minnesota); 3) Hill Air Force Base Operable Unit 10 (Utah); 4) Hopewell Junction (New York); and 5) Tooele Army Depot (Utah). Samples were filtered and EAPs for aromatic oxygenase enzymes (phenylacetylene, 3-hydroxyphenylacetylene and cinnamonitrile) and one for soluble methane monooxygenase (coumarin) were applied. Bacteria labeled with the aromatic oxygenase enzymes were quantified using epifluorescence microscopy, while sMMO was assayed using a fluorometer. TCE degradation rates were determined using ^{14}C-labeled compounds to determine the fate of TCE and the identity of the products formed. Results from these analyses will also be compared to other molecular biological tools, such as qPCR and metagenome analyses.

Results/Lessons Learned. Experimental results were broken up into four categories: EAP response and no TCE degradation rate, EAP response and TCE degradation rate, no EAP response and TCE degradation rate, and no EAP response and no degradation rate. Four monitoring wells showed EAP activity despite lack of observable TCE degradation rates. These results indicate that there is some type of inducer (e.g., humic acids) leading to production of the enzymes that is not stimulating TCE degradation rates above background. Phenylacetylene appeared to be the best EAP for predicting TCE degradation rates, with three sites showing presence of this EAP and TCE degradation. Inducers of enzyme activity are likely present in these groundwater samples. Nearly half of the monitoring well samples showed no EAP activity and no TCE degradation rates. Two phenylacetylene, three cinnamonitrile and four 3-hydroxyphenylacetylene probe samples produced results below the established 8×10^3 cell density, but TCE degradation rates were shown for the samples. While the results are below the previously selected threshold for significance relative to MNA, cells with the desired activity were still present. Coumarin results were positive for five of the 19 sites, but do not provide numbers of positive cells, so were not compared directly to TCE degradation rates. Results

from qPCR analyses supported the EAP results. These results show that EAP can be a powerful tool for predicting the potential for implementing MNA at sites contaminated with chlorinated solvents. Results can be used with other groundwater analyses and TCE degradation rate studies to predict potential, or actual ongoing MNA.