

FACT SHEET

Environmental Molecular Diagnostics: Chemical-Based Tools



Introduction

Environmental molecular diagnostics (EMDs) are a group of advanced techniques used to analyze biological and chemical characteristics of environmental samples. EMDs facilitate decision making throughout the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) process (Figure 1). This fact sheet will focus on the chemical-based tools including compound specific isotope analysis (CSIA), stable isotope probing (SIP), and carbon 14-labeled assays (^{14}C).

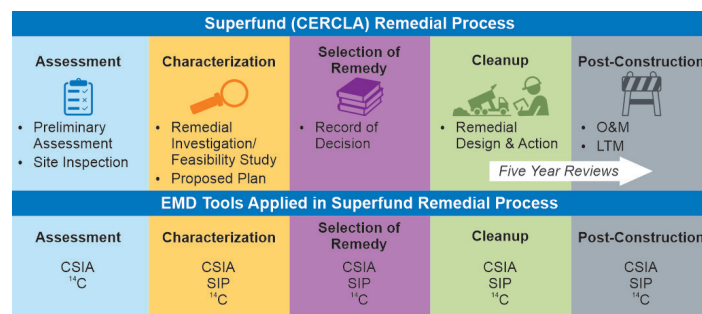


Figure 1. CERCLA Process (Courtesy of Battelle)

Technology Background

Chemical-based techniques rely on isotopes to determine if degradation is occurring.

CSIA

CSIA is a laboratory method that measures the ratios of naturally-occurring stable isotopes in a sample. This method can provide information on contaminant source, degradation mechanism and degradation rate. CSIA can be used on a wide range of contaminants in a number of applications such as environmental forensics, biodegradation, and abiotic degradation (Interstate Technology and Regulatory Council [ITRC], 2011).

SIP

SIP uses contaminants artificially enriched with high levels of stable isotopes to detect and quantify biodegradation processes and to characterize the microorganisms responsible for these activities. SIP also analyzes changes in the isotopic composition of biomolecules such as lipids, nucleic acids and proteins derived from microorganisms. SIP does not require any prior knowledge of the microorganisms, genes, or enzymes involved in a specific biodegradation process (ITRC, 2011). SIP is specifically used for contaminants that are electron donors (e.g., petroleum hydrocarbons).

^{14}C

^{14}C radiolabeling technique, an emerging technology, involves modifying molecules containing carbon (^{12}C) atoms with ^{14}C atoms. Isotopically distinct atoms serve to mark the molecule (or a fragment thereof) for later detection. The degradation products also become labeled making them easy to be tracked (Remediation Innovative Technology Seminars [RITS], 2020).



How Does It Work?

Chemical-based EMDs are used to characterize and quantify the changes in geochemical and microbiological characteristics of soil, groundwater or surface water as contaminants degrade (ITRC, 2011). CSIA uses isotopes (typically carbon, hydrogen, or chlorine) in contaminants to determine the extent of specific chemical and biochemical reactions impacting the contaminant. SIP measures changes in isotopes in biomolecules – usually phospholipid fatty acids (PLFAs) resulting from biodegradation of isotopically-labeled contaminants. ^{14}C labeling allows the determination of the fate of a parent compound, the rate of degradation, and the products formed.

Use of EMDs in the CERCLA Process



How Can It Help?

EMDs provide multiple lines of evidence to help make decisions throughout the CERCLA process, specifically (Figure 1):

- Identify specific sources of contamination during the characterization phase,
- Determine the effectiveness of existing remediation approaches and identify the need for enhancements such as chemical amendments or bioaugmentation during cleanup or post-construction phases,
- Identify degradation pathways and their degree of completion during cleanup or post-construction phases,
- Estimate degradation rates to determine whether site-specific cleanup goals will be attained within an acceptable timeframe during post-construction and five-year reviews, and
- Provide complementary data to support site closure.

What CERCLA questions does the tool answer?

Table 1 provides examples of information generated by application of CSIA, SIP, and ¹⁴C labeling within the CERCLA framework.

Restoration Phase	CSIA	SIP	¹⁴ C
Site Characterization	<ul style="list-style-type: none"> • To what extent has biological or abiotic degradation occurred? • Is there evidence of multiple sources of contamination? 	<ul style="list-style-type: none"> • Are microorganisms that can degrade the contaminant present? 	<ul style="list-style-type: none"> • Are parent compound and degradation products present?
Remediation	<ul style="list-style-type: none"> • Is monitored natural attenuation (MNA) feasible? • What is the estimated degradation rate for the contaminant present? 	<ul style="list-style-type: none"> • Can biodegradation of a contaminant occur under existing environmental conditions? • Is the contaminant completely mineralized to carbon dioxide? 	<ul style="list-style-type: none"> • Is degradation occurring? • Is the contaminant completely mineralized to carbon dioxide? • What is the kinetic degradation rate? • Will an in situ amendment enhance biodegradation of a contaminant?
Monitoring	<ul style="list-style-type: none"> • Is degradation of a contaminant occurring? 	<ul style="list-style-type: none"> • Is biodegradation of a contaminant occurring? 	<ul style="list-style-type: none"> • Is degradation of a contaminant occurring?

Table 1. CERCLA Questions



Involve regulatory agencies during all phases of site restoration.



Monitoring of MNA at Naval Air Station North Island



Project Objective: CSIA was performed on sediment porewater samples to determine: (a) whether natural attenuation occurred through degradation and (b) if vertical upward enrichment was occurring.

Site Background: Site 9 is a 50-acre former chemical waste disposal area, consisting of unpaved land and two buildings, where elevated concentrations of contaminants of concern (COCs) have been detected in groundwater monitoring wells (Figure 2). An estimated 32 million gallons of liquid wastes were disposed at Site 9 between the 1940s and mid-1970s. Areas 1, 3, and 8 were identified as former disposal areas containing liquid wastes with chlorinated volatile organic compounds (cVOCs).



Figure 2. Site 9 Location (Courtesy of Battelle)

Results: The results of this microcosm study show that 99% or more mass of the chloroethenes (CEs) at all three test locations was removed via intrinsic biodegradation occurring in offshore sediments. The ^{13}C enrichment in trichloroethene (TCE), dichloroethene (DCE), and vinyl chloride (VC), as shown in the data below (Table 2), was attributed mainly to the activity of the *Dehalococcoides* bacteria (Dhc) within the microbial community. For example, vinyl chloride reductase activity (*vcrA*) resulted in enrichment of VC. The data from the T5-11 location show a significant decrease in TCE concentration which is confirmed by the isotopic fractionation data (^{13}C accumulates and the ^{13}C increases or becomes less negative in comparison to the non-fractionated compound). The natural TOC concentrations detected are within a range that has been shown to support natural dechlorination in other published microcosm studies. Microcosm results along with results of the supplemental analyses and CSIA provide multiple lines of evidence showing that CEs attenuate naturally via reductive dechlorination at all locations. The pattern of upward decreasing COC concentrations between 8-foot and 1-foot depth, combined with the multiple data sets from the microcosm study, molecular analyses, and CSIA, indicated that natural biodegradation is acting to degrade and reduce COC concentrations to levels below California Toxic Rules at the 1-foot depth. Based on the results of the microcosm study, it was concluded that MNA is a viable remedial strategy for addressing chlorinated VOC impacts in the offshore sediments at IR Site 9 (NAVFAC, 2019).

Parameter		Location T5-11		
Depth bgs (ft)		1	5	8
Chlorinated ethene reduction (%)		>99	>99	>99
TOC (g/kg)		0.9	0.6	0.8
Microorganisms/genes (qPCR)		<i>Dhc, vcrA</i>		<i>Dhc</i>
	^{13}C Enrichment Baseline (Literature values - Eccarius et al., 2012; Hunkeler et al., 2008; and Pirkle, 2006)	^{13}C Enrichment Post-Treatment		
TCE	-33.40	-16.51	-19.12	-20.60
DCE	-27.5	-11.85	-23.62	-26.63
VC	-25.0	-19.05	-29.21	U

U – Either there was no peak corresponding to the target analyte or the peak did not produce a reliable CSIA result

Table 2. Site 9 Results (Source: Adapted from NAVFAC, 2019)



CSIA measures change in isotope ratios in parent compounds and daughter products; provides conclusive evidence that degradation of COCs is occurring.



CASE STUDY 2 - ^{14}C Assay

Determining First-Order Rates of TCE Co-Oxidation



Project Objective: A ^{14}C -labeled TCE assay was developed to help quantify degradation rates.

Approach: Aerobic co-oxidation of TCE in the environment is difficult to quantify by simply measuring changes in the concentration of TCE and the presence of aerobic co-oxidation degradation products in the field. The monitoring approach used for this study included an assay employing ^{14}C -labeled TCE (Figure 3). Groundwater samples collected in the field were shipped on ice via an overnight courier to the lab. Highly purified ^{14}C -labeled TCE was added to the groundwater samples. The disappearance of ^{14}C -labeled TCE and the accumulation of aerobic co-oxidation ^{14}C -labeled degradation products, including $^{14}\text{CO}_2$ and soluble ^{14}C -labeled compounds such as formate, glycolate, and oxalate, were quantified. The high precision of these measurements due to the strong signal emanating from ^{14}C made it possible to estimate pseudo-first order rate coefficients for aerobic TCE degradation over a relatively short timeframe.

Results: The results of the study were (Figure 4):

- ^{14}C -TCE assay quantified pseudo-first order rate constants in groundwater samples from eight of the 19 wells evaluated (half-life of 0.26 to 105 years)
- Rate could not be determined in 11 wells (i.e., no statistical difference from control)
- $^{14}\text{CO}_2$ constituted the majority of the ^{14}C product quantified, followed by non-volatile aerobic degradation products (RITS, 2020); (Environmental Security technology Certification Program [ESTCP, 2017])

Disclaimer

This publication is intended to be informational and does not indicate endorsement of a particular product(s) or technology by the DoD, nor should the contents be construed as reflecting the official policy or position of any of those Agencies. Mention of specific product names, vendors or source of information, trademarks, or manufacturers is for informational purposes only and does not constitute or imply an endorsement, recommendation, or favoring by the DoD.

References

Eccarius, B., U. Desery, D. Wanty, S. Sacco. 2012. Differentiation of Several PCE Plumes in Groundwater by CSIA, Eighth International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA. May.

Environmental Security Technology Certification Program (ESTCP). 2017. Providing Additional Support for MNA by Including Quantitative Lines of Evidence for Abiotic Degradation and Co-metabolic Oxidation of Chlorinated Ethylenes. ER-201584.

Interstate Technology & Regulatory Council (ITRC). 2013. Environmental Molecular Diagnostics, New Site Characterization and Remediation Enhancement Tools. April.

ITRC. 2011. Environmental Molecular Diagnostics Fact Sheets. November.

Remediation Innovative Technology Seminar (RITS). 2020. Advances in Environmental Molecular Diagnostic (EMD) Tools for Site Assessment. Presented by Tony Danko, NAVFAC EXWC. June.

Hunkeler, D., R. Meckenstock, B. Sherwood-Lollar, T. Schmidt, and J. Wilson. 2008. A Guide for Assessing Biodegradation and Source Identification of Organic Ground Water Contaminants Using Compound Specific Isotope Analysis (CSIA). EPA 600/R-08/148.

Naval Facilities Engineering Command (NAVFAC). 2019. Final Offshore Sediments Natural Attenuation Microcosm Study Technical Memorandum, Site 9. June.

Pirkle, R. 2006. The Science, Technology and Selected Examples from the Literature with Application to Fuel Oxygenates and Chlorinated Solvents. White paper. Microbial Insight Inc., July.

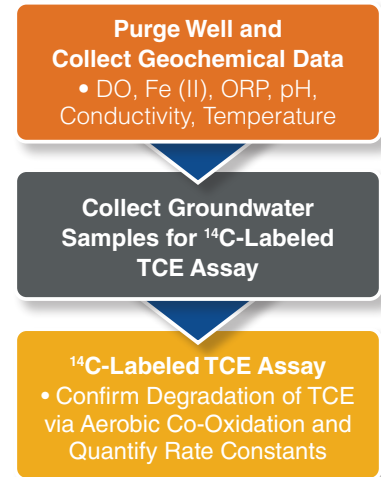


Figure 3. ^{14}C -labeled TCE Assay Approach (Adapted from ESTCP, 2017)

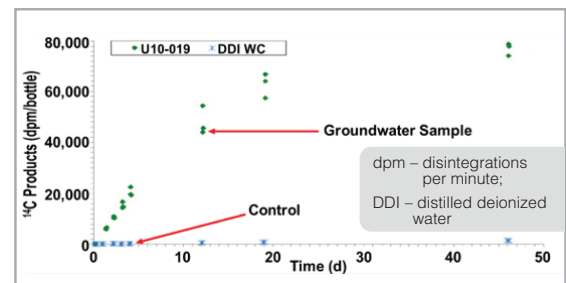


Figure 4. Study Results (Source: ESTCP, 2017)



The ^{14}C assay provides a tool to determine if TCE is undergoing transformation and, if so, at what rate; provides information in a familiar format that is needed to assess natural rates of attenuation.

