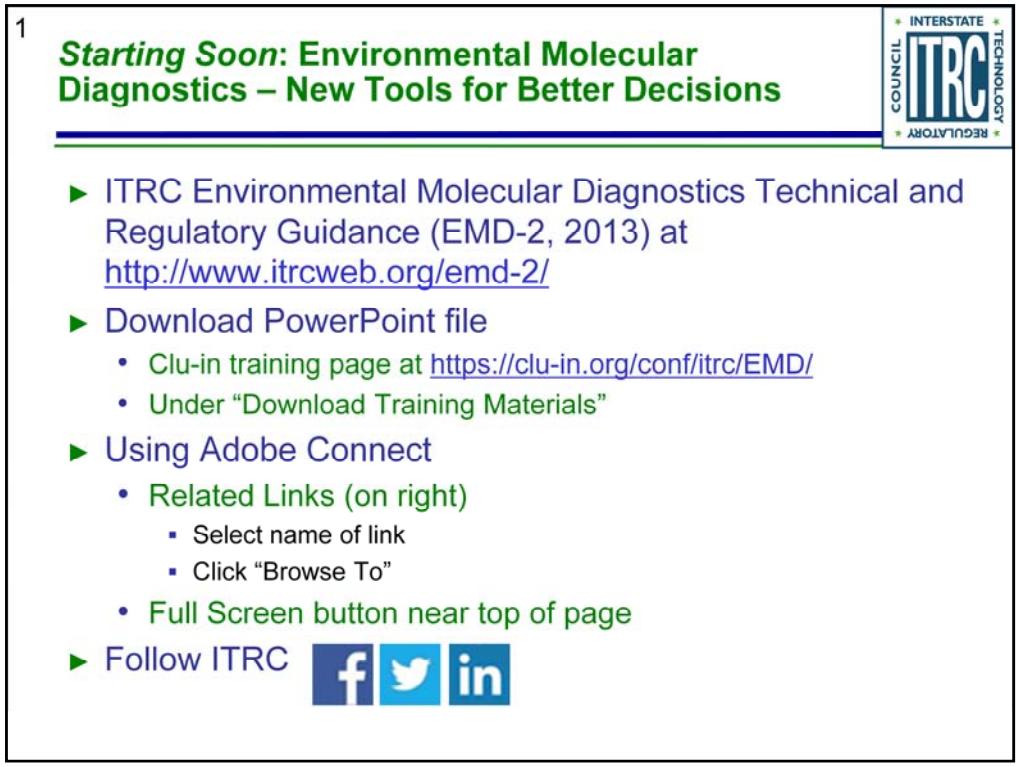


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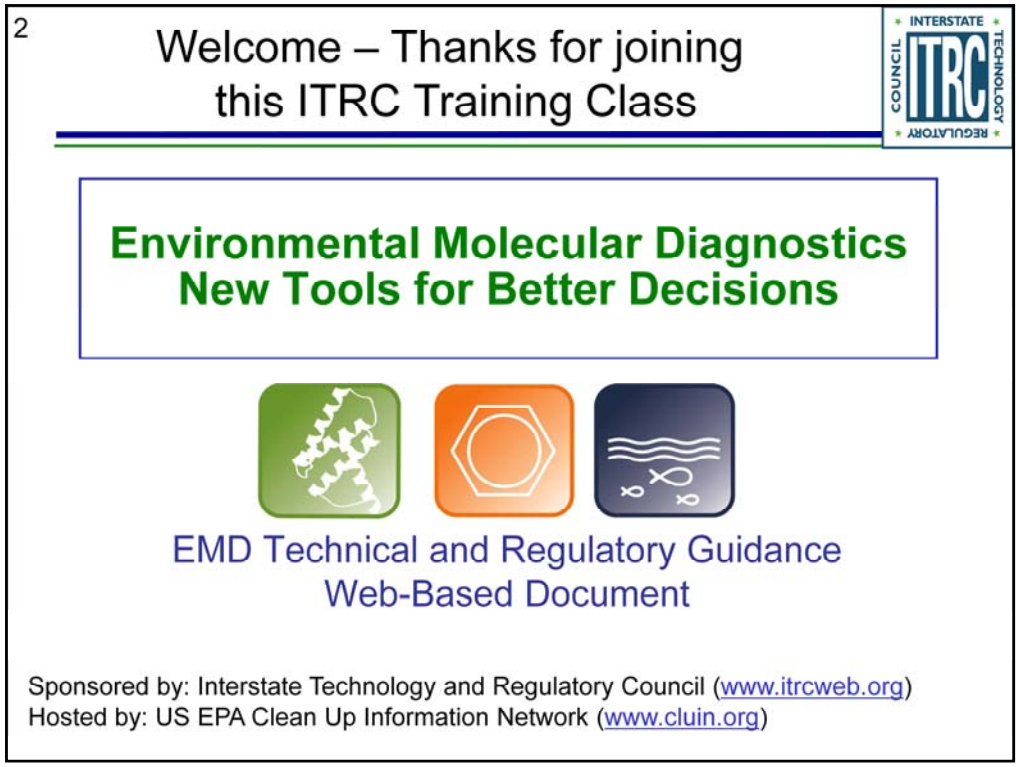
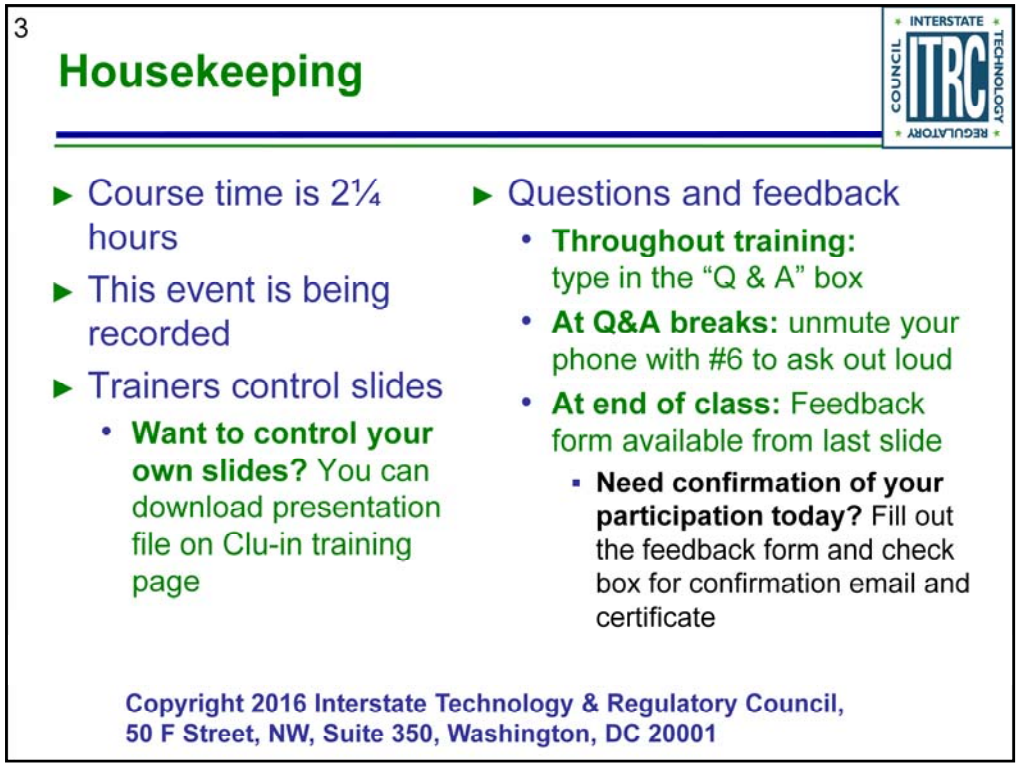



Environmental molecular diagnostics (EMDs) are a group of advanced and emerging analytical techniques used to analyze biological and chemical characteristics of environmental samples. Conventional data (e.g., hydrogeological data, chemical, and geochemical analyses) often provide only indirect data regarding the mechanisms and rates of key attenuation or treatment processes. EMDs can complement these data by providing direct measurements of the organisms, genes or enzymes involved in contaminant biodegradation, of the relative contributions of abiotic and biotic processes, and of the relative rates of various degradation processes. The information provided by EMDs can improve estimates of attenuation rates and capacities and improve remedy performance assessments and optimization efforts. Improved understanding of the biological and non-biological degradation processes also can lead to greater confidence in MNA or closure decisions. EMDs have application in each phase of environmental site management (including site characterization, remediation, monitoring, and closure activities), address a wide variety of contaminants (including PCE, PCBs, radionuclides, perchlorate, fuels), and work with various media (including groundwater, soil, sediments, soil vapor).

Although EMDs have been used over the past 25 years in various scientific fields, particularly medical research and diagnostic fields, their application to environmental remediation management is relatively new and rapidly developing. The ITRC Environmental Molecular Diagnostics Fact Sheets (EMD-1, 2011), ITRC Environmental Molecular Diagnostics Technical and Regulatory Guidance (EMD-2, 2013) and this companion Internet-based training will foster the appropriate uses of EMDs and help regulators, consultants, site owners, and other stakeholders to better understand a site and to make decisions based on the results of EMD analyses. At the conclusion of the training, learners will be able to determine when and how to use the ITRC Environmental Molecular Diagnostics Technical and Regulatory Guidance (EMD-2, 2013); define when EMDs can cost-effectively augment traditional remediation data sets; and describe the utility of various types of EMDs during remediation activities.

ITRC (Interstate Technology and Regulatory Council) www.itrcweb.org
Training Co-Sponsored by: US EPA Technology Innovation and Field Services Division (TIFSD) (www.clu-in.org)
ITRC Training Program: training@itrcweb.org; Phone: 402-201-2419

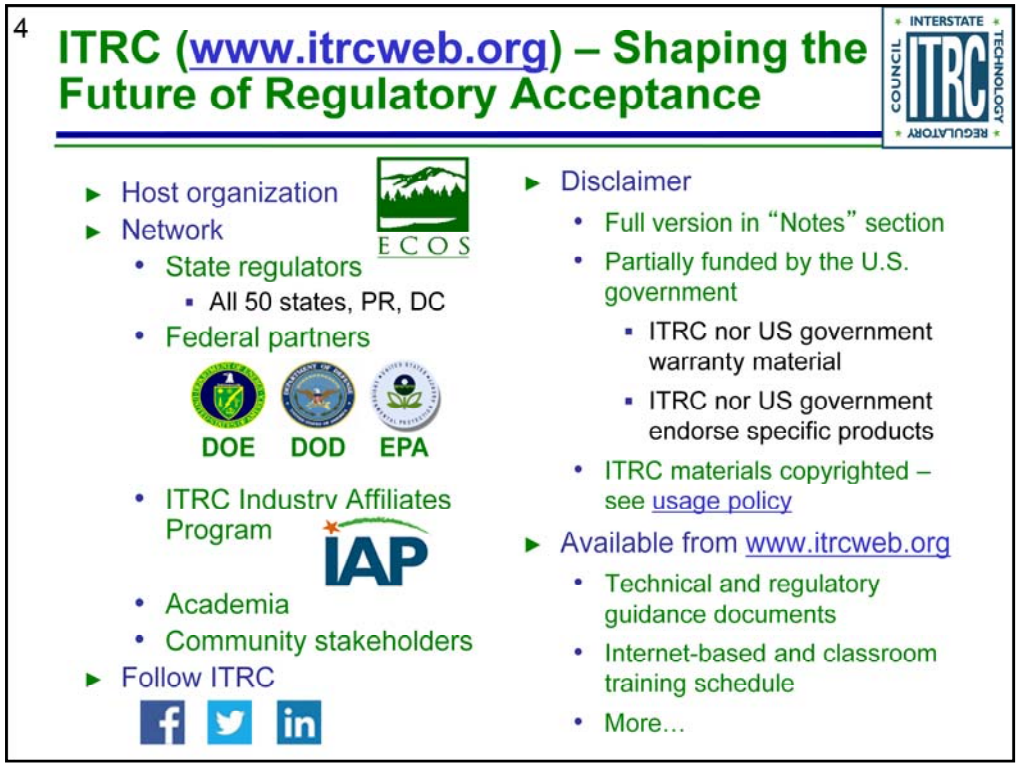



Although I'm sure that some of you are familiar with these rules from previous CLU-IN events, let's run through them quickly for our new participants.

We have started the seminar with all phone lines muted to prevent background noise. Please keep your phone lines muted during the seminar to minimize disruption and background noise. During the question and answer break, press #6 to unmute your lines to ask a question (note: *6 to mute again). Also, please do NOT put this call on hold as this may bring unwanted background music over the lines and interrupt the seminar.

Use the "Q&A" box to ask questions, make comments, or report technical problems any time. For questions and comments provided out loud, please hold until the designated Q&A breaks.

***Everyone*** – please complete the feedback form before you leave the training website. Link to feedback form is available on last slide.




The Interstate Technology and Regulatory Council (ITRC) is a state-led coalition of regulators, industry experts, citizen stakeholders, academia and federal partners that work to achieve regulatory acceptance of environmental technologies and innovative approaches. ITRC consists of all 50 states (and Puerto Rico and the District of Columbia) that work to break down barriers and reduce compliance costs, making it easier to use new technologies and helping states maximize resources. ITRC brings together a diverse mix of environmental experts and stakeholders from both the public and private sectors to broaden and deepen technical knowledge and advance the regulatory acceptance of environmental technologies. Together, we're building the environmental community's ability to expedite quality decision making while protecting human health and the environment. With our network of organizations and individuals throughout the environmental community, ITRC is a unique catalyst for dialogue between regulators and the regulated community.

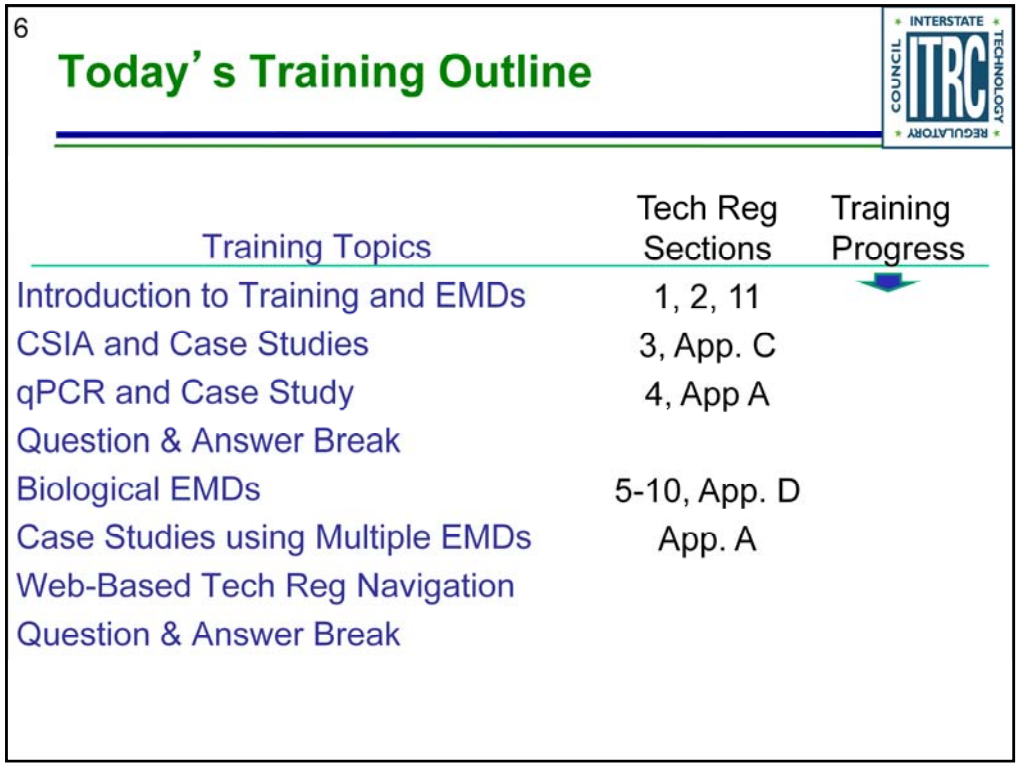
For a state to be a member of ITRC their environmental agency must designate a State Point of Contact. To find out who your State POC is check out the "contacts" section at www.itrcweb.org. Also, click on "membership" to learn how you can become a member of an ITRC Technical Team.


**Jennifer Weidhaas** is an Associate Professor of Civil and Environmental Engineering at University of Utah. Previously, she was an Assistant Professor of Civil and Environmental Engineering at West Virginia University, from 2010-2016 where she focused on determining the fate, transport and bioremediation of emerging contaminants. Prior to taking a position at WVU, Jennifer worked for six years with North Wind Inc. as a professional environmental engineer on hazardous waste remediation projects. At North Wind, Inc. she directed a molecular biology laboratory which routinely analyzed groundwater, soil and sediment samples by various EMD methods for microorganisms involved in contaminant bioremediation. Additionally she has work on numerous groundwater, soil and sediment bioremediation projects throughout the western United States. She has been a member of the Environmental Molecular Diagnostics Team since 2010. Jennifer earned a bachelor's degree in Civil Engineering in 1999 from Montana State University at Bozeman and a master's in 2002 and PhD in 2006 from the University of California at Davis in Civil and Environmental Engineering. Jennifer is a registered professional engineer in Idaho.

**Paul Hatzinger** is a senior research scientist working with SERDP in Lawrenceville, NJ. Paul's current areas of research focus on the development of *in situ* and *ex situ* bioremediation technologies for emerging contaminants and the use of stable isotope methods to distinguish contaminant sources and to document contaminant biodegradation. Paul has served at the Principal Investigator on several research grants focused on the use of stable isotopes to delineate natural from anthropogenic perchlorate in the environment, and to document the biodegradation of perchlorate and explosives in groundwater. He has authored more than 50 peer-reviewed research papers and book chapters, including several on perchlorate forensics. Paul has been a member of the ITRC Environmental Molecular Diagnostics (EMD) team since its inception. He earned a bachelor's degree in Biology and Environmental Science from St. Lawrence University in Canton, NY in 1986, and he holds both a master's degree (1991) and a doctoral degree (1996) in Environmental Toxicology from Cornell University in Ithaca, New York.

**James Fish** is an Environmental Program Specialist with the Contaminated Sites Program of the Alaska Department of Environmental Conservation (DEC), in Fairbanks, Alaska. He was worked for the Alaska DEC since 2009. As a Project Manager, he oversees the characterization and remediation of contaminated sites across Alaska's Interior. In addition to environmental cleanup, he has worked in many aspects of environmental microbiology, including water quality and treatment processes, aquaculture and fish health. His interests in microbial ecology involve biodegradation, microbial survival, and the activities of microorganisms in cold environments. He is currently the president-elect of the Alaska Chapter of the American Society of Microbiology. He joined the ITRC EMD team in 2011. He earned a bachelor's degree in Fisheries Biology in 1986 and a master's degree in Microbiology in 1991, both from the SUNY College of New York at Buffalo in Buffalo, New York.

**Dr. Aaron Peacock** has worked as an environmental scientist at Haley & Aldrich Inc. in Oak Ridge, Tennessee since 2006. Since 1995, he has worked both in academia and industry, specializing in biotechnology and environmental molecular diagnostics (EMDs). He has worked extensively in the development, evaluation, and implementation of new technologies for environmental surveillance, monitored natural attenuation (MNA), and enhanced bioremediation of metals and organic contaminants. His current focus has been on the use and development of electronic sensors and EMDs to aid in the evaluation of sites for in-situ bioremediation/MNA potential, performance of remediation programs, and forensics of non-performing sites. Aaron has been a member of the ITRC Environmental Molecular Diagnostics team since 2010. He earned a bachelor's degree in Soil Science in 1997, a master's degree in Biosystems Engineering Technology in 2000, and a doctoral degree in Environmental Science in 2007, all from The University of Tennessee in Knoxville, TN.

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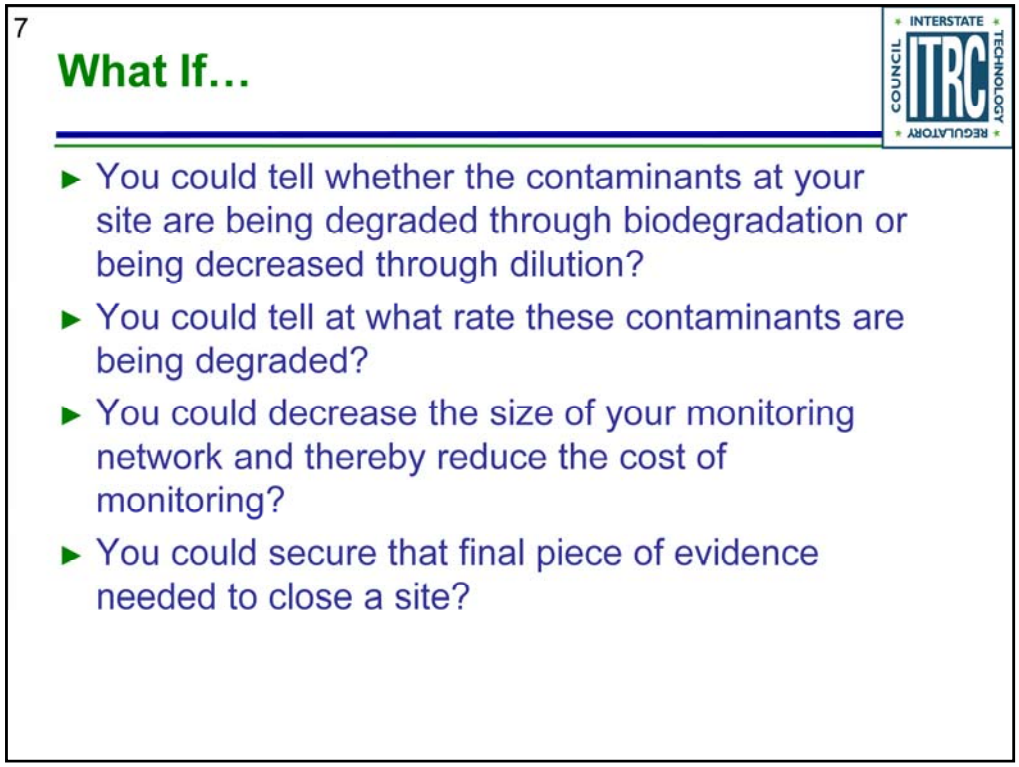
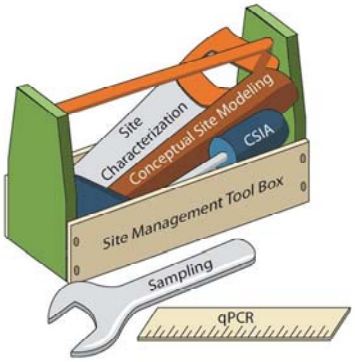



No associated notes.

8

# What Are Environmental Molecular Diagnostics (EMDs)?

- Group of analytical techniques
  - Used to analyze biological and chemical characteristics of soil, sediment, and water samples
- Developed for medicine
  - Adapted for environmental site management
- Two major categories
  - Chemical techniques
  - Molecular biological techniques (MBTs)

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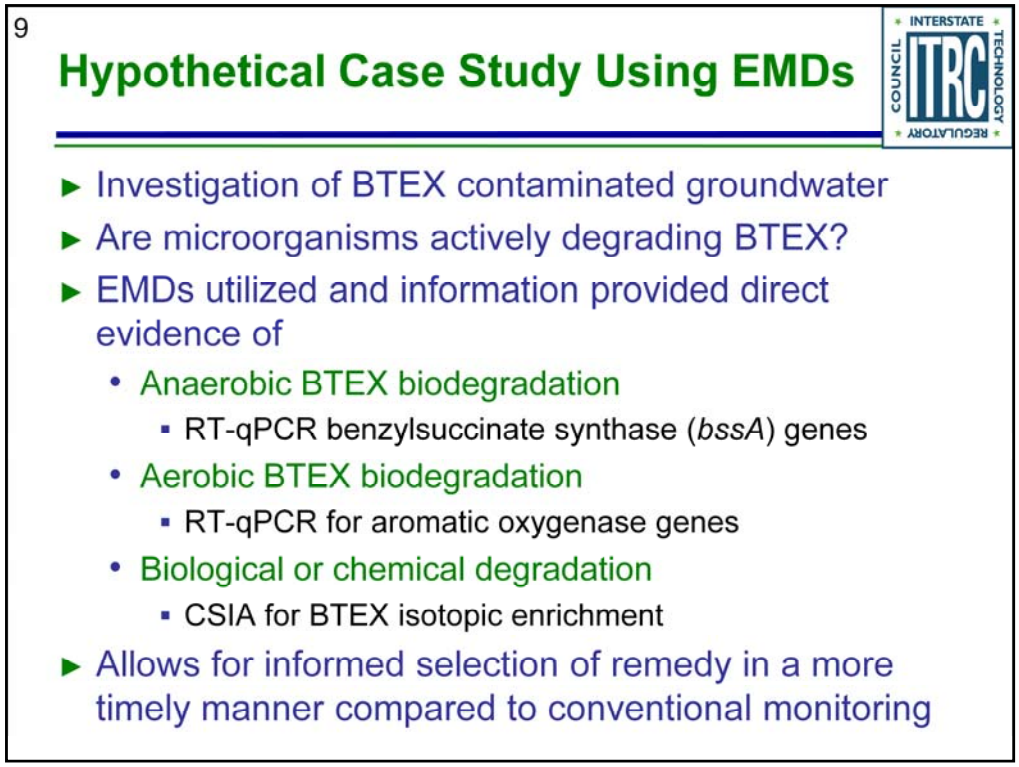
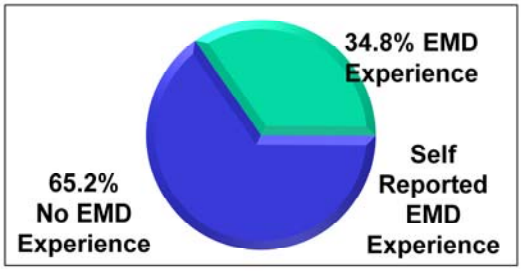


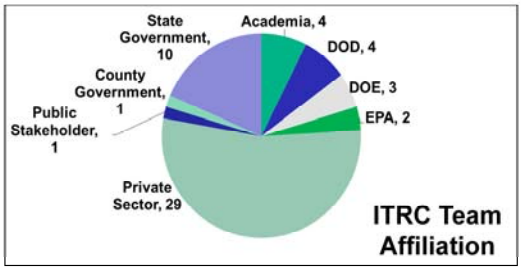

No associated notes.

10

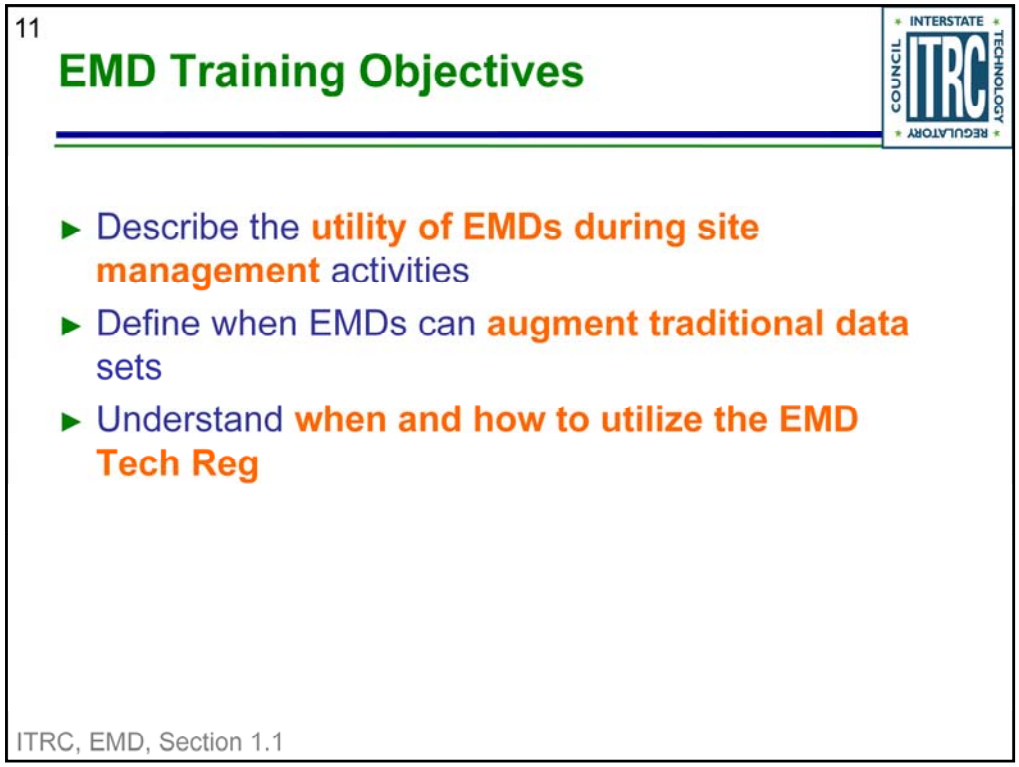
# What is the Level of Regulatory Knowledge and Acceptance?

- Survey results
  - 47 regulators from 29 states
  - Most EMDs are new to regulators
  - Expressed willingness to become educated
  - Agreed that EMDs can contribute toward regulatory decision making
  - Expressed concern about site implementation

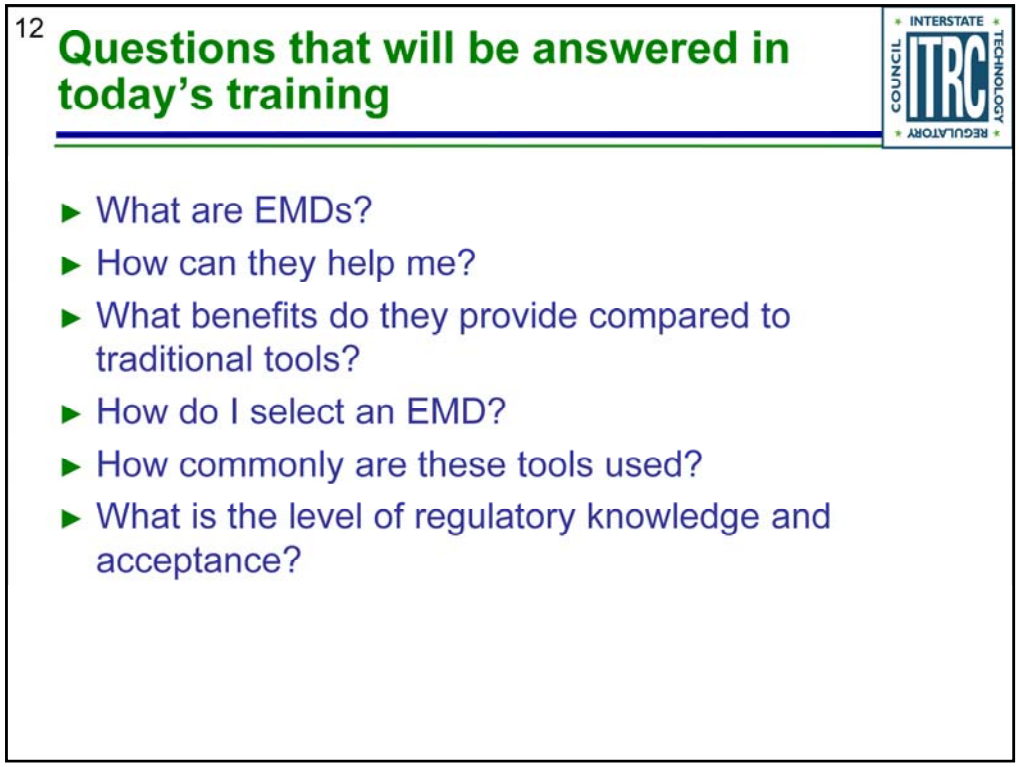



Survey results:
78 people completed the survey
60% were regulators, 21% were consultants
29 states were represented.




More information on ITRC is available at www.itrcweb.org.

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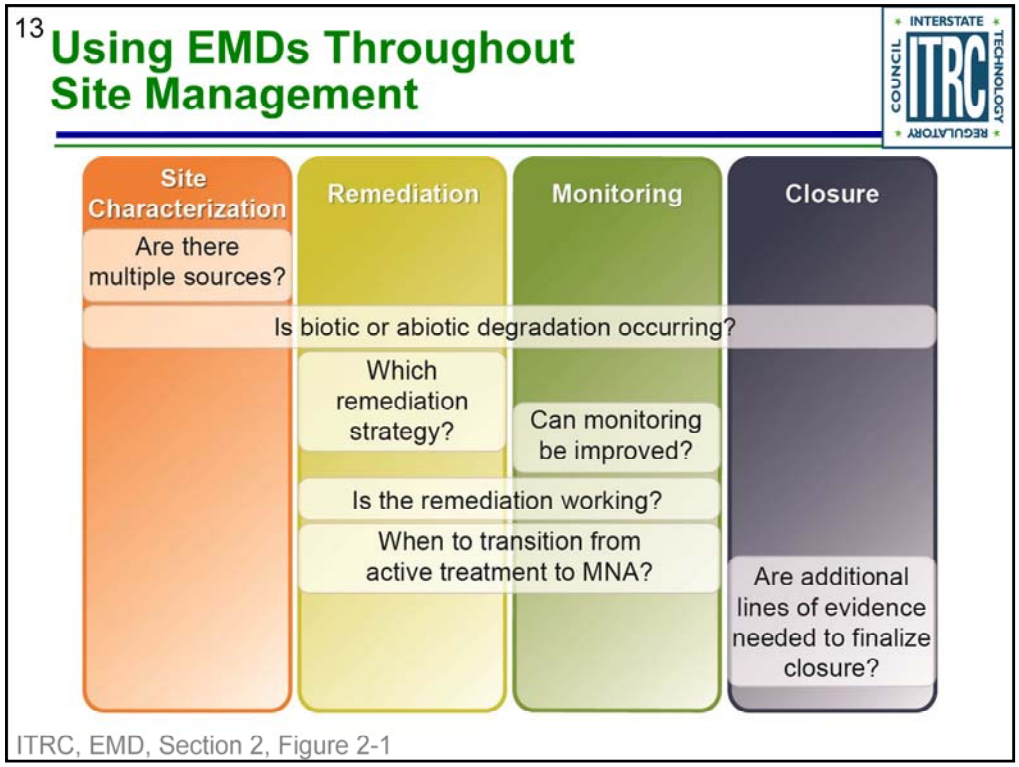
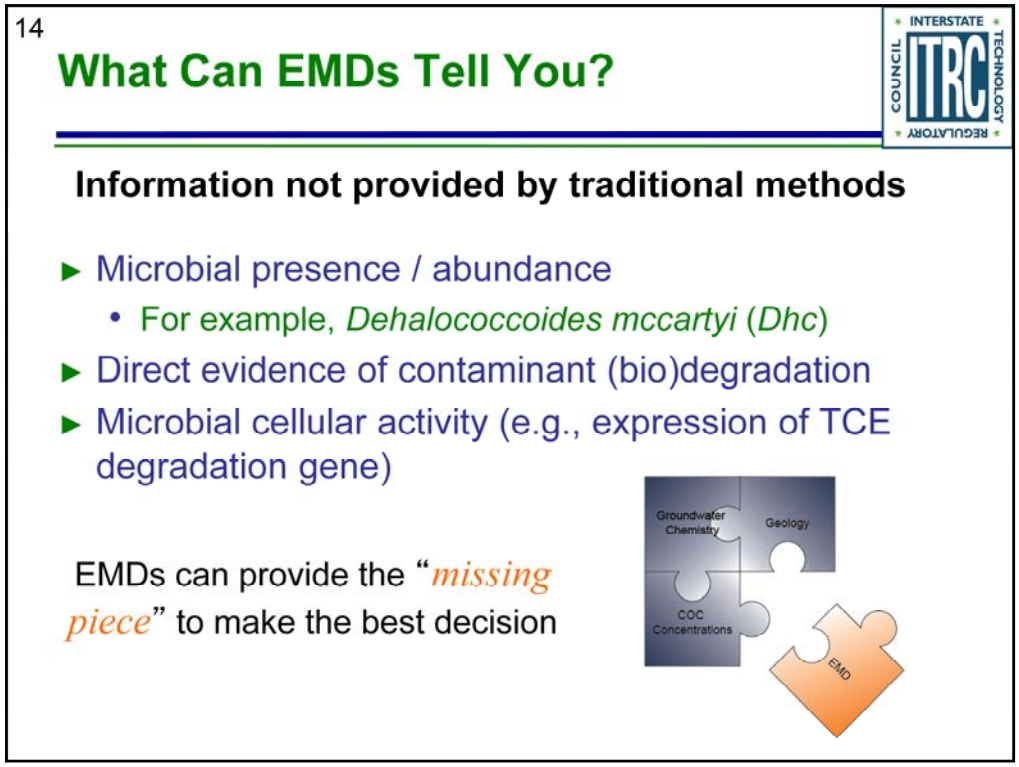



EMDs have applications throughout the life cycle of environmental cleanup projects. The terminology and regulatory framework for the stages of the project within its life cycle, however, often vary under different regulatory programs. For simplicity, this document organizes the discussion of site management around four main technical tasks: site characterization, remediation monitoring and closure.

The questions presented are only examples of the types of questions that EMDs can help to answer by providing supplemental information to the site-specific characterization data and information. The project life cycle stages are depicted as linear steps for simplicity. Often at sites the stages overlap; for example, some tasks under monitoring are conducted during what might be considered the remediation phase and some characterization tasks continue throughout the life of an environmental cleanup project.




The biological degradation pathway for the chlorinated solvent tetrachloroethylene (by Dehalococcoides spp.) is as follows:

tetrachloroethylene (PCE) to trichloroethylene (TCE) to dichloroethylene(DCE) to vinyl chloride (VC) to ethene

Dehalococcoides: Recent Developments

The biodegradation of chlorinated contaminants in the environment is an active area of research. The first Dehalococcoides isolate capable of complete dechlorination of PCE,'Dehalococcoides ethenogenes' strain 195, is capable of reductive dehalogenation of mono- and poly-chlorinated and brominated aromatic compounds, alkanes, and alkenes. (Maymó-Gatell et al. 1997).

Many Dehalococcoides strains have been isolated from geographically distinct freshwater locations (such as river sediments and aquifer materials), and exhibit differing dechlorination abilities, but share greater than 98% 16S rRNA gene sequence similarity (the cutoff typically used to classify two organisms as the same species). Specific reductive dehalogenase genes distinguish these different strains and confer distinct dechlorination capabilities among the strains.

Dehalococcoides mccartyi (Dhc) was recently published as the type species of the genus Dehalococcoides, which includes all characterized strains including strains 195, BAV1, CBDB1, FL2, GT and VS (Löffler et al. 2013). This species is the only known species with strains capable of complete dechlorination of tetrachloroethene (PCE) to ethene and inorganic chloride. More than a single Dhc strain may be included in commercially available bioaugmentation consortia. In the EMD-2 document, "Dhc" refers to Dehalococcoides mccartyi, including all of the strains that reductively dechlorinate chlorinated ethenes to environmentally benign ethene and inorganic chloride.

ADD Loeffler 2013 citation.

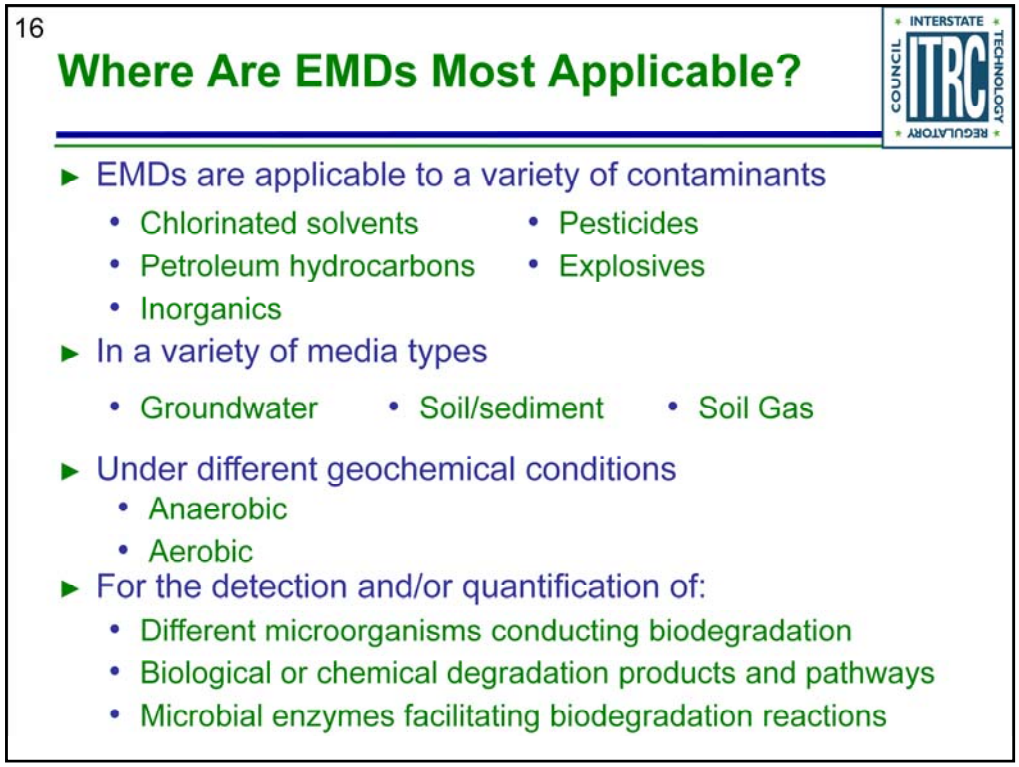
15

## Which EMDs Support Site Management Decision Making?

- Cells make a living by
  - Eating
  - Breathing
  - Getting rid of waste
  - Reproducing
  - Adapting to environment
- Cells use enzymes to facilitate these processes

- Molecular biological techniques (MBTs)
  - Quantitative polymerase chain reaction (qPCR)
  - Fingerprinting methods (DGGE, TRFLP, PLFA)
  - Microarrays
  - Stable isotope probing (SIP)
  - Fluorescence in situ hybridization (FISH)
  - Enzyme activity probes (EAPs)
- Chemical techniques
  - Compound specific isotopic analysis (CSIA)

No associated notes.

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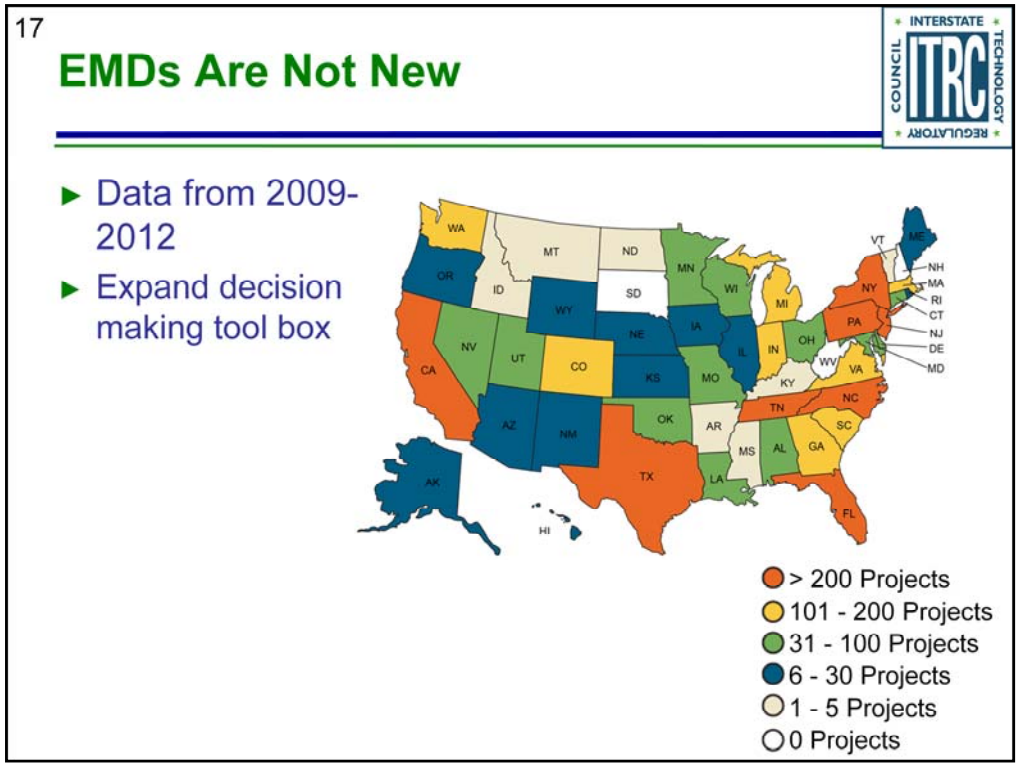



Environmental molecular diagnostics (EMDs) are a group of advanced and emerging analytical techniques used to analyze biological and chemical characteristics of environmental samples. Many of these techniques were originally developed for applications in medicine, defense, and industry.

Over the last decade, however, EMDs have proven effective in environmental site management. EMDs have applications in each phase of environmental site management and provide additional lines of evidence for making better remediation decisions. EMDs provide key information not available using traditional analytical methods (e.g., groundwater analysis for volatile organic compounds).

Decision makers pursue traditional strategies because they lack sufficient data to support alternatives that could result in equal or more efficient remediation at a lower cost and perhaps in less time.

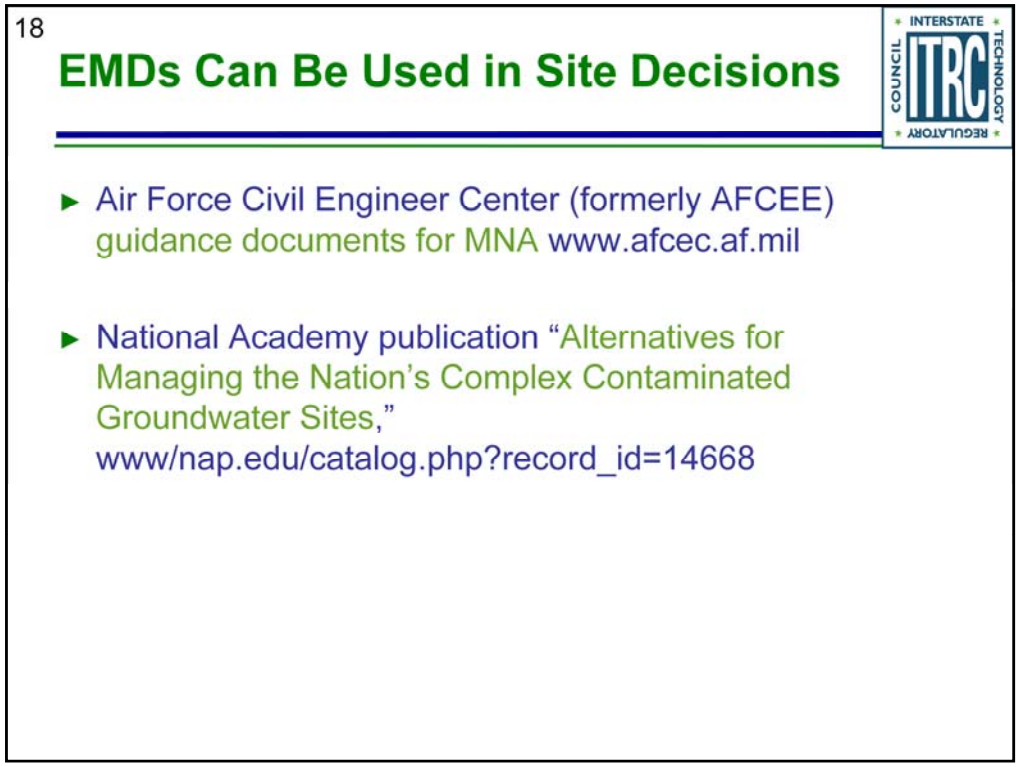
What EMDs can tell you:
•Microbial presence / abundance
•Microbial cellular activity (e.g., transcription)
•Biodegradation activity
•Direct evidence of contaminant biodegradation

The data are cumulative numbers from 2009-2012

The data are from two commercial labs, so they don't represent all of the EMD work that was completed in that time period

The states are assigned based on the information available to the labs, there is uncertainty in some of the project locations due to client confidentiality.

The map is intended to provide a relative understanding of the usage of EMDs and not provide definitive numbers of projects.




Many may be familiar with technical protocols or guidance for MNA; based on three lines of evidence: contaminant concentrations, geochemistry, and microbiology.
Microbiological information has traditionally comes form laboratory microcosms based on culturing microorganisms. EMD are culture-independent, can often provide in situ information, and can provide information on contaminant molecules (e.g., CSIA). Thus, additional lines of evidence are provided and strengthened with EMDs.

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## EMD Cost and Availability

| EMD | Cost per sample |
|---|---|
| **Widely commercially available** | |
| Quantitative Polymerase Chain Reaction (qPCR) | $ 275 - 425 |
| Compound Specific Isotope Analysis (CSIA) | $ 100 - 2,500 |
| Microbial Fingerprinting Methods | $ 300 - 570 |
| **Minimally commercially available** | |
| Microarrays | $ 1,250 - 5,000 |
| Stable isotope probing (SIP) | $ 1,500 and up |
| Enzyme activity probes (EAPs) | $ 250 - 2,500 |
| Fluorescence in situ hybridization (FISH) | $ 250 - 5,000 |

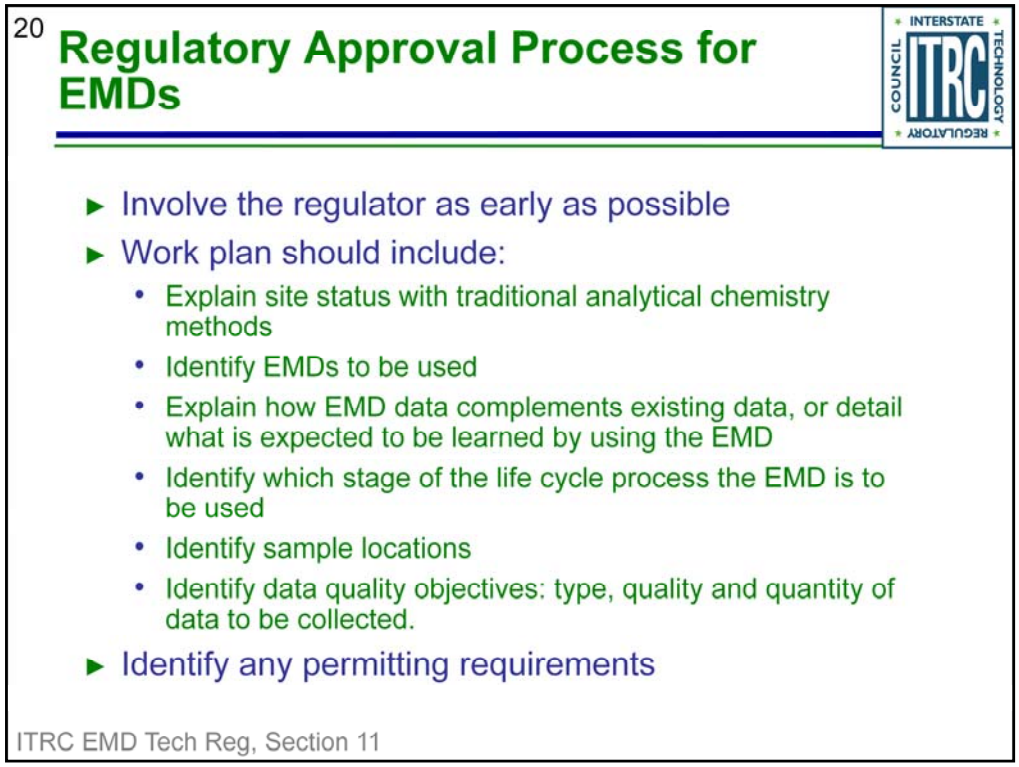
ITRC EMD-1, Introduction to EMDs Fact Sheet

Costs are per sample. Depends on how many samples and how many analyses are being done for the project. The low end of the cost range represents a very restricted analysis and may not be applicable to every site; for example for only one compound or target with very limited quality control documentation.

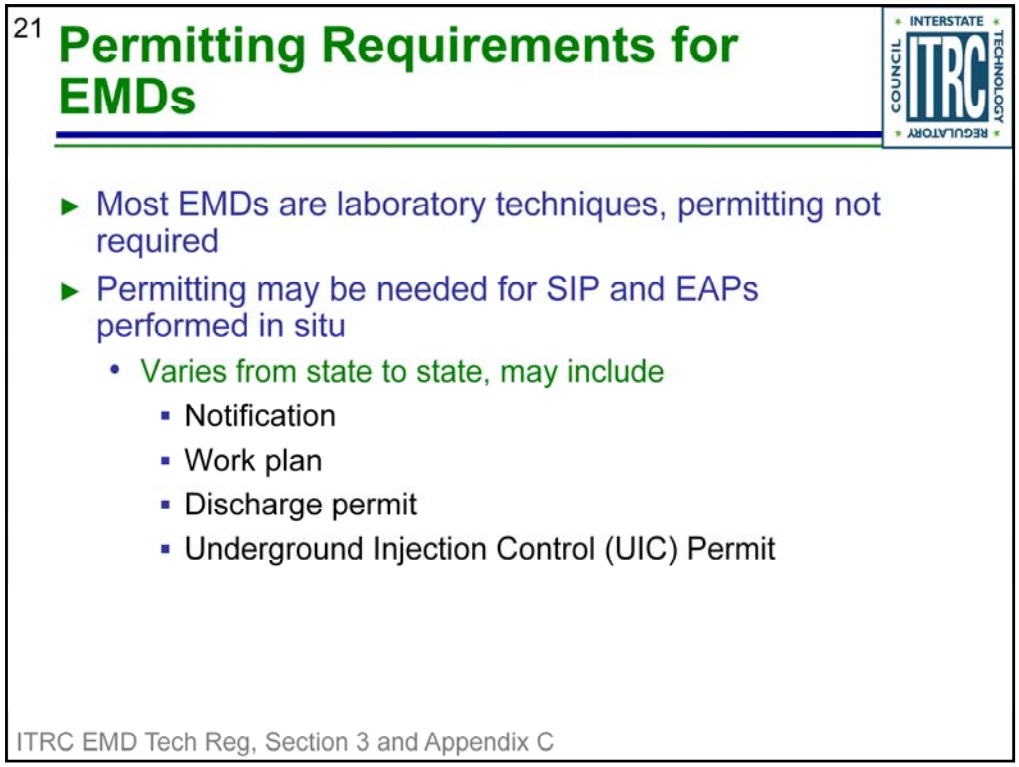
Some analyses for some methods (for example for some compounds or targets) are currently available through university or research laboratories.

CSIA costs per sample depend on the number and type of isotopes and the number of compounds being analyzed.

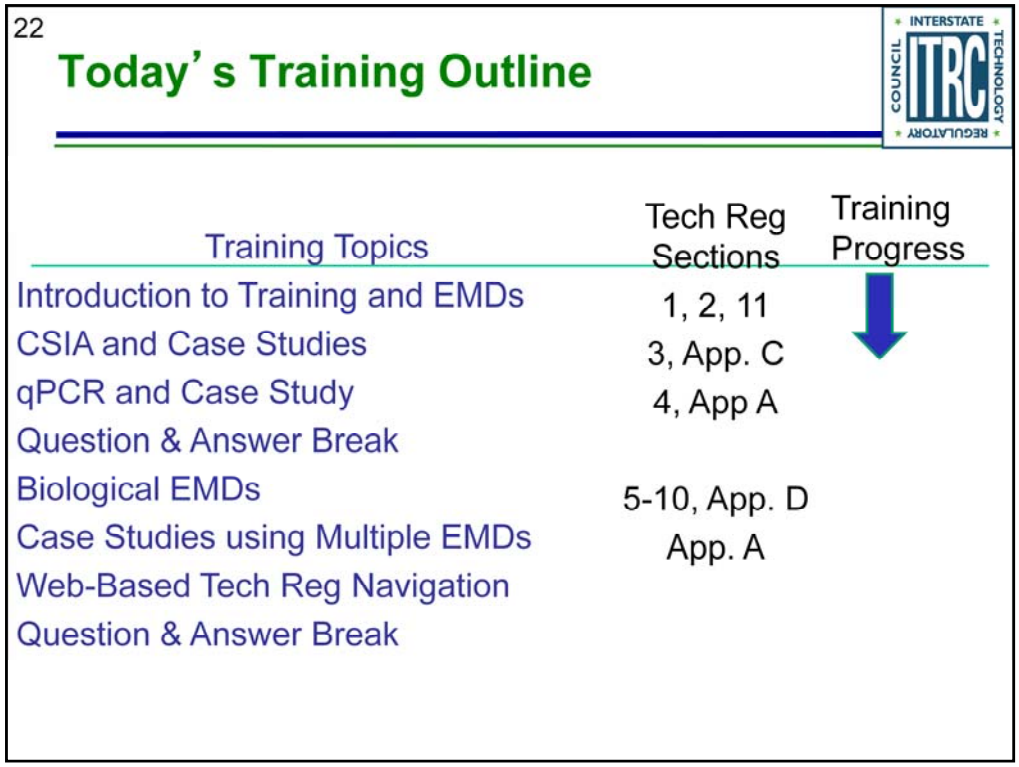
Availability and costs will be changing going forward. Information as of 2011.




The work plan can easily be adjusted to reflect all agreements reached during the meeting and can be submitted later as a final document.




The ITRC EMD team used survey results and results from a questionnaire completed by the states' POCs to identify permitting/regulatory concerns that may be raised when the use of EMDs is proposed. As expected, the responses varied from state to state. However, one can expect at a minimum to acquire approval for one or more, or all of the following: notification, a work plan, a discharge permit or a UIC Permit. The use of amended EMD sampling devices, such as stable isotope probing (SIP) and in-situ enzyme activity probes (EAPs), involve the introduction of contaminant-bearing materials into the subsurface. Although the introduced contaminants are small in quantity and are intended to stay in place, these in situ evaluations may require additional regulatory review and approval, or a UIC Permit. In cases where groundwater discharges to surface water, a discharge permit may be required. In cases where drinking water wells could potentially be impacted, it may be necessary to notify drinking water regulatory programs, or even end-users or well owners. A thorough review of permitting requirements and regulatory approval is encouraged on a site-specific basis whenever the use of EMDs is proposed.

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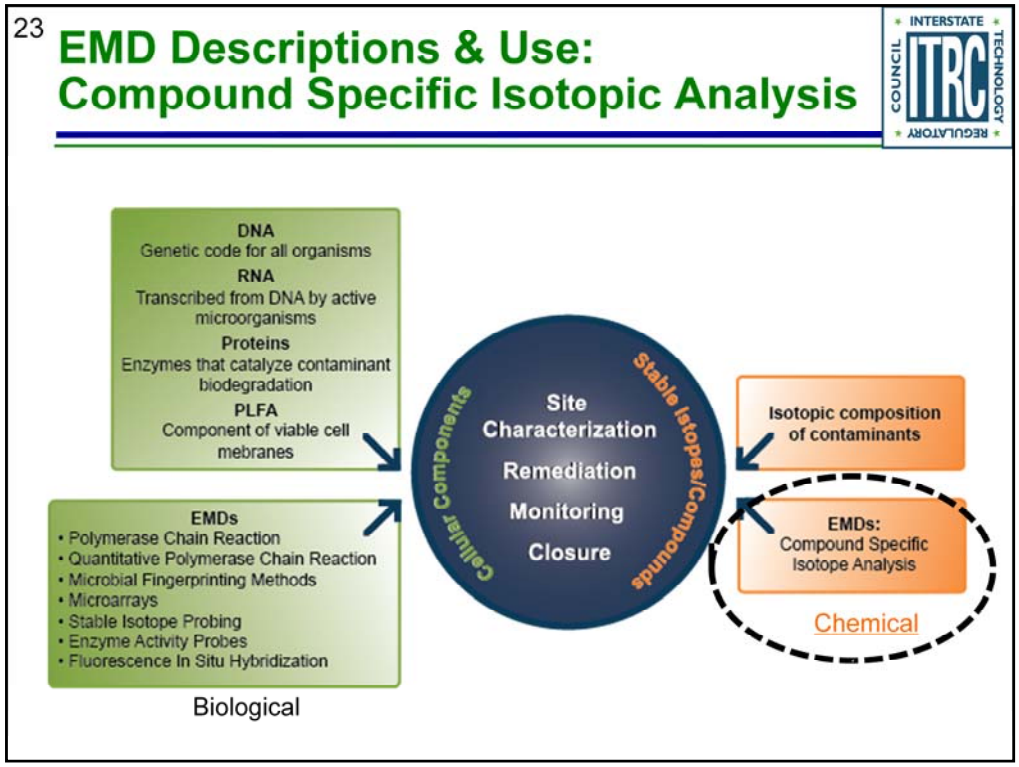
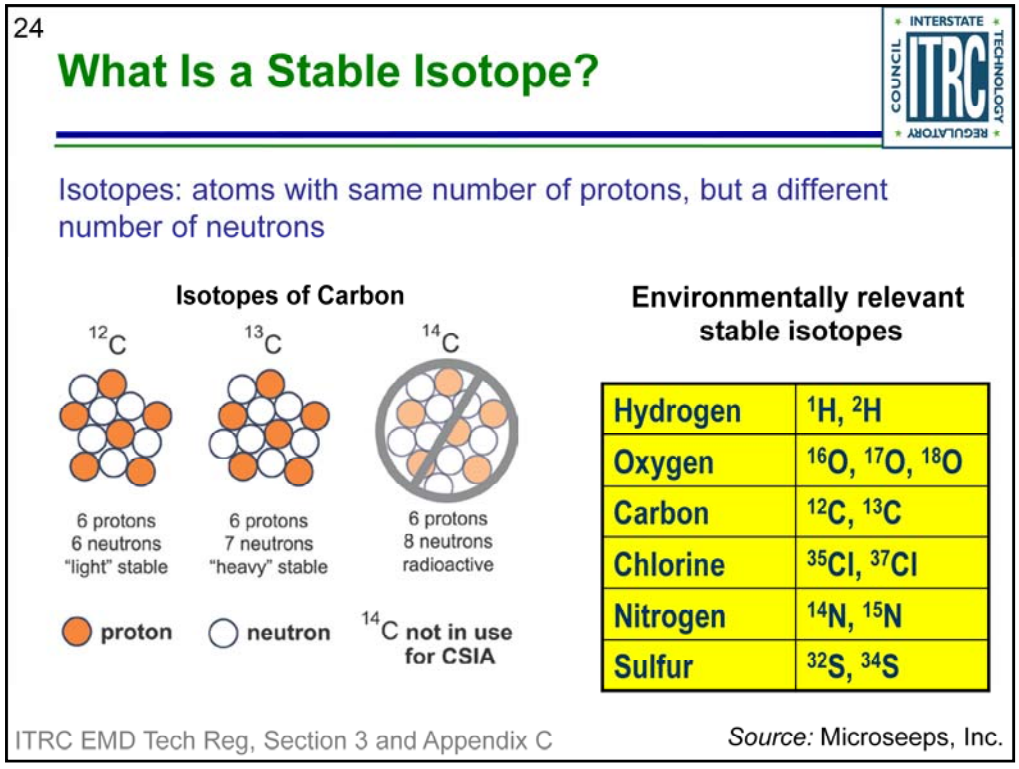
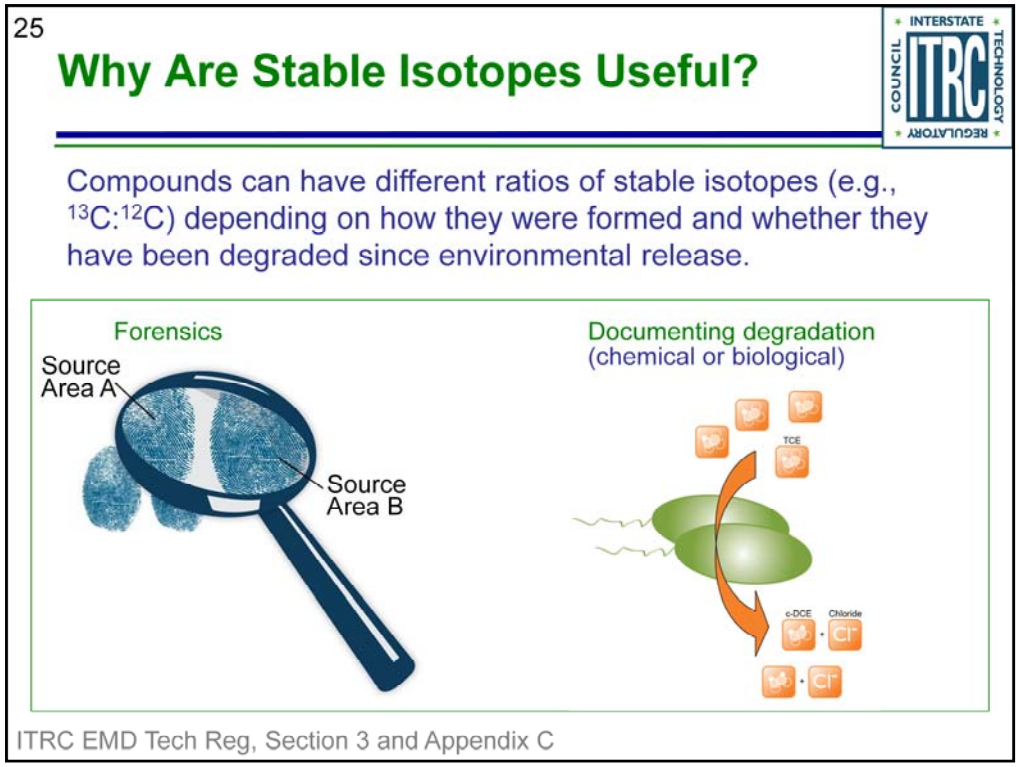



EMDs can be classified into two major categories of analytical techniques: chemical techniques, in particular compound specific isotope analysis (CSIA), and a variety of molecular biological techniques (MBTs).

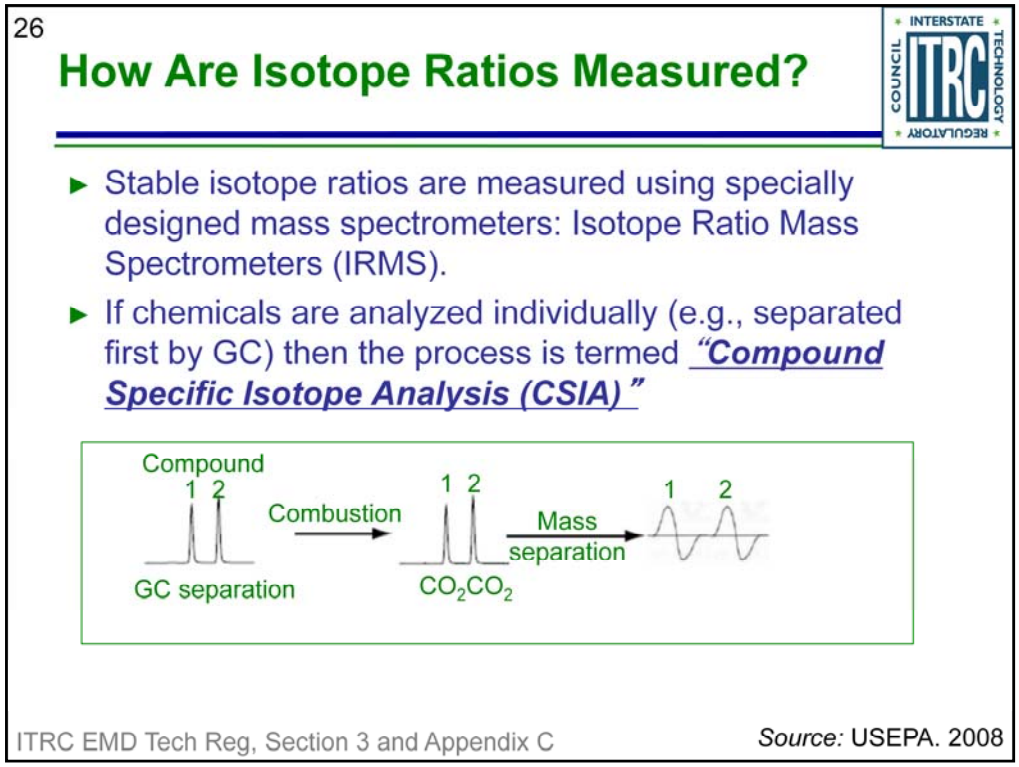
What can CSIA do for you?




Stable isotopes have the same number of protons and electrons, but a different number of neutrons. Many environmental contaminants contain elements that have multiple stable isotopes and for which CSIA may have useful applications.

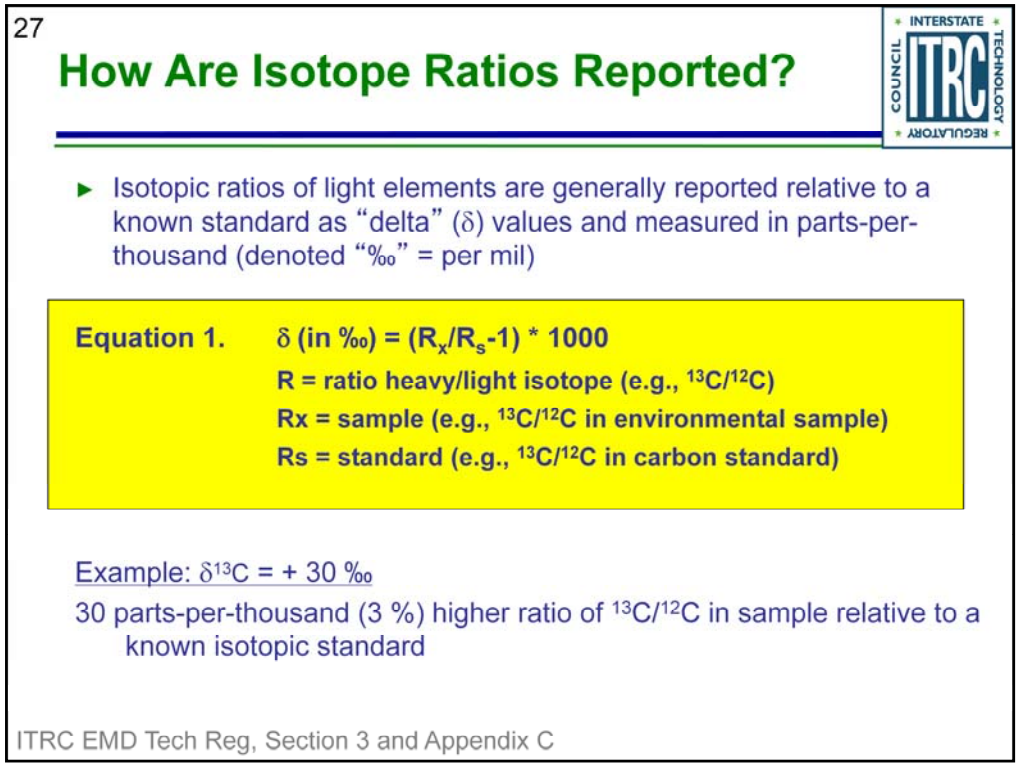



CSIA can be useful for forensic applications and for documenting degradation. Predictable shifts in stable isotope ratios occur during degradation of many pollutants by biological or abiotic methods.




*Source:* USEPA. 2008. A Guide for Assessing Biodegradation and Source Identification of Organic Groundwater Contaminants using Compound
Specific Isotope Analysis (CSIA). U.S. EPA National Risk Management Research Laboratory, ADA, OK ; EPA 600/R-08/148, 67 pg.

Bulk analysis is addressed in the Tech Reg in section 3.3.2.2 and in Appendix C.5.

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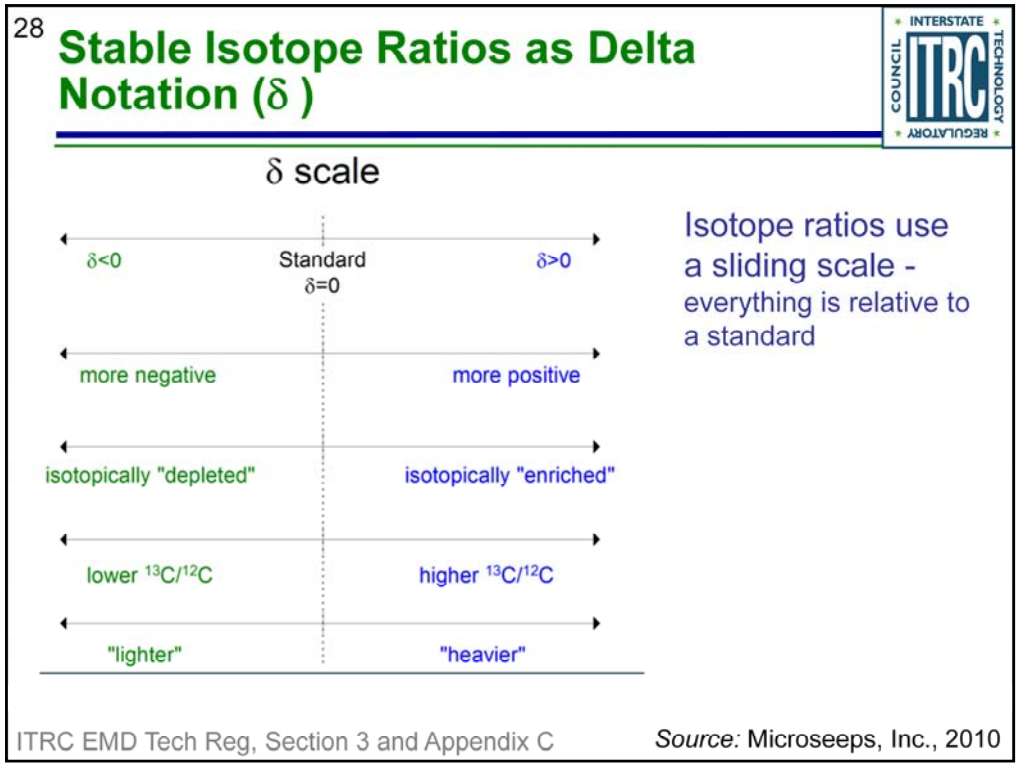
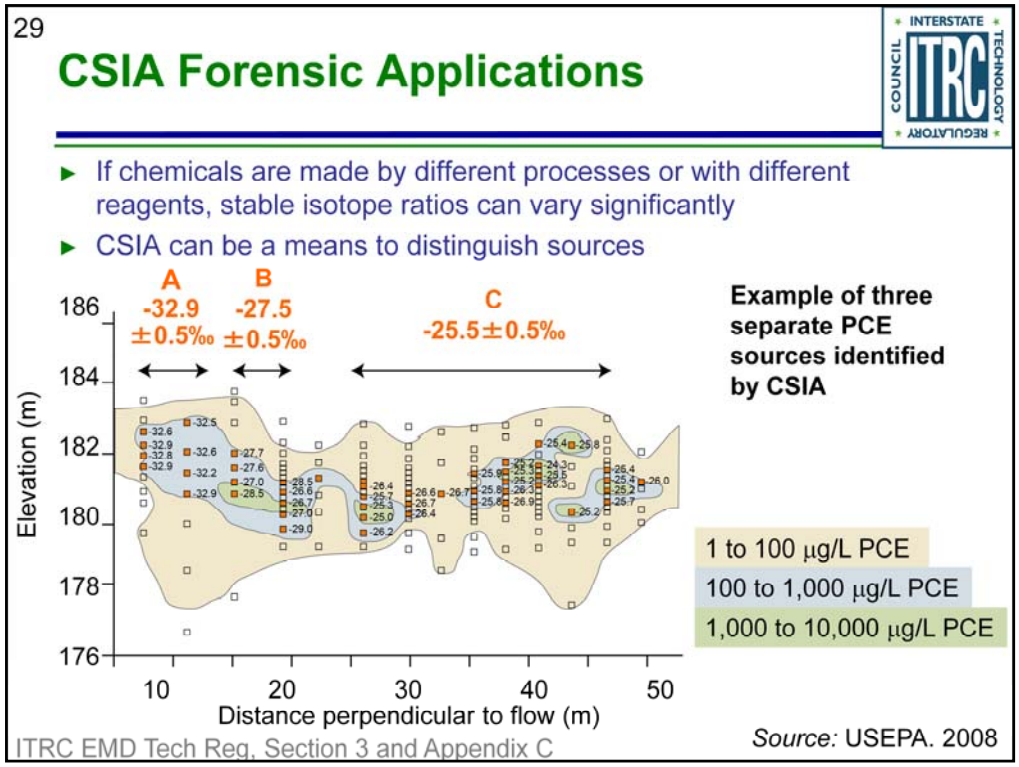



All elements have reference standards for stable isotope ratio analysis. Generally the stable isotope ratio (δ value)of these standards is set at 0. For example the reference standard for Carbon is Vienna Pee Dee Belemnite (VPDB).

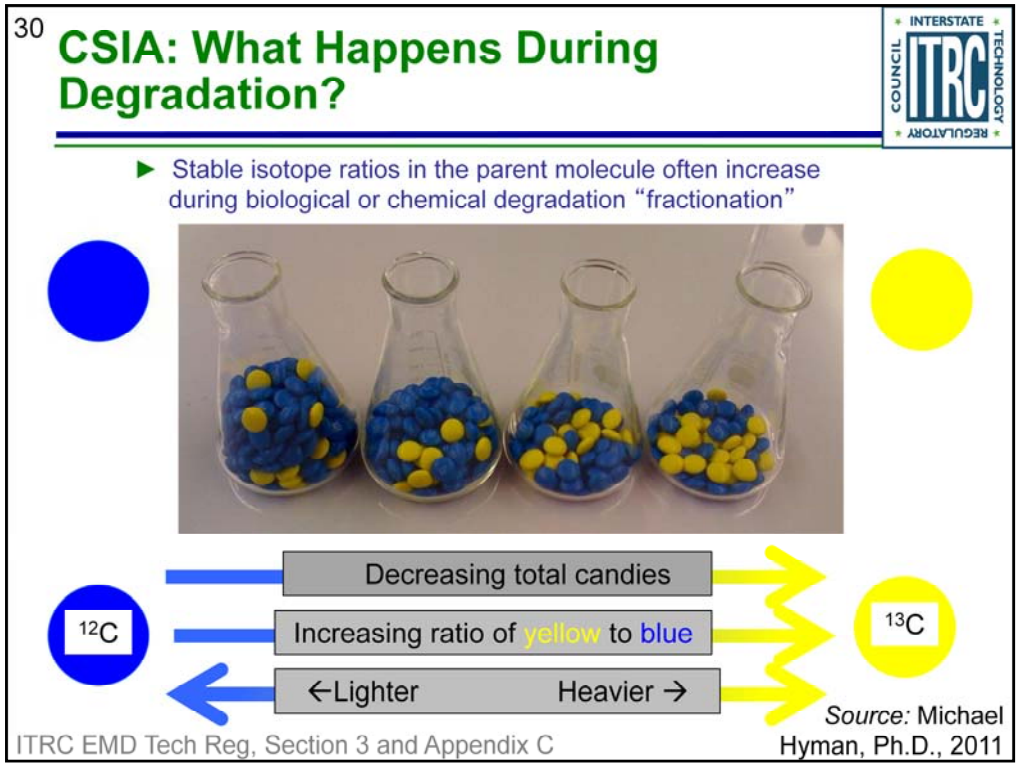
The δ value in a sample is compared to that in the standard for that element with values often being described as “heavier” or “lighter” than the relevant standard. Stable isotope ratios can be positive or negative. Table C-2 in Appendix C of the Tech. Reg. document includes reference standard information for common elements in environmental applications.




*Source:* USEPA. 2008. A Guide for Assessing Biodegradation and Source Identification of Organic Groundwater Contaminants using Compound
Specific Isotope Analysis (CSIA). U.S. EPA National Risk Management Research Laboratory, ADA, OK ; EPA 600/R-08/148, 67 pg.

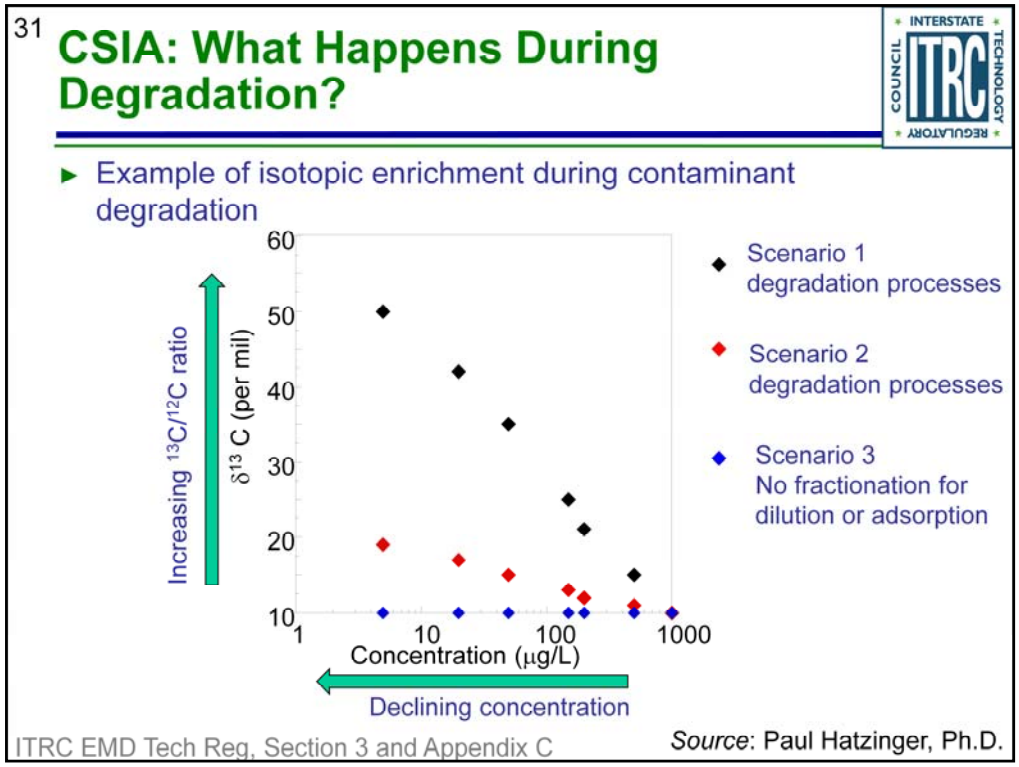
Further information available on precision of isotopic analyses in the EPA 2008 document.

On the graph above, the plume is flowing out of the page toward the reader. Delta values of PCE in nested wells are provided in the figure along with concentrations (see colors). The delta values (δ) for C in PCE collected from these wells suggest that there are three distinct sources (Zone A, B, C).

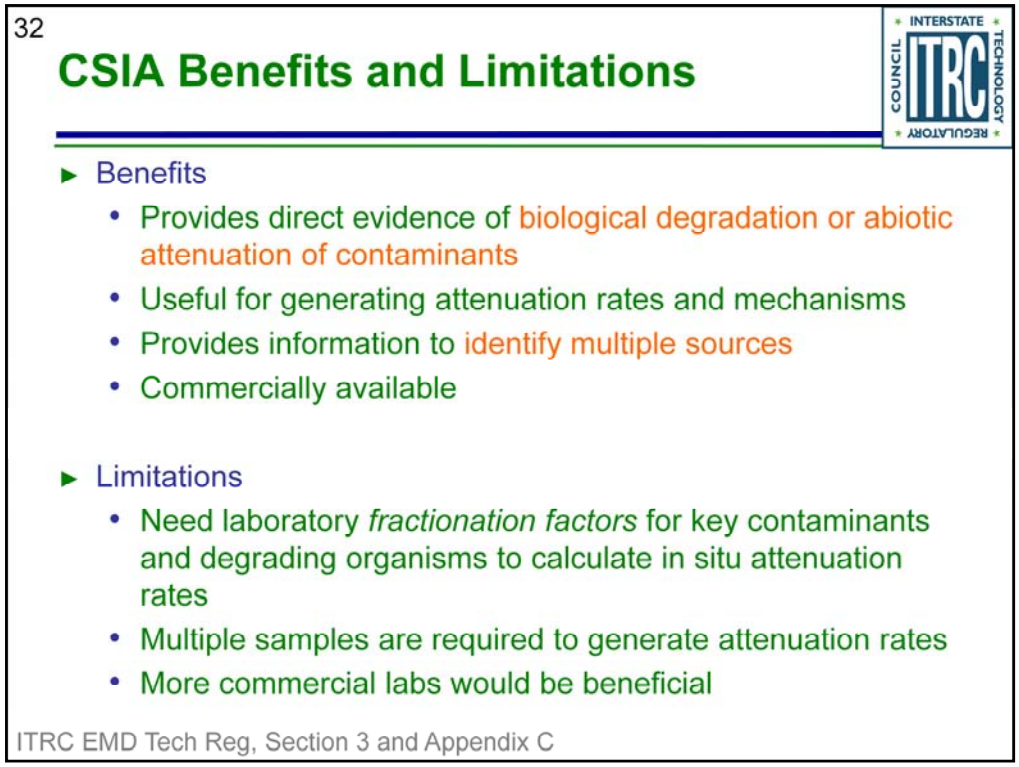



Bonds between heavier isotopes are stronger than those between lighter isotopes – broken less readily

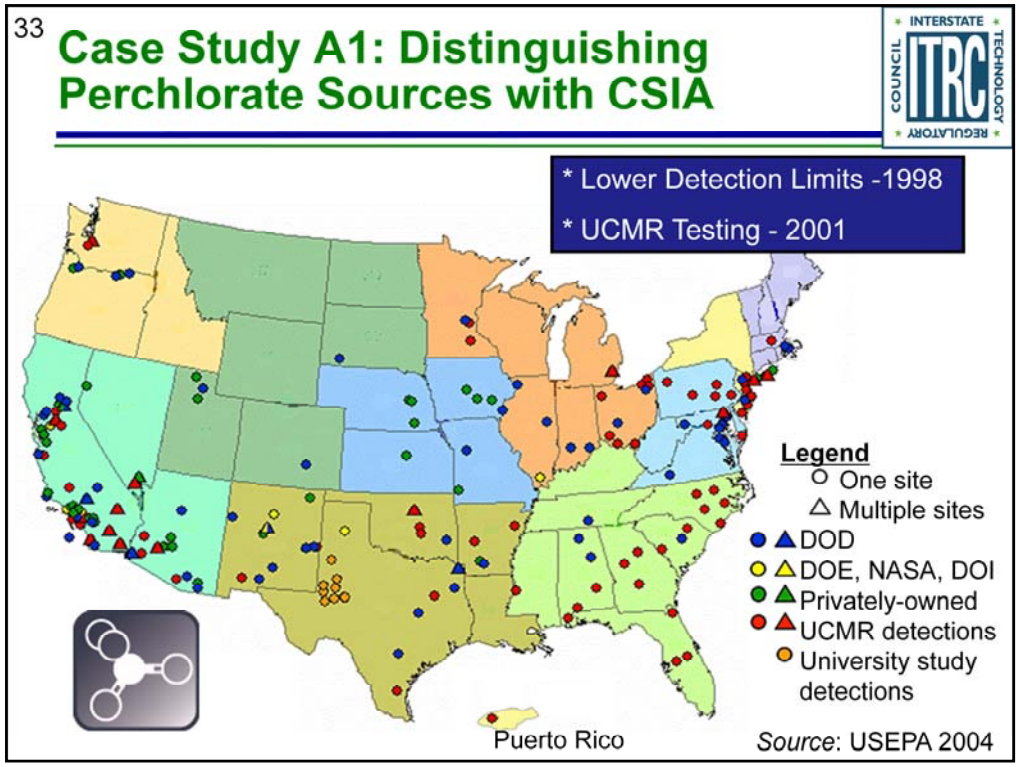
As a result, isotope ratios often increase in parent molecules when biodegradation or chemical degradation occurs – "fractionation"




The extent of isotope fractionation during degradation is often measured by plotting chemical concentration vs. the δ value for the isotope. This approach can be used to show that degradation is occurring (increasing δ value with declining chemical concentration) and get an indication of mechanism (e.g., by evaluating slope of the lines in the figure above – more vs. less fractionation), particularly if you can measure isotopes of multiple elements in a molecule (e.g., Cl and C in TCE).



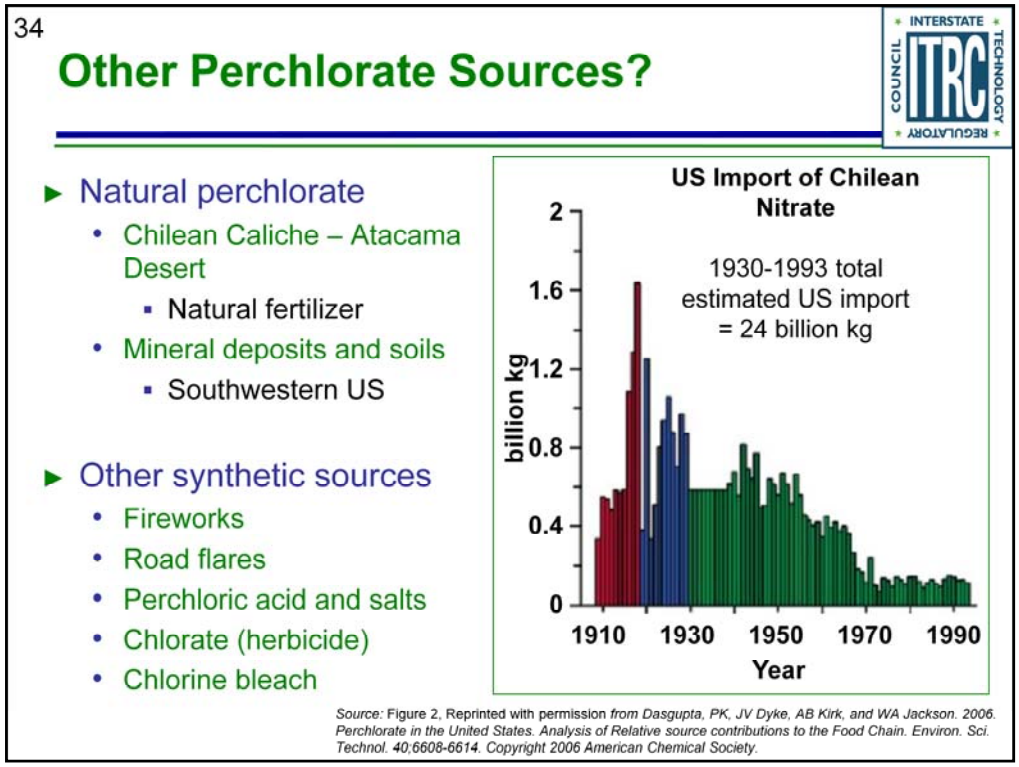

No associated notes.




Source USEPA 2004. “National Perchlorate Detections as of September 23, 2004.” Federal Facilities Restoration and Reuse Office. See also ITRC Perchlorate: Overview of Issues, Status, and Remedial Options (PERC-1, Sep-05).

Perchlorate is a solid oxidant that is widely used in rocket motors (e.g., space shuttle rocket boosters contain 380 tons each) as well as various other military items.
Historically, perchlorate was considered to be a contamination issue unique to the military and aerospace industries with a limited number of potential sites.

With improved detection limits and required testing under the Unregulated Contaminant Monitoring Rule in 2001, it became apparent in the early 2000’s that perchlorate in groundwater was much more prevalent than expected based on the previous paradigm? In many instances, there was no apparent correlation between perchlorate detected in groundwater and any military, aerospace, or manufacturing facility.




*Source:* Figure 2, Reprinted with permission *from Dasgupta, PK, JV Dyke, AB Kirk, and WA Jackson. 2006. Perchlorate in the United States. Analysis of Relative source contributions to the Food Chain. Environ. Sci. Technol. 40;6608-6614. Copyright 2006 American Chemical Society.*

Data references are included in Dasgupta et al 2006 – reference numbers 36, 38 and 39.

Several sources other than military/aerospace use of propellants are potentially important, including commercial synthetic sources and natural perchlorate derived from Chilean nitrate fertilizer or atmospheric production and accumulation. Perchlorate associated with Chilean nitrate fertilizer averaged 750 metric tons per year in imported materials from 1930 -1993 (Dasgupta et al). Natural perchlorate that has formed in the atmosphere and accumulated in dry areas of the Southwest is also a potential source – not going to be a focus of this case study.

35

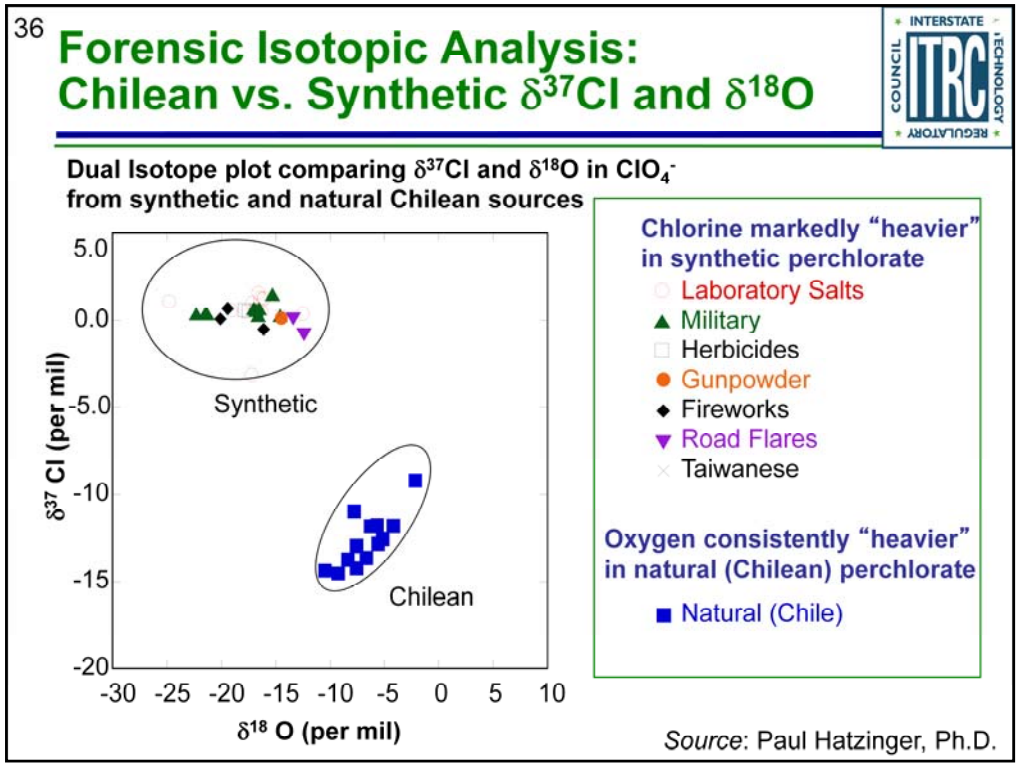
## CSIA for Perchlorate

- Methods developed for analysis of both O and Cl isotopes in perchlorate ($ClO_4^-$)
- Analyzed by Isotope Ratio Mass Spectrometer (IRMS) with a precision of about ± 0.1 to 0.5 ‰
- Special columns developed to collect perchlorate from groundwater

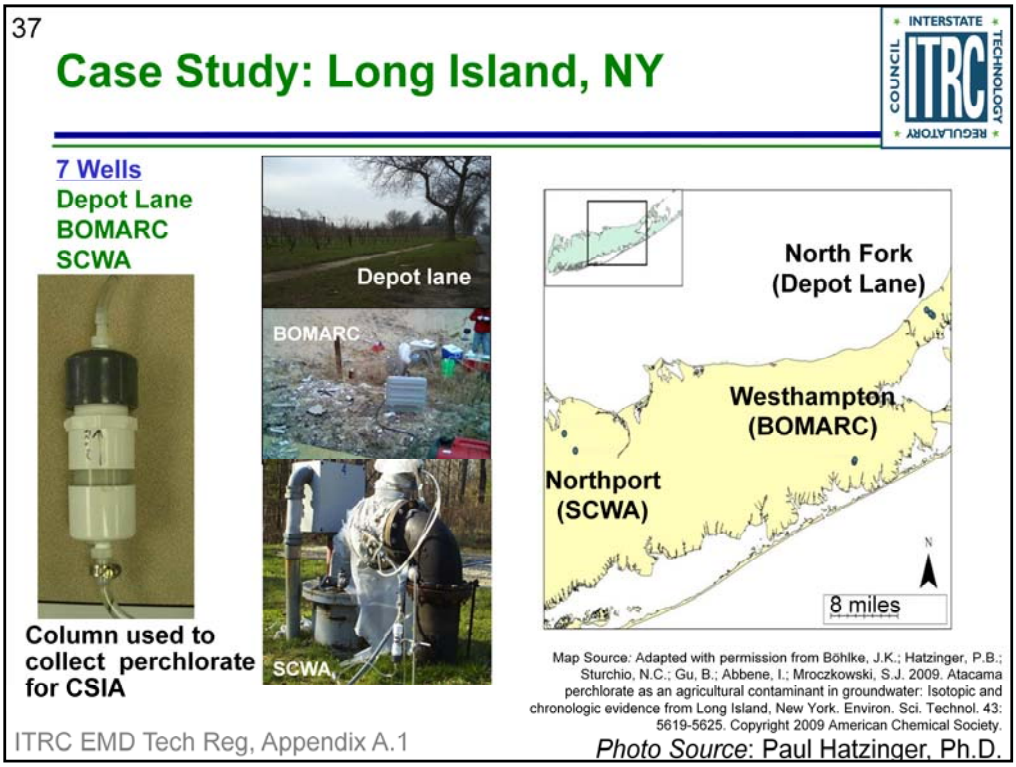
Elements in a compound can have widely different isotopic ratios based on mode of Formation. Stable isotope ratios can provide a unique "fingerprint"

| | |
|---|---|
| Hydrogen | $^{1}H$, $^{2}H$ |
| Oxygen | $^{16}O$, $^{17}O$, $^{18}O$ |
| Carbon | $^{12}C$, $^{13}C$ |
| Chlorine | $^{35}Cl$, $^{37}Cl$ |
| Nitrogen | $^{14}N$, $^{15}N$ |
| Sulfur | $^{32}S$, $^{34}S$ |

Methods have been developed to analyze both Cl and O isotopes in perchlorate with good precision. Oxygen has three stable isotopes (16-O, 17-O, 18-O) and chlorine has two (35-Cl, 37-Cl). The ratios of isotopes in compounds can vary widely based on their mode of formation, which is why stable isotope ratio analysis is sometimes useful for forensic evaluations.




When measuring stable isotope ratios of Cl and O in source materials, all synthetic perchlorate samples (from military sources, flares, fireworks, lab reagents, and other sources) have very similar Cl isotope ratios, with a $\delta^{37}$Cl value very near O (similar to the seawater standard used for comparison). The d$^{18}$O for synthetic perchlorate varies more widely, presumably based upon the source water used to make perchlorate in an electrochemical cell (water is the source of oxygen in perchlorate manufacture). In contrast, perchlorate derived from Chilean materials (surface salts and imported fertilizers) has a much lower $\delta^{37}$Cl value, and a significantly heavier average d18O value. When plotted on a dual isotope plot, these sources are clearly distinguishable based on their stable isotope values of Cl and O.




Map Source*:* Adapted with permission from Böhlke, J.K.; Hatzinger, P.B.; Sturchio, N.C.; Gu, B.; Abbene, I.; Mroczkowski, S.J. 2009. Atacama perchlorate as an agricultural contaminant in groundwater: Isotopic and chronologic evidence from Long Island, New York. Environ. Sci. Technol. 43: 5619-5625. Copyright 2009 American Chemical Society.

This case study of perchlorate sources in groundwater was conducted on Long Island, NY. There have been a number of perchlorate detections on Long Island in monitoring and public supply wells, with unknown sources. This area has a long agricultural history, as well as potential commercial and military sources. Seven different wells with unknown sources of perchlorate were samples on Long Island, NY. These included three wells in an agricultural area on the North Fork (Depot Lane), two wells at a former BOMARC missile site (BOMARC; land now used for training by the Suffolk County PD), and two large public supply wells in Northport owned by the Suffolk County Water Authority (SCWA). Perchlorate was collected by passing water through small ion exchange columns, to trap~ 5-10 mg per well. A number of other supporting parameters were also collected in each well, including anions and cations, nitrate isotopes, groundwater age dating parameters, dissolved gases, and others. It is always important to utilize CSIA in conjunction with other supporting evidence in forensic investigations.

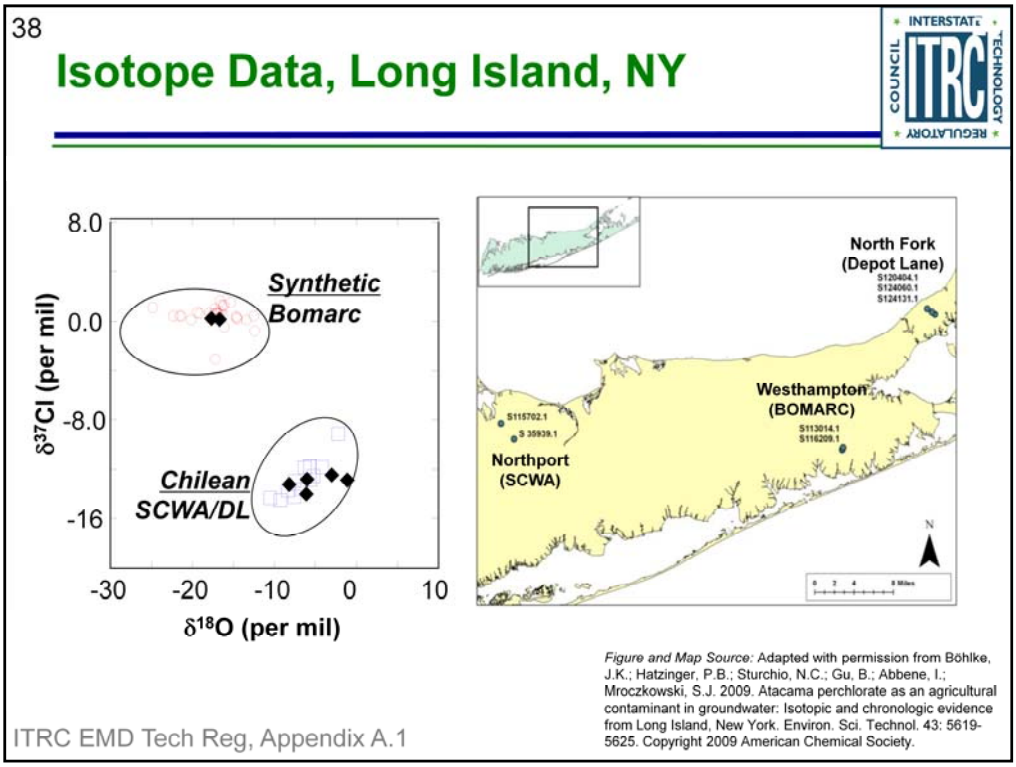
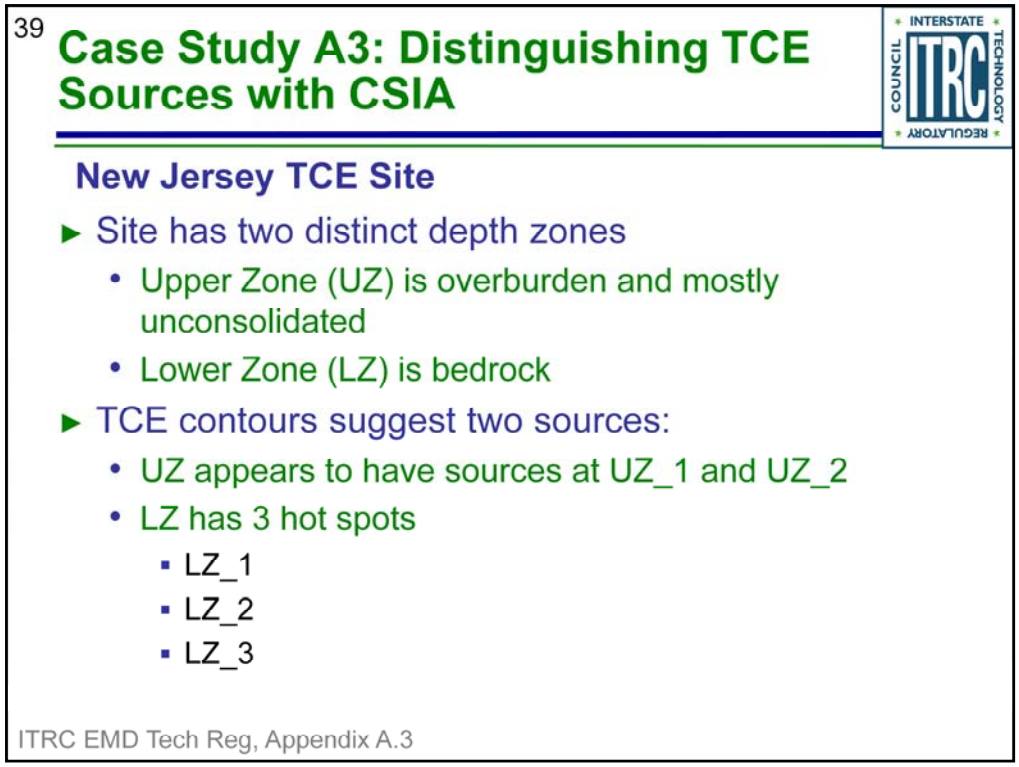
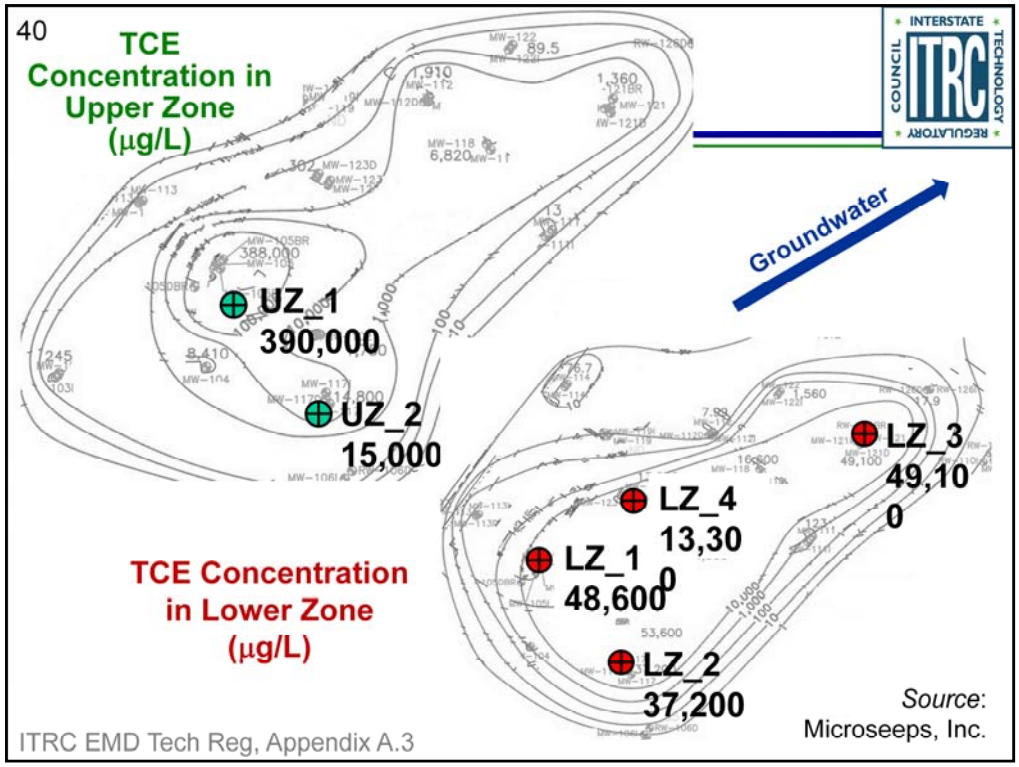



*Figure and Map Source:* Adapted with permission from Böhlke, J.K.; Hatzinger, P.B.; Sturchio, N.C.; Gu, B.; Abbene, I.; Mroczkowski, S.J. 2009. Atacama perchlorate as an agricultural contaminant in groundwater: Isotopic and chronologic evidence from Long Island, New York. Environ. Sci. Technol. 43: 5619-5625. Copyright 2009 American Chemical Society.

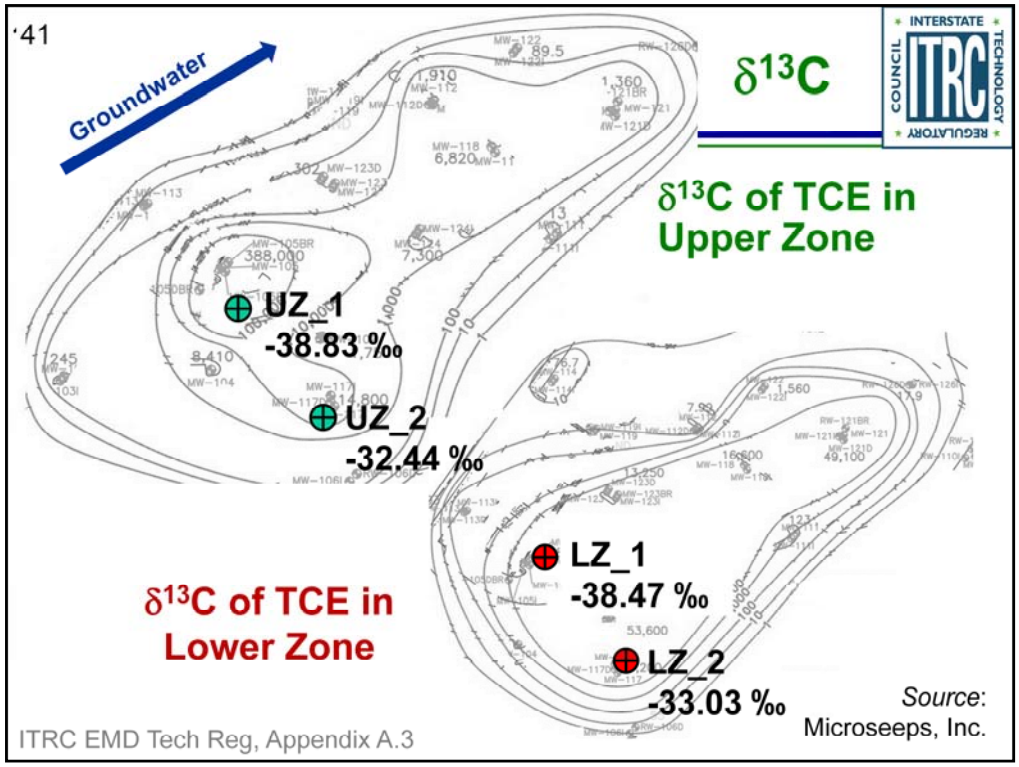
The isotope data clearly showed two distinct sources in the wells tested. Two of the wells had perchlorate with Cl and O isotope values that were indistinguishable from synthetic sources that has been previously analyzed. These wells, at the former BOMARC site, are believed to have been impacted by disposal of fireworks by the Suffolk County Police Department, who currently use this area as a training facility (and dispose of illegal fireworks there). The other 5 wells tested had perchlorate with isotopic signatures similar to that of perchlorate in Chilean nitrate fertilizers. Thus, the unknown source in this region appears to be past application of Chilean nitrate fertilizers. A large number of wells in California have also been shown in recent years to have perchlorate form this source using CSIA.



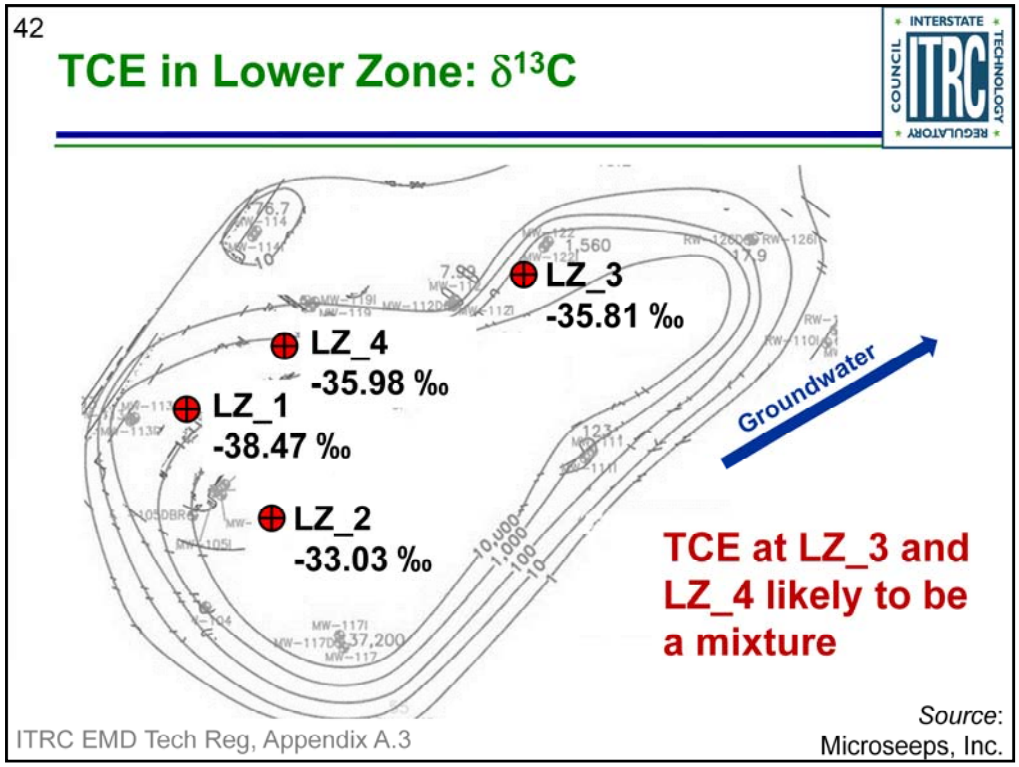

New Jersey Site. Stable isotope analysis of C in TCE was conducted to evaluate whether one or two sources were present at a NJ site.




Well UZ_1 is in an alluvial aquifer and is located above LZ_1, which is screened in bedrock. Similarly, well UZ_2 is screened in alluvium above bedrock well LZ_2. Wells LZ_3 and LZ_4 are downgradient bedrock wells.

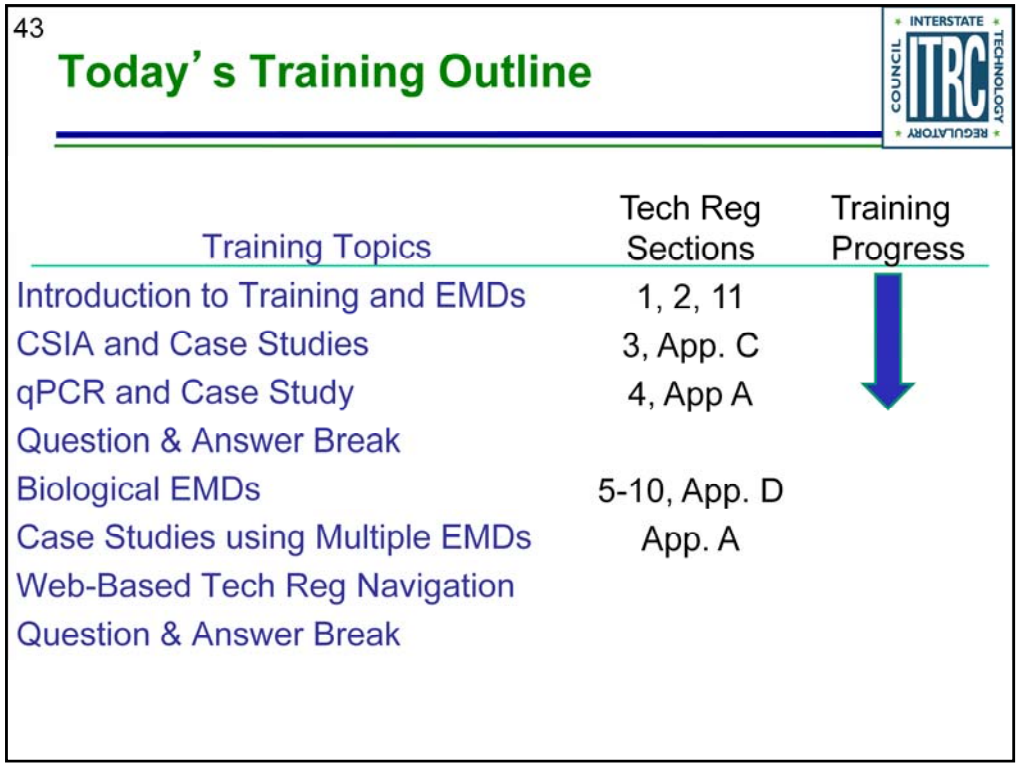



Stable isotope data are very similar for TCE in UZ_1 and LZ_1. The source appears to be the same in this region in alluvium and bedrock.
The isotopic signature of C in TCE at UZ_1 and LZ_1 is also very negative. The stable isotope value of C in TCE at UZ_2 and LZ_2 is similar, but distinctly different than that at UZ_1 and LZ_1. The data suggest two distinct sources on this site, one affecting UZ_1/LZ_1 and one affecting UZ_2/LZ_2.




The stable isotope ratios in LZ_3 and LZ_4 are between those of the two sources at LZ_1 and LZ_2, suggesting that the TCE in these wells is a mixture of the two sources.

Later site assessment work using a membrane interface probe (MIP) supported the CSIA results, showing two distinct sources. Determining whether two sources were present was critical for site planning and management decisions.

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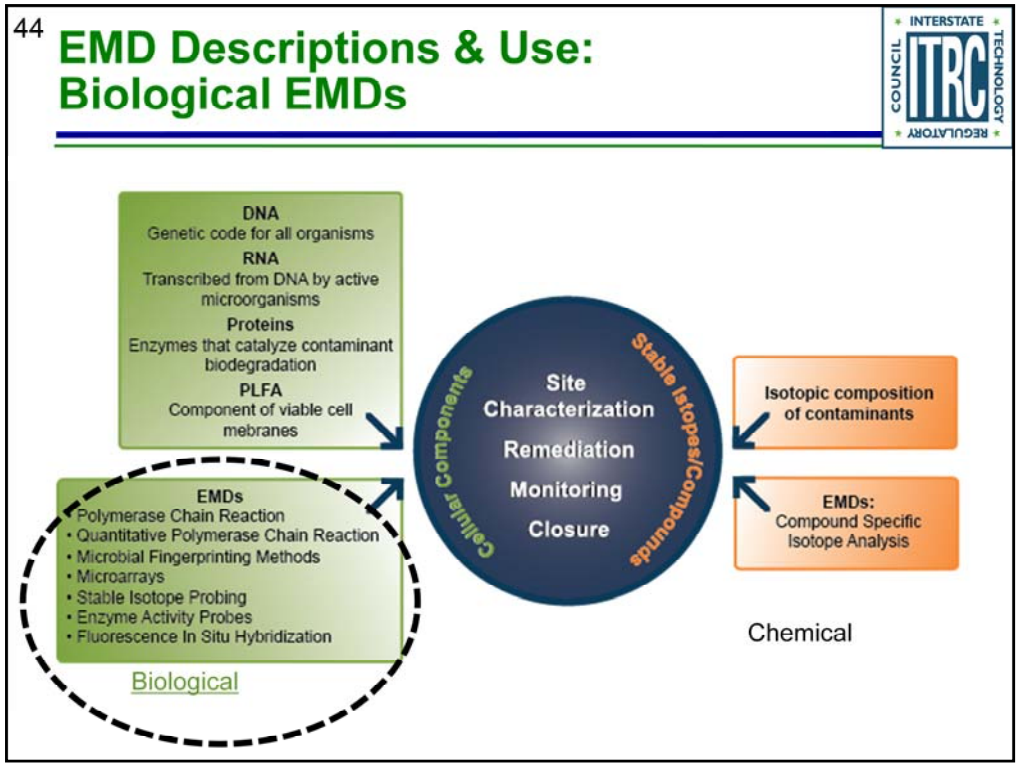
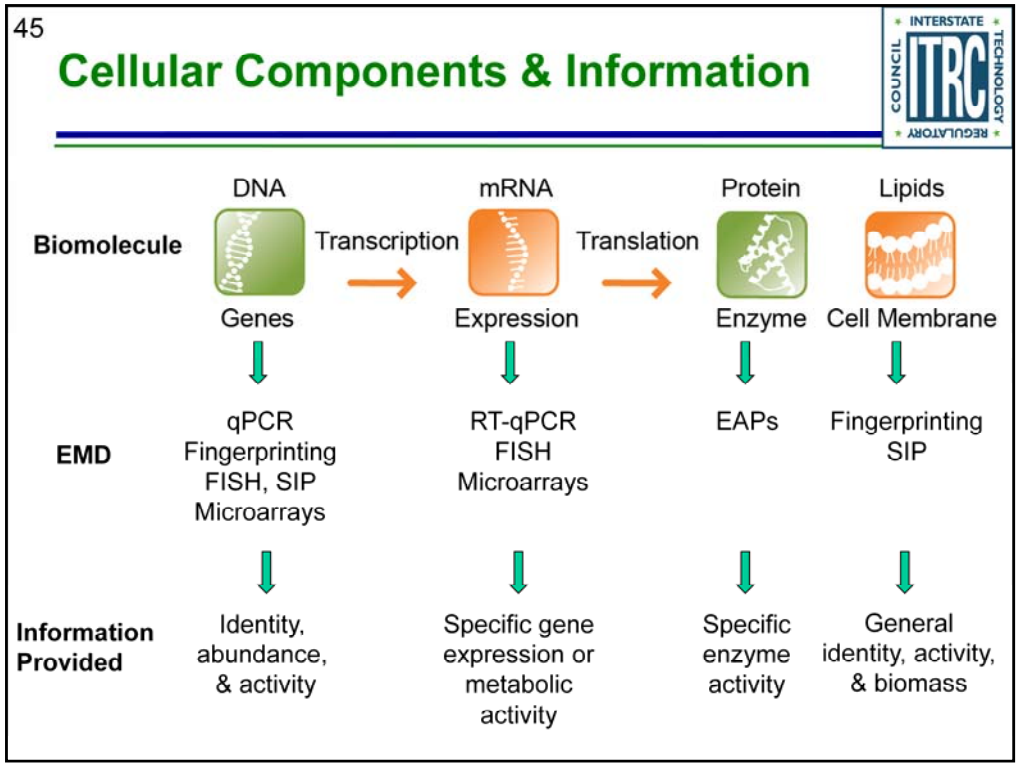



EMDs can be classified into two major categories of analytical techniques: chemical techniques, in particular compound specific isotope analysis (CSIA), and a variety of molecular biological techniques (MBTs).

Biological EMDs provide information about the biodegradation of contaminants




This figure depicts the biomolecules used in EMD methods, the EMD that uses that biomolecule, and information gained from the EMD analysis

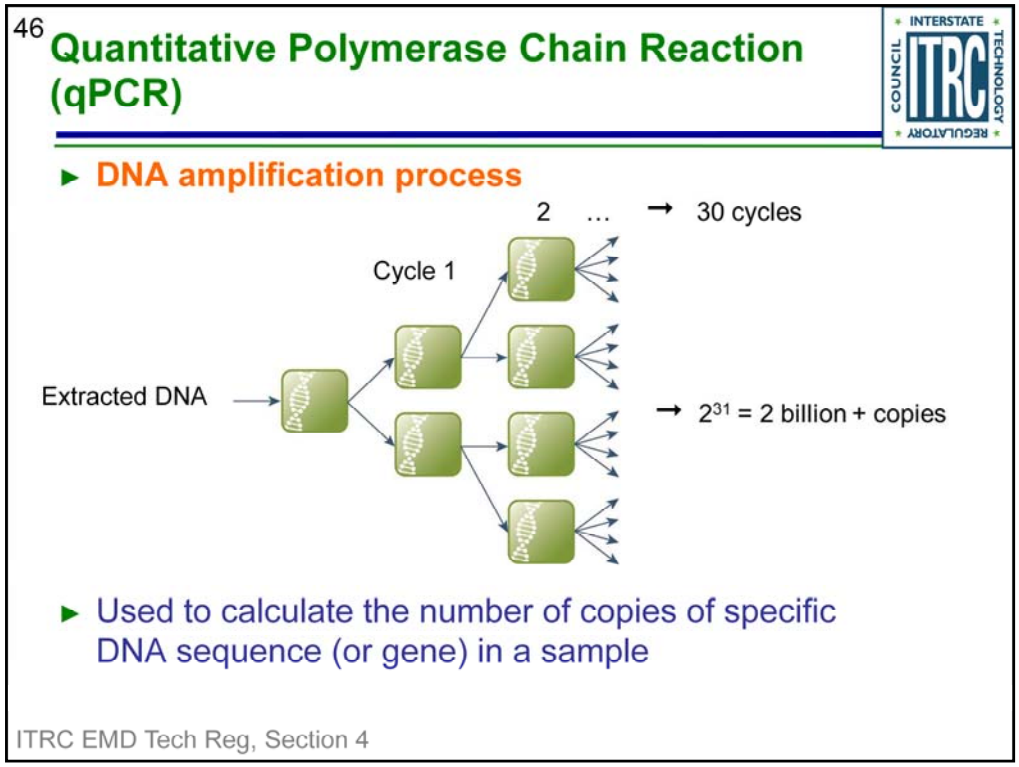
DNA & RNA are nucleic acids that encode the production of proteins that enzymatically break down contaminants, but they are also biomolecules that can gives some specific information

DNA can be used to identify microorganisms, and count their numbers in a sample. The EMDs that use DNA include qPCR , Fingerprinting methods, Stable isotope probing, Fluorescent Insitu hybridization, and Microarrays.

Analysis of mRNA can give information concerning the expression of certain genes, and thus some insight to specific metabolic activity

Proteins and lipids can be assessed to measure activity - proteins are the biological agents that are actually involved in contaminant biodegradation

Lipids can be used to identify major groups of microorganisms and assess the standing biomass of the microbial community at a site.

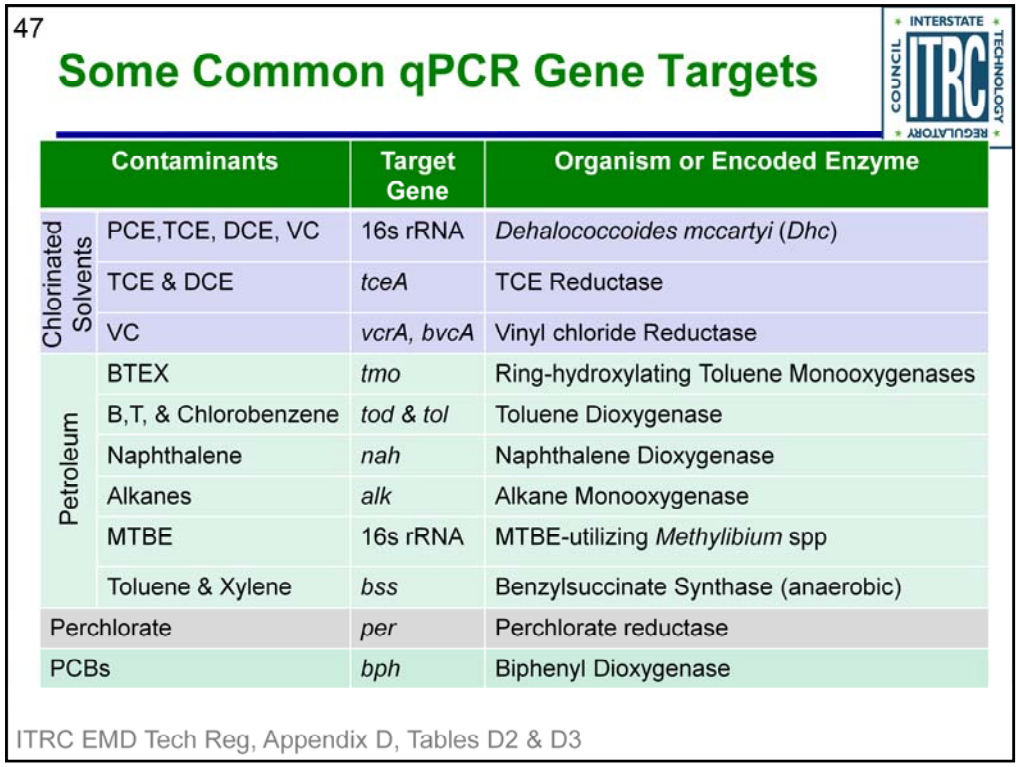



The most common biological EMD regulators will likely encounter is the polymerase chain reaction or PCR

PCR is a DNA amplification method; an automated laboratory method to create many copies of a specific fragment of DNA by repeated rounds of DNA replication

Through repeated rounds of DNA replication the target DNA is amplified to eventually produce millions to billions of copies of the target DNA.

Quantitative PCR (or qPCR) is a type of PCR that can relate the amplified DNA back to the number of copies in the original sample – thus determining the number of genes – or specific microorganisms – in a sample.

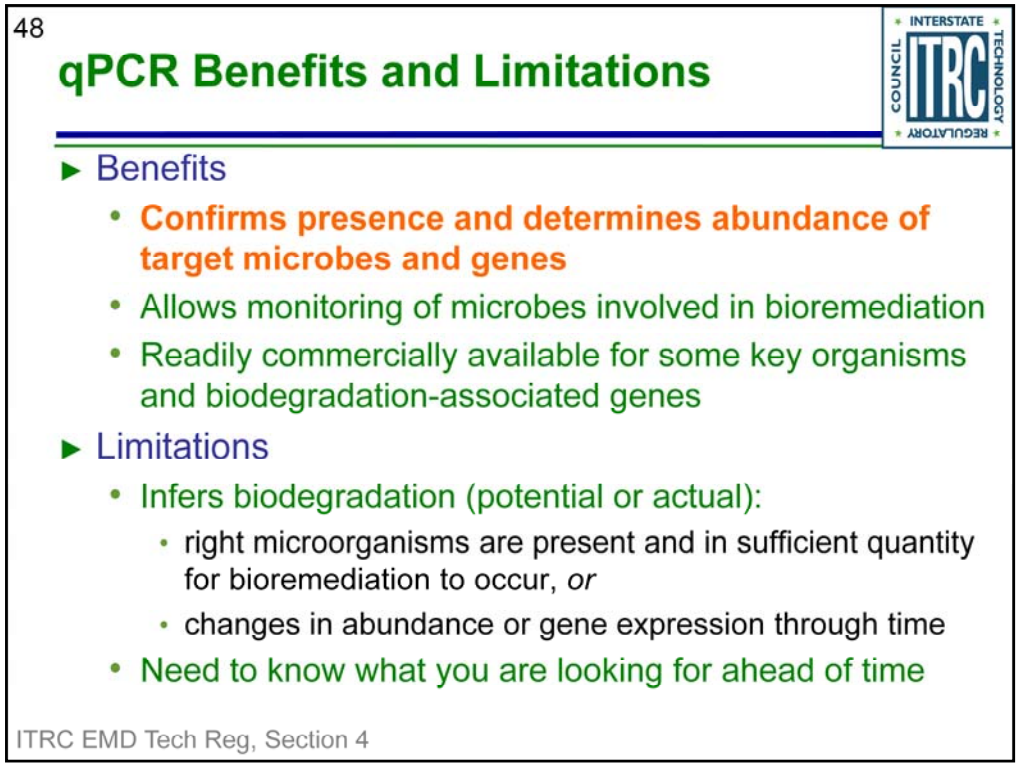



Contaminants are degraded by the actions of enzymes, and the genes that encode for the production of these enzymes are known and reported in the scientific literature.

qPCR tests are available for known gene sequences, and we can use them to screen for degradative genes in environmental samples.

This slide shows some common contaminants regulators may deal with at contaminated sites as well as the target gene that encodes the functional enzymes that's involved in their degradation.

Please Note that some gene targets are phylogenetic genes – typically the 16s rRNA gene of microorganisms used to infer phylogenetic relations and identity – while other targets are functional genes that encode for specific degradative enzymes, such as a reductase or an oxygenase.



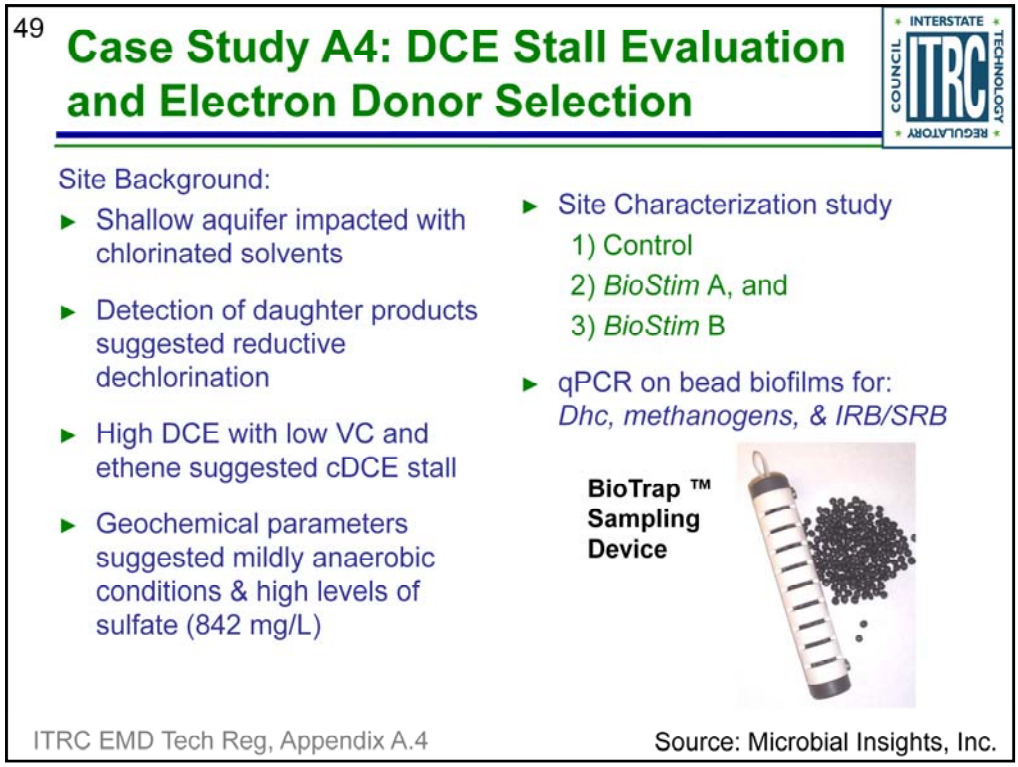

qPCR confirms presence, but can also determine the abundance of target microorganisms or their particular genes involved in biodegradation & can be used as a monitoring tool to assess changes in microbial population growth & distribution

qPCR  tests for degradative genes are commercially available

The presence of microorganisms – or particular genes – does not always mean they are active in contaminant degradation, but rather the potential is there.

Following changes in abundance of degradative genes through time provides strong inferential evidence that biodegradation is occurring.

Primer sets used in the PCR reaction are necessary to be known ahead of time.




qPCR used to evaluate cis-DCE stall at a chlorinated solvent site, and help select an appropriate electron donor for groundwater remediation

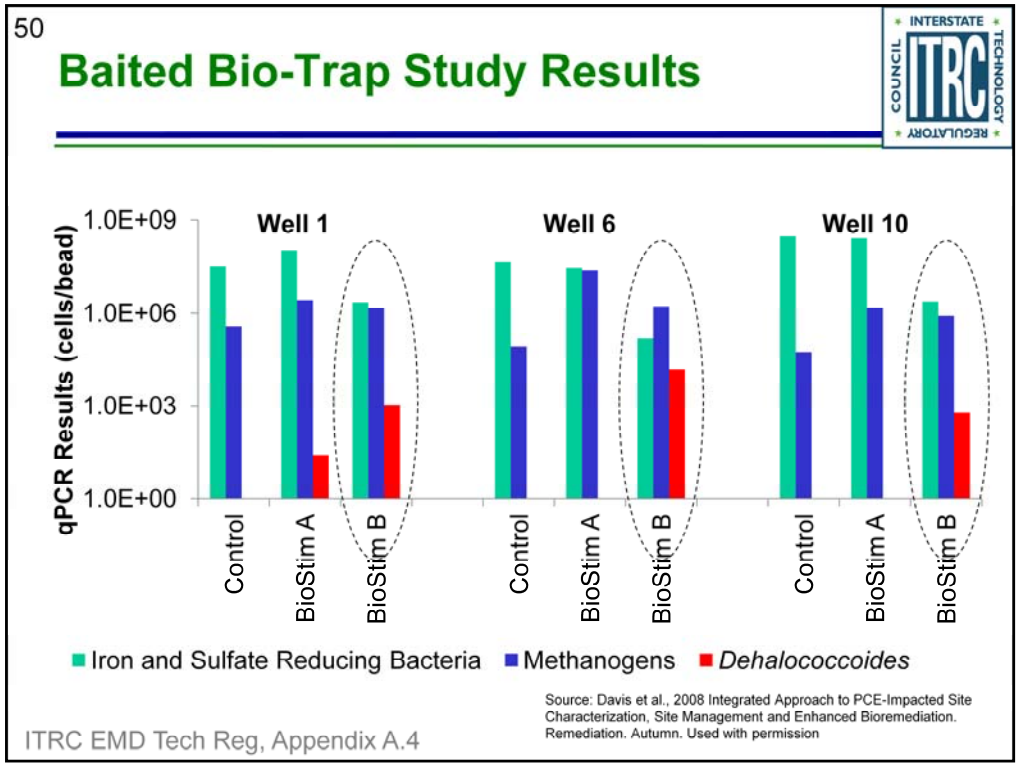
An *in situ bio*remediation approach was sought to further reduce VOCs in groundwater, and attain site-closure goals

Chlorinated ethene daughter products in groundwater suggested some Reductive Dechlorination was occurring, but accumulation of cDCE with little detection of VC & Ethene suggested a classic cDCE stall.

During site characterization (Phase 1), some additional information was necessary to identify the most promising remediation (either MNA, Biostim A or Biostim B) – qPCR was used to identify microbes and genes involved in Reductive Dechlorination stimulated by amendment additions.

Each application involved the use of a BioTrap sampling device (some amended with biostimulants A or B, or some unamamend ) followed by qPCR using biofilm recovered from the sampling device beads.

Please see EMD Sampling Methods Fact Sheet (EMD-1) for more information on BioTrap samplers:
http://www.itrcweb.org/Guidance/GetDocument?documentID=32




Source: Davis et al., 2008 Integrated Approach to PCE-Impacted Site Characterization, Site Management and Enhanced Bioremediation. Remediation. Autumn. Used with permission

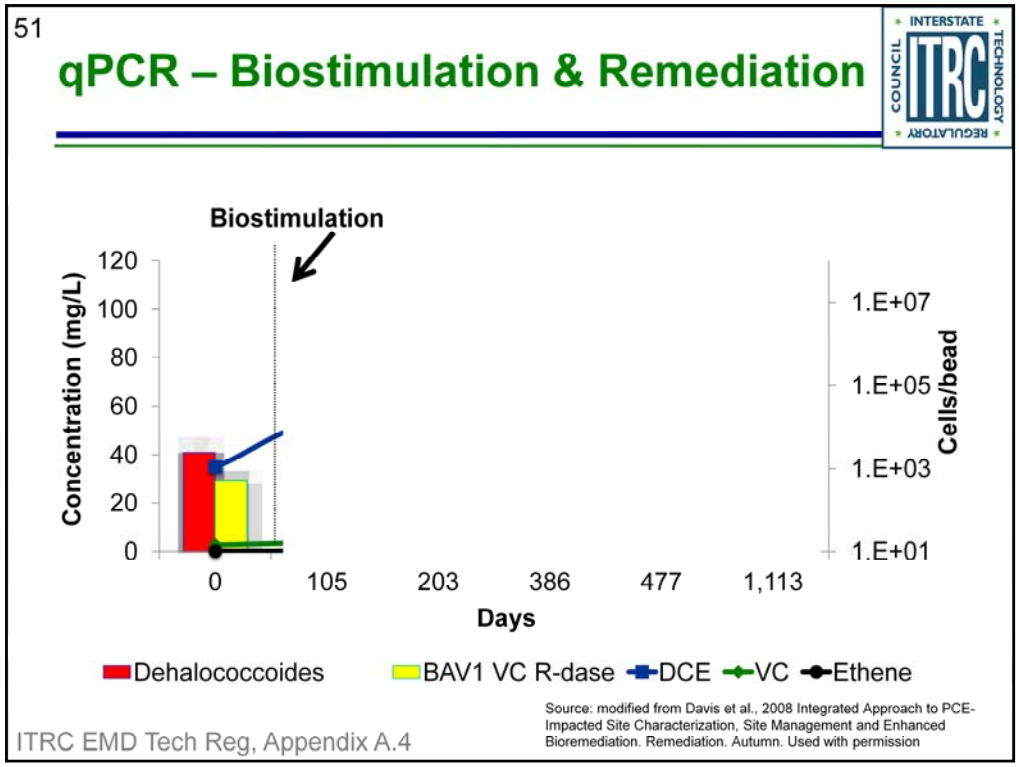
Results from 3 different wells during site characterization BioTrap study for amendment selection

On unamended biotraps, Iron and sulfate reducers (light blue) were typically greater in numbers than methanogens (dk blue), reflecting the mildly reducing geochemical conditions in the aquifer.

Dhc populations were below the laboratory detection limit on control BioTraps, indicating complete reductive dechlorination was unlikely

Dhc responded best with Biostimulant amendment B, and did not respond to amendment A in all wells.

Based on qPCR results, both MNA and biostimulant donor A were eliminated as potential remediation strategies



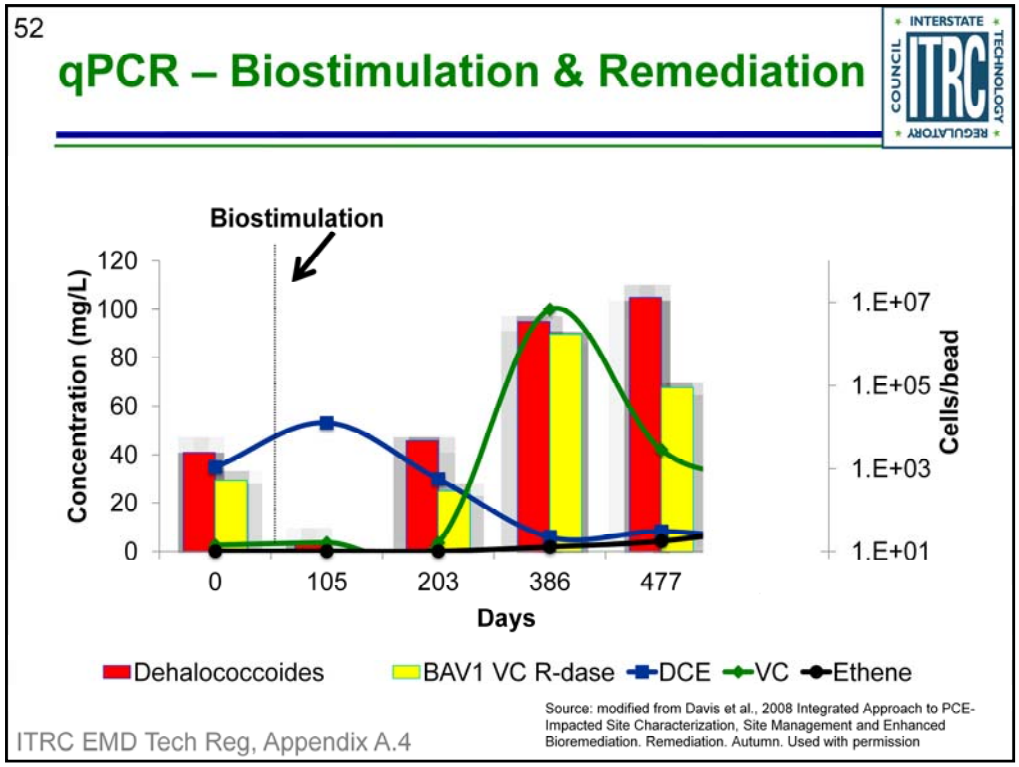

Modified from: Davis et al., 2008 Integrated Approach to PCE-Impacted Site Characterization, Site Management and Enhanced Bioremediation. Remediation. Autumn. Used with permission.

Next, a pilot injection of BioStimulant B was conducted near a single MW, followed by full-scale implementation as a remedy for the site.

The pilot test lasted 492 days, after which full-scale remediation followed

Results:

• Prior to injection (Day 0), *Dhc* populations were on the order of 10^3 cells/bead.

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Results:

• Prior to injection (Day 0), *Dhc* populations were on the order of 10^3 cells/bead.

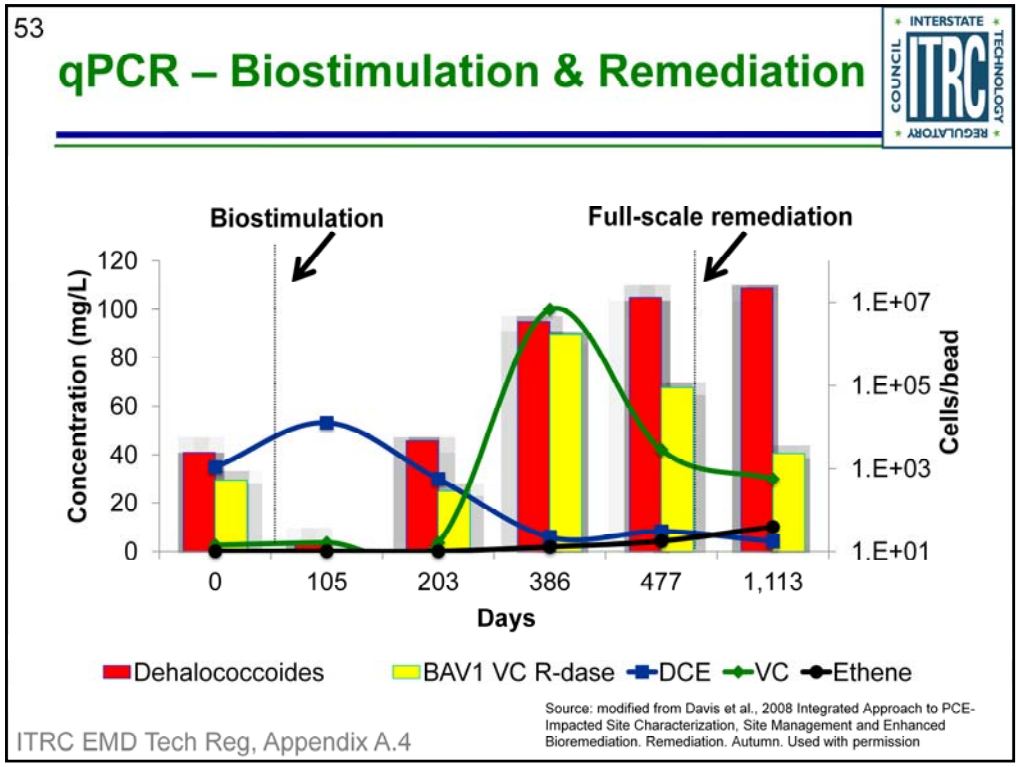
• During the first 105 days after injection, *Dhc* decreased to 10^1 cells/bead and cis-DCE conc. increased

• After Day 105, *Dhc* abudance returned to 10^3 cells/bead, and the cis-DCE conc. decreased by day 203

• After day 203 (at 386), cis-DCE conc. continued to decrease and vinyl chloride conc. temporarily increased due to increased reductive dechlorination of cis-DCE.

During this period, *Dhc* also increased to 10^6 to 10^7 cells/bead with corresponding increases in vinyl chloride reductase genes (BAV1 vcr)

• *Dhc* numbers maintained after 400 days, even when [VC] decreased (shown at day 477).

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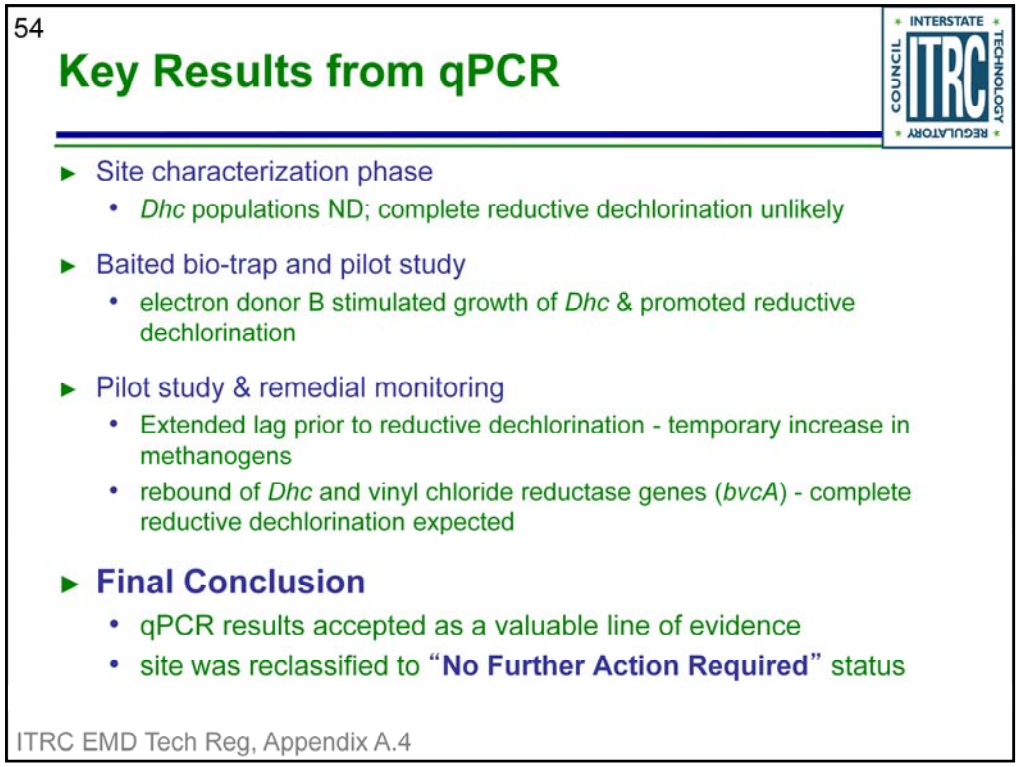
• *Dhc* numbers maintained after 400 days, even when [VC] decreased (shown at day 477).

During full-scale remediation, Biostim B was injected in the source zone and in downgradient areas of the dissolved plume

- *Dhc* numbers were maintained past 1000 days

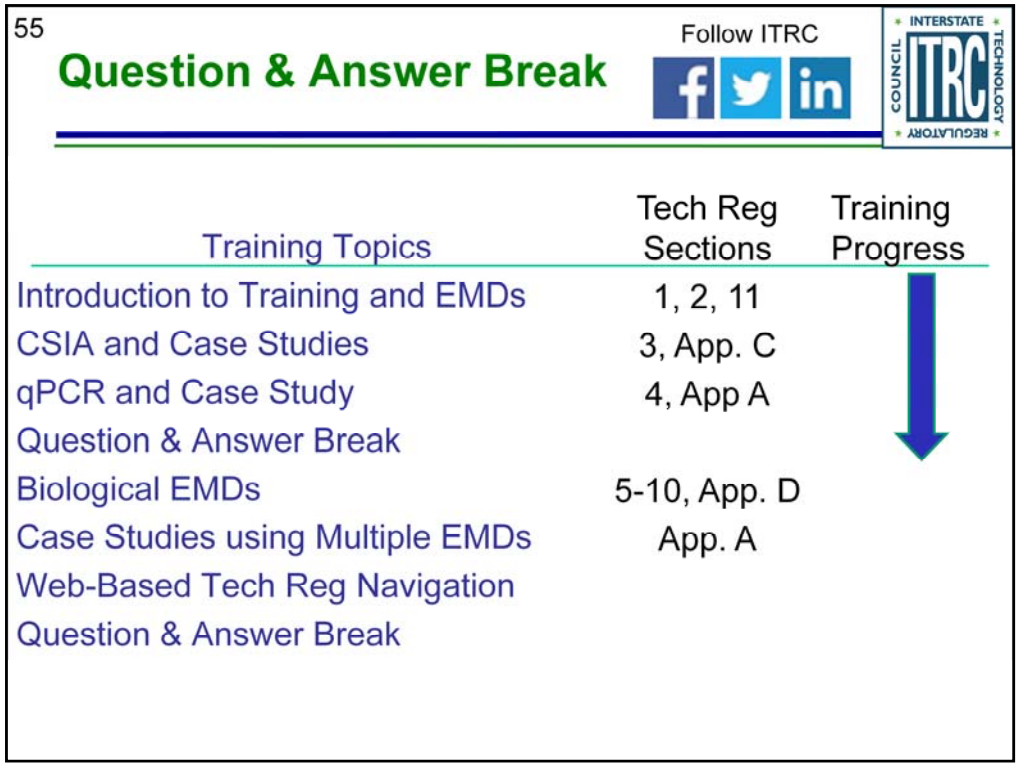



Site characterization, qPCR showed Dhc was ND and complete Reductive Dechlorination was unlikely; qPCr also showed electron donor B stimulated the growth of Dhc and promoted Reductive Dechlorination

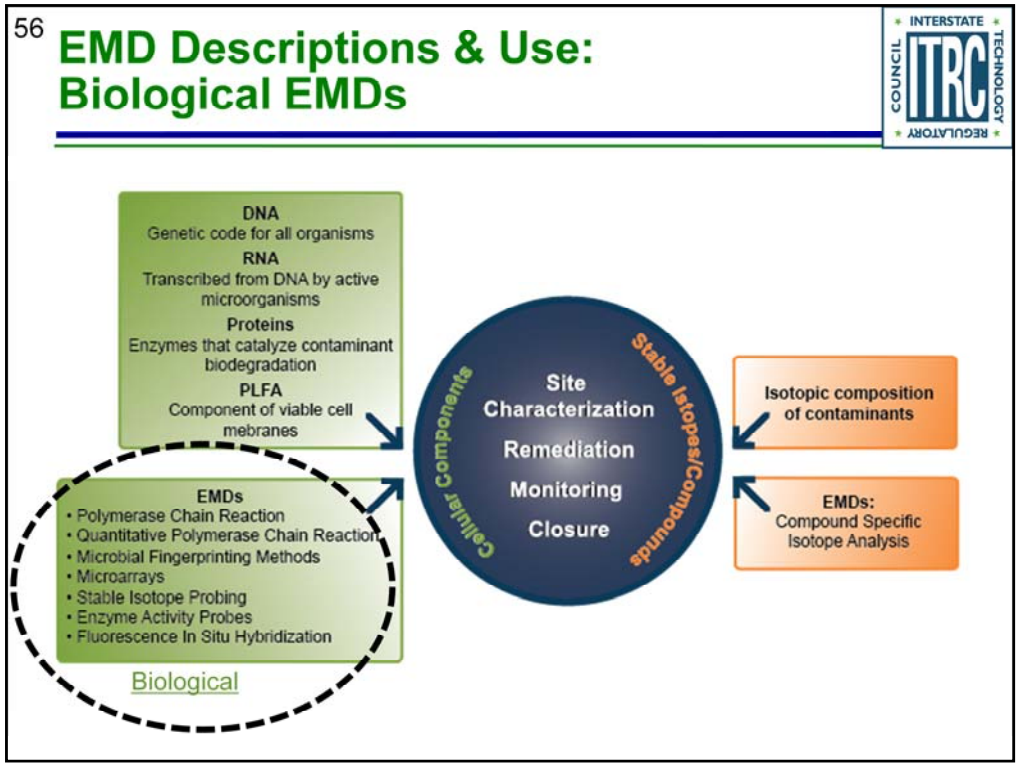
Pilot study, qPCR showed an extended lag phase for growth of Dhc populations, a temporary increase in MGNs, and a rebound of Dhc, its VC-reductase genes, and an increase in Reductive Dechlorination –

Remedial Action: complete Reductive Dechlorination during full scale remediation

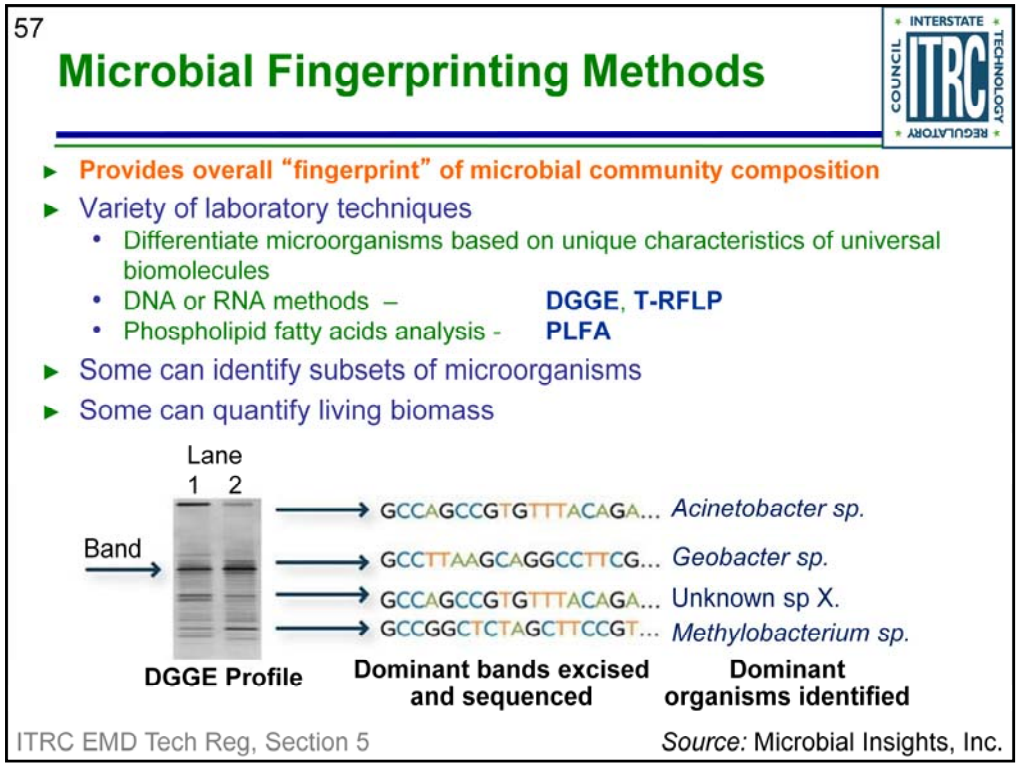
qPCR cost-effectively identified the corrective action which lead to the site being reclassified to No Further Action required




Question and answer break.




Continuing with descriptions of biological EMDs - or molecular biological tools…




Microbial Fingerprinting methods understand microbial community make-up without fully to species level

For example, microbial fingerprinting methods can identify a group microorganisms, such as sulfate reducers, that may be involved in biodegradation

Three methods profiled in the Tech Reg include:
denaturing gradient gel electrophoresis & terminal restriction fragment length polymorphism, (both using DNA), and Phospholipid fatty acid analysis (using cell membrane lipids).

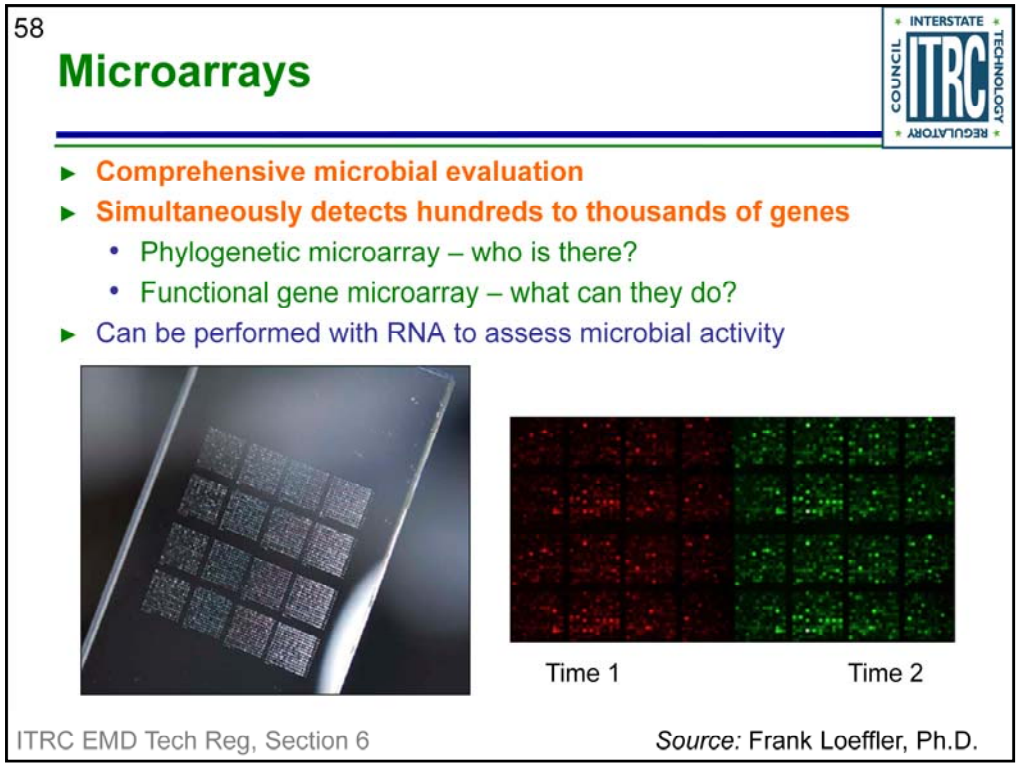
The figure shows the bands on a denaturing gradient gel (a method to separate DNA sequences) that correspond to DNA of a particular groups of microorganisms, who's identity could be further explored through DNA sequencing.

Fingerprint from restriction enzyme digest

Fingerprint from PLFA of membrane lipids from cells

All generate a community profile - or fingerprint

Use this method as a monitoring tool to follow changes in microbial community profiles




*Source:* Frank Loeffler, Ph.D., University of Tennessee, used with permission

Microarrays allow detection or tracking a large quantity & variety of genes simultaneously – for example hundreds to thousands of genes at a time – allowing for a more comprehensive look at the community all at once.

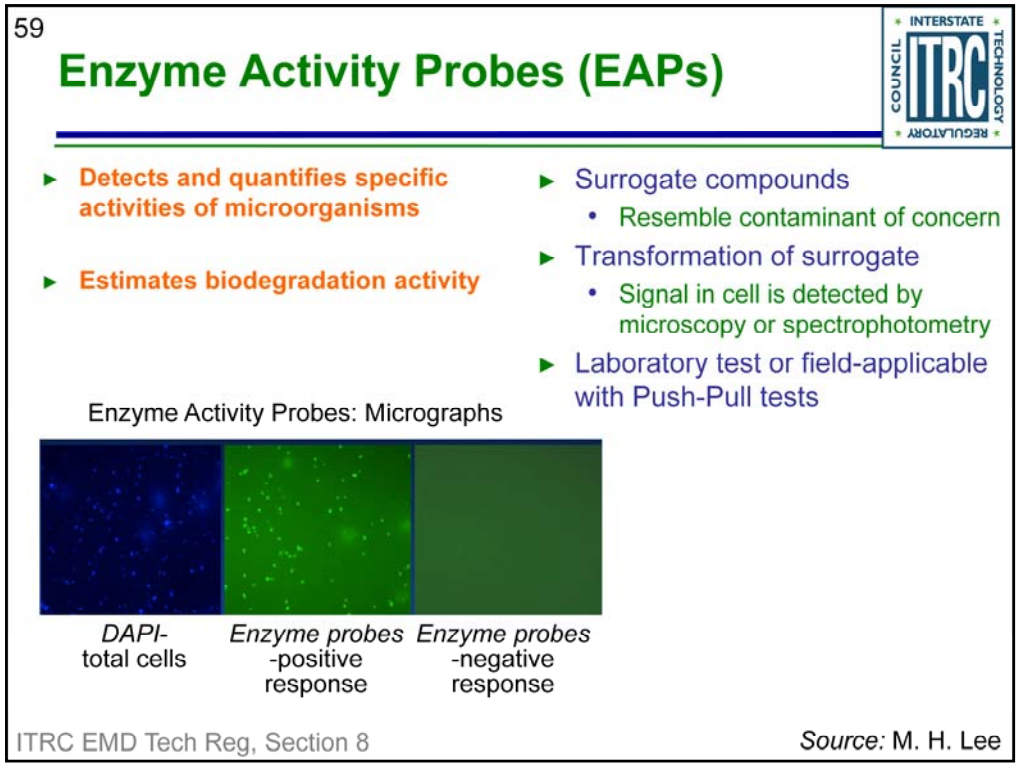
Basically a slide with ss DNA, that's washed with a sample, & if that sample contains complementary DNA, it will bind and fluoresce & the whole slide can be scanned & read by a computer

A commercially-available microarray contains up to 45,000 genes involved in biogeochemical reactions as well as biodegradation activities. Other commercially available microarrays are also available.

Microarrays can track large numbers of individual genes or organisms within a community over time, and assess changes in community structure (shown in T1 & T2, above)

Can also assess changes in activity - as gene expression - over time, by using RNA instead of DNA in the method

Microarrays are also customizable



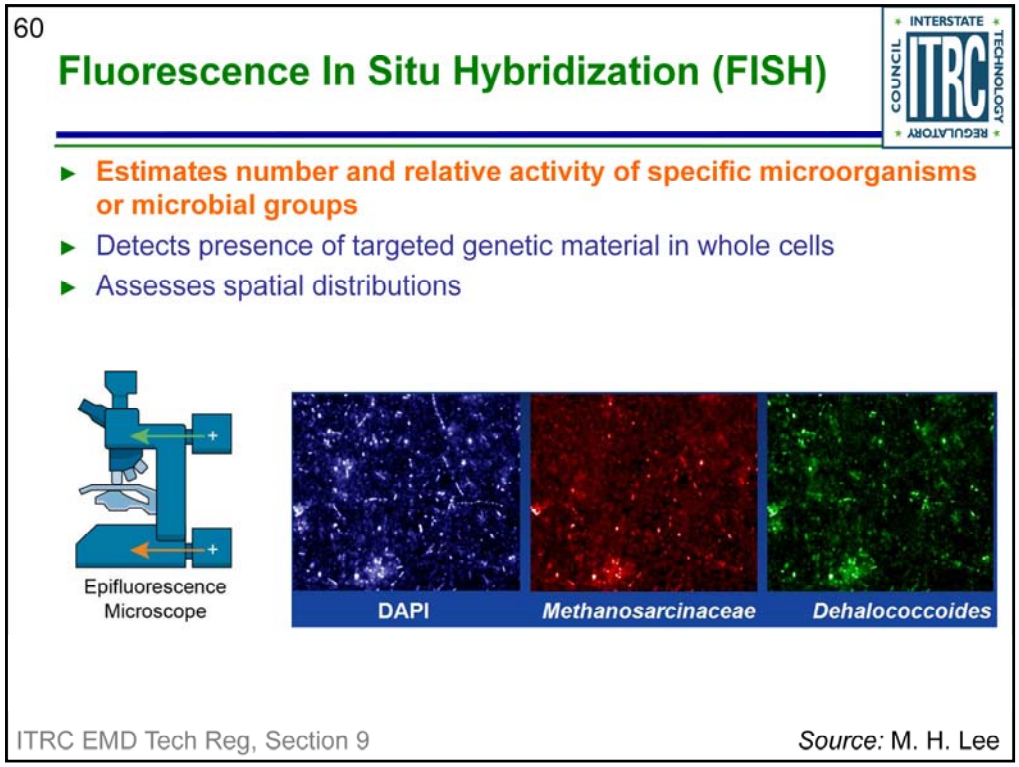

*Source:* M. H. Lee, PNNL, used with permission.

EAP method exposes surrogate compounds – or compounds that resemble contaminants of concern –to elicit the actions of degradative enzymes in the same manner as the presence of contaminants.

When combined with a fluorescent label, cells become fluorescently labeled when the enzyme reacts with the substrate and we can observe or detect enzyme activity with epifluorescent microscopy or spectrophotmetry **– this methods Provides direct evidence of the activity of enzymes involved in biodegradation of contaminants**.

When using microscopy (as shown in photomicrographs), this method is a Direct and quantitative measure of enzymatic activity, that can distinguish active cells (cells with active enzymes) from total cells and inactive cells & can be used estimate biodegradation rates.

EAPs can be used in the laboratory, but can also be applied to field sites with a common push-pull test with monitoring wells.



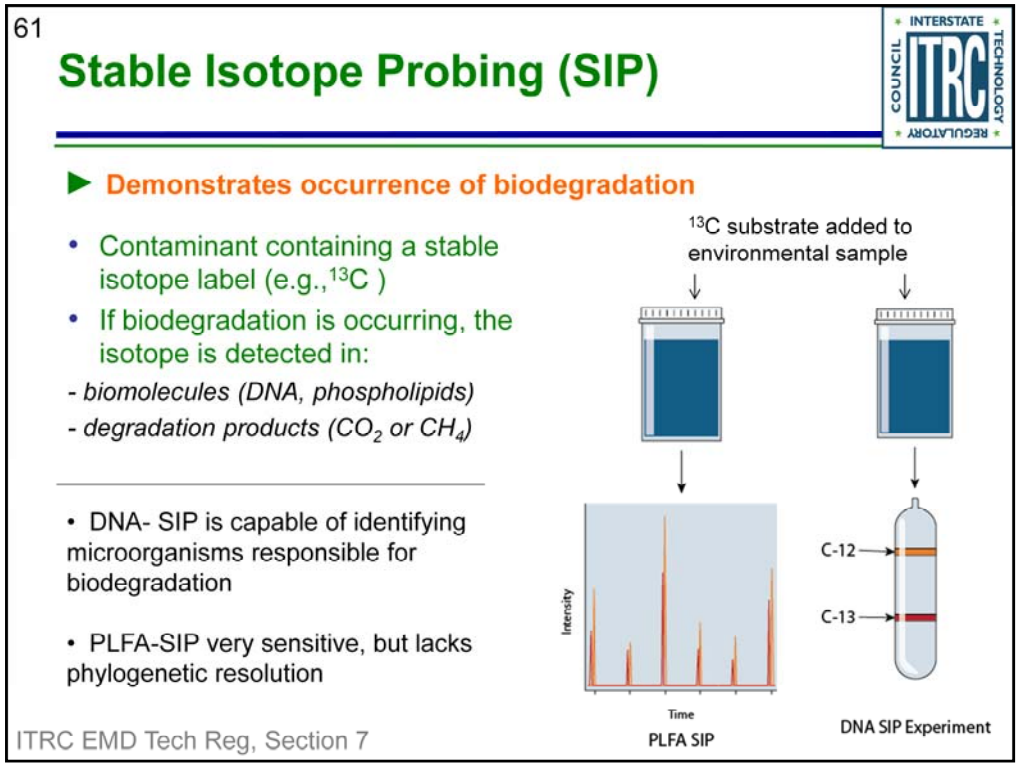

*Source:* M. H. Lee, PNNL, used with permission.

Fluorescence In Situ Hybridization – or FISH uses microscopy to assess the presence and activity of specific microorganisms

This method relies on the hybridization or binding of complementary sequences of DNA or RNA – where a fluorescently-labeled gene sequence (as a marker or PROBE) is hybridized with the genetic material inside a whole cell, and viewed using epifluorescent microscopy.

Direct counts of microorganisms carrying specific genes can be measured to determine the presence, abundance and in some case the activity of organisms of interest.

Microscopy also provides visual information of the spatial distribution of microorganisms, and can allow for biomass determinations.




SIP involves the use of stable isotopes as a tracer to follow the uptake or assimilation of the stable isotope into bio-molecules such as DNA or cell membrane lipids.

Stable isotopes that originally came from the contaminant molecules taken up into bio-molecules, **thus demonstrating the occurrence of biodegradation**.

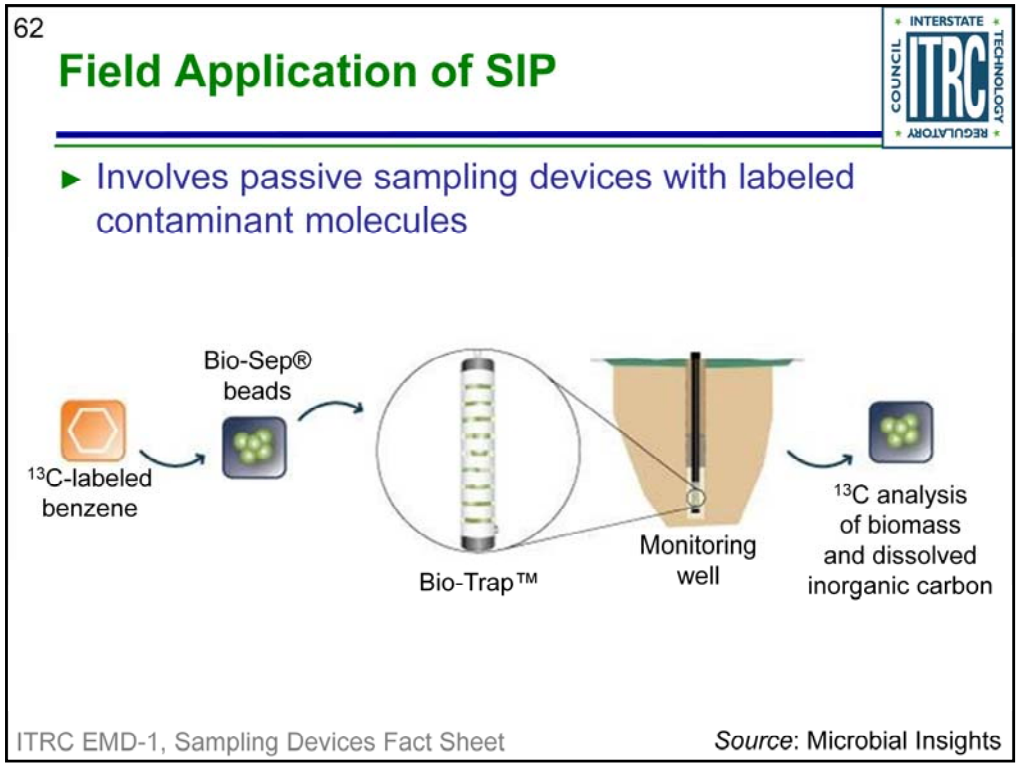
Those stable isotopes can also be manifested in cellular degradation products, such as carbon dioxide and methane.

Our figure is showing an environmental sample being exposed to substrate (or contaminant) that contains a known quantity of stable isotope – typically 13C

separate the heavy DNA isotopic fraction from the lighter DNA isotopic fraction with density gradient centrifugation – **shown in the figure on the right**) and analyze the labeled DNA by a number of methods to ID microorganisms.

Or extract the PLFAs from cell membranes and analyzing to identify uptake of 13C into membrane lipids, and to identify the group of microorganisms involved in its uptake (**shown in figure on left**).

PLFA - SIP is limited to identifying only major groups of microorganisms; DNA methods can identify to the species and isolate level.



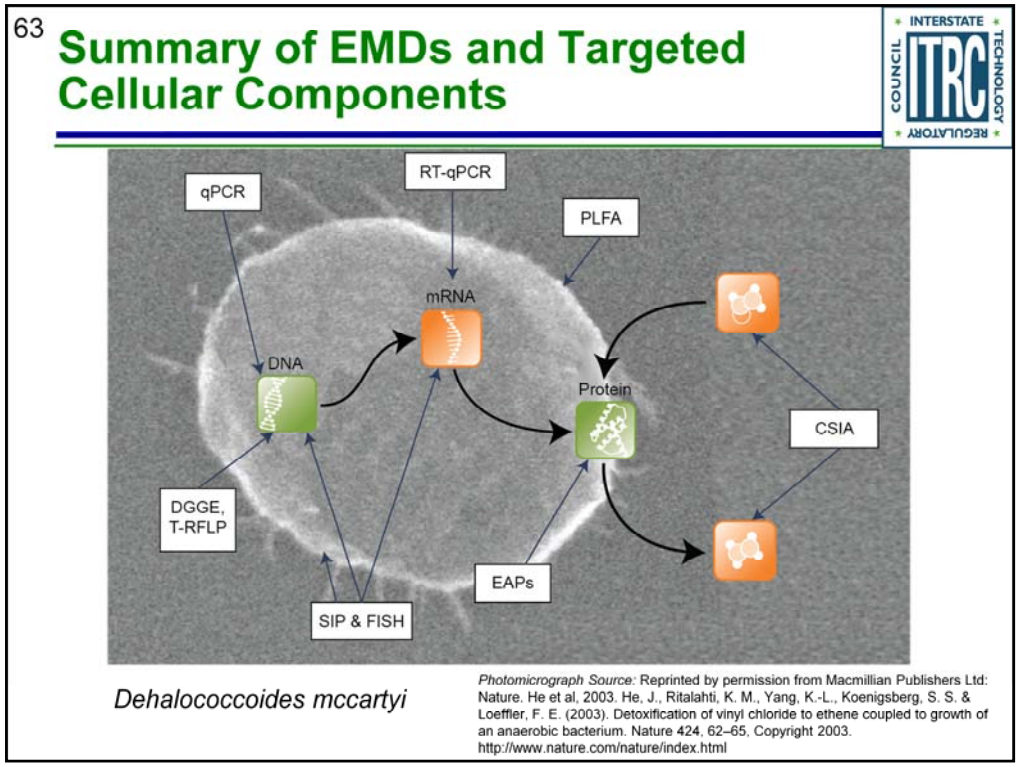

Source: Microbial Insights, used with permission

A variation of a stable isotope probing analysis involves the use of passive sampling devices – in this case the BioTrap sampling device mentioned previously –to measure biodegradation in-situ at a field site – in this figure, benzene biodegradation is explored.

This method provides direct evidence of biological degradation of contaminants and requires no prior knowledge of the microorganisms, genes, or enzymes involved in a specific biodegradation process

The analysis at the end of diagram can be DNA-based or involve the analysis of PLFAs.

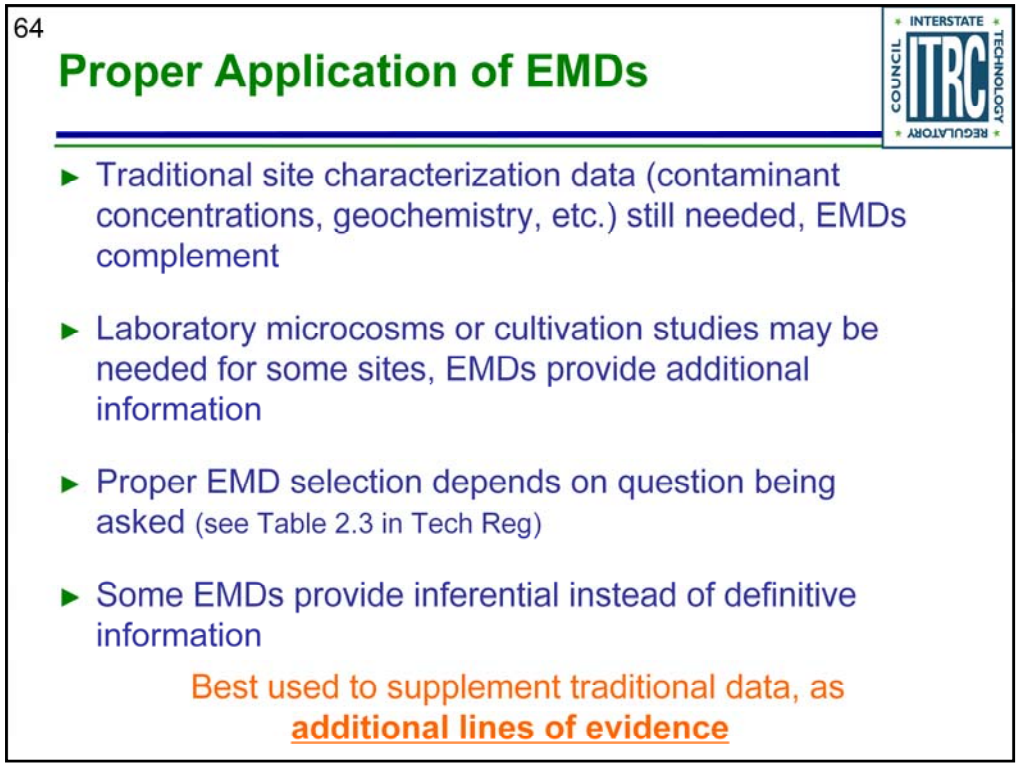
**This method is very powerful to identify if biodegradation is occurring in-situ at your site.**




*Photomicrograph Source:* Reprinted by permission from Macmillian Publishers Ltd: Nature. He et al, 2003. He, J., Ritalahti, K. M., Yang, K.-L., Koenigsberg, S. S. & Loeffler, F. E. (2003). Detoxification of vinyl chloride to ethene coupled to growth of an anaerobic bacterium. Nature 424, 62–65, Copyright 2003. http://www.nature.com/nature/index.html

This figure shows *Dehalococcoides* mccartyi – the only microbe known to completely transform PCE into ethene (formerly known as Dhc ethenogenes)

But it also shows, as a summary, where each EMD functions and the cellular components that are targeted by EMD methods (shown with the blue arrows).




Some concepts of proper EMD applications

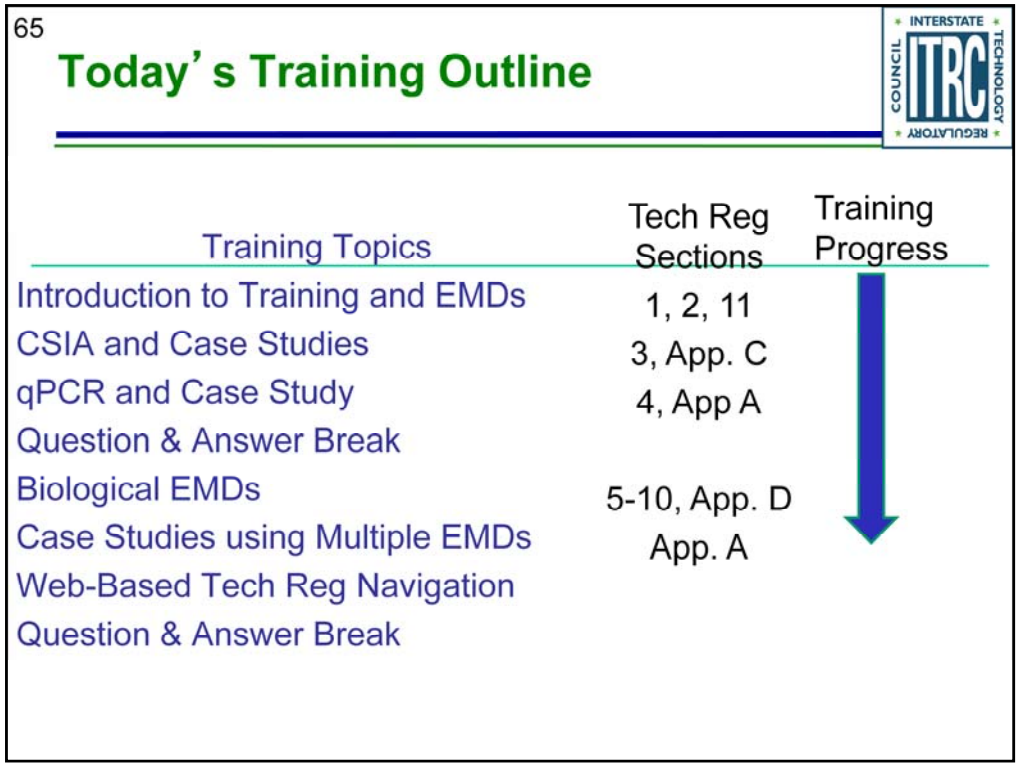
• EMDs should complement (and not replace) traditional SC data

• EMDs provide additional information – but laboratory microcosms or cultivation studies still may be needed to understand biodegradation

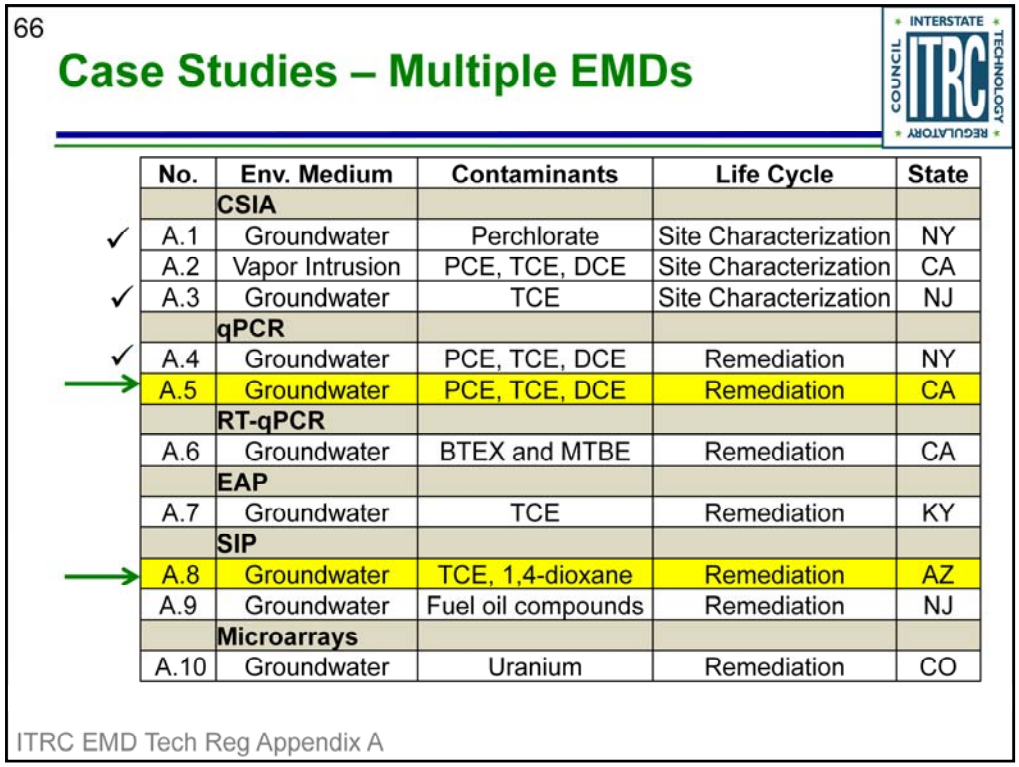
• Selecting the proper EMD depends on the question being asked – see Table 2.3

• Some EMDs provide inferential data while some provide more definitive data

EMDs are best used as **additional lines of evidence** to supplement traditional data that's typically collected at a contaminated site




No associated notes.




| | No. | Env. Medium | Contaminants | Life Cycle | State |
|---|---|---|---|---|---|
| | | **CSIA** | | | |
| ✓ | A.1 | Groundwater | Perchlorate | Site Characterization | NY |
| | A.2 | Vapor Intrusion | PCE, TCE, DCE | Site Characterization | CA |
| ✓ | A.3 | Groundwater | TCE | Site Characterization | NJ |
| | | **qPCR** | | | |
| ✓ | A.4 | Groundwater | PCE, TCE, DCE | Remediation | NY |
| → | A.5 | Groundwater | PCE, TCE, DCE | Remediation | CA |
| | | **RT-qPCR** | | | |
| | A.6 | Groundwater | BTEX and MTBE | Remediation | CA |
| | | **EAP** | | | |
| | A.7 | Groundwater | TCE | Remediation | KY |
| | | **SIP** | | | |
| → | A.8 | Groundwater | TCE, 1,4-dioxane | Remediation | AZ |
| | A.9 | Groundwater | Fuel oil compounds | Remediation | NJ |
| | | **Microarrays** | | | |
| | A.10 | Groundwater | Uranium | Remediation | CO |

This table in Appendix A shows all of the case studies and includes the complementary EMDs used at case studies.

Each method section in the Tech Reg includes short application summaries with paper citations for more case studies.

67 **Case Study A5: qPCR and CSIA to Evaluate Culture Distribution and Confirm Degradation**

- Seal Beach Naval Weapons Station
- ESTCP Project ER-0513
- Demonstration area had TCE concentrations as high as 190 mg/L
- Compared passive and active bioaugmentation

ITRC EMD Tech Reg, Appendix A.5

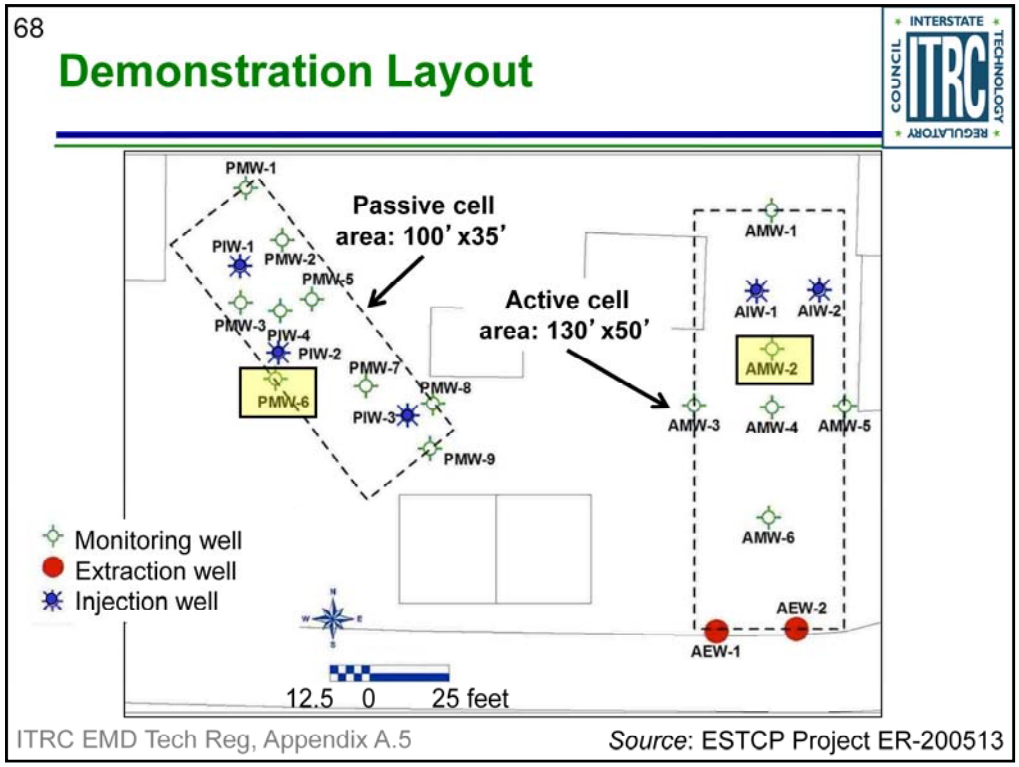
Site photo courtesy of J. Trotsky, US Navy.

Site photo courtesy of J. Trotsky, US Navy.

Web location for Environmental Security Technology Certification Program (ESTCP) project reports: www.serdp.org

Passive = Injection wells located throughout treatment area and microbial culture is distributed via ambient groundwater flow

Active = Recirculation system and culture is distributed via forced advection




Source: Figure 5-1 of the Final Report for A Low-Cost, Passive Approach for Bacterial Growth and Distribution for Large-Scale Implementation of Bioaugmentation (ESTCP Project ER-200513)

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## Use of EMDs

- qPCR was used to
  - Determine if *Dhc* existed at the site and select an appropriate bioaugmentation culture
  - Evaluate bacterial growth and distribution throughout both treatment cells
- CSIA was used to
  - Determine the extent of dechlorination in both treatment cells during the demonstration period

ITRC EMD Tech Reg, Appendix A.5

No associated notes.

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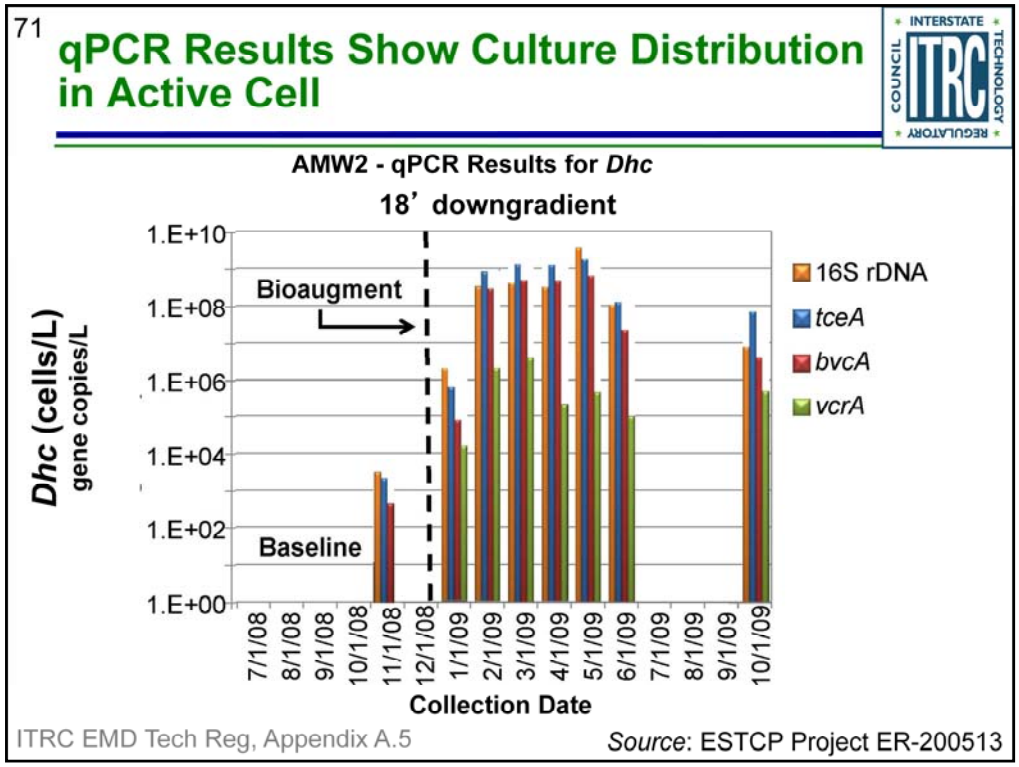
# Project Approach

- Aquifer pre-conditioned
- Pre-bioaugmentation monitoring
  - *Dhc* detected, but *Dhc* functional gene *vcr*A was not detected
- Performed bioaugmentation using microbial culture containing the *vcr*A gene
- *vcr*A was used as a "tracer"
- Continued electron donor addition
  - Active cell: Weekly injections
  - Passive cell: Monthly injections

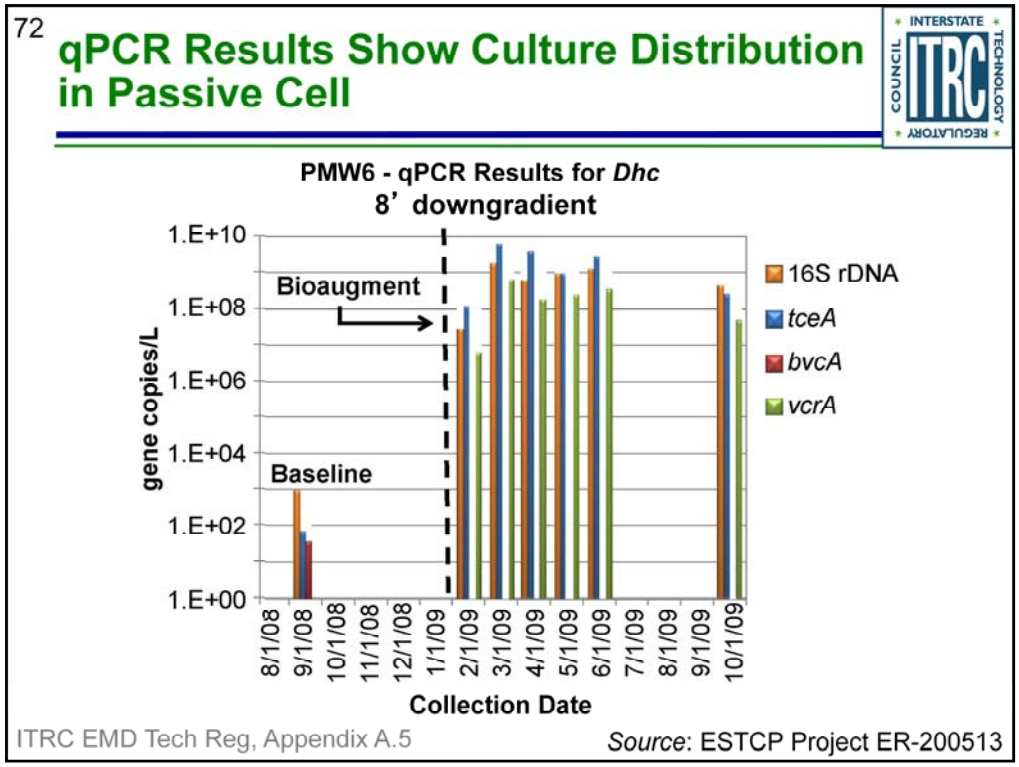
*Source*: Final Report for ESTCP Project ER-200513

ITRC EMD Tech Reg, Appendix A.5

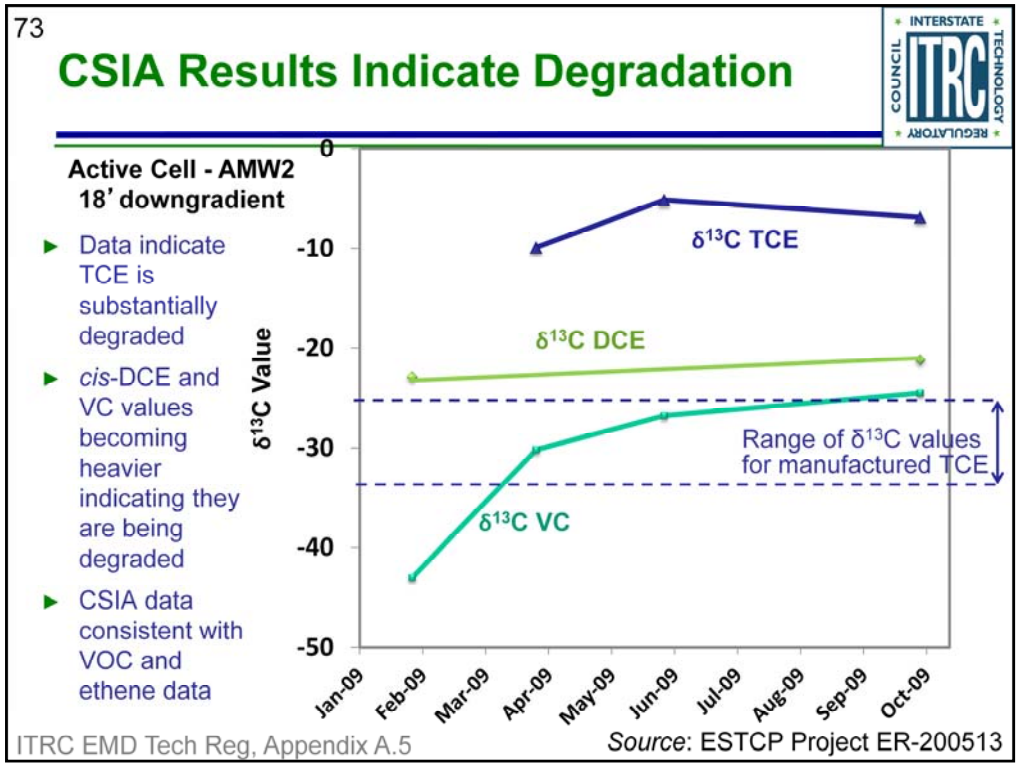
Source: Final Report for ESTCP Project ER-200513,




Source: Final Report for ESTCP Project ER-200513

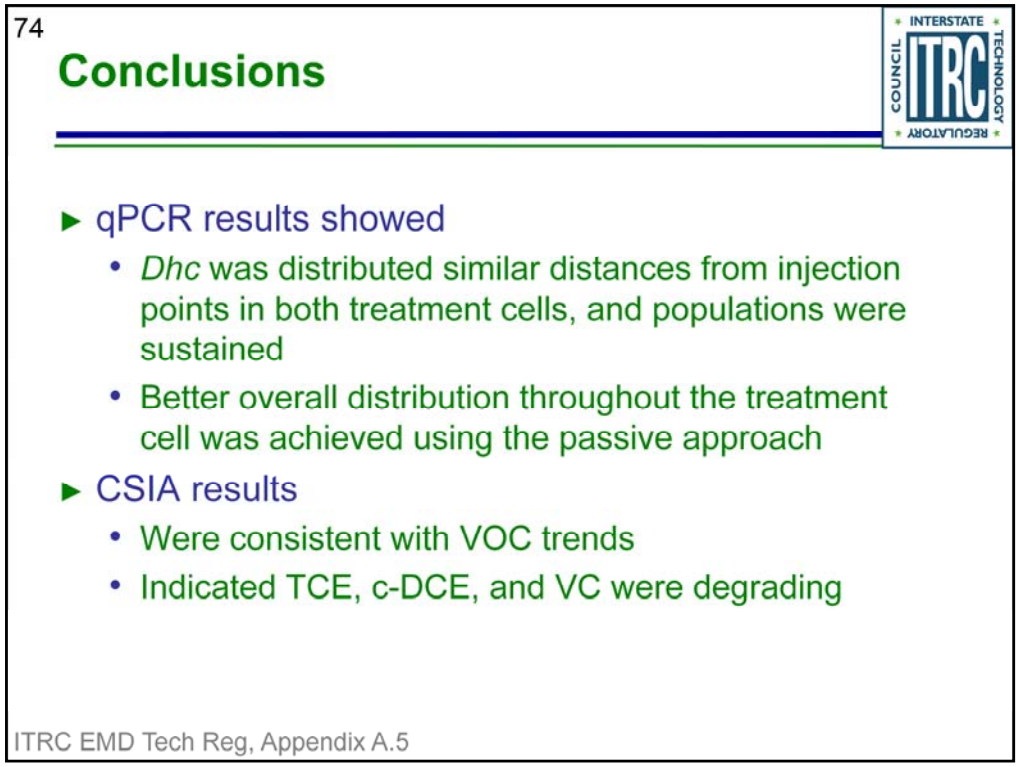



Source: Final Report for ESTCP Project ER-200513




Source: Final Report for ESTCP Project ER-200513

www.serdp.org




No associated notes.

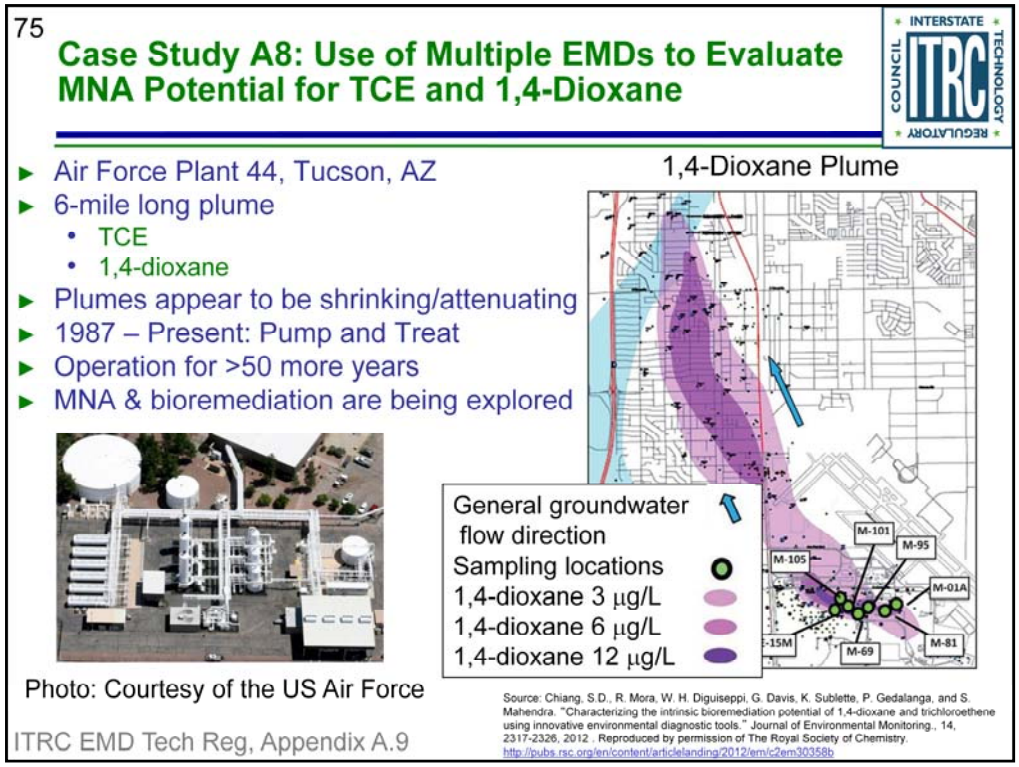



Photo: Courtesy of the United States Air Force.

Source: Chiang, S.D., R. Mora, W. H. Diguiseppi, G. Davis, K. Sublette, P. Gedalanga, and S. Mahendra. "Characterizing the intrinsic bioremediation potential of 1,4-dioxane and trichloroethene using innovative environmental diagnostic tools." Journal of Environmental Monitoring., 14, 2317-2326, 2012 . Reproduced by permission of The Royal Society of Chemistry.

http://pubs.rsc.org/en/content/articlelanding/2012/em/c2em30358b

TCE historic max = 1,100 ug/L
Dioxane historic max = 7,000 ug/L

P&T = 2,400 gpm system

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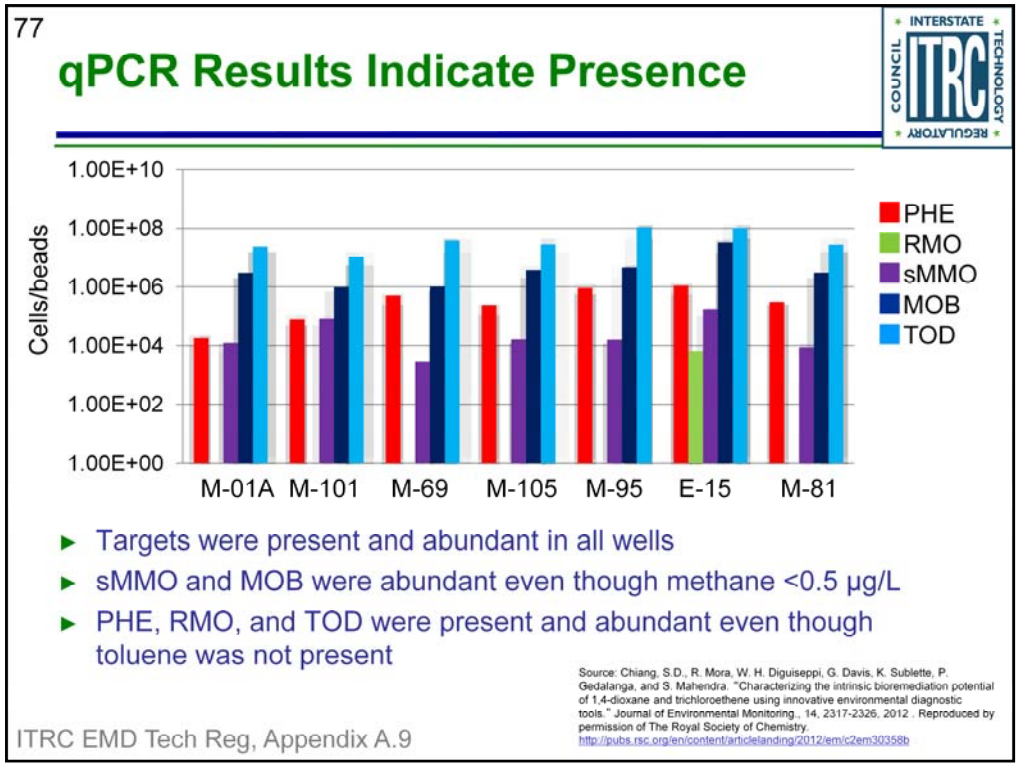
# Approach Using EMDs

| Step | Objective/Question Answered | Measurements |
|---|---|---|
| 1 | Geochemical conditions? | Field measurements<br>Geochemical parameter analyses |
| 2 | Contaminant-degrading bacteria and enzymes present ? | **qPCR** :<br>• Methanotrophs (MOB)<br>• Soluble methane monooxygenase (sMMO)<br>• Toluene monooxygenases (PHE and RMO)<br>• Toluene dioxygenase (TOD for TCE only) |
| 3 | TCE and/or 1,4-dioxane being aerobically degraded? | **Compound Specific Isotope Analysis (CSIA)** for TCE<br><br>**Stable Isotope Probing (SIP)** |
| 4 | Contaminant-degrading enzymes metabolically active? | **Enzyme activity probes (EAPs)** |

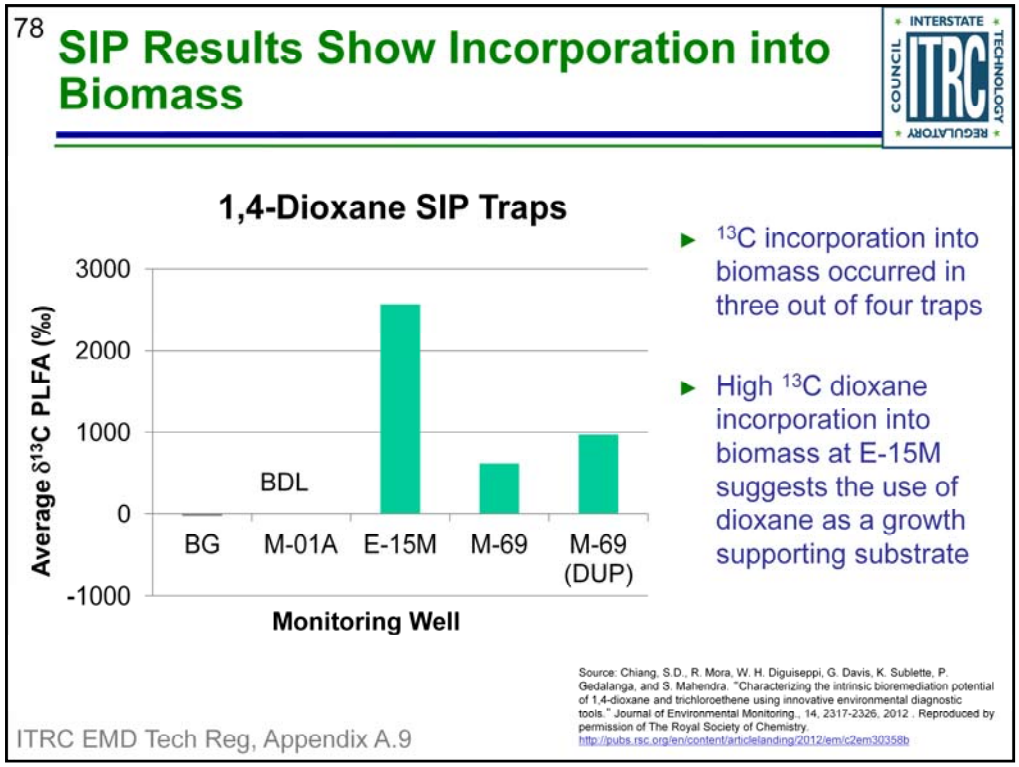
ITRC EMD Tech Reg, Appendix A.9

No associated notes.




Source: Chiang, S.D., R. Mora, W. H. Diguiseppi, G. Davis, K. Sublette, P. Gedalanga, and S. Mahendra. "Characterizing the intrinsic bioremediation potential of 1,4-dioxane and trichloroethene using innovative environmental diagnostic tools." Journal of Environmental Monitoring., 14, 2317-2326, 2012 . Reproduced by permission of The Royal Society of Chemistry.

http://pubs.rsc.org/en/content/articlelanding/2012/em/c2em30358b




Source: Chiang, S.D., R. Mora, W. H. Diguiseppi, G. Davis, K. Sublette, P. Gedalanga, and S. Mahendra. "Characterizing the intrinsic bioremediation potential of 1,4-dioxane and trichloroethene using innovative environmental diagnostic tools." Journal of Environmental Monitoring., 14, 2317-2326, 2012 . Reproduced by permission of The Royal Society of Chemistry.

http://pubs.rsc.org/en/content/articlelanding/2012/em/c2em30358b

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## CSIA Results Serve as Baseline

- CSIA groundwater sampling before SIP trap deployment
- CSIA data used to establish baseline isotopic fractionation and evaluate whether TCE degradation has occurred
- $\delta^{13}C$ of undegraded TCE ranges from ~ -24 to -34 (EPA, 2008)
- Comparison between wells shows a difference of up to 3 ‰
- Even robust aerobic co-metabolism of TCE results in small shifts in $\delta^{13}C$

| Loc ID | M-101 | E-15M | E-15M (DUP) | M-69 | M-69 (DUP) |
|---|---|---|---|---|---|
| Conc (µg/L) Feb 2009 | 330 | 110 | 110 | 390 | 390 |
| $\delta^{13}C$ (‰) | -25.12 | -25.22 | -25.55 | -28.02 | -27.92 |

ITRC EMD Tech Reg, Appendix A.9

CSIA results do not indicate degradation, but are useful as a baseline.

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# EAP Results Indicate Enzymes Are Active

Units = cells per milliliter

| | Probes for Toluene Oxygenases (PHE, RMO, TOL, TOD) | | | | Probe for sMMO |
|---|---|---|---|---|---|
| Sample | PA | 3-HPA | CINN | 3EB | Coumarin |
| M-69 | - | 1.05E+04 | - | - | 15.22 |
| M-69 | 8.21E+03 | 1.25E+04 | - | - | - |
| M-01A | 2.54E+04 | - | 2.14E+04 | 8.12E+03 | - |
| M-81 | 2.15E+04 | 2.04E+04 | - | - | 42.11 |
| M-105 | 2.68E+04 | 2.21E+04 | 1.12E+04 | - | - |
| M-101 | 3.54E+04 | - | - | - | - |
| M-95 | 2.45E+04 | - | 1.42E+04 | - | - |

- At least one positive result in all wells
- Enzymes are active and intrinsic aerobic biodegradation is occurring

ITRC EMD Tech Reg, Appendix A.9

No associated notes.

81

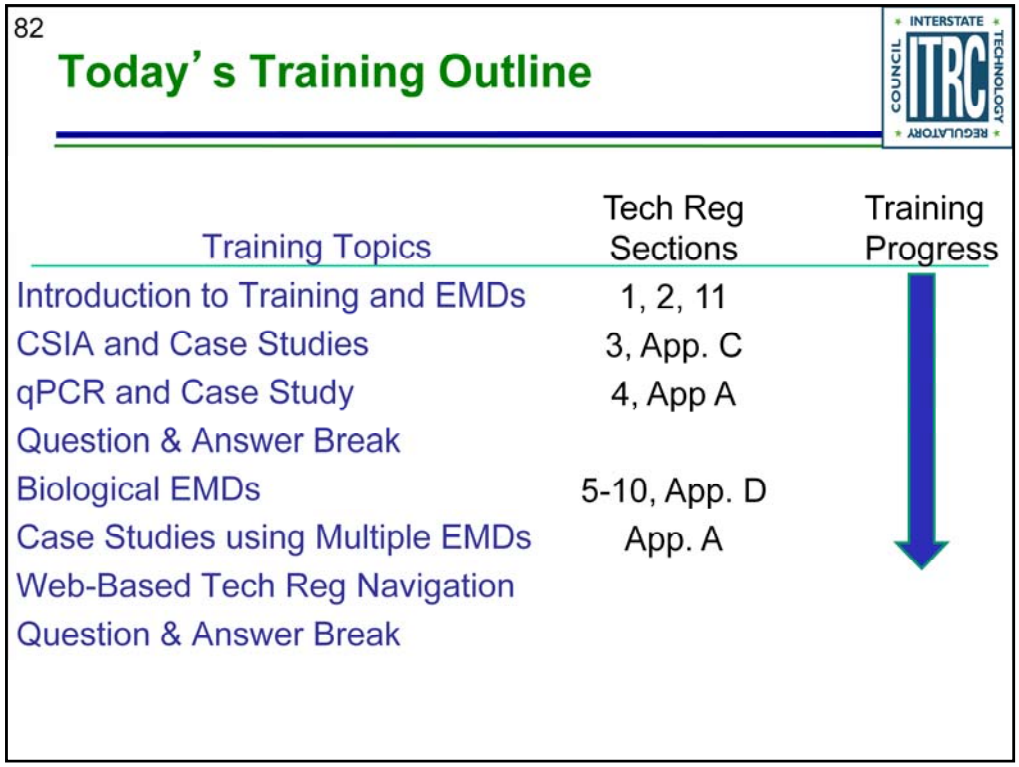
## Conclusions

- qPCR results showed presence and abundance
- CSIA results did not confirm TCE degradation, but did serve as a baseline
- Incorporation of $^{13}C$ into biomass suggests 1,4-dioxane may serve as a growth supporting substrate
- EAP results confirmed that enzymes are active

Multiple EMDs and lines of evidence showed that MNA can be considered as part of the site remedial strategy

ITRC EMD Tech Reg, Appendix A.9

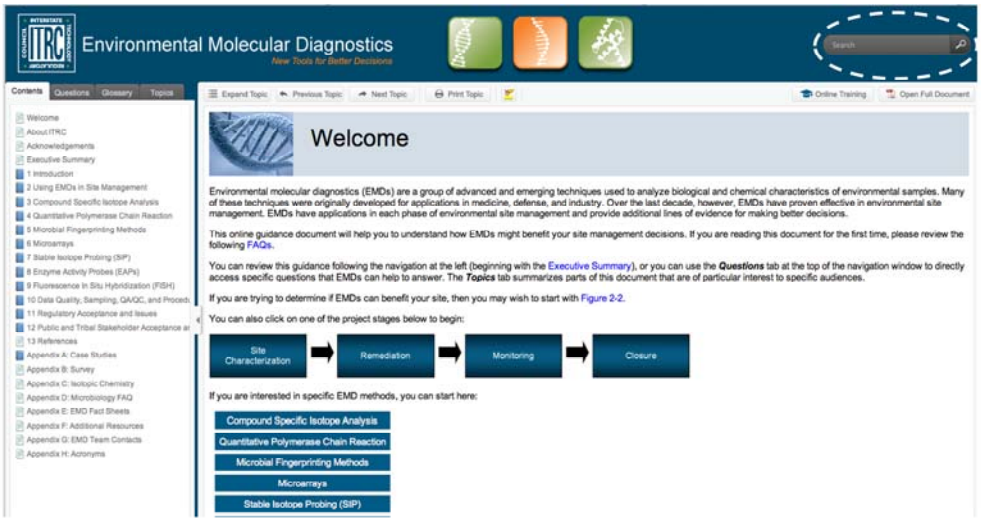
No associated notes.




No associated notes.

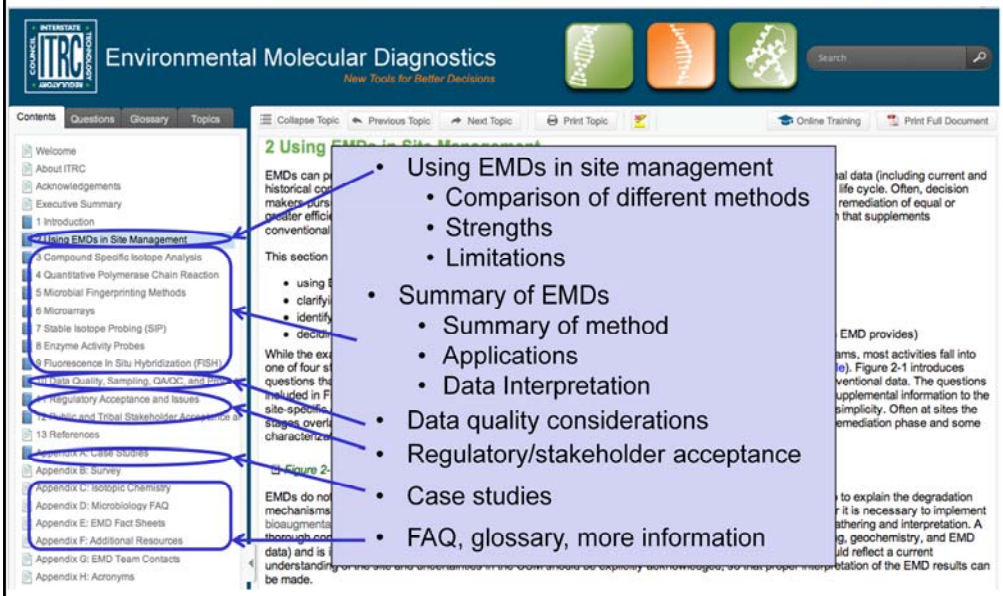
83

# EMD Technical Regulatory Guidance Document (Tech Reg)



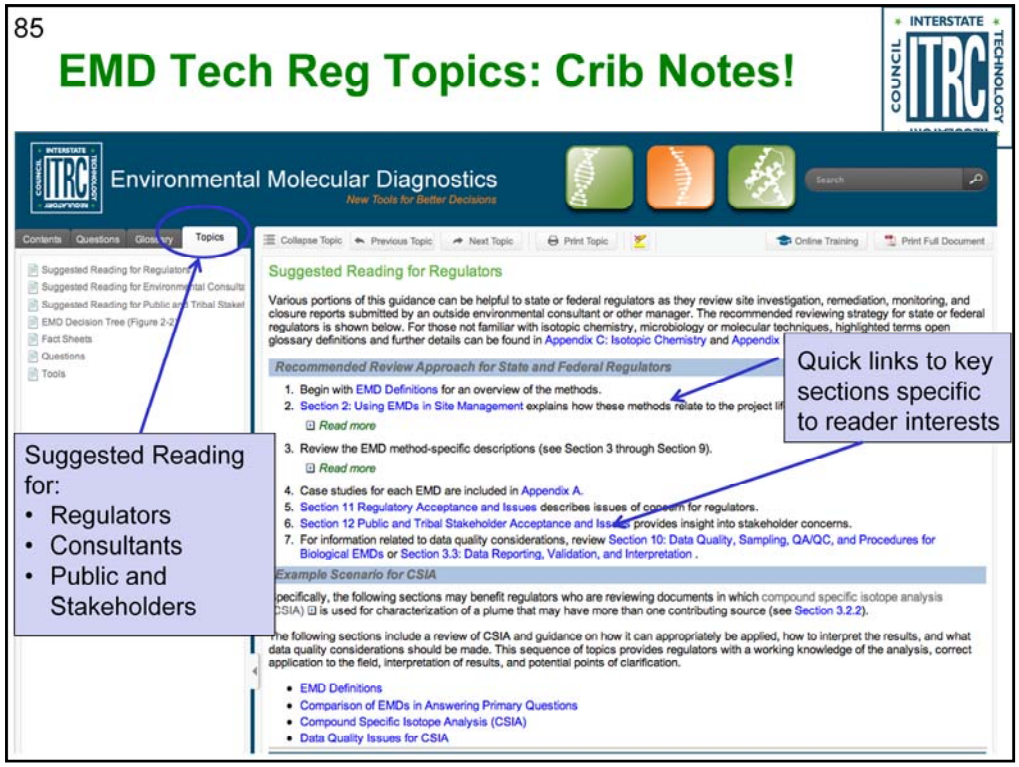

Electronic EMD Technical and Regulatory Guidance
--streamlined navigation based on table of contents
--search feature
--print PDF feature
--collapsible/expandable sections

# EMD Tech Reg Contents




No associated notes.

# EMD Tech Reg Topics: Crib Notes!

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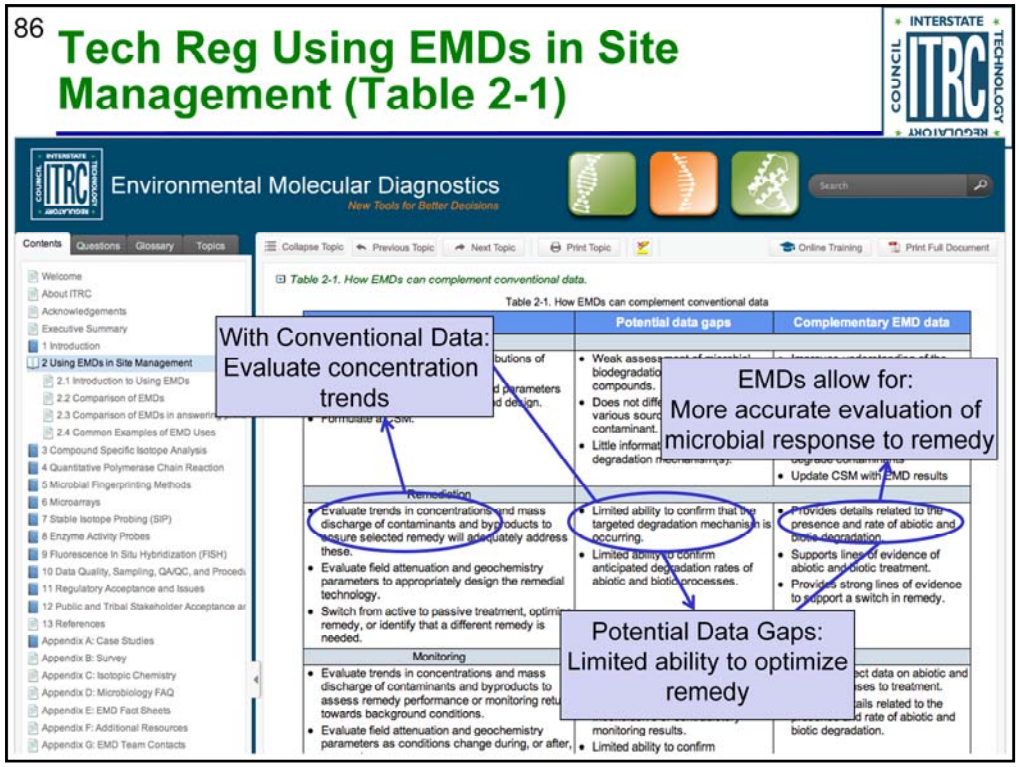


Conventional data do not allow you to determine the microbial response to remediation activities
--e.g. no information on growth versus decay of key organisms, are key organisms actively degrading contaminants or are they just hanging out?
--can use microbial information for optimal placement of remediation amendments, timing of amendment additions, evaluating dechlorination stall

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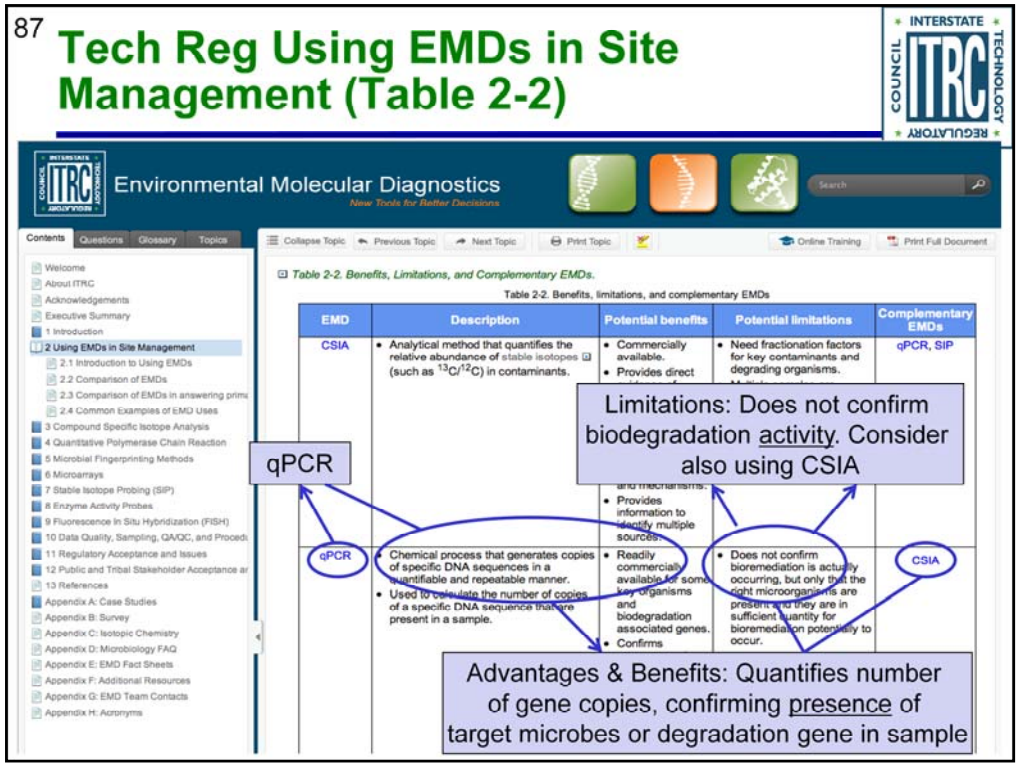
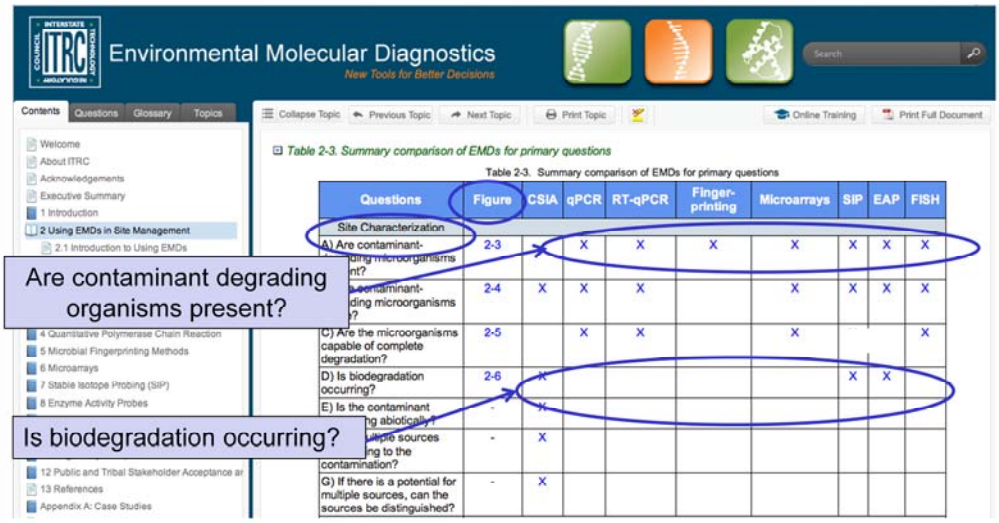
# Tech Reg Using EMDs in Site Management (Table 2-2)




No associated notes.

88

# Tech Reg Using EMDs to Answer Primary Questions (Table 2-3)

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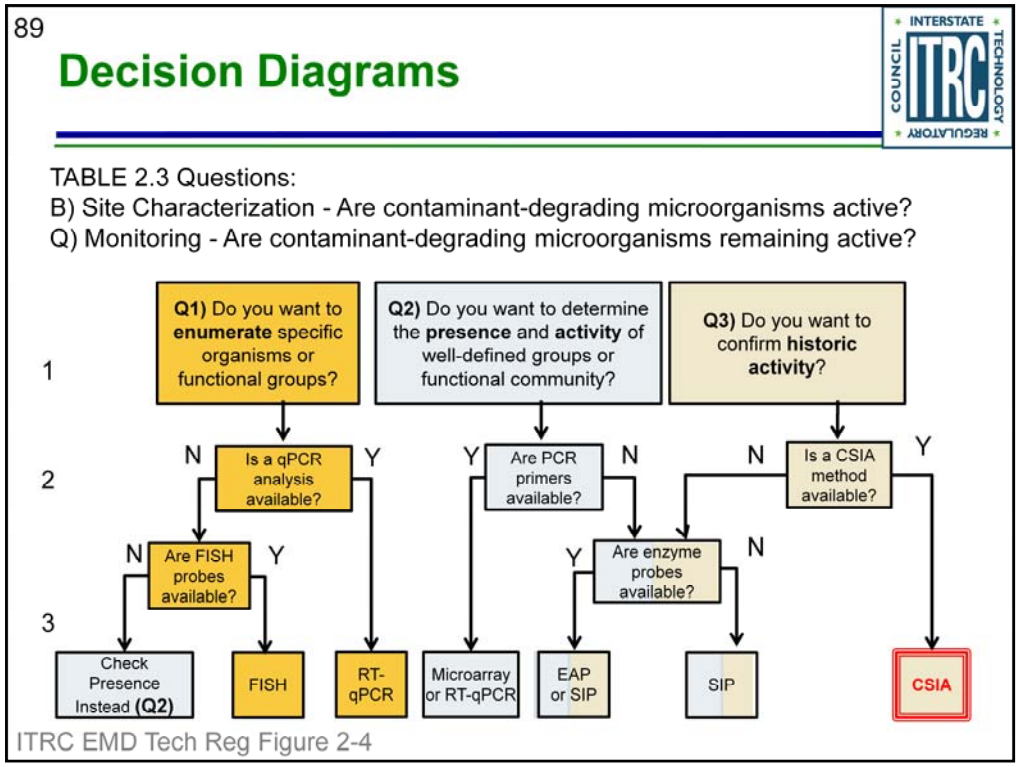


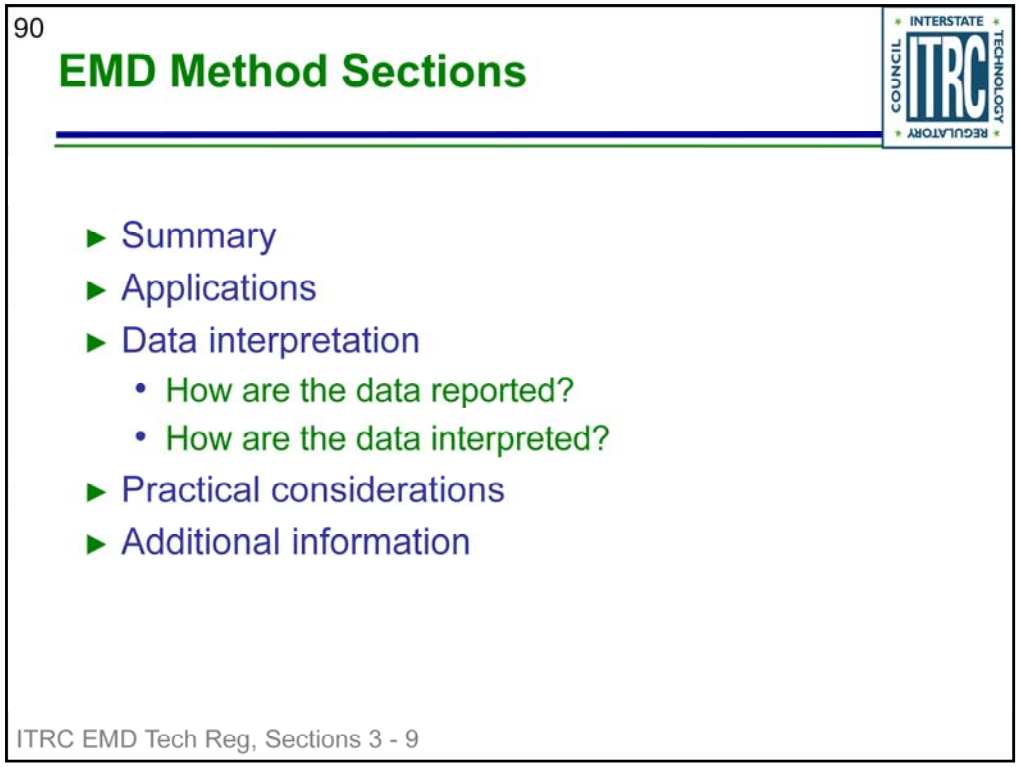

1- Supplemental Questions

2- Decision Steps

3 – EMD to Consider

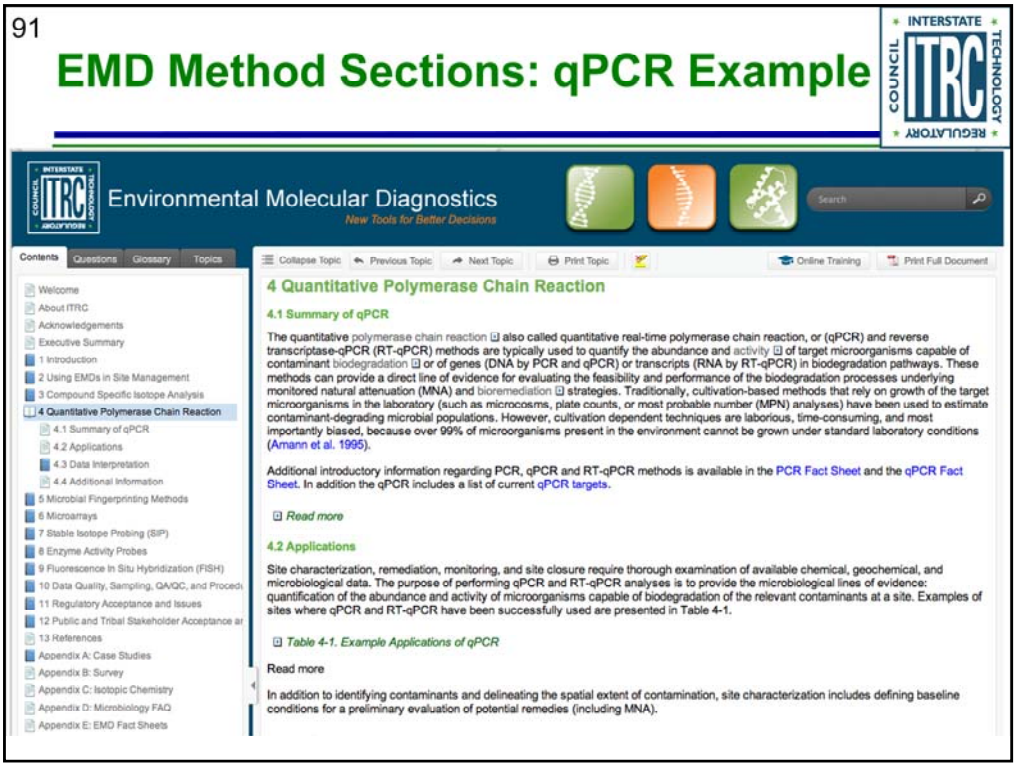
CSIA is widely commercially available.

Questions are discussed on the Questions Tab.




No associated notes.

# EMD Method Sections: qPCR Example




No associated notes.

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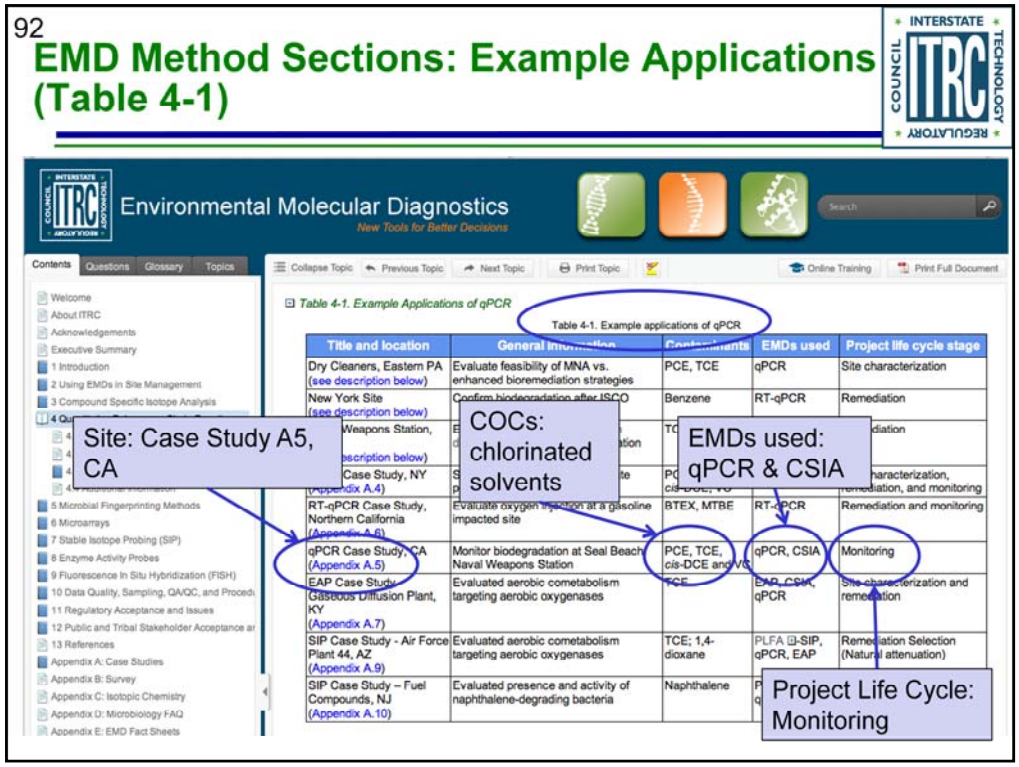
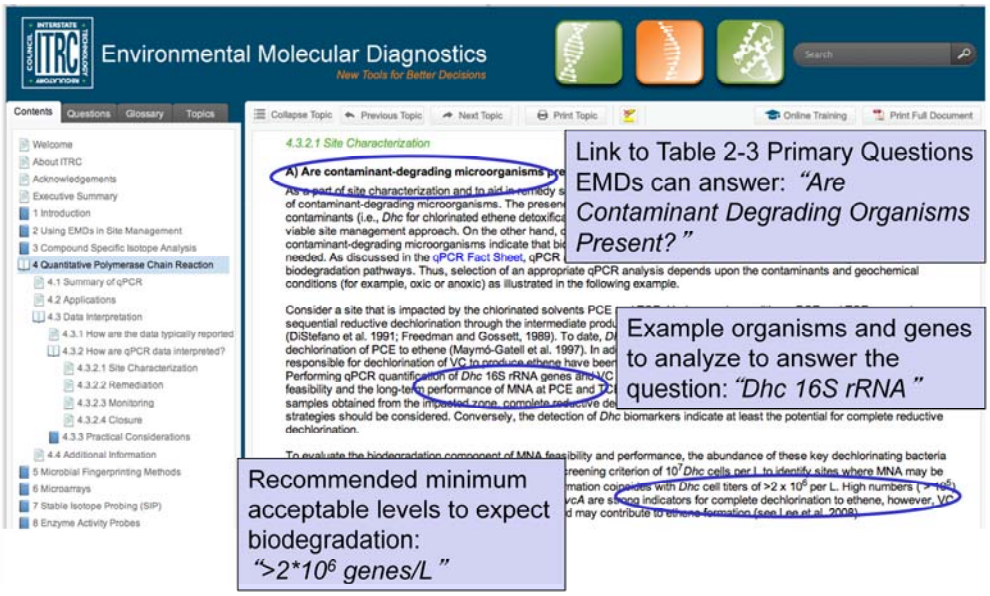
# EMD Method Sections: Example Applications (Table 4-1)




| Title and location | General information | Contaminants | EMDs used | Project life cycle stage |
|---|---|---|---|---|
| Dry Cleaners, Eastern PA (see description below) | Evaluate feasibility of MNA vs. enhanced bioremediation strategies | PCE, TCE | qPCR | Site characterization |
| New York Site (see description below) | Confirm biodegradation after ISCO | Benzene | RT-qPCR | Remediation |
| RT-qPCR Case Study, Northern California (Appendix A.6) | Evaluate oxygen injection at a gasoline impacted site | BTEX, MTBE | RT-qPCR | Remediation and monitoring |
| qPCR Case Study, CA (Appendix A.5) | Monitor biodegradation at Seal Beach Naval Weapons Station | PCE, TCE, *cis*-DCE and VC | qPCR, CSIA | Monitoring |
| EAP Case Study Gaseous Diffusion Plant, KY (Appendix A.7) | Evaluated aerobic cometabolism targeting aerobic oxygenases | TCE | EAP, CSIA, qPCR | Site characterization and remediation |
| SIP Case Study - Air Force Plant 44, AZ (Appendix A.9) | Evaluated aerobic cometabolism targeting aerobic oxygenases | TCE; 1,4-dioxane | PLFA-SIP, qPCR, EAP | Remediation Selection (Natural attenuation) |
| SIP Case Study – Fuel Compounds, NJ (Appendix A.10) | Evaluated presence and activity of naphthalene-degrading bacteria | Naphthalene | | |

No associated notes.

# EMD Method Sections: Data Interpretation




No associated notes.

94

# EMD Method Sections: Data Reports

- Recommended and Desirable Information for Lab Reports

Typical reporting limits
- 100 gene copies per L or g soil

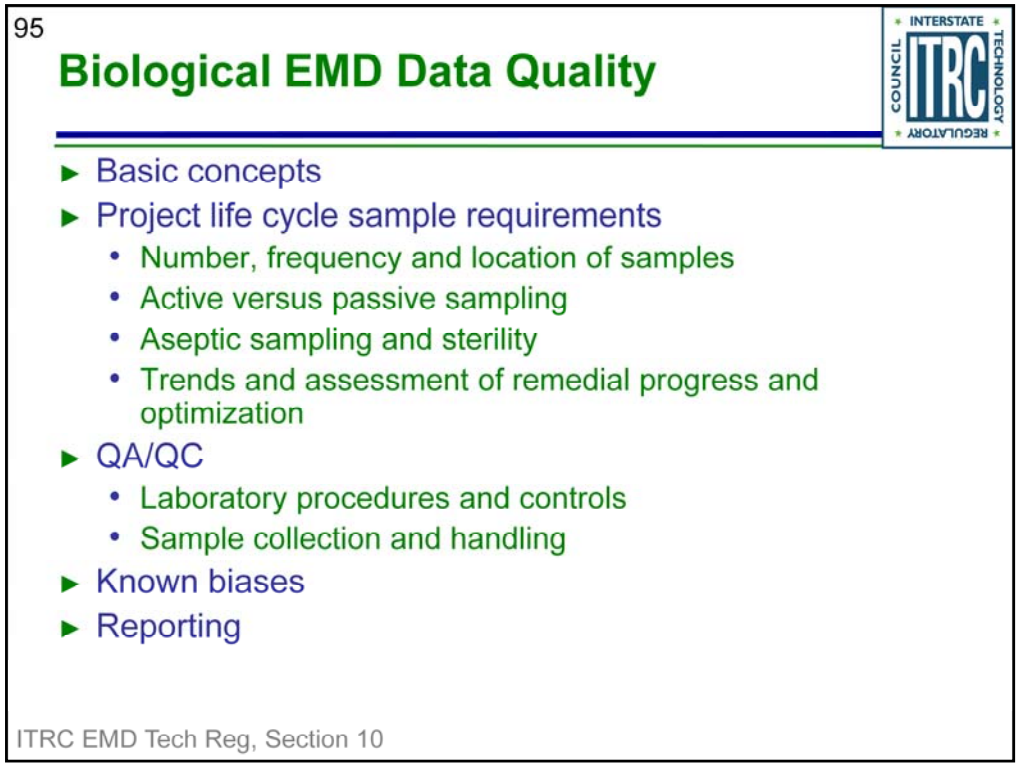
Laboratory Control Sample Results
- DNA extraction blank
- laboratory qPCR positive and negative

Table 4-2. Recommended and desirable information for qPCR laboratory report

| Report information | Typical information or acceptable ranges |
|---|---|
| **Common Laboratory Report Information** | |
| Site Identifier | Location, name, monitoring well |
| Sample type or matrix | Water, sediment, soil, Bio-Trap® |
| **Specific qPCR Information** | |
| *Recommended* | |
| Results | Number of gene copies present in the environmental sample. In some cases, number of organisms if they contain one copy of the 16s rRNA gene, such as *Dhc*. |
| Reporting Units | Gene copies per volume of water or mass of soil |
| Typical Reporting Limits | 100 gene copies per sample volume or mass |
| Limit of Detection | Varies, should be adequate for the application. |
| Sample storage/transportation | |
| Gene Target, Specificity | VC reductase |
| Primer name or sequence | |
| DNA extraction method | |
| Detection Chemistry | TaqMan or SYBR Green |
| Extraction blank | |
| Laboratory Control Sample | |
| No Template Controls (NTC) | |
| Inhibition testing | Dilutions, Spiked samples |
| *Desirable* | |
| Analysis Method | |
| Volume Extracted | 0.5 to 2 L (see Note 1) |
| Processing time after sampling | Preferably within 24 hours |

ITRC EMD Tech Reg Table 4-2

No associated notes.



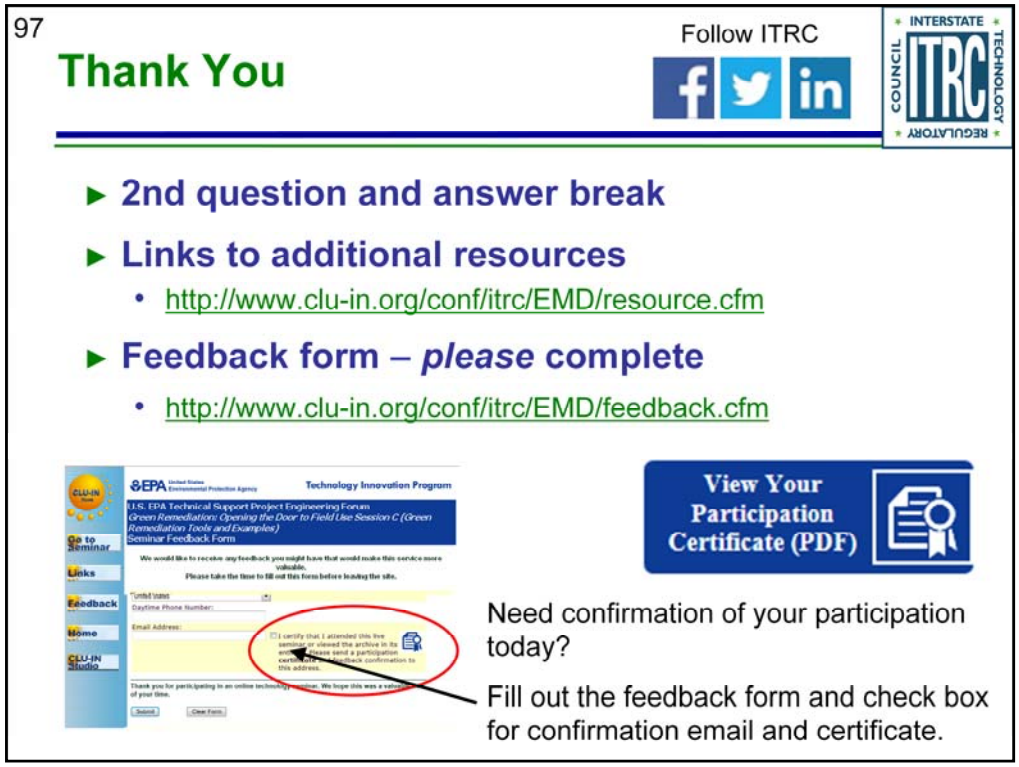

No associated notes.

96

# Wrap Up: Ready to…


- Describe the utility of EMDs during remediation activities
- Define when EMDs can cost effectively augment traditional data sets
- Determine when and how to utilize the EMD Tech Reg

EMD Tech Reg web site www.itrcweb.org/EMD-2




Links to additional resources:
http://www.clu-in.org/conf/itrc/EMD/resource.cfm

Your feedback is important – please fill out the form at:
http://www.clu-in.org/conf/itrc/EMD/feedback.cfm

**The benefits that ITRC offers to state regulators and technology developers, vendors, and consultants include:**

✓Helping regulators build their knowledge base and raise their confidence about new environmental technologies

✓Helping regulators save time and money when evaluating environmental technologies

✓Guiding technology developers in the collection of performance data to satisfy the requirements of multiple states

✓Helping technology vendors avoid the time and expense of conducting duplicative and costly demonstrations

✓Providing a reliable network among members of the environmental community to focus on innovative environmental technologies

**How you can get involved with ITRC:**

✓Join an ITRC Team – with just 10% of your time you can have a positive impact on the regulatory process and acceptance of innovative technologies and approaches

✓Sponsor ITRC's technical team and other activities

✓Use ITRC products and attend training courses

✓Submit proposals for new technical teams and projects