

**What's New in the Site Characterization Tool Box:
Molecular Biological Tools to Identify Microorganisms that Degrade Contaminants
and Contaminant-Specific Isotope Analysis to Identify Sources and Document Degradation**

John T. Wilson and Ann Keeley, EPA/ORD/Ada

In the past, it was necessary to conduct intensive sampling and monitoring of concentrations of contaminants in ground water to trace plumes back to their true source. Now it is possible to determine the ratio of stable isotopes of carbon, hydrogen or chlorine in the contaminants in ground water. The analysis is called compound specific isotope analysis or CSIA. The particular ratios of isotopes in the contaminants in the plume and in a suspected source can be compared to associate the plume with its source (under favorable conditions).

In the past, the only way to determine whether microorganisms that can degrade a contaminant were present at a site was to isolate and characterize the microorganisms. It was necessary to make the organisms grow in culture media, and this is often difficult. Now it is possible to determine the number of copies of particular genes for an active enzyme or the characteristic sequences of DNA from a known degrader organism without requiring growth. The DNA is isolated, and then the polymerase chain reaction or PCR is used to copy particular sequences of DNA repeatedly until the concentrations of DNA are high enough to be analyzed chemically.

In the past the only evidence for degradation of a contaminant was attenuation in concentrations of the contaminant with distance from the source. Uncertainties associated with sampling ground water at field scale makes this approach problematic at many sites. Now it is often possible to use changes in the ratio of stable isotopes of carbon, hydrogen and chlorine to recognize degradation of a contaminant at field scale, and estimate the extent of degradation that has already occurred. Under some conditions, it is possible to determine if a daughter product (such as *cis*-DCE or Vinyl Chloride produced from TCE) is degrading in ground water.