

FINAL REPORT

U. S. Department of Energy

**Stable Isotopic Investigations of In-Situ Bioremediation  
of Chlorinated Organic Solvents**

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### **3. Executive Summary**

The purpose of this project was to investigate the potential applications of stable isotope ratio measurements in characterization of the source terms, the transport, and the fate of chlorinated solvents in groundwater aquifers. The approach to this research was threefold: to develop methods for the sampling and isotopic analysis of chlorinated solvents in groundwaters; to perform laboratory experiments to measure equilibrium and kinetic isotope effects associated with biological and physical transformation processes of chlorinated solvents; and to perform field investigations at well-characterized, contaminated aquifer sites to demonstrate the applicability of the isotopic approach in real-world situations.

New methods were developed for the extraction and isotopic analysis of chlorinated solvents in water samples. Our method for isotopic analysis of carbon and chlorine in chlorinated organic compounds was awarded a U. S. Patent. The new methods were applied to a series of laboratory experiments assessing the isotope effects associated with biological and physical transformation processes. We found that a large kinetic isotope effect was caused by microbial degradation of chlorinated solvents, under both aerobic and anaerobic conditions. The evaporation of chlorinated solvents also caused a large isotope effect, but in that case the carbon isotopic fractionation was in the reverse sense. Isotopic fractionation associated with adsorption and chemical exchange of chlorinated solvents was negligible.

We performed field investigations at five present and former industrial sites undergoing various stages of remediation. These investigations were collaborative with Lockheed-Martin Energy Systems, Inc., and ENSR, Inc., the companies responsible for the remedial work at these sites. Application of our new sampling and analytical methods at these sites showed that stable isotopic measurements yield powerful insights into underground processes. In particular, we found positive

evidence for in-situ microbial degradation of chlorinated solvents at all five sites, evidence for volatilization of chlorinated solvents at three sites, and evidence for multiple sources of chlorinated solvents at two sites. At a site where six-phase heating was applied, we found evidence for increase solubilization of pure phase chlorinated solvents during heating. The general conclusion of the field investigations is that stable isotope measurements should become an essential component in site characterization and remediation of sites contaminated with chlorinated solvents.

#### **4. Research Objectives**

The research objectives of this program are threefold: to develop methods for measuring stable isotope ratios of carbon and chlorine in chlorinated aliphatic hydrocarbons (CAHs); to apply these methods to experimental determinations of kinetic and equilibrium isotope effects during biological, chemical, and physical transformation of CAHs; and to apply these methods to CAHs extracted from ground water at well-characterized, contaminated aquifer sites. The overall objective is to develop an understanding of the environmental isotopic behavior of CAHs and to apply this understanding to better characterize, monitor, and evaluate natural and engineered bioremediation. This is an important problem because of the magnitude and frequency of ground water contamination by CAHs and the resultant health risks imposed to the population, as well as the enormous costs involved in cleaning up such contamination.

This project is innovative as it represents the first systematic effort of its kind. Since its inception, a number of other scientists have also started research into the stable isotope chemistry of CAHs in both laboratory and field investigations (e.g., Jendrzewski et al., 1997; Beneteau et al., 1999; Sherwood Lollar et al., 1999; Poulson and Drever, 1999; Hunkeler et al., 1999). Complementary experimental and theoretical studies of the equilibrium stabilities and kinetics of CAH degradation by microbial and abiotic mechanisms continue to be a major field of research (e.g., Leisinger et al., 1994; Freedman et al., 1997; McCarty, 1997; Yager et al., 1997; Knauss et al., 1999; Haas and Shock, 1999; Haston and McCarty, 1999; Pagan et al., 1999; Butler and Hayes, 1999; Erbs et al., 1999).

During the field demonstration, this project received support from two collaborating institutions: (1) Lockheed Martin Energy Systems, Inc., at the Paducah Gaseous Diffusion Plant in Kevil, Kentucky, and (2) ENSR, Inc., at a number of industrial sites in the central and southeastern United States where ENSR manages cleanup operations.

#### **5. Methods and Results**

The experimental methods used in this work have either been published (Holt et al., 1997; Heraty et al., 1999; Huang et al., 1999) or will be published during the next year. Three principal methods were developed. The first method, which was awarded a U. S. Patent in 1999, is for isotopic analysis of carbon and chlorine in chlorinated volatile organics (Holt et al., 1997 – see Appendix A for details). This method has been used routinely for hundreds of analyses in our laboratory and has been adopted by other laboratories in the United States, Canada, and Europe. In this high-temperature, low-pressure procedure, the chlorinated volatile organic sample is heated with CuO in a sealed reaction tube for 2 hours at 550°C to form, among other products, CO<sub>2</sub> and CuCl. The CO<sub>2</sub> and the excess CuO are removed, and the CuCl is then heated with excess CH<sub>3</sub>I in the same reaction tube for 2 hours at 300°C to form CH<sub>3</sub>Cl. The yields and purities of both the CO<sub>2</sub> and the CH<sub>3</sub>Cl are suitable for mass spectrometric isotope ratio analyses without further treatment. The precision is generally better than +0.1‰ for both d<sup>13</sup>C and d<sup>37</sup>Cl in the analysis of the pure compounds.

The second method we developed was for near-quantitative extraction of trace amounts of CAHs from ground water samples for isotopic analysis. This is a combined ultrasonic vacuum extraction, helium sparging, cryogenic distillation technique that is based on our earlier work on dissolved gases in ground water (Holt et al., 1995). It will be described in a manuscript that is

currently in preparation. The third method we developed is studies of kinetic isotope effects in microbial degradation (Heraty et al., 1999 – see Appendix B for details).

The results of our research have been published in part (see Appendices). Our investigations include: a field study of natural attenuation of trichloroethene (TCE) at the Paducah Gaseous Diffusion Plant (Sturchio et al., 1998 – see Appendix C for details); an experimental study of kinetic isotope effects for carbon and chlorine during aerobic microbial degradation of dichloromethane (Heraty et al., 1999 – see Appendix B for details); an experimental study of kinetic isotope effects for carbon and chlorine during evaporation of TCE and dichloromethane (Huang et al., 1999 – see Appendix D for details; and an experimental study of the kinetic isotope effect for carbon during reaction of CAHs with metallic iron (Dayan et al., 1999 – see Appendix E for details). Additional data from field demonstration sites have been acquired and will be presented in a manuscript that is in preparation. A collaborative study on the chlorine isotope ratios of semivolatile chlorinated organic compounds is now in press (Reddy et al., 2000).

## **6. Relevance, Impact, and Technology Transfer**

a. The new scientific information generated by this research focuses on the problem of management of chlorinated solvents. The results of this research significantly improved the understanding of the fate and transport of chlorinated solvents in ground water aquifers and vadose zones.

b. The principal benefits to DOE of the new information generated are that: (1) it will improve site characterization by revealing the nature and extent of natural attenuation processes occurring at a site and can be used in conjunction with hydrologic data to estimate attenuation rates and (2) it can be used to monitor cleanup and to help evaluate the most effective engineered remedial strategy for a given site.

c. This knowledge bridges the gap well between fundamental research and its timely application. Data generated in this research have already been used in several real-world applications for chlorinated solvent cleanup.

d. The results of this research have already been used and will continue to be used. Part of this research involved development of a method for precise isotopic analysis of chlorinated solvents. This method was published and awarded a U. S. Patent. A number of other laboratories in the United States, Canada, and Europe have already adopted this method for routine use.

e. The methods and initial results produced by this project have been highly successful. Larger-scale applications of the stable isotope approach to monitoring and evaluation of in-situ bioremediation and natural attenuation processes are warranted. A widely used method for isotopic analysis of chlorinated solvents in ground water has been developed.

f. Collaborating scientists have received training in analytical methods, field sampling methods, and data analysis methods.

g. This research has advanced our understanding of the kinetic isotope effects associated with

biological, chemical, and physical transformations of the CAHs. It has advanced our knowledge of the extent to which various processes affect the isotopic composition of chlorinated solvents in ground water aquifers.

h. Additional scientific hurdles include: (1) establishing a larger database of experimentally measured kinetic isotope effects for microbial degradation of chlorinated solvents and (2) integrating isotopic data more fully into conceptual and numerical ground water and vadose zone transport models for chlorinated solvents.

i. Other agencies that have expressed interest in the project include a variety of environmental consulting firms, law firms, universities, and national laboratories are listed below:

- Lockheed Martin Energy Systems, Inc., Kevil, KY (Mr. Jay Clausen – now at Ogden Environmental and Energy Services, Inc., Westford, MA)
- ENSR, Inc., Westmont, IL (Mr. Greg Smith – now at Radian International, Rolling Meadows, IL).
- Gardere & Wynne, L.L.P., Tulsa, OK (Dr. J. Berton Fisher )
- Leatherwood, Walker, Todd, and Mann, P.C., Greenville, SC (Mr. Eugene McCall)
- Environment Institute of the Joint Research Centre of the European Commission, Ispria, Italy (Mr. Stephan Valley)
- Dr. Richard Hurst, independent consultant, Los Angeles, CA
- Prof. Max Coleman, University of Reading, U.K.
- Dr. Chris Reddy, Woods Hole Oceanographic Institute, Woods Hole, MA
- Dr. Chris Romanek, Savannah River Ecology Laboratory, Aiken, SC
- Dr. Simon Poulson, University of Nevada, Reno, NV
- Dr. Mark Conrad, Lawrence Berkeley National Laboratory, Berkeley, CA
- Dr. Stephane Vuilleumier, Institute of Microbiology, ETH, Zurich, Switzerland

## **7. Project Productivity**

The project accomplished all of its research goals on schedule, except for some of the anaerobic microbial degradation experiments. The delay was the result of the extremely slow kinetics of the anaerobic microbial systems. Furthermore, we observed little or no microbial degradation of chlorinated aliphatics in our matrix of experiments. We chose instead to emphasize experimental

work on aerobic microbial systems and on kinetic isotope effects associated with volatilization.

## 8. Personnel Supported

- Prof. Teofilo Abrajano (visiting faculty, Rensselaer Polytechnic Inst, Troy, NY)
- Ms. Linnea Heraty (Scientific Associate)
- Dr. Lin Huang (Postdoctoral Appointee)
- Dr. Ben Holt (Special Term Appointee)
- Ms. Virginia Inofinada (graduate student)
- Ms. Roxanne Myshkowec (undergraduate student)
- Dr. Neil Sturchio (P.I., Scientist)
- Mr. Jeff Ullian (undergraduate student)

## 9. Publications

### *a. Peer-reviewed journal articles*

Holt B. D., Sturchio N. C., Abrajano T. A., and Heraty L. J, 1997. Conversion of chlorinated organic compounds to carbon dioxide and methyl chloride for isotopic analysis of carbon and chlorine. *Analytical Chemistry* 69, 2727-2733.

Sturchio N. C., Clausen J. C., Heraty L. J., Huang L., Holt B. D., and Abrajano T., 1998. Stable chlorine isotope investigation of natural attenuation of trichloroethene in an aerobic aquifer. *Environmental Science and Technology* 32, 3037-3042.

Dayan, H., Abrajano T., Sturchio N. C., and Winsor L., 1999. Carbon isotopic fractionation during reductive dechlorination of chlorinated solvents by metallic iron. *Organic Geochemistry* 30, 755-763.

Heraty L. J., Fuller M. E., Huang L., Abrajano T., and Sturchio N. C., 1999. Carbon and chlorine isotopic fractionation during microbial degradation of dichloromethane. *Organic Geochemistry* 30, 793-799.

Huang L., Sturchio N. C., Abrajano T., Heraty L., and Holt B. D., 1999. Carbon and chlorine isotope fractionation of chlorinated aliphatic hydrocarbons by evaporation. *Organic Geochemistry* 30, 777-785.

### *b. Proceedings papers*

Sturchio N. C., Heraty L., Holt B., Huang L., and Abrajano T., 1999. Stable isotope investigations of chlorinated aliphatic hydrocarbons. In *Proceedings of the Second International Symposium on Integrated Technical Approaches to Site Characterization*, Argonne, IL, p. 151-156.

*c. Accepted/submitted for publication*

Reddy C. M., Heraty L. J., Holt B. D., Sturchio N. C., Eglinton T. I., Lake J. and Maruya K., 1999. Stable chlorine isotopes in semivolatile organic compounds. Environmental Science and Technology, in press.

## **10. Interactions**

*a. Presentations at meetings*

Holt, B. D. and Sturchio, N. C., 1996. High temperature method for conversion of chlorinated organic compounds to CH<sub>3</sub>Cl and CO<sub>2</sub> for isotopic analysis of chlorine and carbon. Abstracts of Papers of the American Chemical Society, v. 212, pp. 154-ENVR.

Sturchio N. C., Holt B. D., Abrajano T. A., Heraty L. J., Fuller M. E., and Manning J. F., 1997. Carbon and chlorine isotopic studies of biodegradation of chlorinated volatile organics. 18th International Meeting on Organic Geochemistry, Maastricht, The Netherlands, Sept. 22-26.

Sturchio, N. C., Abrajano, T., Heraty, L., Huang, L., and Clausen, J., 1997. Stable isotope investigation of natural attenuation of trichloroethene at the Paducah Gaseous Diffusion Plant. Geological Society of America Abstracts with Programs 29(6), A185.

Dayan, H., Abrajano, T., Heraty, L., Huang, L., and Sturchio, N. C., 1997. Isotopic fractionation during reductive dehalogenation of chlorinated ethenes by metallic iron. Geological Society of America Abstracts with Programs 29(6), A185.

Huang L., Sturchio N. C., Heraty L., and Abrajano T., 1998. Chlorine and carbon isotopic fractionations of aliphatic hydrocarbons in evaporation processes: Implications for biodegradation. 9th International Conference on Geochronology, Cosmochronology, and Isotope Geology. Beijing, China, August 1998.

Sturchio N. C., Heraty L. J., Holt B. D., Huang L., and Abrajano T., 1999. Stable isotope investigations of the chlorinated aliphatic hydrocarbons. Ninth Annual V.M. Goldschmidt Conference, Cambridge, MA, August 22-27, 1999.

Reddy C.M., Heraty L.J., Holt B.D., Sturchio N.C., Eglinton T.I., Maruya K.A., and Lake J.L., 1999. Chlorine isotope ratios of semi-volatile chlorinated organic compounds. Ninth Annual V.M. Goldschmidt Conference, Cambridge, MA, August 22-27, 1999.

Heraty L. J., Sturchio N. C., Holt B. D., Huang L., Abrajano T., and Smith G., 1999. Microbial degradation of chlorinated aliphatic hydrocarbons: Field investigations using stable carbon and chlorine isotopes. Geological Society of America 1999 Annual Meeting, October 25-28, Denver, CO.

Holt B. D., Heraty L. J., and Sturchio N. C., 1999. Extraction of chlorinated aliphatic hydrocarbons from water for isotopic analysis of chlorine and carbon. American Chemical Society 218th National Meeting, New Orleans, LA, August 22-26, 1999.

Sturchio N. C., Heraty L., Holt B., Huang L., and Abrajano T., 1999. Stable isotope investigations of chlorinated aliphatic hydrocarbons. In Proceedings of the Second International Symposium on Integrated Technical Approaches to Site Characterization, Argonne, IL, June 7-9, 1999. p. 151-156.

Sturchio N. C., Heraty L., Holt B. D., Huang L., Abrajano T., and Smith G., 2000. Stable isotope ratios of chlorinated aliphatic hydrocarbons in contaminated aquifers. Second International Conference on Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, May 22-25, 2000.

*b. Collaborations*

· Mr. Jay Clausen (Lockheed-Martin Energy Systems, Inc., Kevil, KY (now at Ogden Energy and Environmental Systems, Inc., Westport, MA), on application of chlorine isotope ratio measurements in an investigation of natural attenuation of trichloroethene at the Paducah Gaseous Diffusion Plant.

· Mr. Greg Smith, ENSR, Inc. (now at Radian International), on application of carbon and chlorine isotopic measurements to solvent cleanup activities at a number of industrial sites.

· Dr. Chris Reddy, Woods Hole Oceanographic Institute, Woods Hole, MA, on application of chlorine isotope measurements to environmental studies of semivolatile chlorinated organics (e.g., PCBs and pesticides).

## **11. Transitions**

a. Our method for stable isotope analysis of carbon and chlorine in chlorinated aliphatic hydrocarbons has been adapted by several laboratories (e. g., University of Nevada, Reno, NV; University of Waterloo, Canada; University of Reading, U.K.; Environment Centre of the Joint European Commission, Ispria, Italy).

b. Our methods were applied in conjunction with remedial activities for TCE-contaminated groundwater aquifers carried out by Lockheed-Martin Energy Systems, Inc. at the Paducah Gaseous Diffusion Plant in Kevil, KY, and by ENSR, Inc. (Westmont, IL) at locations in the Chicago, IL; Kansas City, MO; and Greer, SC areas. The principal hydrogeologist on the Lockheed-Martin project in Kentucky was Jay Clausen (currently with Ogden Energy and Environmental Systems, Inc., Westport, MA) and the principal hydrogeologist on the ENSR, Inc., projects, with whom we interacted extensively, was Greg Smith (currently with Radian International, Rolling Meadows, IL).

## 12. Patents

U. S. Patent # 5,942,439. Ben D. Holt and Neil C. Sturchio. Method for Isotopic Analysis of Chlorinated Organic Compounds (issued 8/28/1999).

## 13. Future Work

The line of inquiry that we have begun has a natural extension in several areas: (1) measurement of additional kinetic isotope effects associated with physical and biological degradation processes, as well as equilibrium isotope fractionation factors; (2) application to additional field investigations of well characterized, contaminated aquifer systems and vadose horizons; (3) integration of isotope data into conceptual and numerical transport models for chlorinated solvents in ground water aquifers and vadose zones.

## 14. Literature Cited

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Dayan, H., Abrajano T., Sturchio N. C., and Winsor L., 1999. Carbon isotopic fractionation during reductive dechlorination of chlorinated solvents by metallic iron. *Organic Geochemistry* 30, 755-763.

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chlorine. *Analytical Chemistry* 69, 2727-2733.

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Yager R. M., Bilotta S. E., Mann C. L., and Madsen E. L., 1997. Metabolic adaptation and in-situ attenuation of chlorinated ethenes by naturally occurring microorganisms in a fractured dolomite aquifer near Niagara Falls, New York. *Environ. Sci. Technol.* 31, 3138-3147.

## **15. Feedback**

A mechanism for renewal of projects should have been available.

## 16. Appendices

A. Holt B. D., Sturchio N. C., Abrajano T. A., and Heraty L. J, 1997. Conversion of chlorinated organic compounds to carbon dioxide and methyl chloride for isotopic analysis of carbon and chlorine. *Analytical Chemistry* 69, 2727-2733.

B. Heraty L. J., Fuller M. E., Huang L., Abrajano T., and Sturchio N. C., 1999. Carbon and chlorine isotopic fractionation during microbial degradation of dichloromethane. *Organic Geochemistry* 30, 793-799.

C. Sturchio, N. C., Clausen, J. C., Heraty, L. J., Huang, L., Holt, B. D., and Abrajano, T., 1998. Stable chlorine isotope investigation of natural attenuation of trichloroethene in an aerobic aquifer. *Environ. Sci. Technol.* 32, 3037-3042.

D. Huang L., Sturchio N. C., Abrajano T., Heraty L., and Holt B. D., 1999. Carbon and chlorine isotope fractionation of chlorinated aliphatic hydrocarbons by evaporation. *Organic Geochemistry* 30, 777-785.

E. Dayan, H., Abrajano T., Sturchio N. C., and Winsor L., 1999. Carbon isotopic fractionation during reductive dechlorination of chlorinated solvents by metallic iron. *Organic Geochemistry* 30, 755-763.