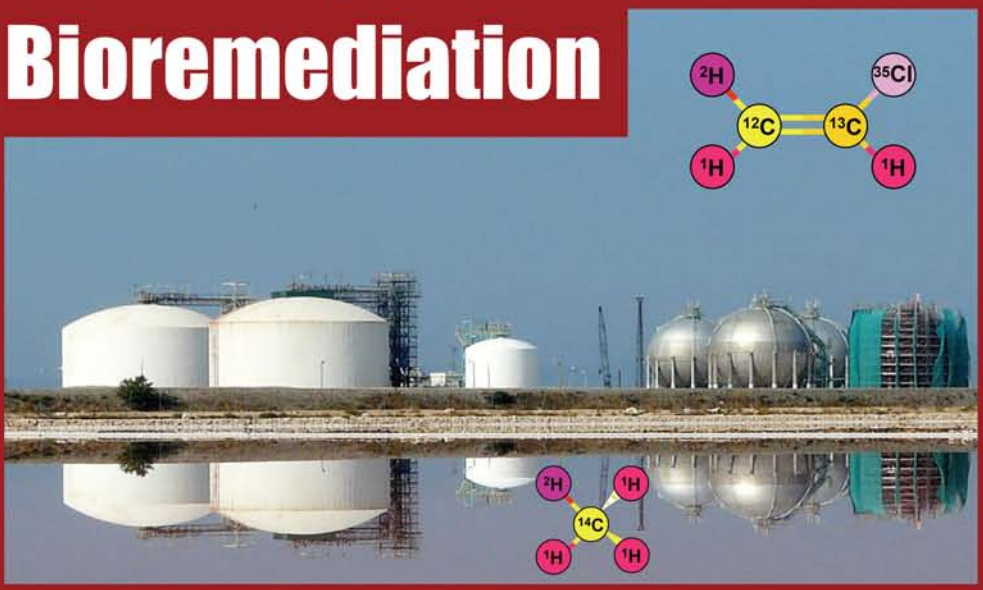
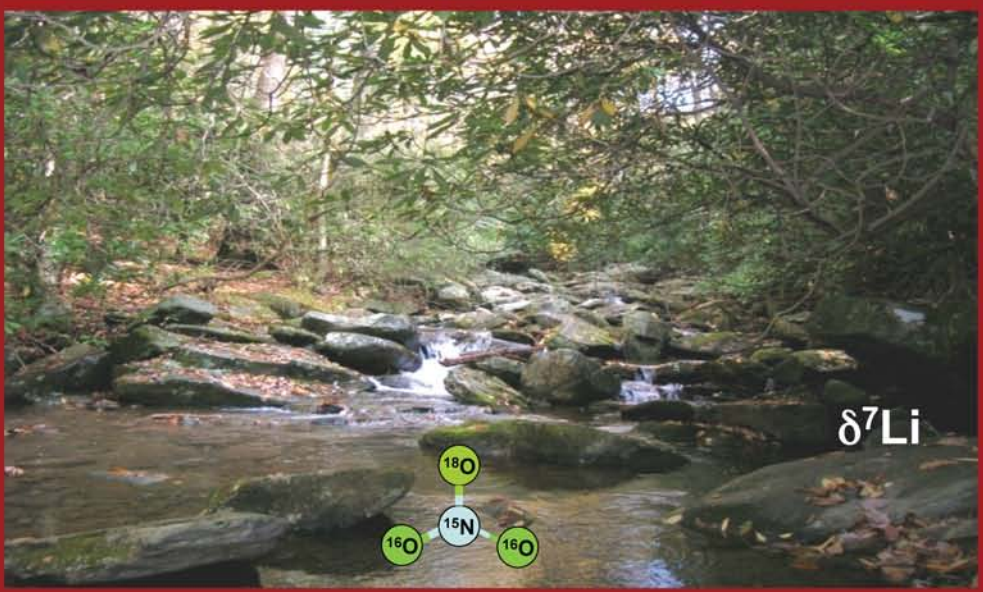
Environmental Isotopes in Biodegradation and Bioremediation





Edited by
C. Marjorie Aelion • Patrick Höhener
Daniel Hunkeler • Ramon Aravena

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Edited by

C. Marjorie Aelion
University of Massachusetts, Amherst, USA
and University of South Carolina, Columbia, USA

Patrick Höhener
Université de Provence, Marseille, France

Daniel Hunkeler
Centre for Hydrogeology, University of Neuchâtel, Switzerland

Ramon Aravena
University of Waterloo, Ontario, Canada

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Preface

The application of isotopes has expanded from the field of geology to biogeochemistry, and on to many areas of environmental sciences. The use of isotopes in natural systems has been described in many excellent books covering marine systems, terrestrial biotic systems, geologic earth science systems, and the atmosphere. Similarly, the role of microorganisms in natural and contaminated environments has been discussed in several excellent books that describe microbial physiology and biochemistry in detail. This is the first book, to our knowledge, that brings the world of isotopes to the world of hydrology and microbial ecology in both natural and contaminated environments. Isotopes are a powerful tool to investigate this world and provide information on the interaction of microorganisms with natural compounds and pollutants in the area of biodegradation and its application in bioremediation and chemical cycling. The development of enhanced analytical capabilities and separation techniques, improvements in detection limits, and accessibility of instrumentation have opened up this world by allowing compound-specific isotope analysis.

This book began at the urging of Brian Kenet, who attended a session at an international meeting chaired by C. Marjorie Aelion in 1999 in which she presented a paper entitled "Soil Gas and Isotopic Assessment of Anaerobic Petroleum Biodegradation." Kenet urged again in 2001 after attending a similar session chaired by Aelion entitled, "Isotopic Approaches in Bioremediation." Kenet was convinced that there was significant and growing interest on the use of isotopes in biodegradation and environmental contaminant studies. Aelion agreed and the book took form; coauthors were garnered, the outline of the book was generated, and the book was contracted in 2002 with the expanded view of natural and contaminated environments. Since that time and during the writing of the book, the field has exploded. Initially in the domain of a relatively small group of specialized academic researchers, the field of isotopic applications to contaminant hydrology and microbial ecology has expanded to great numbers of researchers and to the commercial sector. This was due in part to increased access and availability to commercial analytical facilities and analytical developments. Continued development of the technique will expand the field even further to areas as yet untapped.

This book is intended to provide sufficient technical detail to be used as a textbook for advanced undergraduate students and graduate students in the fields of geology, environmental science, and microbial ecology who want to understand the role of isotopes in microbial ecology with more specific detail on biodegradation and bioremediation. The questions of what amount of a compound comes from anthropogenic release, whether these chemicals undergo degradation in the environment, and whether they persist and accumulate are of great concern and isotopes may help to answer these questions. Advanced students in the field need both theoretical and practical knowledge to thoroughly understand the complexities of the use of isotopes in these fields and an understanding of the potential for future expansion. Additionally the book will be useful to environmental practitioners in the field of

bioremediation and scientists in environmental regulatory agencies who must understand, interpret, and evaluate the data presented to them by the environmental practitioners in the day-to-day realities of site remediation.

To this end in this first edition we have divided the book into four broad areas and the following four sections: "Isotope Fundamentals," "Isotopes and Microbial Processes," "Isotopes in Field Applications," and "Isotope Emerging Areas." The first section covers important background and theoretical information that is necessary for the understanding of later chapters. Chapter 1 defines isotopes, how isotopes were discovered, and how isotope data are reported with respect to international standards. Chapter 2 details how isotopes are analyzed and the importance of understanding the levels of sensitivity and accuracy that can be achieved for the various isotopes. Chapter 3 provides the theoretical basis for understanding how isotopes undergo fractionation in the environment, the extent of fractionation in different fractionation processes, and the fundamental governing equations for these transformations. Chapter 4 highlights the fundamental principles of isotope fractionation during the transformation of organic contaminants and compiles large tables with fractionation factors for stable isotopes in contaminants undergoing various transformation processes.

The second section covers different environmental redox conditions that dictate the predominant microbial processes that will occur. Chapter 5 focuses on aerobic processes in which molecular oxygen (O_2) is the electron acceptor and reactions that allow the highest energy yield for the degrading organisms. Aerobic degradation leads to carbon dioxide as an end product and is important to carbon recycling and the enhanced bioremediation of contaminants. Chapter 6 focuses on methanogenesis that recycles carbon in anaerobic environments such as landfills, lake sediments, and marshes producing methane as an end product. Methanogenesis leads to the largest of all carbon isotope shifts in the processed carbon and in the produced methane, so isotope methods may be particularly useful if sufficient methane is generated. Chapter 7 describes isotope studies focusing on the two stable isotopes ^{15}N and ^{34}S, often combined with ^{18}O in oxygen-bearing compounds. Both nitrogen and sulfur can serve as electron acceptors by nitrate- and sulfate-reducing microorganisms, respectively, to oxidize carbon and can be used to track more reduced forms such as ammonia, elemental sulfur, and sulfides.

The third section describes, more specifically, the transformation of anthropogenic pollutants and the application to field sites. Chapter 8 explains how transformation processes can be assessed using stable isotopes at field sites in completely water-saturated porous media and presents quantitative concepts to estimate the extent of degradation with isotope data. Chapter 9 summarizes those processes that fractionate isotopes in gases and volatile pollutants in the unsaturated zone, with a special focus on gas-phase diffusion that may confound the interpretation of the fractionation caused by biodegradation and may necessitate modeling of both fractionation by gas-phase diffusion and by biodegradation.

The fourth section covers expanding fields that may warrant books in their own right. Chapter 10 summarizes the use of compounds labeled with stable isotopes for degradation studies in natural and contaminated environments including the emerging area of stable isotope probing that combines stable isotope labeling with the

extraction and analysis of molecular biological markers of organisms responsible for degradation. Chapter 11 highlights the use of radiocarbon at natural abundance as an extremely sensitive tool used in combination with stable carbon isotopes to examine carbon in natural and contaminated environments. Finally, Chapter 12 breaks away from the classical focus of many environmental scientists, often limited to the elements of carbon, nitrogen, oxygen, and sulfur, to examine the world of multiple elements. This chapter gives an overview of the use of novel stable isotopes for the elements Li, Cl, Ca, Se, Cr, and Fe, which have potential applications in a wide range of environmental studies, a few of which are highlighted.

Several individuals gave freely of their time to improve the content of this book. Chapters were reviewed by experts in the field and we thank each of the following reviewers: Johannes Barth, University of Erlangen; Stefano Bernasconi, ETH Zurich; Daniel Bouchard, SNC Lavalin; Thomas Boyd, Naval Research Laboratory; Jeffrey Chanton, Florida State University; Keith Hackley, Illinois State Geological Survey; Lawrence Miller, U.S. Geological Survey; Barbara Morasch, EPFL, Lausanne; Elizabeth Guthrie Nichols, North Carolina State University; Egbert Schwartz, Northern Arizona State University; Greg Slater, Woods Hole Oceanographic Institute; Leonard Wassenaar, NWRI, Environment Canada; Tim Buscheck, Chevron; Gwenaël Imfeld, Laboratoire d'Hydrologie et de Geochimie de Strasbourg; and Florian Breider, University of Neuchâtel. Melissa Engle provided extensive support for the illustrations in several chapters. We appreciate and acknowledge the patience and important contributions of Jennifer Ahringer, Kari Budyk, Matt Lamoreaux, and Rachael Panthier of Taylor & Francis, in the production of this book.

To complement this book, we have established a supporting website at www.unine.ch/chyn/isotopes where additional material is posted. We hope that this book and website are well used by a variety of people in a variety of disciplines, and will garner interest around the globe as is evidenced by the geographic distribution of the books' collaborators.

We look forward to receiving comments on new areas for isotopic applications as they develop in the future. Finally, we request that any omissions or errors be excused as unintentional on the part of the authors and we hope these will be brought to our attention.

C. Marjorie Aelion
Patrick Höhener
Daniel Hunkeler
Ramon Aravena

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Many people contributed to this effort and the names of many of them are listed in the preface. In addition to these individuals we would like to thank Mr. Peter Stone of the S.C. Department of Health and Environmental Control who, as a consummate geochemist and inquisitive scientist, initiated a great deal of thought and research into the world of stable isotopes. Dr. Brian Kirtland was a contributor to this world, and Dr. Thomas Leatherman remains a long-term contributor to multiple efforts.

Editors

C. Marjorie Aelion is dean of the School of Public Health and Health Sciences at the University of Massachusetts, Amherst. She worked for the U.S. Geological Survey, Water Resources Division as a hydrologist for three years before beginning her academic career at the University of South Carolina in Columbia from 1991 to 2008. She was an assistant professor, an associate professor, and a professor in the Department of Environmental Health Sciences, and the associate dean for research for the Arnold School of Public Health. She obtained her SMCE from the Massachusetts Institute of Technology in Cambridge, Massachusetts in civil engineering, and her PhD in environmental sciences and engineering, environmental chemistry and biology from the School of Public Health at the University of North Carolina, Chapel Hill. She has been a Fulbright awardee to the Université de Bretagne Occidentale in France, and the University of Wageningen in the Netherlands. Her research is in the area of biodegradation of organic contaminants, tools for assessing remedial technologies, including stable isotopes and naturally occurring radiocarbon, and the application and development of enhanced remediation systems for contaminated groundwater. She has additional interests in the associations of metals in residential soils with negative health outcomes in children. C. Marjorie Aelion is on the editorial board of *Bioremediation Journal* and *Oceans and Oceanography*, and a managing editor for *Biodegradation*. She is the author of more than 70 peer-reviewed scientific articles and one edited book.

Patrick Höhener is a professor in hydrogeochemistry at the University of Provence, Marseille. He obtained a PhD in environmental sciences in 1990 from the Swiss Federal Institute of Technology, where he later had a position as lecturer at Zurich and Lausanne. His research interests lie in the management and remediation of soils and aquifers contaminated with organic chemicals. This includes the development of site investigation methods as well as the development of low-cost remediation techniques such as in situ bioremediation. He is also interested in the biogeochemical processes that control elemental cycling at contaminated sites and the use of partitioning tracers and environmental isotopes as tools for process identification. Patrick Höhener is editor of the *Journal of Contaminant Hydrology* and the journal *Grundwasser* and the author of more than 50 peer-reviewed articles.

Daniel Hunkeler is a professor for groundwater quality at the Centre for Hydrogeology at the University of Neuchâtel, Switzerland, and an adjunct professor at the University of Waterloo, Canada. He obtained a PhD from the Swiss Federal Institute for Technology, Zürich. His research focuses on the development of stable isotope methods and their application to gain insight into the contaminant behavior at the field scale. He also works on the development of methods for the characterization and remediation of contaminated sites and the use of geochemical and isotope tools to characterize the dynamics of groundwater flow systems. Daniel Hunkeler directs the Centre for Hydrogeology Stable Isotope and Hydrochemistry Laboratory and teaches graduate courses on groundwater chemistry, contaminant hydrogeology, stable isotope methods, and water resources management at the University of Neuchâtel as well as international short courses on isotope methods. He is an associate editor of *Organic Geochemistry* and the editor of *Grundwasser.*

Ramon Aravena is a research professor in the Department of Earth Sciences and Environmental Sciences at the University of Waterloo. Dr. Aravena's research has focused on the application of environmental isotopes (^{18}O, ^{2}H, ^{13}C, ^{14}C, ^{3}H, ^{34}S, and ^{15}N) in hydrology, geochemistry, and quaternary geology. He has been involved in numerous groundwater studies in Latin America, Canada, the United States, and Europe related to the evaluation of groundwater resources and groundwater contamination. His current research focuses on groundwater contamination caused by agricultural, urban, and industrial activities. Dr. Aravena consults as part of the expert pool of the International Atomic Energy Agency, Vienna, Austria, for its projects worldwide. He has also developed a strong research collaboration network with European and Latin American universities. His teaching involves isotope hydrology and geochemistry courses at the University of Waterloo, Canada, and courses on isotope hydrology in Latin America organized by the International Atomic Energy Agency, and at the National Groundwater Association professional course program. He has also been involved in teaching short courses on the application of environmental isotopes in contaminant hydrogeology in different European universities. He is the author and coauthor of more than 100 peer-reviewed articles.

Contributors

C. Marjorie Aelion
Department of Public Health
University of Massachusetts
Amherst, Massachusetts

and

Department of Environmental Health Sciences
University of South Carolina
Columbia, South Carolina

Ariel D. Anbar
School of Earth & Space Exploration and the Department of Chemistry & Biochemistry
Arizona State University
Tempe, Arizona

C. Fred T. Andrus
Department of Geological Sciences
University of Alabama
Tuscaloosa, Alabama

Ramon Aravena
Department of Earth and Environmental Sciences
University of Waterloo
Waterloo, Ontario, Canada

Brian Beard
Department of Geology & Geophysics
University of Wisconsin-Madison
Madison, Wisconsin

Stefano Bernasconi
Geological Institute
ETH Zürich
Zürich, Switzerland

Daniel Bouchard
SNC-Lavalin Environnement Inc.
Montréal, Quebec, Canada

Martin Elsner
Helmholtz Zentrum München
Institute of Groundwater Ecology
Neuherberg, Germany

Patrick Höhener
Université de Provence
Laboratoire Chimie Provence
Marseille, France

Edward R. C. Hornibrook
Department of Earth Sciences
Bristol Biogeochemistry Research Centre
University of Bristol
Bristol, United Kingdom

Daniel Hunkeler
Centre for Hydrogeology
University of Neuchâtel
Neuchâtel, Switzerland

Silvia A. Mancini
Golder Associates
Mississauga, Ontario, Canada

Bernhard Mayer
Department of Geoscience
University of Calgary
Calgary, Alberta, Canada

Barbara Morasch
Environmental Chemistry Laboratory
Swiss Federal Insitute of Technology Lausanne (EPFL)
Lausanne, Switzerland

R. Sean Norman
Department of Environmental Health Sciences
University of South Carolina
Columbia, South Carolina

Christopher S. Romanek
Department of Earth and Environmental Sciences
University of Kentucky
Lexington, Kentucky

Section I

Isotope Fundamentals

1 Fundamentals of Environmental Isotopes and Their Use in Biodegradation

Patrick Höhener and C. Marjorie Aelion

CONTENTS

1.1 WHAT ARE ENVIRONMENTAL ISOTOPES?

This chapter explains what isotopes are, what their average occurrence in nature is, how isotope measurements are reported with respect to international standards, and how isotope data can be used in biodegradation. The focus is on environmental isotopes, a loosely defined class of primary isotopes in biological and hydrological systems. Because hydrogen, carbon, oxygen, nitrogen, and sulfur are the major elements that make up organic matter, biologists are most interested in the isotopes of these elements. All of these elements are relatively light and, therefore, their isotopes have relatively large mass differences and are subject to fractionation by biochemical or physical processes—which makes them interesting tracers. However, the isotopes of chlorine, calcium, iron, and others are relevant to microbial processes and are candidates to figure among the environmental isotopes. The notion of environmental isotopes has been used in other textbooks (Fritz and Fontes 1980; Clark and Fritz

1997; Mook and De Vries 2000) that focus on tracing the hydrological cycle and its associated biogeochemical processes. It should be noted, however, that environmental isotopes does not signify that these isotopes were all produced by natural reactions (see ^{14}C as an example of an isotope that is of natural and anthropogenic origin). Also, some of the heavier isotopes that are used in geology for dating of rocks or tracing rock-forming processes may be called environmental isotopes. Therefore, there is no exact definition of what environmental isotopes are and this book does not make any attempt to put forward such a definition. We will focus on stable and radioactive isotopes of the elements: hydrogen, carbon, nitrogen, oxygen, sulfur, silica, and chlorine. Chapter 12 will give supplementary information on lithium, chlorine, calcium, selenium, chromium, and iron.

1.2 DISCOVERY OF ISOTOPES

Until the late nineteenth century, chemists were convinced that each chemical element has a distinct and integer molar mass. The elements were ordered in the periodic table of elements according to their molar masses, and minor deviations from integer molar masses were thought to be caused by analytical errors. Only after the discovery of radioactivity by Henry Becquerel and Marie and Pierre Curie at the end of the nineteenth century (Nobel Prize in physics in 1903), the ideas about the nature of elements began to change. The discovery of radioactivity soon led to the description of the different modes of radioactive decay, and it became recognized that decay causes elements to produce other elements of lower molecular weights. This led to the description of the uranium and thorium decay series and to the recognition that elements like uranium and thorium are composed of molecules with different molecular weights (e.g., for uranium, the mass could be either 235 or 238 g/mol). However, this and the fractional molecular masses found for some other elements remained somewhat of an enigma until the 1930s when the neutron was discovered. With the understanding of the role of neutrons in atoms and the help of modern analytical techniques, it became clear that an element can be composed of molecules with variable numbers of neutrons and that only the number of protons is invariable for each element. Molecules of different masses of the same element were thereafter called isotopes, a term first used by Frederick Soddy in 1913. The name isotope comes from the Greek *isos* meaning equal and *topos* meaning place and reflects the fact that isotopes are at the same place on the periodic table. Today, more than 2200 isotopes of the 92 naturally occurring elements are known. Not long after the discovery of neutrons came the first precise measurements of isotope abundance ratios, which were achieved using a mass spectrometer by Alfred Nier in 1936. This marked the start of a new research era in geosciences. As the mass spectrometric techniques became ready for routine analyses, scientists began to explore the natural variations of isotopes in air, water, soils, rocks, organic matter, and various other materials, and also began to investigate the fractionation processes that lead to small differences in isotope abundance ratios. Since then, isotope techniques have become standard tools to evaluate problems in paleoclimatology, hydrology, hydrogeology, oceanography, biochemistry, organic geochemistry (Galimov 2006), and many other branches of

science. A number of excellent textbooks on isotope techniques are available also (Fritz and Fontes 1980; Clark and Fritz 1997; Kendall and McDonnell 1998; Cook and Herczeg 1999; Mook and De Vries 2000; Hoefs 2004; Johnson 2004; Flanagan, Ehleringer, and Pataki 2005).

It is no surprise that isotope techniques are also becoming attractive tools to study problems in environmental microbiology such as the microbial breakdown of organic matter or environmental pollutants, the biogenic formation of greenhouse gases, the cycling of nitrogen, the mobilization of iron, or the bioremediation of soils and aquifers. However, the samples that need to be analyzed to study such problems are often small, heterogeneous, or of complex chemical composition and need an advanced processing before being transferred to a mass spectrometer. The techniques of sample introduction to mass spectrometers evolved only very little during the 50 years after the introduction of mass spectrometry (see Chapter 2). Although it was possible, from the 1970s on, to couple elemental analyzers to mass spectrometers and measure stable isotopes (e.g., carbon, nitrogen, sulfur, and hydrogen) in various samples after combustion of the bulk material, the isotopic composition of single molecule species in the bulk matrix remained unresolved. A major analytical breakthrough was achieved in 1978 by coupling a gas chromatograph to a mass spectrometer (Matthews and Hayes 1978) and later by the invention of a combustion interface (Merritt et al. 1995). This technique called gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), allows routine measurements of stable isotope abundances in separated organic molecules. The technique profits from the massive progress that has been made in gas chromatography, as this is the most widely used technique for separation and analysis of organic pollutants since its invention in the late 1940s. Since the late 1990s, GC-C-IRMS is commercially available and widely applied in many laboratories. As a consequence, many stable isotope studies on the fate of pollutants in environmental systems appeared in literature within the last 10 years.

Although stable isotope biogeochemistry based on mass spectrometry is a recent branch of science, it must be noted that physical chemists were able to study the characteristics of isotope-labeled compounds by more than 50 years ago. These chemists manufactured isotope-labeled compounds (e.g., fully deuterated hydrocarbons) using organic synthesis and measured their physicochemical properties such as vapor pressure, molar volume, or viscosity relative to unlabeled counterparts in order to understand molecular interactions in solvents. By 1953, it was recognized that ^{13}C-labeled solvents (benzene, chloroform, and carbon tetrachloride) have a higher vapor pressure than ^{12}C-labeled solvents (Baertschi, Kuhn, and Kuhn 1953), and isotope vapor pressure effects from over 600 studies were known and reviewed more than 30 years ago (Jancso and Van Hook 1974). The measurement techniques for vapor pressure were based on distillation apparatuses and high-precision mercury manometers (Davis and Schiessler 1953), and the results obtained, for example on the vapor–liquid equilibrium effects of solvents, compare well with present-day GC-C-IRMS techniques (Wang and Huang 2003). However, the old physical-chemical literature is often not well known to present day environmental scientists.

1.3 WHAT ARE ELEMENTS, NUCLIDES, ISOTOPES, ISOTOPOMERS, AND ISOTOPOLOGUES?

Elements are defined by the number of protons (Z) in their nucleus. The arrangement of elements according to their proton number and the number of electron shells around the nucleus is depicted in the periodic table of elements. The mass number or atomic weight (A) of an element is equal to the sum of both protons (Z) and neutrons (N) in the nucleus, or

$$A = N + Z. \tag{1.1}$$

A single element can have two or more different atomic weights (A) due to differences in the number of neutrons that can exist in the nucleus. The resulting different atoms are called nuclides. The periodic table of elements may give information on some different nuclides of selected elements. Nuclides of the same number of protons (Z) but different atomic weights (A) are called isotopes. While protons have a positive charge, neutrons have no charge, so the number of neutrons does not affect the charge of an atom. Conventional short notation for nuclides and isotopes use a superscript of the atomic weight preceding the elemental symbol (e.g., ^{12}C, ^{13}C). An exception to this general notation rule is made for the hydrogen isotopes: ^{2}H is called deuterium (and noted sometimes like an element as D), and ^{3}H is called tritium (sometimes abbreviated as T). When the names of isotopes have to be given in full text (e.g., in manuscript titles), the element's full name is written, followed by a hyphen and the A number (e.g., carbon-14, radon-222).

To date, about 270 stable nuclides and about 2,000 radionuclides have been identified (Clark and Fritz 1997). Radionuclides can decay by different modes, including the α-, β-, and γ-decays, which are the most important. Radionuclides that emit alpha particles (helium nuclei) or beta particles (electrons) decay into an isotope of another element, which may or may not be stable. For example, the decay series of uranium-238 proceeds via 14 different decay reactions involving 8 elements and coming to a halt only when the stable isotope lead-206 is formed.

Charts of nuclides are available which display isotopes in horizontal rows (Figure 1.1). The charts have the atomic number Z as y-axis and the number of neutrons N as x-axis. In Figure 1.1, only the nuclide chart for the elements with 1 to 8 protons is shown. For heavier elements, the International Atomic Energy Agency (IAEA) publishes a nuclide chart (http://www-nds.iaea.org/nudat2/). As can be seen in Figure 1.1, the number of stable isotopes per element within this section of the nuclide chart ranges between 1 and 3, whereas the number of radioactive isotopes can be as high as 15 for oxygen. However, many of these radioisotopes have half-lives that are too short to be of use in environmental studies. Look at the 15 isotopes of nitrogen as an example: ^{10}N to ^{12}N and ^{16}N to ^{24}N have half-lives shorter than 10 seconds. If we wished to use these isotopes as tracers, we would need to synthesize the isotopes on our study site, fuel them into a process that we want to study, and measure them immediately. It becomes easily understandable that only isotopes with longer half-lives are potentially useful, particularly outside laboratories. In the case of nitrogen, there is only one such radioactive isotope, ^{13}N with

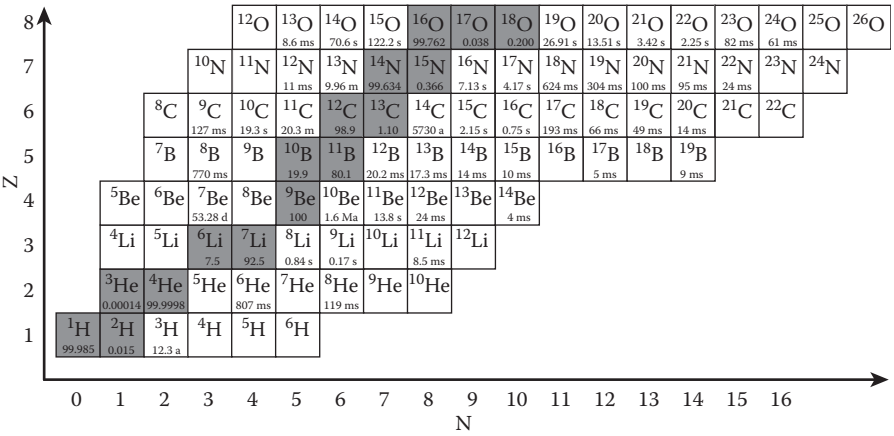

FIGURE 1.1 Nuclide chart of isotopes with proton numbers (Z) from 1 to 8 (*y*-axis) versus neutron numbers N (*x*-axis). Grey squares designate stable isotopes and the number below each isotope is its mean global natural abundance. White squares show radionuclides, with their half-life times. Half-life times are <1 ms when not given below isotope.

a half-life of about 10 minutes. Still, this half-life is at the limit of being a useful environmental tracer, although attempts have been made to study denitrification in soils (Tiedje et al. 1981), or sewage sludge (Kaspar, Tiedje, and Firestone 1981), or to study fertilizer uptake by plants in the field (Gerwing, Caldwell, and Goodroad 1979) by using ^{13}N. However, such ^{13}N-based studies remain unrepeated since they were too costly. Thus, for nitrogen, we are basically left with only the two stable isotopes ^{14}N and ^{15}N, which are very useful for environmental purposes. A similar problem is noted for oxygen: there are three stable isotopes (^{16}O to ^{18}O), but the 12 radioactive oxygen isotopes ^{12}O to ^{15}O and ^{19}O to ^{26}O are too short-lived to be of practical use for environmental studies (Figure 1.1). For hydrogen and carbon, however, we are fortunate, since both elements have two stable isotopes and radioactive isotopes (^{3}H, ^{14}C) with half-lives that are well suited for many purposes. Many inorganic or organic compounds labeled with ^{3}H or ^{14}C are commercially available and there is no need to use the short-lived ^{11}C or other radioactive carbon isotopes as tracers.

When looking at isotope distribution in chemical compounds, one must introduce the notions of isotopomers and isotopologues, which should not be confused. Isotopomers (isotopic isomers) are isomers having the same number of each isotopic atom but differ in their positions. For example, CH_3CHDCH_3 and $CH_3CH_2CH_2D$ form a pair of constitutional isotopomers of propane. Isotopomers undergo different processes in the environment, which could cause isotope fractionation. Isotopologues are chemical species that differ only in the isotopic composition of their molecules or ions. An example is water, where three of its hydrogen-related isotopologues are: HOH, HOD, and DOD. Simply, the isotopologue of a chemical species has at least one atom with a different number of neutrons.

1.4 NATURAL ABUNDANCE OF STABLE ISOTOPES

The original isotopic compositions of solar systems are a result of nuclear processes in stars and planets. Isotopic compositions in terrestrial environments change gradually by the processes of radioactive decay, cosmic ray interactions, mass-dependent fractionations that accompany inorganic and biological reactions, and anthropogenic activities such as the processing of nuclear fuels, reactor accidents, and the testing of nuclear weapons. Stable isotopes are nuclides that do not appear to decay to other isotopes on geologic time scales but may themselves be produced by the decay of radioisotopes. However, the rate of formation of the elements that are discussed in this book are far below any measurable rate, and the global mean abundance of stable isotopes of H, C, N, O, S, and Cl can be regarded as constant.

Elements usually have one common isotope and that is the form found in greatest abundance in nature. Table 1.1 summarizes the natural occurrence of the stable isotopes of interest in this book, giving values recommended by the International Union of Pure and Applied Chemistry (De Laeter et al. 2003). For instance, 98.93% of carbon in nature occurs as ^{12}C and only 1.07% of carbon occurs as ^{13}C. These numbers are depicted in the nuclide chart (Figure 1.1) in the squares for stable isotopes below the isotope name. Similarly, 99.9885% of hydrogen is found as ^{1}H, but a second stable isotope (^{2}H, or deuterium, D) is present in 0.0115% (Table 1.1). For sulfur, carbon, nitrogen, and oxygen the average terrestrial abundance ratio of the heavy to the most abundant light isotope ranges from 1:22 (sulfur) to 1:500 (oxygen), whereas the ratio $^{2}H/^{1}H$ is much lower at 1:6410. Note that for most elements discussed in this book, the lightest stable isotope is the most abundant. This is not the case, however, for some of the heavier elements like chromium or iron (Table 1.1).

Since the chemical behavior of an atom is largely determined by its electrons, isotopes exhibit nearly identical chemical behavior. The electronic structure of atoms

TABLE 1.1
Average Terrestrial Abundance of the Stable Isotopes of H, C, N, O, Si, S, Cl, Cr, and Fe

Element	Stable Isotopes and Natural Abundance (%)			
Hydrogen	^{1}H (99.9885)	^{2}H (0.0115)		
Carbon	^{12}C (98.93)	^{13}C (1.07)		
Nitrogen	^{14}N (99.636)	^{15}N (0.364)		
Oxygen	^{16}O (99.757)	^{17}O (0.038)	^{18}O (0.205)	
Silica	^{28}Si (92.223)	^{29}Si (4.685)	^{30}Si (3.092)	
Sulfur	^{32}S (94.99)	^{33}S (0.75)	^{34}S (4.25)	^{36}S (0.01)
Chlorine	^{35}Cl (75.76)	^{37}Cl (24.24)		
Chromium	^{50}Cr (4.345)	^{52}Cr (83.789)	^{53}Cr (9.501)	^{54}Cr (2.365)
Iron	^{54}Fe (5.845)	^{56}Fe (91.754)	^{57}Fe (2.119)	^{58}Fe (0.282)

Source: De Laeter, J. R., Bohlke, J. K., De Bievre, P., Hidaka, H., Peiser, H. S., Rosman, K. J. R., and Taylor, P. D. P., *Pure and Applied Chemisry,* 75:683–800, 2003.

and molecules is essentially independent of isotopic distribution of nuclear mass and within the limits established by the Born–Oppenheimer approximation (Jancso and Van Hook 1974). However, measurable and important small differences occur for a number of specific processes. Due to their larger masses, heavier isotopes tend to form shorter and more stable chemical bonds (Urey 1947; Bigeleisen and Wolfsberg 1958; Elsner et al. 2005), tend to occupy smaller molar volumes (Bartell and Roskos 1966), and tend to diffuse more slowly than lighter isotopes of the same element (Craig 1953) because molecular diffusion is inversely proportional to the square root of the molecular mass. This mass effect is most pronounced for ^{1}H and ^{2}H because ^{2}H has twice the mass of ^{1}H. For heavier elements, the relative mass difference between isotopes is much less but not always negligible. Environmental processes such as microbial transformation, photosynthesis, evaporation, and gas-phase diffusion have slightly different rates with respect to different isotopes. This leads to stable isotope fractionation—a phenomenon described in detail in Chapter 3. Fractionation processes lead to small but measurable differences in different pools of elements and compounds in nature. An example is given for the ranges of the isotope ratios in different pools of carbon (Figure 1.2). Isotope ratios are usually reported as ratios R, which is defined as the abundance of the heavy isotope relative to the abundance of the light isotope ($R = {}^{13}C/{}^{12}C$ for carbon). The mean global isotope ratio $^{13}C/^{12}C$ is $R = 0.011237$ (Table 1.1). According to Figure 1.2, deviations from this mean global ratio occur. Often, carbon is found to be somewhat enriched in ^{12}C ($R < 0.011237$), but marine bicarbonate and some carbonate rocks are also found to be enriched in ^{13}C

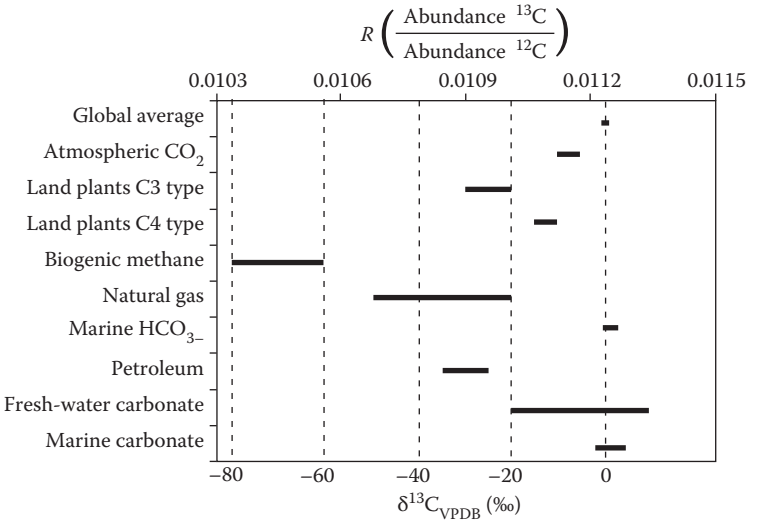

FIGURE 1.2 Ratios of isotope abundance $^{13}C/^{12}C$ in various carbonaceous materials (upper x-axis). Also shown are the values of the more convenient delta notation (lower x-axis). Data from (Mook, W. G., and J. J. De Vries, *Environmental isotopes in the hydrological cycle: Principles and applications.* Vol. 1 *Introduction—Theory, methods review, IAEA*, Isotope Hydrology Section, Vienna, 2000; Clark, I., and P. Fritz, *Environmental isotopes in hydrogeology.* CRC Press, Boca Raton, FL, 1997.)

(Figure 1.2). The variations in *R* are large in comparison to the analytical uncertainty of modern mass spectrometers and these variations can be traced reproducibly. As a consequence, it is important to note that the natural abundances of stable isotopes in Table 1.1 are not universal constants but global means. The analytical detection of slight changes of the natural abundance of stable isotopes and the comparison of local measurements with global means is thus a powerful tool to trace specific biologic or chemical physical processes in the environment.

Reporting isotope abundances as in Figure 1.2 as *R* values is unpractical since small and uneven numbers have to be compared to each other. A much more convenient way is to compare measured *R* values to an international standard and report only the deviation from this standard. To do so, standards—to which everybody relates the measurement—and an internationally accepted notation to report isotopic data of samples relative to these standards are needed. This will be introduced in the next section.

1.5 STABLE ISOTOPES: DELTA-NOTATION AND INTERNATIONAL STANDARDS

The measurement of absolute isotope ratios or abundances is not easily done and requires very sophisticated mass spectrometric equipment (see Chapter 2). However, for most studies, the monitoring of relative variations in stable isotope concentrations rather than absolute abundances provides the necessary information about the processes. By measuring a known reference material under the same conditions as our sample, we can then express isotopic concentrations as the difference between sample and standard.

Stable isotope variations at or near natural abundance levels are usually reported in the delta (δ) notation, a value given in parts per thousand (‰, per mil). Isotope ratios (*R*) are measured for samples and standard by mass spectrometry and the relative measure delta (δ) is calculated as written here for the ^{13}C and ^{12}C (Coplen 1996):

$$\delta^{13}C = \frac{R_{sample} - R_{standard}}{R_{standard}} .1000 \tag{1.2}$$

with R_{sample} being the $^{13}C/^{12}C$-ratio determined by mass spectrometry and $R_{standard}$ is the abundance ratio of the international standard (Table 1.2). Delta values are not absolute isotope abundances but differences between sample and the international standard that, by definition, has a delta value of 0‰. For instance, if a biomass sample is found to have a $^{13}C/^{12}C$-ratio R_{sample} greater than the $R_{standard}$ by five parts per mil, this value is reported as $\delta^{13}C = +5‰$. A positive δ value means that the isotopic ratio of the sample is higher than that of the standard; a negative δ value means that the isotopic ratio of the sample is lower than that of the standard. Many isotope geochemists advocate always prefacing the δ value with a sign, even when the value is positive, to distinguish between a true positive δ value and a δ value that is merely missing its sign (a frequent occurrence with users unfamiliar with isotope terminology).

As a basis for global comparison of stable isotope data, international standards have been introduced. Two organizations are taking the lead in distributing these standards: the National Institute of Standards and Technology (NIST, www.nist.gov) in the

TABLE 1.2
The International References and their Abundance Ratios for Most of the Stable Isotopes Discussed in this Book

Element	Ratio of Isotopes	Standard(s)	Abbreviation	Abundance Ratio $R_{standard}$[a]
Hydrogen	$^{2}H/^{1}H$	Vienna Standard Mean Ocean Water	VSMOW	1.5575×10^{-4}
Helium	$^{3}He/^{4}He$	Atmospheric helium	AIR He	1.38×10^{-6}
Lithium	$^{7}Li/^{6}Li$[b]	Lithium carbonate from Spodumene deposits in North Carolina	L-SVEC	12.1735[b]
Boron	$^{11}B/^{10}B$	Boric acid	NBS 951	4.04362
Carbon	$^{13}C/^{12}C$	Carbonate from Vienna Pee Dee Belemnite	VPDB	0.011237
Nitrogen	$^{15}N/^{14}N$	Atmospheric nitrogen	AIR N_2	3.677×10^{-3}
Oxygen	$^{18}O/^{16}O$	Vienna Standard Mean Ocean Water	VSMOW	2.0052×10^{-3}
		Carbonate from Vienna Pee Dee Belemnite	VPDB	2.0672×10^{-3}
Silicon	$^{30}Si/^{28}Si$	Silica sand NBS-18 (= RM8546)	NBS-18	0.03365761[c]
		Silicon crystals	IRMM-018a	0.03365602[c]
Sulfur	$^{34}S/^{32}S$	H_2S in Vienna Ca–on Diablo Troilite	VCDT	4.5005×10^{-2}
Chlorine	$^{37}Cl/^{35}Cl$	Chloride ion in ocean water Standard Mean Ocean Chlorine	SMOC	0.324

[a] From Clark, I., and P. Fritz, *Environmental isotopes in hydrogeology*. CRC Press, Boca Raton, FL, 1997; see Chapter 12 for information on calcium, selenium, chromium, strontium, iron, and molybdenum isotopes and http://www.ciaaw.org/ for reference materials.

[b] Reporting of $^{6}Li/^{7}Li$ should be discontinued (Coplen, T. B., J. K. Bohlke, P. De Bievre, T. Ding, N. E. Holden, J. A. Hopple, H. R. Krouse, et al., *Pure and Applied Chemistry*, 74, 1987–2017, 2002).

[c] Valkiers, S., K. Rußе, P. Taylor, T. Ding, and M. Inkret, *International Journal of Mass Spectrometry*, 242, 321–23, 2005.

United States and the International Atomic Energy Agency (IAEA, www.iaea.org). Reference material is available through either of these sources. A complete list of reference materials and the recommendations for the reporting of isotopic compositions of the various elements is also given by the Commission of the International Union of Pure and Applied Chemistry (IUPAC, http://www.ciaaw.org/). Different protocols for reporting isotope data created some confusion before 1996. Since that time, the isotope community has adopted the IUPAC recommendations for reporting isotope data (Coplen 1996). The international standards for C, H, O, N, S, Cl, and Si are briefly introduced in the following and standards of other elements supplementary information is given in Chapter 12.

$^{13}C/^{12}C$: The international standard for $^{13}C/^{12}C$ introduced in 1957 (Craig 1957) was the internal calcite structure (rostrum) of the fossil *Belemnitella americana* from the Cretaceous Pee Dee Formation in South Carolina, in many publications referred to as the Pee Dee Belemnite (or PDB). The short notation $\delta^{13}C_{PDB}$ refers to this standard. It had an abundance ratio $R_{standard}$ $^{13}C/^{12}C$ of 0.011237 (Table 1.2). The IAEA in Vienna

subsequently defined the hypothetical VPDB scale (Vienna PDB, considered as identical to the PDB) as reference for stable carbon analysis. Before the limited PDB supply was exhausted, a crushed white marble designated as NBS-19 was calibrated and forms the basis for the definition of VPDB. The material has a slightly positive $\delta^{13}C$:

$$\delta^{13}C_{NBS\text{-}19} = +1.95‰\ \delta^{13}C_{VPDB} = +1.95‰\ \delta^{13}C_{PDB}. \quad (1.3)$$

The $\delta^{13}C$ values of various carbonaceous reference materials are reported in the new guidelines for $\delta^{13}C$ measurements (Coplen et al. 2006b). Furthermore, a normalization procedure for reporting $\delta^{13}C$ data is described (Coplen et al. 2006a).

$^{2}H/^{1}H$ and $^{18}O/^{16}O$: The international standard for $^{2}H/^{1}H$ and $^{18}O/^{16}O$ used to be the Standard Mean Ocean Water (SMOW), as defined by Craig (1961). In fact, Craig's SMOW never existed. It was a hypothetical water calibrated to the isotopic content of NBS-1, a water sample from the Potomac River (Clark and Fritz 1997). The IAEA prepared standard water from distilled seawater that has an isotopic composition close to SMOW. The reference is identified as Vienna Standard Mean Ocean Water (VSMOW) and has been used for about four decades as the accepted reference. The abundance ratios are given in Table 1.2. Although there is some debate whether VSMOW = SMOW (Clark and Fritz 1997), $\delta^{2}H_{VSMOW}$ and $\delta^{18}O_{VSMOW}$ are defined as 0. In order to supply another water that is highly depleted compared to ocean water, NIST distributes rainwater (Standard Light Antarctic Precipitation, SLAP) with a $\delta^{2}H_{VSMOW}$ of –428.0‰ and a $\delta^{18}O_{VSMOW}$ of –55.50‰ (Coplen 1994).

For $\delta^{18}O$ in carbonates, the isotope community refers to the $\delta^{18}O$ that had been measured in the Pee Dee Belemnite, noted $\delta^{18}O_{VPDB}$. The reference material is a white marble NBS-19, with $\delta^{18}O_{NBS\text{-}19} = –2.20‰\ \delta^{18}O_{VPDB}$.

$^{15}N/^{14}N$: The international standard for $^{15}N/^{14}N$ is atmospheric nitrogen from air (AIR), with $\delta^{15}N_{AIR} = 0$ and an abundance ratio as given in Table 1.2. Reference materials are available as gas (N_2) or as ammonium or nitrate salts.

$^{34}S/^{32}S$: For several decades, troilite (FeS), from the Cañon Diablo meteorite, (CDT) has been employed as the international standard for $^{34}S/^{32}S$. This material had a $^{34}S/^{32}S$ abundance ratio of 0.0450, but is not available anymore. Reference materials are silver sulfides IAEA-S-1 ($\delta^{34}S_{VCDT} = –0.3‰$), IAEA-S-2 ($\delta^{34}S_{VCDT} = +22.67‰$) and barium sulfate NBS-127 ($\delta^{34}S_{VCDT} = +21.1‰$). Since 1995, the IUPAC (Krouse and Coplen 1997) recommends that abundances relative to $^{34}S/^{32}S$ ratios of all sulfur bearing substances be expressed on the VCDT scale, defined by assigning an exact $\delta^{34}S$ value of –0.3‰ (relative to VCDT) to the silver sulfide reference material IAEA-S-1. Confusions concerning the absolute values of some sulfur reference materials have been clarified by measurements (Qi and Coplen 2003) in comparison to elemental sulfur (IAEA-S-4-Soufre de Lacq, $\delta^{34}S_{VCDT} = +16.90‰$). Reporting of $\delta^{34}S$ measurements relative to CDT should be discontinued.

$^{37}Cl/^{35}Cl$: The reference for chlorine isotopes is Standard Mean Ocean Chloride (SMOC), as the isotopic composition in seawater chloride is very constant. The $^{37}Cl/^{35}Cl$ ratio of this standard is 0.324. Two reference materials are available, IRMM-641 and IRMM-642, both made from NaCl in pure water at different concentrations (Ostermann et al. 2001). It should be noted that Cl^- has to be transformed to gaseous chloroform (or to cesium chloride) to be measured using a mass spectrometer. This is

done by precipitation from the water sample by adding $AgNO_3$. The resulting AgCl is purified and reacted with CH_3I to methylchloride, CH_3Cl. This gas is suitable for measuring the $^{37}Cl/^{35}Cl$ ratio by normal IRMS.

$^{29}Si/^{28}Si$ and $^{30}Si/^{28}Si$: The international standard for stable silica isotopes is NBS28 silica sand (also named NIST RM 8546) with $\delta^{29}Si = 0‰$, $\delta^{30}Si = 0‰$ and $\delta^{18}O = +9.6‰$ (Valkiers et al. 2005). A new material (SiO_2 crystals, IRMM-018a) with an isotopic composition that is identical to NBS28 is distributed now (Valkiers et al. 2005).

1.6 NOTATIONS USED IN TRACER STUDIES

For environmental studies using tracers enriched in isotopes, labeled compounds are available with the heavy isotope making up to 99% of the tracer element (e.g., 99 atom % ^{13}C-acetate, 99 atom % ^{15}N-ammonium sulfate). To biologists the principal advantage of stable isotopes is that they are not radioactive and are therefore unrestricted in use and do not generate radioactive waste. A restriction occurs only for laboratories that routinely analyze the natural abundance of rare isotopes (e.g., ^{2}H, ^{13}C). For these laboratories, a certain danger of contamination of the measurement system or of sampling equipment exists when samples with very high abundance ratios are measured. Results from tracer studies are usually reported in units of atom percent (atom %). This value gives the absolute number of atoms of one isotope in 100 atoms of total element, as in the example for ^{13}C:

$$\text{atom } \% = \frac{^{13}C}{^{13}C + ^{12}C} \times 100 \tag{1.4}$$

for the ^{13}C atom % calculation, the trace amounts of naturally present ^{14}C are usually negligible and the sum of ^{12}C and ^{13}C is taken to be total C.

Medical tracer studies of human physiology are most often reported in units of atom percent excess (APE), which is the enrichment level of a sample above a given background reading considered as zero. The background reading in atom % is subtracted from the experimental value to give APE:

$$\text{atom \% excess (APE)} = (\text{atom \% sample} - \text{atom \% baseline}) \times 100. \tag{1.5}$$

1.7 RADIOISOTOPES

Radioisotopes are unstable nuclides that decay with specific half-life times to other nuclides under the emission of radiation. The most important decay modes are α-decay (emission of a helium atom), β-decay (emission of an electron β^- or a positron β^+), and γ-decay (emission of electromagnetic energy). Before exploring the tracer potential of radionuclides, let us first review the rules governing radioactive decay. The disappearance of radioactive atoms is a statistical logarithmic process in which a subset of atoms undergoes decay in a given time period. The activity, A, decreases according to the law of radioactive decay (Equation 1.6)

$$A_{(t)} = A_{(0)} \exp^{(-\lambda t)}. \tag{1.6}$$

For carbon-14, 1 atom out of 8,266 atoms decay each year, and the decay constant λ thus, is 1.2097×10^{-4} yr^{-1}. However, most books and tables on radioisotopes do not give decay constants λ, but rather half-life times $T_{1/2}$, which is related to λ via Equation 1.7:

$$T_{1/2} = \ln 2/\lambda. \tag{1.7}$$

In our example, the half-life time for ^{14}C is 5730 years. The most commonly employed radioisotopes, their half-life times, and decay modes are listed in Table 1.3. Environmental concentrations of radionuclides are determined as radioactive activities and are given in Becquerel (Bq) per volume or mass. One Becquerel equals one decay per second. The unit used before the Bq was Curie, with $1\ Ci = 3.7 \times 10^{10}$ Bq.

Carbon-14 takes a special place in the naturally occurring radioisotopes because of its long half-life time. Willard Frank Libby described the use of this nuclide as a tool for archeological dating in his Nobel Prize–winning work (1952). However, ^{14}C is equally important as a tool to study the mixing of oceans or to discriminate old and recent carbon sources in microbial studies. A typical application is to distinguish the origin of carbon in an ecosystem from either fossil sources (petroleum or natural gas) or recent sources (biogas). Natural ^{14}C is produced in the upper atmosphere when cosmic neutrons hit nitrogen atoms and knock out one proton (the nitrogen isotope ^{14}N with 7 protons +7 neutrons is thus transformed into the carbon isotope ^{14}C with 6 protons and 8 neutrons, Figure 1.3). The production of ^{14}C in the atmosphere is balanced by its decay. However, explosion of nuclear weapons from 1950 to 1963 significantly disturbed that balance and in 1964 the ^{14}C atmospheric activities had almost doubled.

The ^{14}C activities are referenced to an international standard known as modern carbon (mC). Its activity is defined as 95% of the ^{14}C activity, in 1950, of the NBS oxalic acid standard. This is close to the activity of wood grown in 1890 in a fossil CO_2-free environment and equals 226 Bq kg^{-1} carbon (Clark and Fritz 1997). Measured ^{14}C activities are expressed in percent modern carbon (pMC). Like stable isotopes, ^{14}C undergoes fractionation and to maintain universality for dating purposes, pMC must be normalized to a common $\delta^{14}C$ value of –25‰ (Clark and Fritz 1997).

TABLE 1.3
Radioisotopes of Some Elements of Interest in Biodegradation and Bioremediation

Isotope	Half-Life	Decay Mode	Sources
^{3}H (Tritium)	12.43 years	β^-	Cosmogenic, weapons testing
^{13}N	9.965 minutes	β^+	Synthesized tracer
^{11}C	20.3 minutes	β^+	Synthesized tracer
^{14}C	5,730 years	β^-	Cosmogenic, weapons testing
^{35}S	0.239 years	β^-	Industrial use
^{36}Cl	301,000 years	β^-	Cl^-

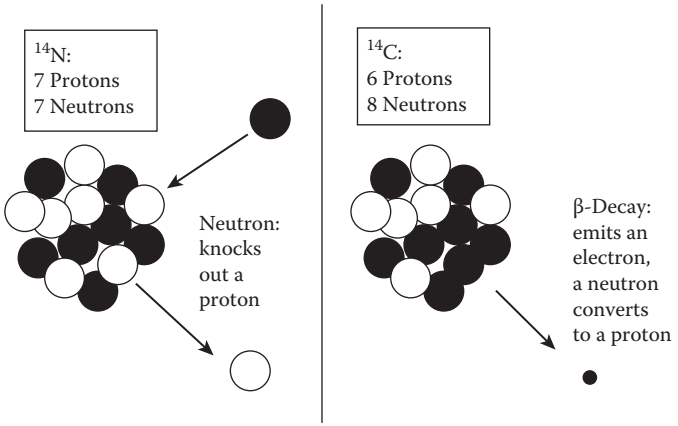

FIGURE 1.3 Atmospheric production of ^{14}C from ^{14}N by cosmic rays (neutron impact, causing a loss of a proton, left-hand side), and radioactive decay of ^{14}C to ^{14}N by β-decay (emission of an electron, generating a proton from a neutron, right-hand side).

After carbon, tritium is the next radioisotope of environmental relevance in the context of this book. Tritium activities are usually expressed as absolute concentrations, using tritium units (TU) as reporting units. So no reference standard is needed. One TU corresponds to one ^{3}H atom per 10^{18} atoms of hydrogen, which is 0.118 Bq/L of water. Due to thermonuclear bomb testing, tritium concentrations in the atmosphere and in rainwater peaked in 1963 worldwide, and tritium concentrations have been used to check the age of groundwater in aquifers or pore water in sediment cores to understand the local water cycle.

1.8 WHY SHOULD ONE USE ISOTOPES IN BIODEGRADATION AND BIOREMEDIATION?

Biodegradation is perhaps the most important process on earth. Without biodegradation, the organic matter synthesized by plants and other lithotrophic organisms would accumulate and the carbon dioxide in the atmosphere would not be regenerated. Biodegradation and biotransformation include the cycling of important elements, including carbon and nitrogen that are both essential molecular building blocks and components of contaminants. Without biodegradation, nutrients fixed in organic matter would not be recycled and chemicals produced by humans and released carelessly into the environment would not be transformed, mineralized, and detoxified. Bioremediation is applied biodegradation, put into action by scientists and engineers, most often with the aim to decontaminate soil or groundwater polluted by anthropogenic chemicals and has become a business of considerable economic importance. Thus the study of biodegradation is of great economic importance and for the sustainable development of people on earth.

As more tools are developed to study biodegradation, more scientific questions can be answered. Is soil organic matter respired to CO_2? Will chlorinated solvents be

degraded in groundwater before migrating to a well for drinking water? Will sulfate-reducing microorganisms oxidize methane that is seeping through sediments at the seafloor and prevent volatilization of this greenhouse gas into the atmosphere? Will nitrate that runs off of agricultural lands into groundwater be reduced to the harmless nitrogen gas by denitrifying microorganisms? There is often a need to assign responsibility for contamination, for example whether a pollutant found downgradient of an industrial site in groundwater is actually originating from this site or from another source? Additional areas of interest are whether nitrate contamination originates from the use of chemical fertilizer or application of manure, and whether it can be guaranteed that 95% of the mass of a spilled chemical is removed by a certain remediation technique. Fundamental questions include: Is a microbial degradation reaction initiated by a certain biochemical reaction? Do the carbon atoms from a certain pollutant end up in microbial biomass, proteins, lipids, or even in the DNA of microbes?

To study these types of questions, scientists historically relied on measurements of concentrations of the compounds of interests and their reaction partners in the specific environment. This was not always successful and often led to uncertainty.

Stable or radioactive isotopes offer a great added value in studies on biodegradation and bioremediation for many reasons. One is that concentration measurements may ambiguously explain certain processes especially at field sites. For example, dilution of dissolved compounds decreases concentrations that are often misinterpreted as being caused by degradation. Furthermore, improper sampling techniques may cause loss of the target compounds and result in misinterpretation, which is of particular concern for gases and volatile pollutants and that can be lost during the sampling or during the transport and storage of samples before analysis. Less volatile compounds also can be lost when adsorbed onto materials used for sampling and storage, like tubing, pumps, vials, and stoppers.

The measurement of isotopes is less affected by the above mentioned problems. Stable isotope data are reported as isotope ratios and are ratios of concentrations of two compounds, one of which carries a rare stable isotope. Isotope ratios in gaseous or aqueous samples are therefore not affected by dilution or by sampling artifacts such as volatilization or sorption since those processes affect both compounds almost equally and thus the ratio remains stable. Ratios of stable isotopes are often an excellent tool to check the consistency of interpretations that are based on concentration data. In addition, radioisotopes contribute the factor of time as additional information to prove or falsify hypotheses. For example, carbon-14 allows one to distinguish between fossil and modern carbon. However, only carbon and hydrogen have natural radioactive isotopes with half-life times well suited for practical studies; unfortunately oxygen, nitrogen, and sulfur either do not have such natural radioactive isotopes or the radioactive isotopes have half-lives that are too short for practical purposes.

Historically, the limitation of using stable isotopes as a tool to study biodegradation was due to difficulties in introducing sample materials into an isotope mass spectrometer. The key development made in the last 10–20 years was the direct coupling of isotope mass spectrometers to classical analytical systems used in laboratories such as gas chromatographs and elemental analyzers that made isotope analyses

faster and cheaper. Also, the detection limits for the analysis of isotope ratios in compounds or pollutants in air or water samples have been drastically lowered by developments of new injection techniques like solid-phase microextraction (SPME) or the Purge & Trap technique.

1.9 TYPE OF INFORMATION OBTAINED BY ISOTOPES

The variations in the abundance of stable isotopes in natural compounds or anthropogenic pollutants can be used in different ways for assessing different types of information. These types can be grouped into at least seven classes. The first possibility is to identify sources of compounds by direct analysis of the compound in a given environment and compare the isotopic abundance to a number of potential sources that have distinct but constant isotope abundances. A prerequisite for this approach is that the concentration of a compound in the environment only changes owing to dilution or phase transfer processes. Hence, its isotopic composition will remain largely constant and thus can be used to elucidate the source of the compound. Preferably, two or three different isotopes are measured, for example ^{15}N and ^{18}O in nitrate, or ^{2}H, ^{13}C, and ^{37}Cl in a chlorinated solvent. For example, one might compare the multi-isotope pattern of hydrogen, carbon, and chlorine in dissolved chloroethene in groundwater to those of the solvent used by a client suspected to be the polluter (Poulson and Drever 1999). Additionally, one might trace the origin of nitrate pollution in groundwater by comparing its ^{15}N and ^{18}O analysis with those of frequently applied fertilizers and with atmospheric nitrogen.

A second type of information that can be gained by stable isotope analysis is the origin of a compound of interest that was formed in a given specific environment and by comparing its isotope signal to the ones of potential precursors. A typical application is determining whether CO_2 found either in soil air or dissolved in groundwater originates from pollutant degradation from a natural process. Radiocarbon can help here most often, since pollutants are often derived from petroleum, which is ancient and therefore free of ^{14}C, whereas natural CO_2 contains modern ^{14}C signals (see Chapter 11). However, stable carbon isotopes also can help to track the origin of dissolved CO_2 in ground water. Stable carbon isotope ratios have been used to determine the percentages of total dissolved CO_2 in groundwater derived from degradation of heating oil versus dissolution of carbonates (Bolliger et al. 1999), or from sugar refinery wastewater versus CO_2 from carbonates (Wersin, Abrecht, and Höhener 2001). Stable isotopes also are used to identify the substrate in methanogenesis because the two major substrates, acetate and CO_2, react with hydrogen gas and are easily distinguished based on stable carbon and hydrogen analysis of methane (see Chapter 5).

A third type of information that is obtained with stable isotope data concerns the assessment of transformation pathways. This is now often applied to resolve the question of whether a contaminant at a given site undergoes aerobic or anaerobic transformation. The initial biochemical reaction in aerobic or anaerobic contaminant transformation is different, and this leads to different kinetic isotope effects that are observable in the remaining unreacted contaminant, provided that the transformation progressed to a certain extent. Initial reactions under aerobic conditions often

involve a mono- or dioxygenase and leads to the opening of carbon-carbon or carbon-hydrogen bonds as is discussed in Chapters 4 and 7. In contrast, under anaerobic conditions, the initial reactions are different and may include a fumarate addition, a reaction that is associated with quite a large carbon isotope effect. Under field conditions, often mixed aerobic–anaerobic conditions prevail, for example in oxic soil with soil aggregates that are anoxic microniches. Several studies have successfully shown that it is possible to assign the prevailing degradation pathways for environmental pollutants in mixed aerobic–anaerobic environments using a dual isotope approach, for example with toluene (Vogt et al. 2008), with methyl tertiary-butyl ether (MTBE) in groundwater (Zwank et al. 2005), with the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in soil (Bernstein et al. 2008), and with nitrobenzene (Hofstetter et al. 2008). Based solely on carbon isotope analysis, it was possible to distinguish between two aerobic degradation processes for 1,2-dichloroethane in the laboratory, since the oxidation creates small fractionation, whereas hydrolytic dehalogenation creates large isotope fractionation (Hirschorn et al. 2004).

The fourth type of information that can be obtained by isotope data is used to distinguish between abiotic and biotic processes that have acted on a contaminant along its migration from a source to a receptor or during a specific treatment, especially to distinguish between biochemical reactions and abiotic transformation or phase transfer processes. This is useful for verifying mineralization of contaminants at sites undergoing natural attenuation or biostimulation. Isotope data often can show that the pollutants were not simply transferred to another phase, since phase transfer processes discriminate rather poorly between isotopes compared to biodegradation. For example, sorption of dissolved contaminants to geosorbents causes fractionation of stable hydrogen or carbon isotopes that is either below detection limits (Slater et al. 2000; Schuth et al. 2003) or that is at the edge of detection limits (Kopinke et al. 2005). Nevertheless, there are cases where phase-transfer processes fractionate isotopes significantly. Recent work summarized in Chapter 9 presented evidence that gas-phase diffusion and volatilization through soil discriminates, quite importantly, for light isotopes in volatile pollutants of low molecular weight, (e.g., in gasoline hydrocarbons) and that the remaining fraction of compounds in liquid gasoline gets progressively heavier during volatilization.

The fifth class of application of stable isotope techniques is the quantification of the extent of transformation. The classical Rayleigh equation (Chapter 3) has always been in the center of such quantitative assessments, classically to describe variations of isotopes during distillation. Progress has been made to explore the use of the Rayleigh equation in groundwater and its limitations when applied to complex groundwater systems, for example, where dilution and biodegradation are physically separated (Abe and Hunkeler 2006; Van Breukelen 2007; Van Breukelen and Prommer 2008). Chapter 8 of this book is devoted to the Rayleigh equation and its limitations with respect to applications in groundwater. It gives the reader a tool for a quantitative assessment of the percentages of biodegradation versus dilution in ideal aquifers using stable isotope measurements in dissolved contaminants along plumes. Chapter 9 addresses how volatilization of pollutants can be quantitatively assessed with stable isotope measurements in the liquid pollution source that has not yet volatilized.

The sixth type of information that can be obtained by exploiting stable isotope data is the verification of reactive transport models. These models are used frequently for the prediction of the length, duration, and environmental impact of contaminant plumes in groundwater or vapor plumes in soil air. Such models can be quite complex, may contain many parameters, and need to be validated, which is usually done using concentration data. Isotope data can provide a further data set for model validation, with the advantage that isotope data may be less biased by sampling errors as discussed previously. A recent comparison of two different numerical models predicting the extension of a plume of chloroethenes at Dover Air Force Base in Delaware suggested that both models were able to correctly predict the concentration contours of chloroethenes, but only one model closely predicted stable carbon isotope ratios in the ethenes (Atteia, Franceschi, and Dupuy 2008). The other model gave completely different shapes of isotope contours. Isotope data identified that one model apparently predicted the concentration data correctly but the incorporated transport processes incorrectly.

The final type of information derived from the use of stable isotopes is associated with their use as both a chemical and a molecular tracer in radioisotope and stable isotope labeling studies that are useful for examining both natural and contaminated environments. Stable isotope labeling of nitrogen has enhanced our ability to quantify nitrogen cycling on time scales of minutes, and to identify the incorporation of carbon into macromolecular organic matter identified in different sediment organic fractions. Finally, as with the advancement of analytical instrumentation for the analysis of isotope ratios, significant advancements have been made in the extraction, purification, and analysis of isotopically labeled molecular biomarkers such as DNA and RNA. The combination of isotopic and bimolecular analyses has opened up future areas of research not only in biodegradation studies but also in the broader field of microbial ecology.

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2 Analysis of Stable Isotopes

Daniel Hunkeler and Stefano Bernasconi

CONTENTS

2.1 INTRODUCTION

This chapter discusses the most important methods to measure light stable isotopes in compounds that are of interest when investigating biogeochemical cycles and contaminated sites. A particular emphasis is given to methods for isotope analysis of specific compounds such as organic contaminants that rely on separation techniques such as gas chromatography. However, methods for analysis of bulk samples where the entire sample is analyzed without prior separation are covered as well. The general principles of the methods are outlined. For detailed procedures, the reader is referred to the specific publications and protocols developed by different laboratories. The procedures for calculation of isotope ratios from raw data of the instruments are also covered here although this is automatically performed by most software packages.

2.2 PRINCIPLES OF ISOTOPE ANALYSIS

The isotope ratios of light elements are most commonly measured with gas-source magnetic sector mass spectrometers often denoted as isotope ratio mass-spectrometers (IRMS). Their measurement principle is different from that of mass-spectrometers commonly used for identification of organic compounds. The first type of these instruments was developed in 1947 by Alfred Nier. With these instruments, the molecules of interest are introduced in gaseous form into the ion source, are ionized, and travel through a magnetic field (Figure 2.1). The ions are deflected with a different radius depending on their mass/charge ratio making it possible to detect them in separate collectors, usually Faraday cups. Thus, what is measured is not directly the abundance of isotopes of an element but the abundance of small molecules with different mass (denoted as isotopologues) containing the element of interest. To be able to calculate the isotope ratio from the ion ratios, the measurement molecules should contain as few atoms as possible and at the same time it should be as easy to transform the molecules of interest into the measurement gas.

The types of molecules that are more commonly used for analysis of different isotopes are listed in Table 2.1. For carbon and oxygen, molecules with three different masses are generally measured using a universal triple collector setup to be able to calculate isotope ratios despite of the existence of multiple combinations of isotopes giving the same mass (see 2.9 for details). For hydrogen, nitrogen, and sulfur, two different masses are sufficient because the abundance of other isotopologues is very low. To be able to measure the isotopic composition of an element (e.g., carbon) in a compound (e.g., benzene), the compound has to be first transformed to the corresponding molecule of measurement (e.g., CO_2). With this approach, the mass spectrometer can be optimized for a few molecules allowing for a high precision of measurement. The measurement of isotope ratios always takes place relative to the isotope ratio of a reference gas (same type of molecules with a known isotopic composition) and thus, potential mass discrimination during transfer, ionization, or counting of the molecules cancels out. Although isotope ratios are usually expressed relative to an international standard using the delta notation (see Chapter 1), the international standard is not usually used as reference gas because it is not available in sufficient quantity. Rather, a laboratory working standard (e.g., a tank of CO_2 gas) is calibrated against the international standard. Thus, the measured isotope ratios relative to the laboratory standard have to be transformed to isotope ratios relative to the international standard.

More recently, new method for isotope analysis based on laser spectroscopy rather than mass spectrometry have been developed (e.g., Lis, Wassenaar, and Hendry 2008). The method relies on differences in vibrational frequencies of molecules depending on the isotope that is present in the chemical bond. The higher the mass of the isotope, the lower the vibrational frequency. By measuring the absorption of electromagnetic radiation at different wavelengths, the abundance of different isotopes can be quantified. The method is available to measure isotope ratios in H_2O, CO_2, and CH_4 and generally less expensive equipment is required than for mass spectrometry. While first instruments coupled to

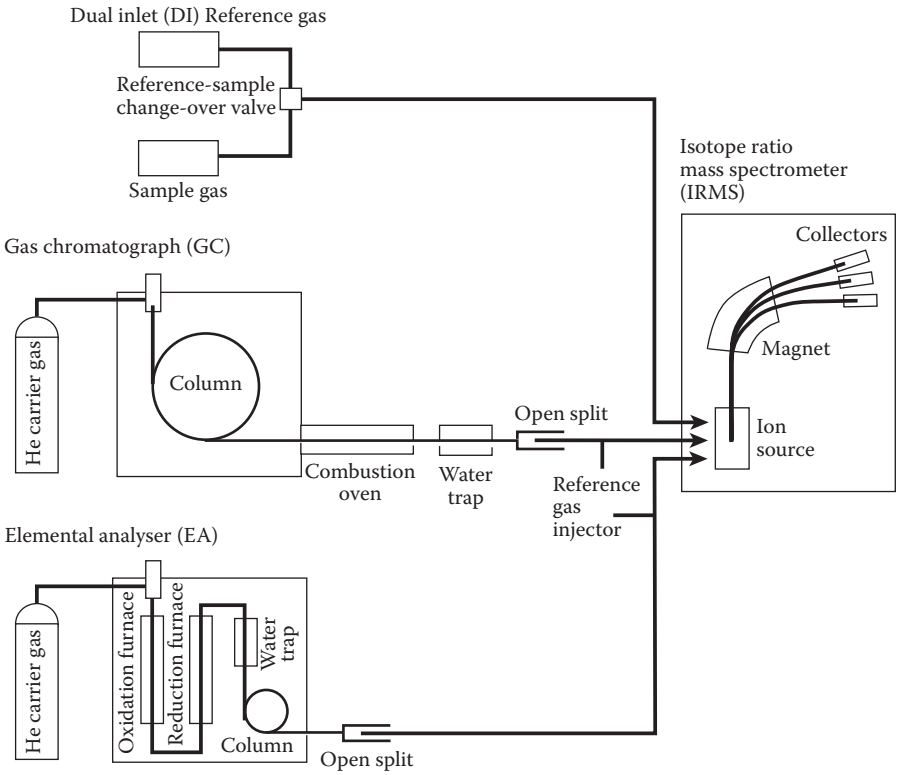

FIGURE 2.1 Schematic setup of dual inlet isotope ratio mass spectrometer (DI-IRMS) and continuous flow systems consisting of a gas chromatograph (GC) connected via a combustion interface to an IRMS or an elemental analyzer (EA) connected to an IRMS.

elemental analysers (EA) are emerging, these instruments cannot (yet) be linked to gas chromatography (GC) systems.

2.3 DUAL INLET AND CONTINUOUS-FLOW SYSTEMS

There are two different approaches of introducing the gaseous molecules into the ion source of an IRMS. The classic dual inlet approach dual inlet-isotope ratio mass spectrometer (DI-IRMS) uses two reservoirs containing pure reference and sample gas, respectively (Figure 2.1) at the same pressure to avoid pressure-dependant mass discrimination effects. Several pulses of sample and reference gas are injected alternately and the isotope ratio of the sample is calculated relative to the reference gas. While the dual inlet method allows for very precise measurements, it requires a time-consuming transformation of the compounds of interest to the measurement gas using preparation lines. Beginning in the 1970s, a new way of introducing sample and reference gas has been developed whereby the measurement molecules are carried to the ion source as pulses of measurement gas in

TABLE 2.1
Measurement Gas and Isotopologues Evaluated When Analysing Isotope Ratio of Different Elements. Minimal Amount of Compound Required for GC-IRMS Applications

Element	Isotope Ratio	Measurement Gas	Minimal Amount nmol of Element for GC-IRMS Analysis and Typical Uncertainty	Isotopologues and Thier Mass	
H	$^{2}H/^{1}H$	H_2	30 (5‰)	2	$^{1}H^{1}H$
				3	$^{1}H^{2}H$
C	$^{13}C/^{12}C$	CO_2	1 (0.5‰)	44	$^{12}C^{16}O^{16}O$
				45	$^{13}C^{16}O^{16}O$, $^{12}C^{16}O^{17}O$
				46	$^{12}C^{16}O^{18}O$, $^{13}C^{16}O^{17}O$, $^{12}C^{17}O^{17}O$
N	$^{15}N/^{14}N$	N_2	3 (0.5‰)	28	$^{14}N^{14}N$
				29	$^{14}N^{15}N$
				30	$^{15}N^{15}N$
O	$^{18}O/^{16}O$	O_2	5 (0.8‰)	32	$^{16}O^{16}O$
				33	$^{16}O^{17}O$
				34	$^{16}O^{18}O$, $^{17}O^{17}O$
		CO_2		See above	
		CO		28	$^{12}C^{16}O$
				29	$^{13}C^{16}O$, $^{12}C^{17}O$
				30	$^{12}C^{18}O$,$^{13}C^{17}O$
S	$^{34}S/^{32}S$	SO_2		64	$^{32}S^{16}O^{16}O$
				66	$^{32}S^{18}O^{16}O$
		SF_6		146	$^{32}S\ ^{19}F_6$
				148	$^{34}S\ ^{19}F_6$

a continuous stream of helium (Figure 2.1). This method is denoted as continuous-flow isotope ratio mass spectrometry (CF-IRMS). These instruments can be directly coupled with other instruments that convert the substance of interest into the measurement molecules (e.g., CO_2). Thus, the preparation of the measurement gas and analysis of its isotopic composition can be performed in one single process, which greatly reduces the time necessary for sample analysis. Furthermore, the required sample amount is much smaller compared to DI-IRMS making it possible to analyze isotope ratios of environmental compounds that can be present at relatively low concentrations.

The development of CF-IRMS for an increasing number of elements and coupled to an increasing number of instruments has led to a much wider application of isotope methods in many disciplines. The most common combinations are: The coupling of a GC via a conversion interface to the IRMS to measure isotope ratios of individual compounds in mixtures (e.g. benzene in gasoline), the coupling of an EA to an IRMS to quantify the average isotope ratio of solid or liquid samples (bulk

samples), the coupling of an IRMS with a preparation system that makes it possible to generate the measurement gas with simple chemical reactions (e.g., liberation of CO_2 from carbonates by addition of phosphoric acid), and, more recently, the coupling of a high performance liquid chromatography (HPLC) system with an IRMS. These combinations are commercially available from several manufacturers. Other combinations have been developed as well by research laboratories such as the combination of a dissolved organic carbon/total organic carbon (DOC/TOC) analyzer with an IRMS (St-Jean 2003).

In CF-IRMS systems, the sample gas travels a relatively long distance being subject to separation and transformation processes, while the reference gas is usually injected immediately before the helium stream enters the IRMS via a reference gas injection box (Figure 2.1). As a result of the different pathways, the reference and sample gas peaks have a different shape and often a different height, in contrast to dual inlet systems, where both the reference and sample gas produce a signal of similar intensity and similar rectangular peak shape (Figure 2.2). Sample peak shapes of CF-IRMS systems are typically Gaussian instead of square shaped due to dispersion effects and the intensity of the sample peak is generally variable depending of the amount of sample introduced for example into the GC or EA (Figure 2.2). However, newly developed mass spectrometers provide accurate isotope ratio values despite these differences in peak shape and intensity between sample and reference gas.

A particular challenge for CF-IRMS systems is the measurement of H_2 and HD with a mass of 2 and 3 in a stream of helium with a mass of 4. Due to the collision of He ions, some of them will deviate from their trajectory and fall into the mass 3 detectors complicating accurate measurements of HD to H_2 ratios. Electrostatic filters added in front of the mass 3 collector were developed to eliminate the contribution of helium to mass 3. A further difficulty of H_2 isotope analysis, common to dual inlet and continuous-flow systems, is that in the ion source H_2 molecules react with H_2^+ to produce H_3^+ ions, which have the same mass as HD (Sessions, Burgoyne, and Hayes 2001). The amount of produced H_3^+ is proportional to the square of the pressure of H_2 in the source. Fortunately, the H_3^+ production is sufficiently consistent and stable so that it can be corrected for. The correction factor (usually denoted as H_3^+-factor) is usually determined by injecting reference gas H_2 at a different pressure and carrying out a linear regression between apparent mass 3 and signal intensity.

2.4 COMPOUND-SPECIFIC ISOTOPE ANALYSIS (CSIA)

2.4.1 Coupling with Gas Chromatography (GC)

The coupling of gas chromatographs with an IRMS makes it possible to analyze isotope ratios of individual compounds in complex mixtures (Matthews and Hayes 1978; Meier-Augenstein 1999). Such methods are usually referred to as compound-specific isotope analysis (CSIA). The instrument is frequently denoted as isotope-ratio-monitoring gas chromatography-mass spectrometry (IRM-GCMS) or more commonly gas chromatography isotope-ratio mass spectrometry (GC-IRMS). The latter abbreviation may be modified depending on the type of conversion interface for example into GC-C-IRMS if a combustion interface is used. The basic components of

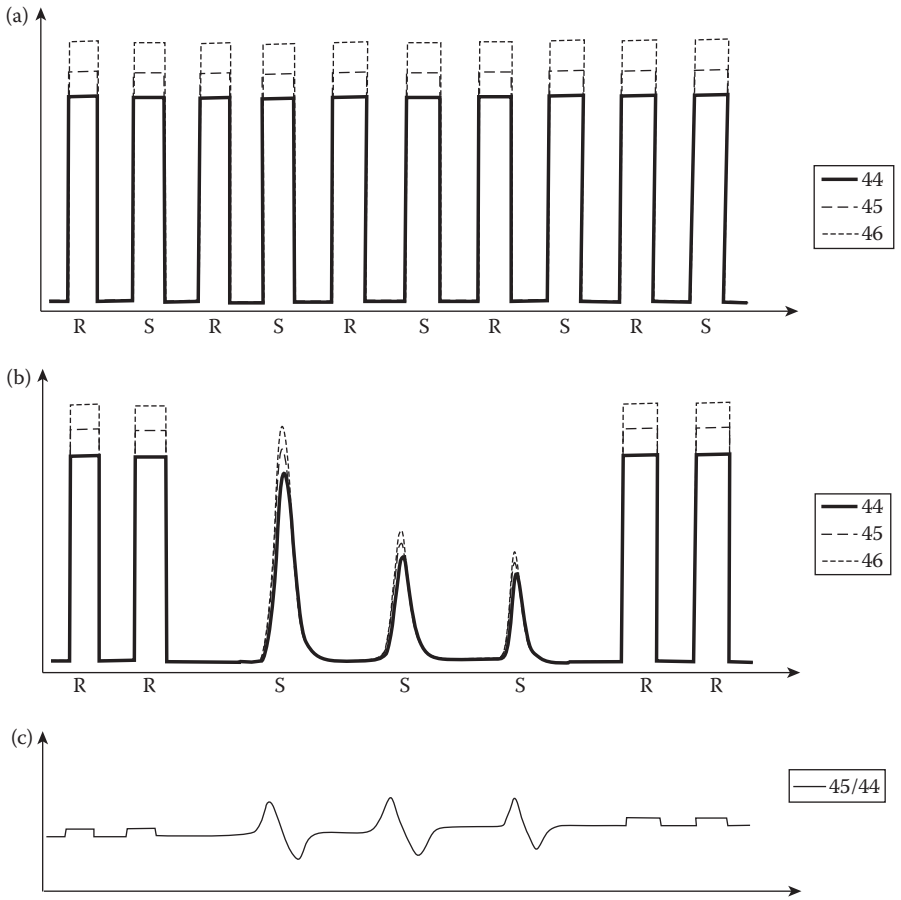

FIGURE 2.2 Signals for different CO_2 masses (see Table 2.1) recorded (a) by dual inlet IRMS, (b) by continuous flow IRMS, and (c) ratio between mass 45 and 44 for continuous flow IRMS. Signals for different masses are scaled to yield peaks of comparable height. R = Reference gas. S = Sample gas.

these analytical systems are illustrated in Figure 2.1. A key component of the coupled system is the interface that converts the compounds eluting from the GC to the measurement gas. The conversion interface is connected to IRMS via an open split. The open split consists of a capillary that is connected to the vacuum of the IRMS and draws in a part of the sample gas stream (typically 0.2–0.5 ml/min) while the remainder is vented. For carbon, the conversion consists of combustion of the compounds to CO_2 using a narrow bore reactor tube (0.5–1 mm bore) that is either packed with Cu-oxide pellets (850°C) or a combination of copper/nickel/platinum wires (940°C). The wire system has a smaller oxidant capacity and therefore needs to be reoxidized regularly with O_2, which can be done automatically. The water from combustion has to be removed because it would lead to protonation of CO_2 to produce HCO_2^+ in the

ion source preventing accurate measurements. Furthermore, condensation of water in the mass spectrometer can shorten electronic contacts. Water removal takes place either by a cryogenic trap with liquid nitrogen or a Nafion membrane tube in a counterstream of helium.

For nitrogen isotopes, usually the same type of combustion reactor is used as for carbon. The combustion leads to the formation of nitrous oxides that are reduced to N_2 in a separate downstream reactor packed with copper and held at 650°C, followed by removal of water and CO_2. During nitrogen isotope analysis there is a higher contamination risk during sample processing and analysis than for CO_2 due to the high concentration of N_2 in the atmosphere. For analysis of hydrogen isotopes, the organic compounds are converted to H_2 either in an empty ceramic tube at a high temperature (typically 1400°C) or at a lower temperature in a tube filled with chromium as a catalyst. The first procedure has been shown to lead to complete conversion of organic compounds to H_2 (Burgoyne and Hayes 1998). For analysis of oxygen isotopes, the organic compounds are transformed to CO in an empty tube with a platinum inner surface at 1280°C. For chlorine isotopes, the common measurement gas is methyl chloride. While continuous-flow methods involving a GC (Shouakar-Stash, Drimmie, and Frape 2005) or EA (Wassenaar and Koehler 2004) were developed, no interface is available yet for on-line conversion of chlorinated hydrocarbons to methyl chloride. However, recently, it was demonstrated that the chlorine isotope ratio of chlorinated ethenes can be measured by directly introducing them into the ion source of an IRMS with a special collector setup after GC separation (Shouakar-Stash et al. 2006). For tetrachloroethene (PCE) the fragment ions 94/96 are measured, for trichloroethene (TCE) the fragment ions 95/97, and for dichloroethene (DCE) the molecular ions 96/98. The method requires only around 4 nmol Cl on the GC column. An alternative method consists of the coupling of a GC with a multicollector-inductively coupled plasma mass-spectrometer (ICP-MS) (Van Acker et al. 2006). While any organic compound that passes through a GC and contains Cl can be analyzed with this approach, the required mass is much higher and the instrumentation more expensive. Furthermore, it was recently shown that Cl isotope ratio can also be derived from ion patterns measured by quadrupole mass spectrometers (Sakaguchi-Soder et al. 2007). However, the precision of the measurement is lower than for IRMS systems.

Some of the main differences of GC-IRMS compared to other GC methods (e.g., GC-MS) are that a larger amount of compound is required and that the compounds should be baseline separated in the GC. The main reason for the second condition is that during separation of the compounds in the GC column, the molecules with heavy isotopes travel slightly faster than molecules with light isotopes due to a small isotope effect associated with partitioning between mobile and stationary phase. As a result, the initial part of the peak is slightly enriched in the heavy isotope (e.g., ^{13}C) while the peak end is depleted in the heavy isotopes. This trend is most apparent when the ratios of the ion traces (e.g., 45/44) are examined (Figure 2.2). The ratio trace first increases as slightly more CO_2 with ^{13}C arrives and then decreases. Precise measurements are only obtained if the entire peak is integrated. Baseline separation can be more challenging for GC-IRMS than for other GC systems because some additional dispersion occurs within the combustion interface and during transfer to the IRMS, widening the peaks.

Approaches to optimizing the GC resolution are not discussed in detail here since they are analogous to those used for any other GC methods and the reader is referred to the extensive literature on the subject. Important considerations are to inject the compounds in a short pulse (= narrow band), to use narrower and longer columns, or the selective purification of the samples before injection. Narrow bands can be reached by using retention gaps and low initial GC temperature, by condensing the sample at the column head by cooling it down (cryogenic refocusing) or by split injection, which ever leads to a loss of mass. When using narrow diameter columns, care has to be taken not to overload the column given that GC-IRMS requires a larger amount of compound. Furthermore, when considering longer columns it has to be kept in mind that the resolution only increases with the square root of column length (e.g., by 1.4 when the column length is doubled from 30 to 60 m).

With GC-IRMS systems, isotope ratios in almost any organic compound that can be introduced on a GC column can be analyzed as long as the requirements regarding amount and resolution described above are fulfilled and the accuracy and precision of the measurement is carefully evaluated. In some cases, the presence of other elements can interfere with the conversion in the interface. For example, the presence of Cl in organic molecules complicates the analysis of H isotopes due to the formation of HCl in the high temperature interface or Cr-chloride in the catalytic interface. However, for compounds containing only C, H, O, and N such interferences are not expected.

2.4.2 Coupling with Liquid Chromatography

The first coupling of liquid chromatography with isotope ratio mass-spectrometers (IRMS) was described by (Mohammadzadeh et al. 2005) and brought on the market in 2005, much later than GC-IRMS. The method opens a wide range of new applications of IRMS because many types of molecules (e.g., pharmaceutical) or many biomolecules (e.g., amino acids, carbohydrates, RNA, DNA) are too polar or thermo-labile to easily pass through a GC. So far only systems for carbon are available. With these instruments, the organic molecules eluting from the HPLC column are transformed to CO_2 by chemical oxidation in the liquid phase. The produced CO_2 is transferred to the gas phase, dried, and introduced into the IRMS. Because wet oxidation is used, only water can be used as mobile phase during HPLC separation restricting the possibilities for optimizing chromatographic resolution. The method requires slightly more mass of compound (around 4 nmol C) than GC-IRMS because the peaks are broader.

2.4.3 Sample Preparation

For environmental samples, a major challenge is often to introduce a sufficient mass of compound for analysis since compound concentrations in the environment can be low. In the following, several approaches for aqueous sample extraction and injection for GC-IRMS analysis are discussed with a special focus on compounds that are of particular interest in environmental studies including CO_2, methane, monoaromatic hydrocarbons (benzene, toluene, ethylbenzene, xylene = BTEX), polyaromatic hydrocarbons (PAH), and chlorinated solvents. For environmental applications, it is often desirable that the analytical method is sufficiently sensitive to analyze concentrations at least as

low as regulatory limits or even lower, for example, to evaluate if low concentrations are a result of dilution or degradation. The most common methods for extracting aqueous samples are compared in Table 2.2. If the necessary equipment is available, the Purge & Trap technique is probably the method of choice for volatile compounds, although the system is susceptible to carry over effects if samples with highly variable concentrations are measured. When installing a purge and trap system (Zwank et al. 2003), it has to be ensured that the entire amount of trapped compounds are transferred to the GC column

TABLE 2.2
Methods for Sample Extraction in GC-IRMS Applications

Method References	Advantage	Disadvantage
Purge and trap	• Large fraction of compound in water can be extracted yielding low detection limit	• Required expensive equipment • Risk of carry over
Solid phase microextraction (SPME)	• Simple, fast, standard injector can be used • Low detection limit for compounds with elevated K_{ow}	• Some sorption competition occur when most sensitive carboxen-PDMS fibers are used for complex mixtures • High detection limit for compounds with low K_{ow} (e.g., vinyl chloride) • Fiber where sorption takes place is fragile
Headspace	• Simple, standard injector can be used • Suited for very volatile compounds such as methane, ethane, ethene	• Only small portion of mass is transferred, leading to high detection limit unless compounds are very volatile
In-needle extraction methods (e.g., ITEX, SPDE)	• Higher sorption capacity than SPME • Simple and robust set-up • Lower detection limits than SPME and headspace	• Less sensitive than purge and trap
Liquid–liquid extraction	• Low detection limit if sufficient volume is extracted • Standard injector can be used	• Time consuming • Separation of very volatile compounds from solvent can be difficult • Only small fraction of what has been extracted can be injected unless large volume injector used • Large volume injector suited for high molecular weight compounds but lead to loss of more volatile compounds possibly associated with fractionation
Twister	• Simple and fast • Higher sorption capacity than SPME	• Requires special desorption unit • Little choice of sorbent material

without losses within a split injector. For very volatile compounds such as vinyl chloride, this may require cryofocusing after desorption. For compounds with an elevated octanol-water partition coefficient, solid phase micro extraction (SPME) is a good alternative (Dias and Freeman 1997; Hunkeler and Aravena 2000; Zwank et al. 2003). With this method, a small volume of sorbent attached to a fiber is exposed to the gas phase or aqueous phase of the sample, followed by desorption directly in the GC injector. The method has the advantage that it can be used with standard injectors and different types of sorbents are available. However, the volume of sorbent quite small and the fiber quite fragile. Several recently developed systems have tried to overcome these limitations, for example, by coating a magnetic stirrer with a larger mass of sorbent (Gerstel-Twister, Mülheim an der Ruhr, Germany) for extraction of dissolved compounds or by packing a syringe needle with sorbent for extracting compounds from the headspace of samples (e.g., In tube extraction, ITEX; CTC Analytics, Switzerland or Solid Phase Dynamic Extraction, SPDE; Chromtech, Germany). For dissolved gases such as methane, ethane, and ethene, headspace extraction is the best suited method. To obtain narrow bands, split injection has to be used, which leads to some loss in sensitivity. Lower detection limit can be reached using a sample loop or by trapping a larger volume of headspace gas using a cryogenic system. Using standard purge and trap systems with 25 ml sample volume, detection limits for carbon isotopes in the range of a few μg/L can be reached for chlorinated hydrocarbons and around one μg/L or lower for BTEX compounds (Jochmann et al. 2006; Zwank et al. 2003). For hydrogen isotopes, the detection limits for petroleum hydrocarbons are about a factor 30 higher, while chlorinated hydrocarbons are difficult to analyze due to the liberation of Cl during pyrolysis.

Sample extraction generally involves one or several phase transfer processes (e.g., from the aqueous phase to the gas phase or from the aqueous phase to the SPME fiber), which can be associated with small isotope fractionation (Hunkeler and Aravena 2000). As long as extraction is performed under identical conditions for all samples, isotope fractionation is usually reproducible and shows little dependence on the amount of compound present in the sample. Further isotope fractionation can occur especially during injection. For example, Schmitt, Glaser, and Zech (2003) observed isotope fractionation during split injection of CO_2 and gaseous BTEX compounds, whereby the amount of fractionation depended not only on the split ratio but also on the amount of compound injected, which complicates corrections. A particularly large isotope fractionation was observed for CO_2 injection at a low split ratio. However, in other studies (Miyajima et al. 1995; Salata, Roelke, and Cifuentes 2000) using a different brand of GC, such fractionation for CO_2 was not observed suggesting that it may depend on the injector design and indicating that results obtained with one analytical system can not necessarily be extrapolated to others. Fractionation during injection can be minimized by using splitless injection, which is not always possible for example when analyzing volatile compounds.

2.4.4 Sampling and Sample Preservation

During sampling, the same precautions have to be taken as with sampling for concentration analysis. Loss of compound due to sorption and degassing has to be avoided even though the effect of these losses may be less critical for isotope then for concentration