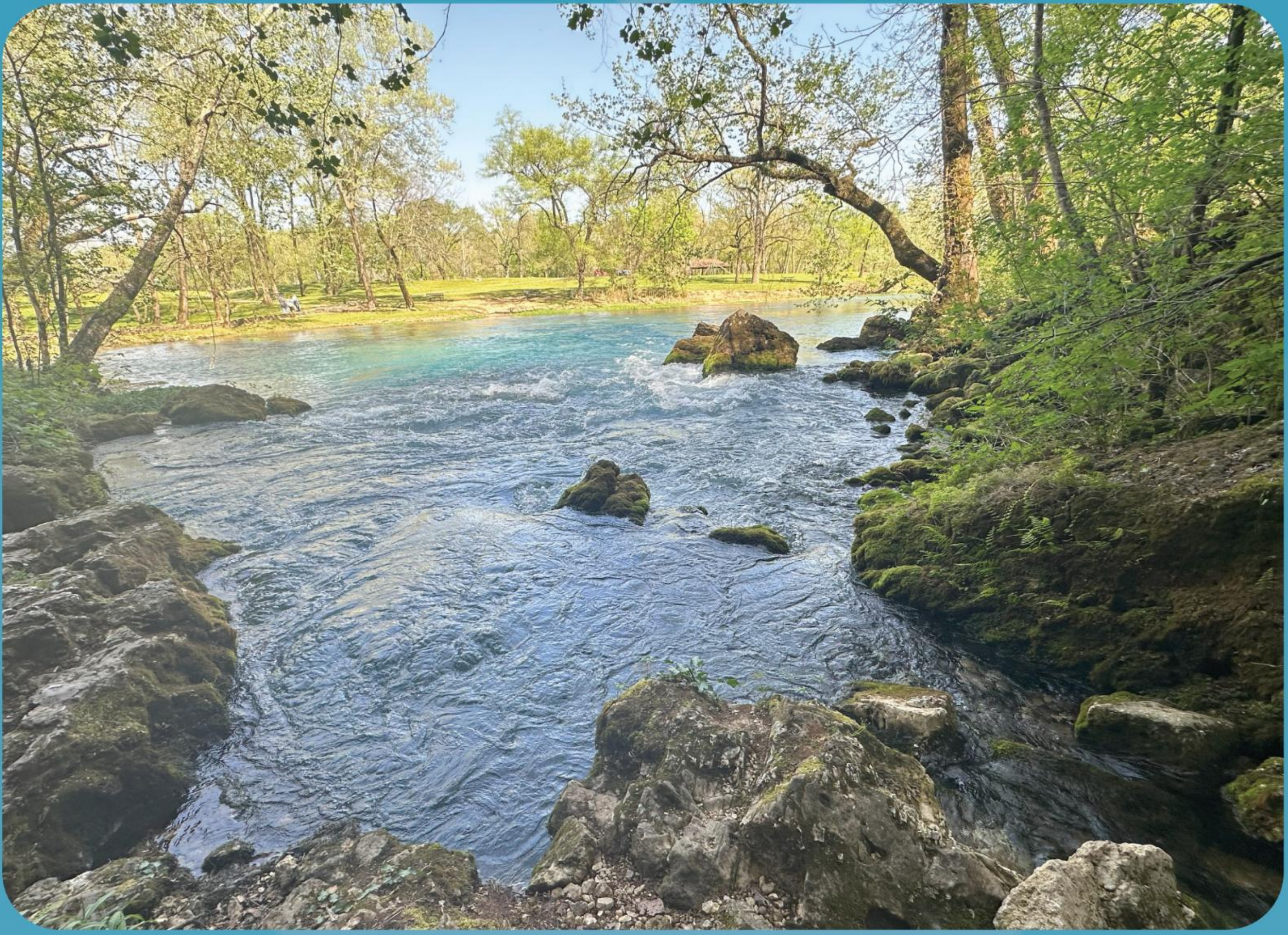

Practical Groundwater Tracing with Fluorescent Dyes

Tom Aley, Trevor C. Osorno, J. F. Devlin,
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THE GROUNDWATER PROJECT

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Practical Groundwater Tracing with Fluorescent Dyes

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THE GROUNDWATER PROJECT

Domain Editors: Eileen Poeter and John Cherry.
Board: John Cherry, Shafick Adams, Gabriel Eckstein, Richard Jackson, Ineke Kalwij, Renée Martin-Nagle, Everton de Oliveira, Marco Petitta, and Eileen Poeter.

Cover Image: Big Spring Missouri at a flow rate of 9.58 m^3/sec (338 ft^3/sec). Multiple long-distance traces have been conducted to this spring using relatively small quantities of dye and methods outlined in this book. Photo credit to Tom Aley.

Dedication

In about 1986 Jim Quinlan, Ralph Ewers, and Tom Aley began planning a text on groundwater tracing with special emphasis on the use of fluorescent tracer dyes. The working title was “The Joy of Dyeing.” For several years it was even cited in some publications as being in preparation. Over the years E. Calvin Alexander, and perhaps another person or two whom Jim Quinlan knew, agreed to help in the effort, but the limited progress ceased with Jim’s untimely death in 1995. Finally, in 2022, with prodding from The Groundwater Project and much more tracing experience, we have the book. Jim, we dedicate this book to you.

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The Groundwater Project Foreword

The United Nations (UN)-Water Summit on Groundwater, held from 7 to 8 December 2022, at the UNESCO headquarters in Paris, France, concluded with a call for governments and other stakeholders to scale up their efforts to better manage groundwater. The intent of the call to action was to inform relevant discussions at the UN 2023 Water Conference held from 22 to 24 March 2023 at the UN headquarters in New York City. One of the required actions is *strengthening human and institutional capacity*, for which groundwater education is fundamental.

The UN-Water website↗ states that *more than three billion people worldwide depend on water that crosses national borders*. There are 592 transboundary aquifers, yet most do not have an intergovernmental cooperation agreement in place for sharing and managing the aquifer. Moreover, while groundwater plays a key role in global stability and prosperity, it also makes up 99 percent of all liquid freshwater—accordingly, groundwater is at the heart of the freshwater crisis. *Groundwater is an invaluable resource*.

The Groundwater Project (GW-Project), a registered Canadian charity with its beginnings in 2018, pioneers in advancing understanding of groundwater and, thus, enables *building the human capacity for the development and management of groundwater*. The GW-Project is not government funded and relies on donations from individuals, organizations, and companies. The GW-Project creates and publishes high-quality books about *all-things-groundwater* that are scientifically significant and/or relevant to societal and ecological needs. Our books synthesize knowledge, are rigorously peer reviewed and translated into many languages. Groundwater is 'hidden' and, therefore, our books have a strong emphasis on visualizations essential to support the spatial thinking and conceptualization in space and time of processes, problems, and solutions. Based on *our philosophy that high quality groundwater knowledge should be accessible to everyone*, The GW-Project provides all publications for free.

The GW-Project embodies a new type of global educational endeavor made possible by the contributions of a dedicated international group of over 1000 volunteer professionals from a broad range of disciplines, and from 70 countries on six continents. Academics, practitioners, and retirees contribute by writing and/or reviewing books aimed at diverse levels of readers including children, youth, undergraduate and graduate students, groundwater professionals, and the general public.

The GW-Project started publishing books in August 2020; by the end of 2024, we have published 55 original books and 77 translations (55 languages). Revised editions of the books are published from time to time. In 2024, interactive groundwater education tools and groundwater videos were added to our website, gw-project.org↗.

We thank our individual and corporate sponsors for their ongoing financial support. Please consider sponsoring the GW-Project so we can continue to publish books free of charge.

The Groundwater Project *Board of Directors*, January 2025

Foreword

The most important physical parameters in the study of groundwater are hydraulic conductivity (permeability), hydraulic head, and groundwater velocity (in essence, groundwater travel time to the locations of interest). Of these parameters, velocity is rarely measured directly, instead being estimated from calculations based on measurements of hydraulic head, hydraulic conductivity, and porosity. Though this is adequate for some investigations, in many cases it is preferable to have direct measurements of velocity or travel time.

Several devices exist for measurement of velocity in wells as described in the Groundwater Project book by Rick Devlin *Groundwater Velocity*, however, the spatial scale of such measurements is so small that many measurements would have to be made to achieve a useful understanding of velocity at the scale of interest. The other approach, which is described in this book, *Practical Groundwater Tracing with Fluorescent Dyes*, is groundwater tracing where a soluble tracer, usually in the form of a dye, enters the groundwater system at a point or a few points and its arrival is observed down gradient at one or more locations.

Not only can groundwater tracing provide a reliable travel time between the two locations and the average velocity along the travel path, but it also establishes that there is a connected path of groundwater flow between the locations. Although groundwater tracing is the most effective way to determine travel time and velocity, the impediments to successfully conducting traces are many and impediments have limited the number of projects in which groundwater tracing is used. This book covers everything that is needed for a person to navigate through and around the impediments. It discusses strategy, test design, and field examples of success and failure.

Dye tracer tests are generally associated with groundwater in karst but this book shows that they are useful in other groundwater domains. There are many types of tracers. This book focusses on fluorescent dyes because they have proven versatile and effective at relatively low cost.

The first author of this book, Tom Aley, has done so many groundwater traces, in so many places over six decades, that he is the global expert. In 1966, Aley established a tracer analysis laboratory at a karst cave system on his own property in Missouri, USA, where Trevor Osorno and Alexa Goers are research scientists. Dr. Rick Devlin collaborated in the creation of this book as an expert in velocity measurement without tracer travel.

John Cherry, The Groundwater Project Leader

Guelph, Ontario, Canada, April 2025

Preface

We frequently want to know where and how groundwater is moving. For example, these questions arise in situations such as following a contaminant spill, at waste disposal sites, at sites where dewatering is required, at dam sites, in connection with groundwater-surface water interaction studies, and the list goes on. A simple approach to answer the questions "Where is the water going?" and "How long will it take to get there?" is to conduct a tracer test.

Arguably, the most famous tracer tests in scientific hydrogeology are those that were performed in the early 1980s and shortly afterward to gain an understanding of solute transport in granular media. The experiments revealed deficiencies in the then-current conceptualization of transport. These experiments were conducted in hydrogeologically simple environments with tracers that could only be chemically analyzed over two orders of magnitude in concentrations without imposing major artifacts in flow due to contrasts in solution density. In many, if not most, aquifers—especially those in karst settings—hydrogeologists have long turned to fluorescent dyes as tracers. These came with the advantage of being *visible tracers*, which when used in sufficient concentration over short distances were less limited by density-induced flow. In addition, with the advent of activated carbon samplers that can sorb dyes over extended time periods, these chemicals could be analyzed down to parts per trillion concentrations. This makes them effective tracers for long distances and permits them to be introduced to the subsurface at reasonably low concentrations. These strengths make fluorescent dyes particularly well suited to answering the questions above: "Where is the groundwater going?" and "How long will it take to get there?"

Without undermining the important contributions of many karst researchers over the past half century, it can be stated fairly that Tom Aley is one of the foremost modern pioneers in the development of fluorescent dye-tracing methods. He has rigorously demonstrated their viability in various challenging hydrologic settings, most particularly in karst aquifers. Tom began using fluorescent dyes in the mid-twentieth century, when methods were relatively unsophisticated. Over the next five decades, he combined scientific experimentation with practical experience to elevate groundwater tracing from a simplistic practice to one suitable for the most demanding projects. He and various co-authors have shared their experiences and insights in published works that appeared regularly throughout the five decades he has been conducting traces. In this volume, all that knowledge is assembled with a wealth of case studies not previously published to pass along the state-of-the art methodology of groundwater tracing to future practitioners.

I first met Tom Aley in 2006 while visiting sites under consideration for a field trip I was putting together. A student of mine, who was an ardent caver, had heard of Tom through the caving community, and we visited him at his home near Protem, Missouri, USA. He gave us a tour of the cave on his property and of the laboratory he had set up to support the tracing work he took on as a consultant. The decision to include his facilities, the Ozark Underground Laboratory (OUL), in the field trip was an easy one. Except for two sabbatical years, I have taken students to Tom's place every year since that first visit. They unfailingly say the OUL stop is their favorite, which is testament to the facilities and, more particularly, to Tom himself. One of the students who participated in the field trip, Trevor Osorno, was so taken with the OUL that he joined Tom's team and now works with Tom doing groundwater tracing—among other things—full time. He is also a co-author on this volume.

When John Cherry spoke to me about his vision for the Groundwater Project, I was quick to mention Tom's name as a possible author, and John was quick to agree. The chance to capture Tom's vast experience in this field in a single book was too important an opportunity to miss. I have been deeply honored to have been part of this initiative with Trevor, Alexa, and Tom. They deserve all the credit for the tracing advice, case studies, and insights. My modest contributions have been aimed at making this work as accessible as possible to hydrologists and other interested readers with a desire to learn about dye tracing for the study of karst hydrology. All four of us hope this work will lead to wider and better-informed use of dye tracing in hydrogeology.

This book provides readers with a rich bank of knowledge about the materials, methods, and steps in conducting groundwater traces with fluorescent dyes. Details concerning the chemistry of the dyes, equipment suitable for chemical analyses down to parts per trillion concentrations, and many case studies illustrating the types of problems that can be addressed with traces are described in easily readable boxes. Interested students and professionals can use this volume to educate themselves on how to perform tracer tests to supplement training they receive from other experienced practitioners and researchers. The book can also serve as a valuable reference for already knowledgeable professionals.

Regardless of who picks up this book, they will come away with a new appreciation of the difficulties of assessing transport issues in groundwater, especially in karst settings. Most importantly, this message of dire challenges is tempered with the offer of a marvelous tool—fluorescent dye tracers—to better understand flow and transport in the subsurface; a sometimes-polluted place that we cannot see, hear, feel, smell, or taste until it is too late.

J. F. Devlin

Acknowledgments

This book contains 35 case studies that should help readers more clearly grasp the wide range of practical applications of tracer studies. The case studies also provide specifics of how the tracing work was conducted and what the results were. While some are from published literature, many are from investigations conducted by the Ozark Underground Laboratory. We greatly appreciate all the people who helped make these investigations possible.

Cathy Aley was responsible for most of the quality assurance work in tracer studies used as case histories; we thank her for the tedious but critical work. We also thank Everett Chaney, Shiloh Beeman, and Philip Moss for their input in developing effective tracer strategies.

We especially thank Dr. E. Calvin Alexander, Jr., Department of Earth and Environmental Sciences, University of Minnesota for decades of help, especially on dye chemistry issues, along with Neal Farmer, Idaho Department of Water Resources, and David Blew, Idaho Power Company, for data on major groundwater traces in lava flows of the Snake Plain Aquifer. The senior author team-taught many professional short courses with Dr. Ralph Ewers, Eastern Kentucky University, and the late Dr. Jim Quinlan. We thank them both for an abundance of absorbed ideas. We also thank John Babcock for some of the artistic illustrations. We are also grateful for the many useful comments and suggestions provided by the three peer reviewers (Craig Divine, Calvin Alexander, and Neal Farmer).

Development of tracing methods and study strategies has benefitted greatly from the work of scores of colleagues and clients; we thank them very much. Finally, the senior author thanks the United States Forest Service for the opportunity to direct the Hurricane Creek Barometer Watershed project from 1966 to 1973. This project developed strategies for water management in rural karst areas, and development of groundwater-tracing strategies was a major component of the work. The longest groundwater trace conducted during that project was 63.4 km (39.5 miles) from the channel of the Middle Fork of the Eleven Point River to Big Spring, on the Current River in Missouri, USA. More information on this and many other traces is found in this book.

We are grateful for Amanda Sills and the Formatting Team of the Groundwater Project for their oversight and copyediting of this book. We thank Eileen Poeter (Colorado School of Mines, Golden, Colorado, USA) for reviewing, editing, and producing this book.

The sources of figures and/or tables are cited in their captions. Where a citation does not appear, the figures and/or tables are original to this book.

1 Introduction

1.1 Objectives

Leibundgut and others (2009) devote 200 pages of their book to identification and discussions of fluorescent dyes and other materials and approaches that have been used to trace water. Many of these are difficult to use or have other practical limitations. In contrast, fluorescent-tracer dyes are relatively easy to use and have a wide range of applications. For these reasons, our text is focused exclusively on groundwater tracing with fluorescent dyes.

Tracing the movement of groundwater with fluorescent-tracer dyes is a simple but underutilized investigative method in hydrogeology. This book, a contribution to the Groundwater Project, is intended to help people involved with groundwater issues appreciate situations where groundwater tracing with fluorescent-tracer dyes is appropriate and to understand practical approaches and methods for conducting tracing work, as shown in Figure 1 and Figure 2.

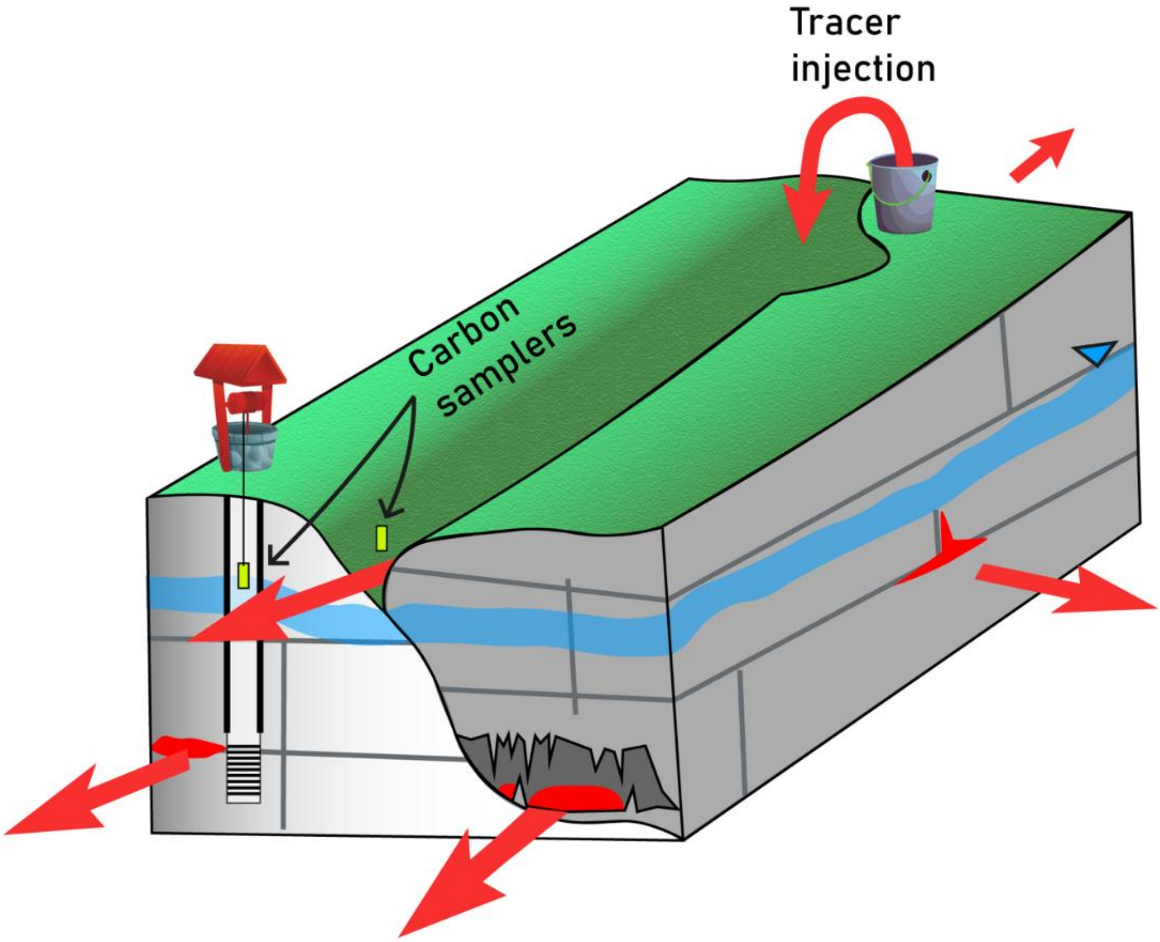


Figure 1 - Dye tracing can show, without doubt, where water is flowing and establish travel times to points of interest.

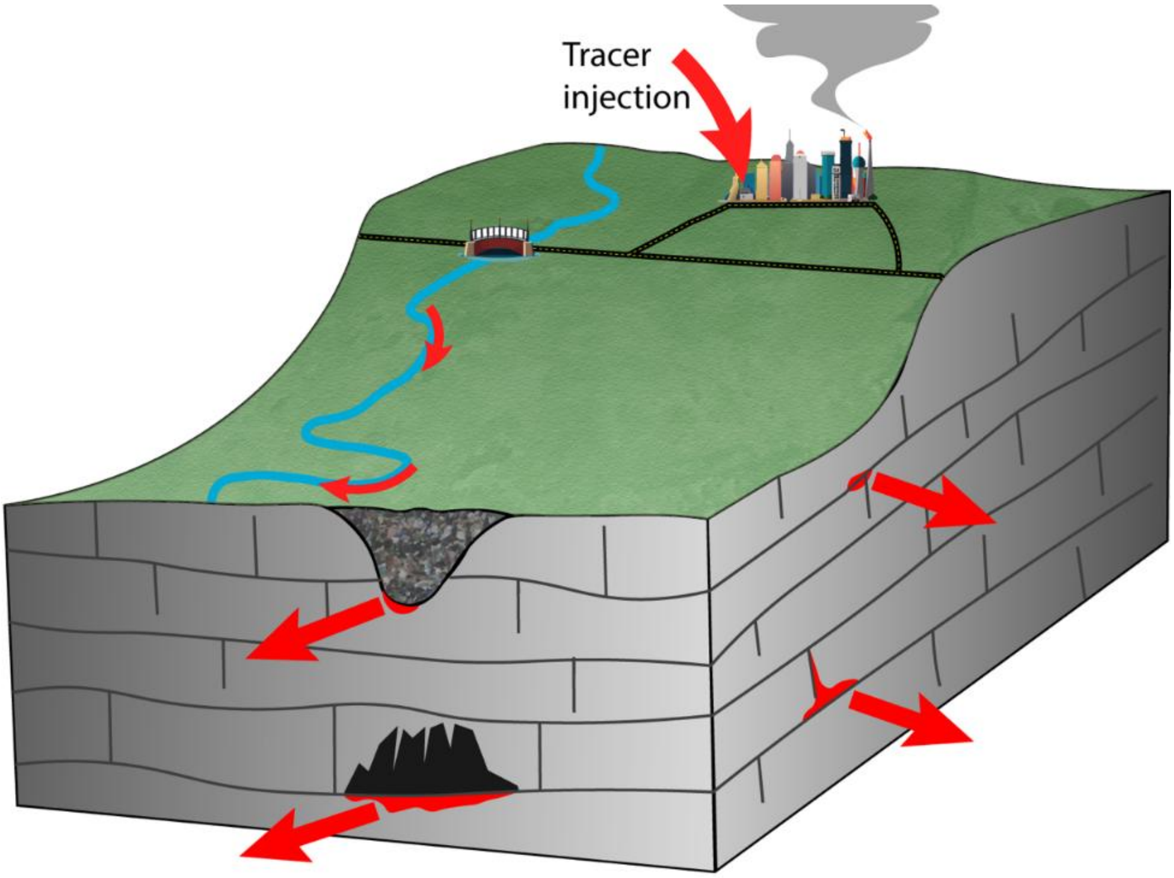


Figure 2 - Tracers can be applied at large or small scales and reveal transport in directions not commonly encountered in non-karstic systems.

This book provides information useful for designing and conducting practical, problem-solving, groundwater-tracing investigations. However, in an era where standardization and procedural manuals are in vogue, it must be remembered that there are multiple ways to approach a problem. The range of conditions and issues that confront those who are considering a groundwater-tracing project are extremely diverse. As a result, no detailed standardized approach or procedure will be ideal in all cases. Accordingly, for essentially every recommendation we present in this book there can be exceptions. We will offer recommendations for ways to conduct various facets of groundwater tracing using fluorescent tracer dyes, but we do want readers to understand that the recommendations are intended as general guidance rather than firm rules.

Tracer dyes are tools. So are hammers. There are dozens of types of hammers and no one hammer is best, although most are well suited to a particular task. The carpenter's hammer is appropriate for driving nails into wood and extracting bent ones. While the geologist's hammer can also drive nails, it is not useful for extracting them. Similarly, there are different dyes and different ways of using them. There are multiple ways to sample for them and to analyze the collected samples. In the final analysis, no single dye, sampling method, or analytical method is always the best one.

This book focuses on well-tested approaches that have been used to solve real-world problems and produce useful information. That is why the word "practical" is in the title. In keeping with the focus on practical information, we have limited our discussions to five dyes. They work well and have a large margin of safety when used appropriately. Several dozen other dyes or fluorescent compounds have, at least occasionally, been used in groundwater tracing; Kass (1998) discusses most of them and is an excellent reference for information on tracer dyes.

Much groundwater tracing has been conducted using visual observations. While it works, especially with a highly visible dye such as fluorescein (also known as uranine), instrumental methods can detect dyes at substantially lower concentrations than possible with the human eye. Much of the instrumental analysis has historically been based on water samples, and the dyes we discuss herein are instrumentally detectable in water samples at concentrations about three orders of magnitude lower than visual detection thresholds. This book gives special attention to the use of activated carbon samplers to detect tracer dyes because it is not as well recognized as other sampling methods and because the samplers dramatically increase detectability of the tracer dyes. The activated carbon samplers are continuous and accumulating devices that adsorp and accumulate all five of the dyes discussed in this book over a chosen sampler-deployment period. Dyes adsorped in the carbon samplers are eluted for analysis. Activated carbon samplers represent a major advance in groundwater tracing that overcomes problems that have long limited groundwater-tracing work dependent on water samples. Carbon samplers are well suited to monitoring multiple sampling points and they routinely decrease the cost of groundwater-tracing studies.

A good cook uses recipes as general guidance. They may use more of one ingredient and less of another to tailor a dish to the palates of those being served and to reflect the cook's experience. A good cook develops their understanding and ideas from studying recipes. This book contains "recipes" and case history experiences related to various facets of groundwater tracing. Our intent is that readers will use these "recipes" to develop their own understanding of how groundwater-tracing techniques can be used to address specific issues with which they are concerned.

Recipes that yield unfavorable results typically are eliminated from food cookbooks. Some of our case histories outline unfavorable results that can occur with certain procedures or under certain conditions—that is, bad recipes. Helping people avoid pitfalls is an important goal of this book.

We have included case histories relevant to particular topics. These case histories are found in Section 8, called Boxes, and are linked from relevant chapters throughout the text. Each case history is briefly described and then discussed to emphasize important points. Most are from Ozark Underground Laboratory (OUL) experiences and are condensed to focus primarily on the topic under consideration. We present these case histories not only to illustrate specific points, but also to help readers understand how successful tracing projects have been designed and conducted. To make it easier for readers to grasp measurements quickly, we have routinely used both metric and British Imperial equivalent values in the text.

While some people may read most of this book, we expect most readers will use only selected portions. To help ensure that critical issues are not missed, some important points are made in multiple sections. We entreat your patience with the redundancy.

1.2 How Complex Is Groundwater Tracing and Can I Do It?

We would not be writing this book if groundwater tracing were typically so difficult or complex that it was of limited general utility. Complex traces and those with potentially major consequences should be done with significant input from a groundwater professional with substantial and relevant tracing experience. However, most traces with fluorescent dyes are relatively simple and straightforward. The following case history discussed in Box 1⤵ illustrates how a simple tracing project was used to solve a groundwater problem that eluded those employing more complicated (and more expensive) approaches.

1.3 The Five Dyes Discussed in This Book

Many kinds of tracing agents can be used in hydrologic investigations, yet many are so esoteric or expensive that they have little routine utility for practical groundwater investigations. Notable exceptions are the five fluorescent dyes discussed in this book. They are commonly known as eosine (eosin), fluorescein (uranine), rhodamine WT, sulforhodamine B, and pyranine. In the experience of the authors, these five dyes are the most useful for groundwater investigations. Four of the five dyes (all except pyranine) can be analyzed with a single analytical scan on a spectrofluorophotometer, making them economical in terms of laboratory costs.

One of the major attributes of these five dyes is that they can be adsorped onto laboratory-grade activated carbon. This carbon (alternately called charcoal) can be conveniently packed into tea-bag-like packets for deployment at locations of interest. Dye adsorped onto the activated carbon can subsequently be eluted in the laboratory with strong bases dissolved in alcohol and water for quantitative analysis. The carbon samplers have three major benefits over water samples in dye-tracing work.

1. They function as continuous and accumulating samplers for the entire period they are in place at a sampling location. As a result, short-duration dye pulses are not missed.
2. Their routine use minimizes the risk of missing tracer-dye occurrences at sampling points when dye concentrations in water are below the detection limit. Carbon samplers accumulate dye, whereas water samples reflect dye concentration in the sample at the time of collection.

3. The accumulation of dye in carbon samplers frequently permits dye traces to be conducted with far less dye than is necessary when detection depends on field fluorometers or grab samples of water. This reduces concerns about visibly colored water and helps prevent failed traces when the mass of dye introduced is underestimated.

The emission fluorescence peaks of the five preferred dyes in carbon sampler eluents occur at markedly different wavelengths, allowing them to be distinguished in groundwater when they are co-introduced (Table 1 and Figure 3). The dyes are pyranine (Py), fluorescein (Fl), eosine (Eos), rhodamine WT (RWT), and sulforhodamine B (SRB). All analyses were done with a spectrofluorophotometer operated under synchronous-scan protocol with a bandwidth separation of 17 nanometers (nm), an excitation slit of 5 nm, and an emission slit of 3 nm. The samples were spiked with dyes to produce peaks of similar heights. While the emission fluorescence peaks are well separated from each other, there is substantial overlap in the widths of the peaks.

Table 1 - Summary of dye mixtures and concentrations producing the emission fluorescence peaks shown in Figure 3.

Dye	% dye in mixture	Concentration of dye mixture producing fluorescence peak (ppb)
Eosine	96	29.22
Fluorescein	50	17.68
Pyranine	77	236.16
RWT	20	1075.76
Sulforhodamine B	35	552.9

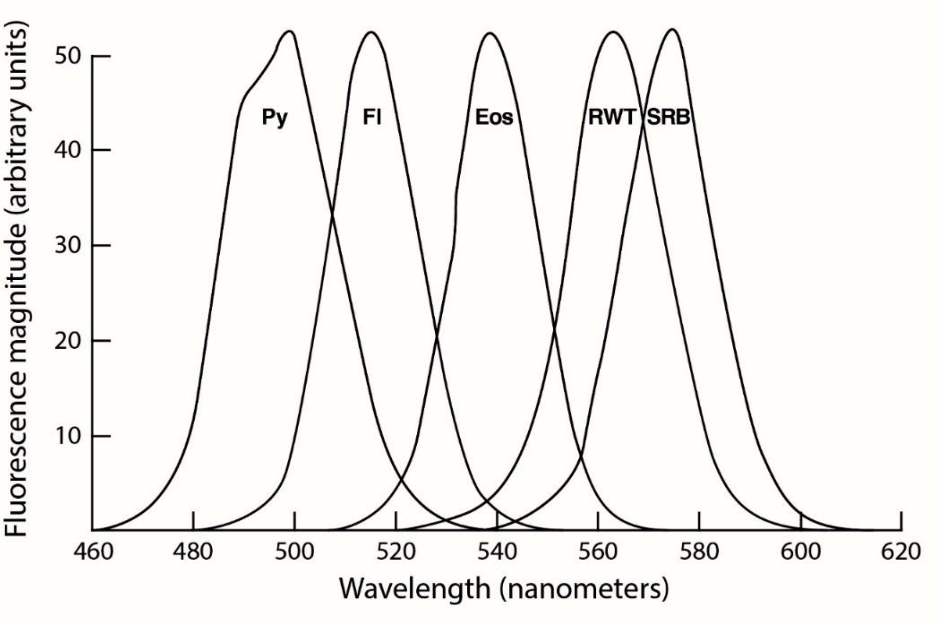


Figure 3 - Fluorescence peaks for five dyes in carbon sampler eluent as described in Table 1. Additional details are given in the text.

All five of the dyes recommended in this book for groundwater tracing are anionic compounds. This is beneficial because most charged earth materials are also anionic in the pH range of groundwater systems and thus tend not to strongly sorb dye molecules. Groundwater traces using Rhodamine B (also known as Basic Violet 10 and Drug and Cosmetic Red 19) have been attempted but generally have failed. Rhodamine B is a cationic dye and experiences substantial adsorption onto earth materials. Rhodamine B also has toxic characteristics, and its use in groundwater tracing is not recommended.

Phloxine B (also known as Acid Red 92 and Drug and Cosmetic Red 28) is another dye that can be adsorped onto activated carbon samplers and eluted from these samplers in the laboratory. It is marketed by at least one firm in the United States as a groundwater-tracing dye. However, phloxine B is a chlorinated compound known to have antimicrobial properties. Phloxine B was not among the 13 dyes evaluated for their adverse properties in Field and others (1995), so background knowledge of this substance is less detailed than other dyes. While phloxine B has sometimes been used even in environmentally sensitive groundwater, the authors of this book discourage its use due to its microbial toxicity. As is the case with eosine dye, phloxine B is probably subject to reductive debromination in some groundwater situations. In the case of eosine, this process degrades the dye and changes its emission fluorescence wavelengths. For those seeking further information on various fluorescent dyes and compounds that have been used in groundwater tracing, we recommend Kass (1998).

1.4 Groundwater Tracing Is Applicable to Many Hydrogeologic Settings

As shown in Figure 4, fluorescent dyes are the most common groundwater-tracing agents and are applicable to a wide range of situations and groundwater systems. There have been thousands of professionally directed groundwater traces conducted over distances ranging from a few meters to at least 64 kilometers (39.5 miles). Many of these have been conducted in the United States and Europe, and we estimate that more than 90 percent of them have used fluorescent tracer dyes. Many of the dye traces that occurred over distances more than a kilometer were performed in karst landscapes. Still, one groundwater trace of 50.43 km (31.3 mi) was conducted through basalt flows in Idaho (USA) by the Idaho Department of Water Resources (N. Farmer, personal communication, on May 5, 2022) as part of a continuing dye-tracing program begun in 2008 in cooperation with the Idaho Power Company. In addition, groundwater traces over a kilometer in length have been conducted in other fractured rock settings.

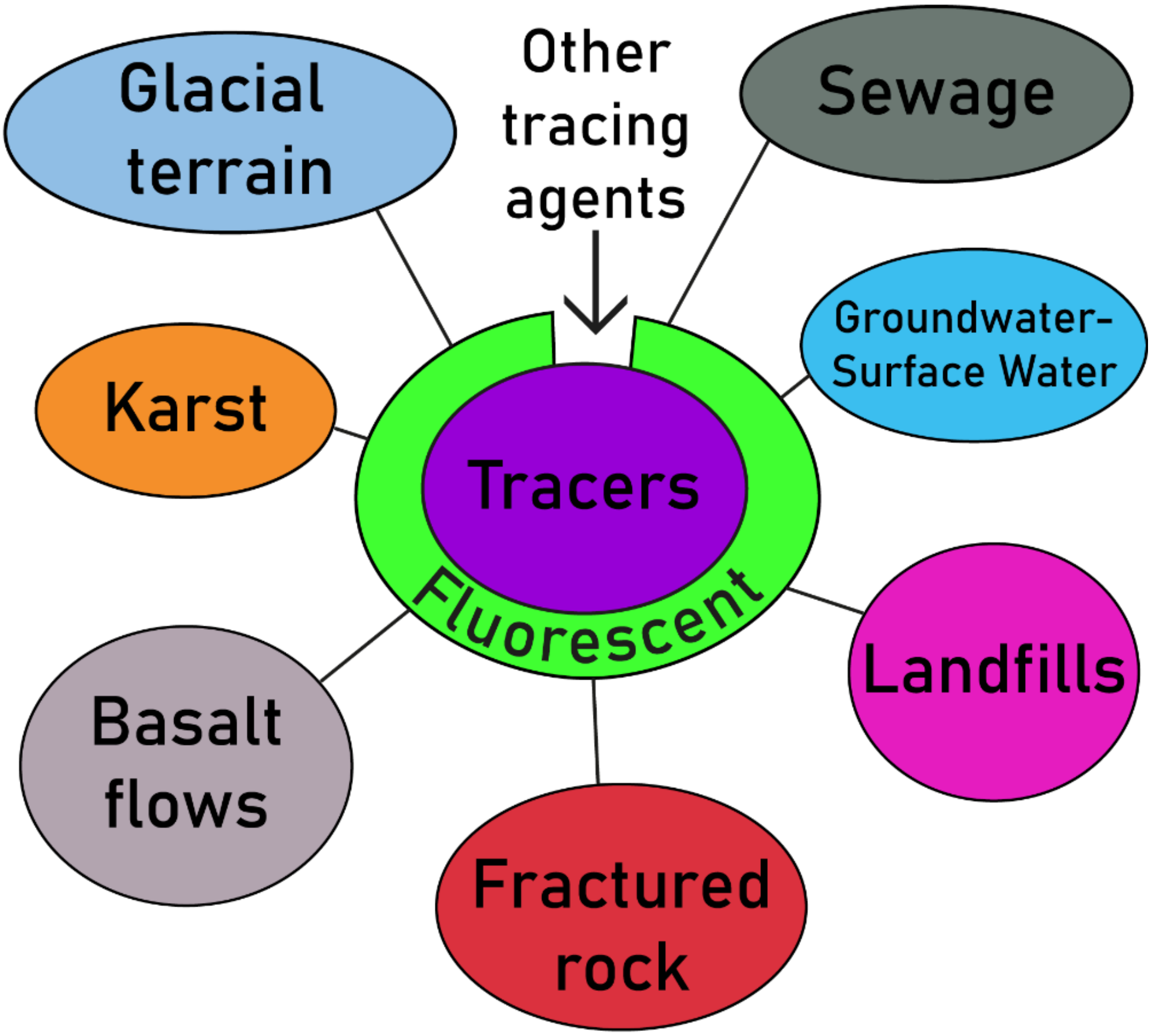


Figure 4 - Overview of applications for fluorescent tracers and the relative proportion of their use in groundwater tracing.

Many important groundwater traces using the dyes discussed in this book have involved identification of preferential flow routes. These occurred in several hydrogeologic settings, including alluvium and glacial outwash. In addition, these dyes have been used in hundreds of dye traces conducted from on-site sewage systems to marine water in Puget Sound, Washington, as discussed in Box 2↴.

The amount of dye used for successful groundwater traces varies with several factors, including characteristics of the geologic materials, travel distances, groundwater volumes, type of dye used, as well as the sampling and analytical methods employed. Box 3↴, Box 4↴, and Box 5↴ illustrate the wide range in dye quantities needed for successful traces. In some cases, the total volume of injected solution is also important.

1.5 A Major Supreme Court Ruling Based on Dye-tracing Results

The US Supreme Court seldom rules on matters concerning groundwater and dye traces but did issue such a ruling in 2020 in a case from the community of Lahaina, on the island of Maui, Hawaii, USA (*County of Maui v. Hawaii Wildlife Fund*, 2020).

1.6 How Much Dye Is Needed for Groundwater Traces?

The amount of dye needed for a groundwater trace is determined by a combination of factors including travel distance, dye type, aquifer characteristics, sampling methods, and analytical method. Sampling for fluorescein dye from the Maui Trace (Glenn et al., 2013) relied on fluorometric analysis of many water samples, the maximum detected dye concentration in the submarine springs was 35 ppb. Sampling for the on-site sewage disposal traces in Washington State, USA, and for the long-distance traces to Big Spring, Missouri, USA, utilized activated carbon samplers that were typically left in place for periods of about a week or sometimes longer. Traces placing primary sampling reliance on activated carbon samplers can be conducted with substantially less dye and fewer samples than is the case with traces dependent upon the analysis of grab samples of water. The Maui tracing efforts were very expensive while the individual traces to Big Spring and the traces from failing septic systems to marine water were inexpensive. Both approaches produce quantitative and credible results, and a combination of the two approaches can strengthen the results. If dye-tracing data are needed, most problems can be adequately addressed by relatively simple investigations that are carefully designed and conducted. An example of a highly effective and cost-conscious groundwater-tracing program that has been underway for 14 years in volcanic rocks in Idaho (USA) is provided in Box 5.

1.7 Relevance of Tracer Studies

In hydrogeological studies, the most common way of understanding water seepage velocities and directions is through the application of Darcy's Law—that is, measuring hydraulic heads at piezometers to determine a hydraulic gradient, then multiplying this by the assumed or measured hydraulic conductivity and dividing by the (usually assumed) porosity. There are many settings where this approach can greatly mislead investigators due to preferred flow pathways or uncertainties inherent in the Darcy approach. Single-well tracer tests or flow measurements can avoid the problems associated with the Darcy approach and provide flow data relevant to local conditions, but leaking barriers, risks to distal receptors, and evidence from directly observable flow pathways and their lateral spatial connectivity are not addressed by these methods. Tracer studies are well suited to fill these gaps.

The five fluorescent tracer dyes that are the focus of this book function as reasonable surrogates for many groundwater pollutants. For this reason, the tracers are extremely useful in the study of contaminated water. Over the last decade fluorescent tracer studies have been increasingly applied at contaminated sites, especially when the use of in situ groundwater remediation agents is being considered. In these applications, the questions are generally about how an injected reagent (rather than the contaminant) will be distributed and transported, and therefore how should an injection-based remediation system be designed. In particular, volumes of injection are very important because transverse dispersion is not a factor for reagent distribution at the time and distance scales of most remediation projects.

Common groundwater questions that tracer dyes can help answer include:

- Where does the water go, or where did it come from?
- Where is the recharge area for an environmentally sensitive area/feature located?
- Where is the zone of influence of a water supply well located?
- How long does it take water to flow between important points?
- What happens to the water and associated contaminants (or remediation agents) as they follow the traced groundwater flow path?
- What is the residence time of remediation agents in contaminated water?
- What are credible estimates of groundwater transport parameters?
- Are estimates from numerical models representative of field conditions?
- How effectively have drilling fluids been removed from wells during well development?

In many cases tracer dyes are the best tool available for directly and credibly answering these questions in a cost-effective manner. Dye tracing is based on observations, not on assumptions. If dye is introduced into groundwater at a particular point and is subsequently detected at one or more other locations, the hydrologic connections and travel rates for that dye under the conditions tested are established.

A common misconception about dye tracing is that it only works in well-developed karst areas. While tracing is often necessary for investigating water related issues in such settings, successful groundwater tracing can routinely be conducted in many other hydrogeologic settings. Tracers are particularly effective in any setting in which there are preferential flow routes, such as exist in many fractured rock aquifers and along macropores in deep soils and residuum. Alluvial aquifers commonly have preferential flow routes with high permeability zones along buried former stream channels. The dyes discussed in this book have been successfully used as groundwater tracers in the following settings:

- in high-yield limestone aquifers including the Edwards Aquifer in Texas, USA, and the Floridan Aquifer in Florida and Georgia, USA;
- in basaltic lava flows over straight-line distances of more than 49.9 km (31 miles) in the East Snake Plain Aquifer, Idaho, USA;
- in fractured andesite and rhyolite for 4.5 km (2.8 mi) in New Mexico, USA;

- through glacial outwash, alluvial deposits, and deep residuum to water supply and monitoring wells at many locations;

and situations to:

- determine paths from highways, rail facilities, and pipeline spills to streams, springs, and wells;
- determine paths from leaky sewers to water supply and monitoring wells, springs, streams, and building sumps;
- determine time of travel at contaminated sites for planning groundwater remediation;
- estimate aquifer properties in-situ;
- detect improper underground connections between stormwater and wastewater pipes (this is especially important at older industrial facilities that have experienced multiple owners and multiple renovations);
- test the integrity of barrier walls;
- identify surface locations that contribute water to both operating and closed/abandoned mines;
- assess groundwater movement and mixing in flooded mines;
- identify discharge points for water draining from mines;
- identify leaks from impoundments (some with synthetic liners) to nearby aquifers and streams;
- determine if wastewater discharges to groundwater are the functional equivalent of direct discharges to surface water;
- assess scenarios where the “worst case” is groundwater flow along preferential paths; and
- assess the accuracy of groundwater models.

Modeling is an important groundwater tool, but if the basic assumptions about the degree of aquifer heterogeneity and anisotropy are not reasonably accurate, then the ensuring calculations will be incorrect. Such errors may have serious consequences. Dye traces can be a practical and cost-effective method of assessing the accuracy of models of groundwater systems likely to contain preferential flow routes and anisotropic conditions. Case histories presented in Box 6⤵ and Box 7⤵ illustrate the value of assessing the accuracy of model results with tracer tests and the relative ease with which this work can often be conducted.

Previous case histories presented in this section have focused primarily on traces conducted in rather heterogeneous aquifers. As illustrated in the following case history, tracer dyes can also work well in more homogeneous aquifers, as discussed in Box 8⤵.

1.8 Summary

1. Groundwater tracing with fluorescent tracer dyes is an under-utilized investigative method that has numerous applications in many different hydrogeologic settings.
2. A common misconception is that dye tracing works only in well-developed karst areas. The case histories in this section and elsewhere in this book clearly demonstrate that groundwater tracing is a valuable tool for addressing many practical issues in many different groundwater settings.
3. Many issues can be addressed with simple traces that many people could conduct. There are businesses that can assist by providing tracing materials and laboratory analysis for the tracer dyes.
4. Activated carbon samplers are a key component of many well-designed groundwater-tracing projects. They function as continuous and accumulating samplers, their routine use minimizes the risk of failing to identify all dye detection sites, and they frequently permit dye traces to be conducted with far less dye than is needed for other sampling approaches. Activated carbon samplers are discussed in detail in Section 3.
5. A US Supreme Court decision resulted from a dye-tracing study on the island of Maui in Hawaii (*County of Maui v. Hawaii Wildlife Fund*, 2020). The court ruled that discharges to groundwater require federal permits just as surface water discharges do if the discharges are the functional equivalent of direct discharges to surface water. Thousands of discharges to groundwater in the United States are likely to require permits that were not required in the past, and groundwater tracing is likely to be a key tool in assessing whether particular discharges are the functional equivalent of direct discharges to surface water.
6. Much simpler and less costly groundwater traces than the one conducted on Maui can provide information necessary for determining if a discharge to groundwater should be viewed as the functional equivalent of a direct discharge to surface water.

2 Fluorescent Tracer Dyes

2.1 Introduction

This section provides information on some of the more important properties of the five subject dyes discussed in this book: eosine, fluorescein (uranine), pyranine, rhodamine WT, and sulforhodamine B. Some traces have failed, or produced incomplete results, because the dye selected for use was poorly suited to existing conditions. However, it is seldom the case that none of the five dyes is suitable for a particular trace. Furthermore, although a dye may not be ideally suited for a given trace, strategies exist that can help make the dye perform adequately.

2.2 Fluorescence

Fluorescence is a critically important characteristic of all five of the subject dyes. Fluorescence is a particular form of luminescence that describes a process in which light is emitted by a particle in response to prior excitation of its molecules. Fluorescence is differentiated from other forms of luminescence (e.g., phosphorescence) by the fact that molecules in the fluorescing material are excited by light at one wavelength but return to ground state immediately ($<10^8$ seconds) by emitting light at a longer wavelength. The process of fluorescence is particularly useful because the fluorescent properties of a given substance are characteristic and can be differentiated from the fluorescence of other materials. The uniqueness of a material's fluorescence characteristics is largely a function of the chemical structure of the substance as expressed in the excitation and emission wavelengths. It is the uniqueness of these fluorescent characteristics that permits highly selective analytical methods for the identification and quantification of the dyes at very low concentrations.

Many natural and manufactured materials exhibit fluorescence and are thus classified as fluorescent materials. The five subject dyes are among these substances. Over a dozen fluorescent dyes and some chemicals used in manufacturing dyes have been successfully used as tracing agents as discussed by Kass (1998). This book focuses on the dyes that the authors have found most useful for solving practical problems with tracer investigations.

2.3 Dye Nomenclature and Its Importance

There are multiple names for each of the five subject dyes. Some of the more common ones are identified in the following paragraphs. For simplicity, we will use the common dye names shown in bold in the following paragraphs. However, it is best to identify the dyes by their Color Index Numbers and Names and/or by their CAS Numbers when purchasing them or describing them in professional reports to ensure they are uniquely identified.

Groundwater tracing is typically done with technical grade dyes. Eosine, fluorescein, and pyranine are also available under Drug and Cosmetic (D&C) names. Dyes purchased under D&C names are routinely more expensive than technical grade dyes, and groundwater-tracing work does not require the use of the more expensive dyes. Pyranine is also available under a solvent dye name (Solvent Green 7) and is usually more expensive when purchased under that name. The chemical structures and colored pictures of the five dyes are shown in Figure 5. The color of some dyes (such as eosine) is a function of their concentration in water.

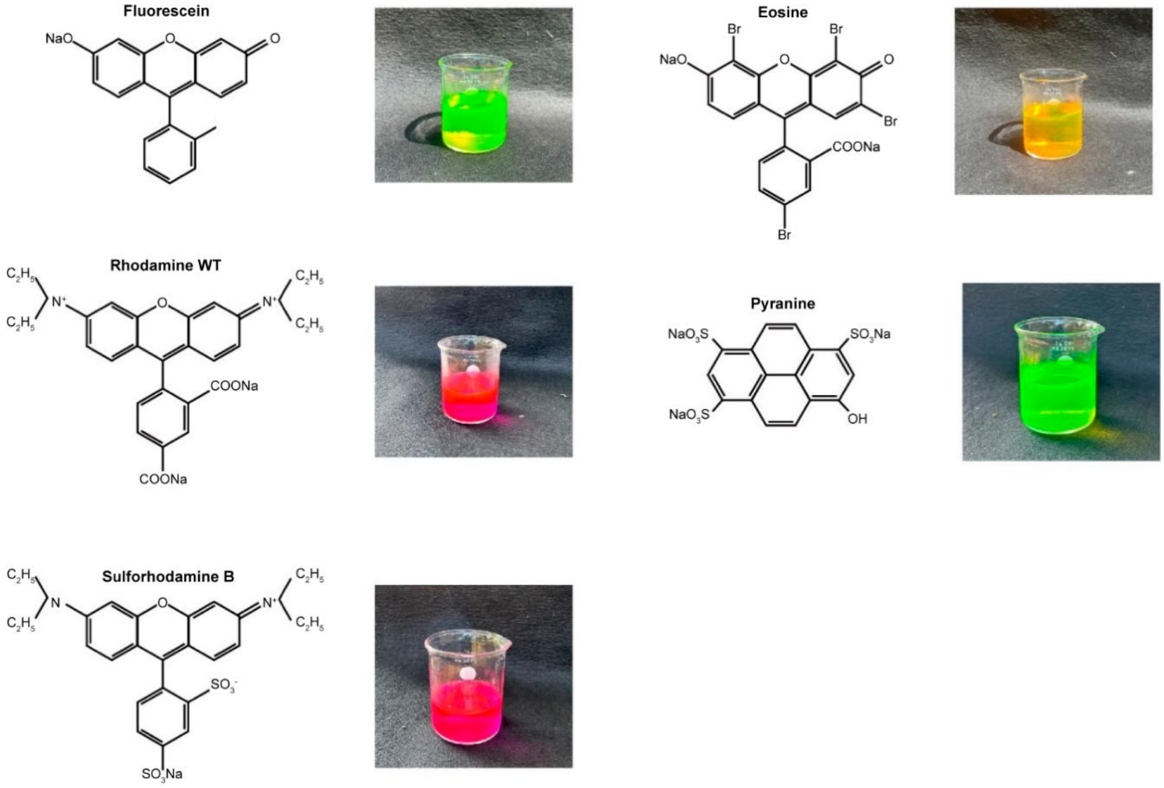


Figure 5 - Chemical structures and visual appearance of the five dyes. The color of eosine is a function of its concentration.

It is important to note that these fluorescent dyes are the sodium salts of organic acids. The parent acids (as large organic molecules) are not very soluble in water. Commercially available liquid dyes are high pH mixtures of the parent organic acids mixed with sodium hydroxide. The dyes are available as solid acids and as their sodium salts, but other than use as primary standards to calibrate the strength of the commercial liquid dyes, trying to use the solid forms of the dyes in the field is a potential disaster. Handling the solid dyes in field or laboratory environments is a high-risk activity.

It is not unusual, as a container of dye is used, to find solid dye crystalized on the inside of the container. The concentrations of the dye may vary from subsample to subsample of a single container of commercial dye.

Eosine (also spelled eosin) has CAS Number 17372-87-1. The Color Index Name is Acid Red 87, and its Color Index Number is 45380. Eosine is also known as Eosin Yellow, Eosin Y, Eosin 3Y, Eosin 3G, Eosin YB, Eosin YS, Eosin DA, Eosin G, Eosin GF, Eosin A, Eosin GS, Eosin BS, Eosin FA, Eosin J, Eosin OJ, and D&C Red 22.

Fluorescein has CAS Number 518-47-8. The Color Index Name is Acid Yellow 73, and its Color Index Number is 45350. This dye is commonly known as fluorescein in the United States and as uranine in Europe. It is also known as Uranine C, Sodium Fluorescein, Fluorescein LT, Fluorescent Yellow/Green, and D&C Yellow 8. The dye is sometimes sold simply as "green fluorescent dye."

Pyranine has CAS Number 6358-69-6. Its Color Index Number is 59040. It is also known as D&C Green 8 and as Solvent Green (SG) 7.

Rhodamine WT has CAS Number 37299-86-8. The Color Index Name is Acid Red 388. It is sometimes sold as Fluorescent Red, but that name is sometimes applied to Rhodamine B (Basic Violet 10) which is a chlorinated compound with carcinogenic properties and is not a suitable dye for groundwater tracing. Rhodamine WT and sulforhodamine B are sometimes sold simply as *red fluorescent dye*. The letters WT in the name are for ***w**ater **t**racing* and the dye was primarily developed for use in surface water studies. Rhodamine WT is sometimes contaminated with a few parts per million fluorescein (Kass, 1998). While this has the potential to create interference if both rhodamine WT and fluorescein are used in a test, the concurrent use of both dyes in a tracing program is generally possible and is commonly a good strategy. Nevertheless, care must be taken in the use of rhodamine WT because of this issue. The OUL once rejected a shipment of rhodamine WT from a supplier due to an excessively large emission fluorescence peak in the normal range of fluorescein in addition to the emission peak typical of rhodamine WT.

The commercially available rhodamine WT dye used in water tracing is composed of equal amounts of two isomers. Isomers of organic compounds have the same empirical formula and the same number of atoms, but they are arranged differently. Isomer 1 is CAS 65392-81-6, and isomer 2 is CAS 75701-30-3. Sutton and others (2001) discuss commercially available rhodamine WT and its utility for groundwater tracing. One of the isomers in commercial rhodamine WT mixtures is mobile in groundwater, but the other experiences a significant retardation factor. This is discussed in more detail later in this section.

Sulforhodamine B has CAS Number 3520-42-1. The Color Index Name is Acid Red 52 and its Color Index Number is 59040. It is also known as sulforhodamine B, pontacyl pink, pontacyl brilliant pink B, lissamine red 4B, kiton rhodamine B, acid rhodamine B, amido rhodamine B, and fluoro brilliant pink. Both this dye and rhodamine WT are sometimes sold simply as red fluorescent dye. If the red fluorescent dye is a powder, it is almost always sulforhodamine B; if it is a liquid, it is commonly (but not always) rhodamine WT.

In this book we will not capitalize any of the dye names. However, the letters "WT" in rhodamine WT will always be capitalized, and the letter "B" in sulforhodamine B will always be capitalized.

2.4 Health and Safety Issues

The five tracer dyes discussed in this book are safe for humans and the environment when used in professionally directed groundwater investigations. Field and others (1995) provide a comprehensive review of tracer dye toxicity and evaluated the five commonly used tracer dyes discussed in this book. They conclude *"The use of tracers for the study of groundwater flow is appropriate if consideration is given to the overall human health and environmental effects. Their use in the environment requires tracer concentrations not exceeding one to two mg/l persisting for a period in excess of 24 hours in groundwater at the point of groundwater withdrawal or discharge"* (Field et al., 1995, p. 75). There are more recently published papers focused on toxicity and environmental concerns for one or more of the tracer dyes. None of the dyes familiar to the authors have indicated problems associated with their use as recommended in Field and others (1995). Their recommendations on dye toxicities are for the concentrations of dye in the dye mixtures. The OUL has conducted approximately 4,000 groundwater traces and seen no evidence of adverse impacts on human health or the environment.

A common concern with tracer dyes is the possibility of colored water at an off-site property, in a well, or in a surface water body. As will be subsequently discussed, the difference between visual detection thresholds for the public and instrumental detection limits for the five dyes in water samples is greater than five orders of magnitude for all dyes except fluorescein; and it is almost five orders of magnitude for fluorescein. This great difference provides a large safety margin for studies to be designed without risking visible dye at sensitive receptors (Exercise 1↴). Colored eosine in surface water is the least visually noticeable of the five tracer dyes and its visual presence is often attributed to algae or other natural materials by the public. Thus, eosine has been introduced into urban surface streams to test for infiltration into nearby sewers or groundwater supplies without any public reports of colored water.

Many tracing projects have failed due to unwarranted concern about creating visually colored water which has resulted in the use of inadequate amounts of dye (Exercise 2↴). A frequent error is basing the amount of dye introduced on an assumption that much of the dye might discharge at a well or surface stream. Based on 20 groundwater traces in the Barton Springs segment of the Edwards Aquifer in Texas (USA), the median percent of introduced dye detected at the discharging karst springs was 4.2 percent of the total mass introduced (Hauwert et al., 2004). Aley (2017) reviewed mass balance data for 15 karst springs in different regions of the US and found that the median percent of introduced dye detected at the discharging karst springs was 4.9 percent of the mass introduced. Solution-enlarged conduits transport much of the water in these karst aquifers and groundwater travel rates are commonly at least several hundred meters per day. Less contact between dyed water and geologic materials is expected in karst aquifers than in most other types of aquifers. As a result, a larger percentage of introduced dye will routinely be adsorped onto aquifer materials in non-karst aquifers than in karst aquifers.

If visual detection of dye at sensitive receptors is a limiting factor in the overall acceptance/implementation of a dye-tracer study, the use of carbon samplers as the primary sampling technique is likely to be the desirable strategy. Tracing projects that place primary sampling reliance on activated carbon samplers routinely require only a fraction of the dye needed for projects dependent upon lab analysis of water samples. Tracing projects reliant upon field fluorometers require the use of greater amounts of dye than those dependent on analysis of water samples. Benefits of tracing projects that use activated carbon samplers is discussed in greater detail in Section 3.

2.5 Minimal Regulatory Controls

Tracer dyes are not harmful materials at the concentrations needed for groundwater studies and their use in such investigations is unregulated in most states in the USA. Some American states require notification to regulatory bodies before conducting traces and a few states have included dyes under provisions of their Underground Injection Control programs. A few localities have provisions requiring that they be informed of planned groundwater traces. In other areas tracer dyes are unfamiliar and regulatory personnel are uncertain as to what actions, if any, might be required. It is a good protocol to check for local or state requirements and to at least inform appropriate agencies of planned dye traces especially if colored water might be visible at any location during the study. This is especially important if dye is added to surface streams to test for hydrologic connections with wells or springs, or if tracer dyes might reach wells supplying private or public drinking water. A benefit of requiring notification of agencies about planned dye traces is that it may prevent traces conducted by different parties from interfering with each other.

A few states in the USA have developed lists of acceptable tracer dyes and concentration limits for each dye. While these well-conceived actions could be helpful, they can be written with a very narrow focus and applied too broadly. At least one such list includes tracer dyes that do not provide robust results, but it fails to include some of the best performing dyes and unnecessarily discourages tracer projects. In many cases, introducing dyes as a pulse rather than at a constant concentration minimizes the amount of dye needed and provides the best time-of-travel information. These benefits can be lost if there are regulatory limits on dye concentrations at dye introduction points. Field and others (1995) in a review of dye toxicity, avoided this problem by recommending maximum dye concentration limits at points where water was withdrawn or discharged. We look forward to the day that poorly conceived regulations will be improved, and new regulations will be drafted by well-informed personnel.

2.6 Dye Mixtures

Dyes routinely used for groundwater tracing are technical grade mixtures; they are not 100-percent pure dye and should be referred to as dye mixtures. The percent of dye in the dye mixture should be indicated. The percent of dye by weight in a dye mixture is called the *dye equivalent*. The remainder of the mixture is diluent; as shown in Figure 6. The diluent is used to standardize the mixture to meet the specifications for the product that the provider is selling, and in some cases to make the resulting mixture dissolve better in water. An example of the diluent allowing for higher solubility of the mixture is illustrated by pure fluorescein being insoluble in water compared to the solubility of technical grade fluorescein mixtures being approximately 500 g/L (Kass, 1998). Field experience indicates that the solubility of eosine, pyranine, and sulforhodamine B mixtures are like fluorescein and that 454 grams (1 lb) of powder dye mixture can be dissolved in 3.79 L (1 gal) of water for field introduction.

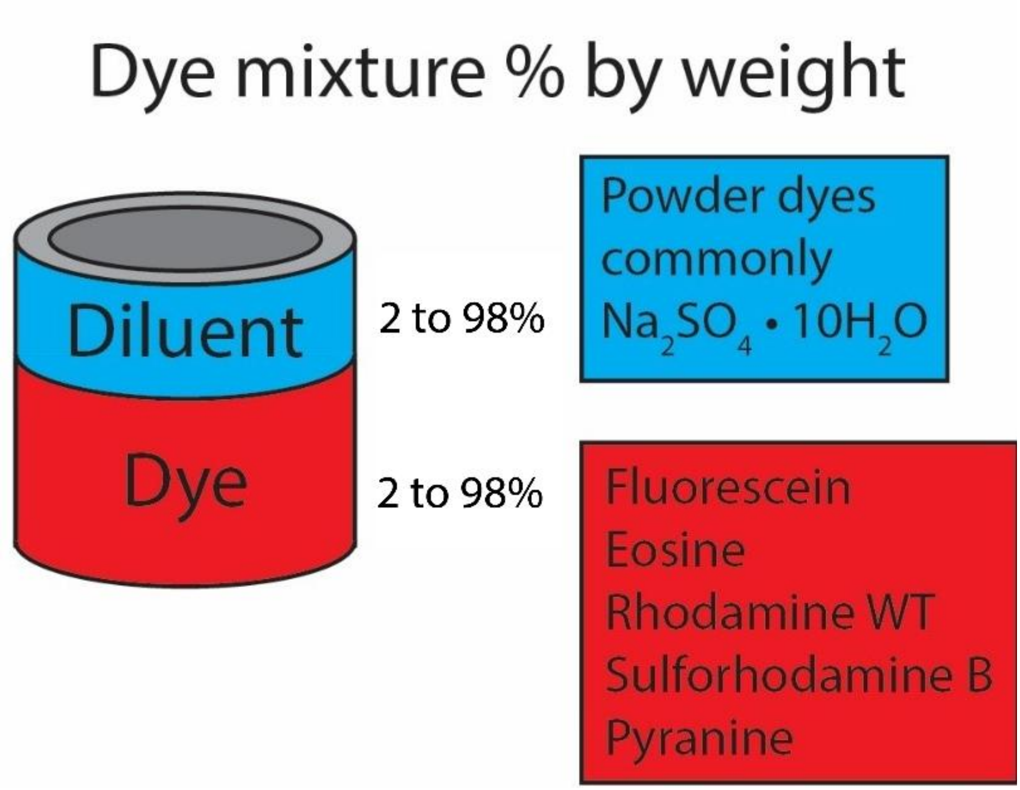


Figure 6 - Dye mixtures are made of dye and diluents.

The most common diluent in technical grade powdered dye mixtures is sodium sulfate decahydrate—also known as Glauber's Salt or mirabilite. Glauber's Salt is a common filler product in laundry detergents and medications. Liquid dye mixtures typically contain Glauber's Salt and water as diluents.

For technical grade dyes it has been the authors' experience that most of the manufacturers or suppliers either do not know or do not wish to readily disclose the approximate dye equivalent percentages in their dye mixtures. There appear to be no regulatory requirements to report dye equivalency values in technical grade dye mixtures. As a result, dye equivalencies vary among suppliers. Additionally, as diluents are standardizing agents, the amount of a diluent in a dye mixture will vary somewhat from batch to batch.

Except for rhodamine WT, which is only sold in liquid form, dye mixtures suitable for tracing projects can be purchased in either powder or liquid form. Powder dye mixtures routinely have higher dye equivalent values than liquid mixtures. The authors have seen dye equivalents in fluorescein mixtures ranging from 2 to 78 percent. As a result, without information on the dye equivalency in a mixture, the amount of dye in use will be unknown. A recommended approach is to purchase dyes through a firm that routinely conducts groundwater traces, has standard supply sources for the dyes they use, knows the performance characteristics of the dye mixtures they use, and will be doing the dye analysis work for the project.

Fluorescein, eosine, and pyranine all have Drug and Cosmetic (D&C) names. If dye is purchased under a D&C name, it will almost always be more expensive than the technical grade mixtures, but in the authors' experience it usually comes with certification as to the dye equivalent in the mixture. This is because the D&C dyes are regulated more stringently, as they are intended for direct human use. These certificates of dye equivalence are useful as calibration standards for technical grade dyes with unreported dye equivalencies (Exercise 3⤵).

Occasionally a regulatory entity unfamiliar with groundwater-tracing investigations recommends or requires that dye used in a tracer study be purchased from retailers who report that their dyes are National Sanitation Foundation (NSF) 60 Certified. In the experience of the authors, dye mixtures sold as *NSF 60 Certified* are no different from technical grade dye mixtures provided by other suppliers. One of the authors of this book attempted to learn approximate dye equivalent values for fluorescein and eosine dye mixtures sold as "NSF Certified" from a prominent seller. Telephone calls yielded an initial response that the percentage was a trade secret and a response from a second company employee that the fluorescein mixture was 100-percent dye and that the eosine was 50-percent dye. Neither of the responses was correct. OUL laboratory analysis indicated that the fluorescence intensity of the NSF certified fluorescein in water was 7 percent lower than the fluorescein dye mixture routinely used by the OUL—thus the NSF dye mixture had approximately a 63-percent dye equivalent—and that the fluorescence intensity of the NSF certified eosine dye mixture was approximately 38-percent dye equivalent—which was 58 percent lower than the eosine mixture the OUL routinely uses. If a person planning a dye trace were to accept the supplier's statements, an insufficient quantity of dye would be used causing weaker responses in samples, poor test results, and potentially false negatives. The prices of the NSF Certified dye mixtures were substantially greater than the prices of the same chemical from a well-established dye supplier. While NSF Certification may be useful for some materials or equipment, its relevance to dyes used for groundwater tracing appears to be negligible at best, especially if sellers do not know, incorrectly report, or will not disclose the fundamentally important dye equivalent percentages in their mixtures. If dye equivalent percentage is unknown, it is best to send the dye to a lab for analysis before deciding on an amount to use for a tracer test.

2.7 Dyes in Different Matrixes

The five dyes discussed in this book can be analyzed in water samples or in *elutants* from activated carbon samplers. Sampling with activated carbon samplers in tracer studies is discussed in detail in Section 3. The carbon samplers adsorp and retain tracer dyes and permit effective groundwater tracing using substantially less dye than is required for studies dependent upon water samples or upon field instruments recording fluorescence intensity. Tracer dyes are eluted from the activated carbon samplers in the laboratory and the resulting elutant is then analyzed with an appropriate instrument such as a spectrofluorophotometer. The following pages provide information related to the five dyes in both water and elutant samples.

Dyes sometimes need to be eluted from soil or sediment samples. This can be accomplished by using the same ratio of eluting solution to the soils or sediment as used with activated carbon samplers. That ratio is 15 ml of eluting solution to 4.25 grams of the moist solid material. In a study in fractured clay, fluorescein and rhodamine WT dyes both moved along the fractures, but fluorescein penetrated further into the clay matrix than did rhodamine WT, so elutant was collected from the clay to capture all of the dye.

2.8 Important Characteristics of Fluorescent Dyes

2.8.1 High Detectability

The fluorescent tracer dyes discussed in this book are highly detectable with modern analytical instruments and mix readily with water. As an illustration, the OUL's detection limits for the five most used tracer dyes in water samples using a spectrofluorophotometer operated under a synchronous-scanning protocol range from 2 to 15 nanograms/liter (ng/L) are shown in Table 2. These detection limits are based on the dye mixtures currently used by the OUL. The instrumental detection limits are based on a signal to noise ratio of three.

Table 2 - Minimum detection concentrations of five tracer dye mixtures under different conditions. Concentrations in micrograms per liter (ppb) of "as-sold" dye mixtures.

Parameter	Eosine	Fluorescein	Pyranine	Rhodamine WT	Sulforhodamine B
Dye equivalent in mixture by weight	96%	70%	77%	20%	35%
SPECTROFLUORPHOTOMETER ANALYSIS*					
Dye in water; instrumental analysis	0.015	0.002	0.010	0.015	0.008
Dye in elutant; instrumental analysis	0.050	0.025	0.015	0.170	0.080
VISUAL ANALYSIS**					
Dye in water; field conditions, experienced person	135	7	175	125	50
Dye in water; field conditions, general public	13,500	140	3,500	2,500	1,000
Dye in water; dark room, experienced person	10	2	3	50	5

*Concentrations in water under spectrofluorophotometer analysis assume pH 9.5 to 11.0 for pyranine, eosine, and fluorescein, and are based on OUL analytical protocol. **Visual analysis presumes clear water with pH≥7.

The differences in detection limits of the five dyes discussed in this book illustrate that a kilogram of one dye mixture is not equal to a kilogram of another. Table 3 compares the relative magnitude of fluorescence for eosine, fluorescein, rhodamine WT, and sulforhodamine B dyes in both water samples and in carbon sampler elutants. All values are based on synchronous scans made on a Shimadzu RF-5301 spectrofluorophotometer operated under a synchronous-scan protocol with a bandwidth separation of 17 nm. For dyes in water the excitation slits were set at 5 nm and emission slits at 3 nm. For carbon sampler elutants the excitation slits were set at 3 nm and the emission slits at 1.5 nm. Pyranine is not compared with the other four dyes because it is analyzed under a substantially different protocol.

Table 3 - Relative fluorescence intensity of four fluorescent dye mixtures routinely used by the OUL. Area of peak is in arbitrary fluorescence units.

Dye type	Water samples		Elutant samples	
	Area of peak	Relative fluorescence intensity	Area of peak	Relative fluorescence intensity
Fluorescein	4278.68	1.00	300.00	1.00
Eosine	1117.55	0.24	233.60	0.77
Sulforhodamine B	834.79	0.18	73.95	0.24
Rhodamine WT	566.02	0.11	43.49	0.14

Values for detection limits vary between laboratories depending on analytical methods and instrument settings. Consequently, comparisons are misleading if the widths of excitation and emission slits are not the same. The wider the slits the lower the detection limit. However, as slit widths increase the ability to discriminate between dyes and other fluorescent compounds decreases. As a result, the lowest reported detection limit does not necessarily imply the best analytical protocol.

Water samples are likely to have smaller concentrations of dyes and other fluorescent compounds than elutants from activated carbon samplers. As a result, it is reasonable to have wider slit settings for water samples than for elutants from carbon samplers. This results in good discrimination between dyes and other fluorescent compounds in elutants and low detection limits for dyes in water samples. The OUL slits used for carbon sampler elutants for all dyes except pyranine are 3 nm for the excitation slit and 1.5 nm for the emission slit. The slits OUL uses for pyranine in carbon sampler elutants are 5 nm for the excitation slit and 3 nm for the emission slit. It has been the experience of the OUL that the amount of pyranine adsorped onto carbon during situations where it is used and then eluted with the standard eluting solution, is less than the amounts typical for other dyes and for this reason wider slits are used for pyranine analysis in carbon sampler elutants. Alternately, more dye could be used for traces with pyranine and emission fluorescence slits equal to those for the other dyes would be reasonable. The magnitude of background fluorescence intensity in typical samples is a major consideration in selecting excitation and emission slit settings.

Values for minimum detection limits between laboratories may appear different based on how dye concentrations are reported. The two most conventional ways to report analytical dye concentrations are based on the weight of the "as-sold" dye mixture or as the weight of dye in the dye mixture. For example, a 1 mg/L concentration of rhodamine WT dye mixture with a 20-percent dye equivalent contains 0.2 mg/L of rhodamine WT dye. Since dye equivalent values may change between batches and may not be precisely known, the best approach is to report dye concentrations based on the weight of the "as-sold" mixture used, as this value is accurately known.

Field instruments exist that can detect a variety of fluorescent dyes in water samples at reported detection limits near those obtained by laboratory instruments. While they are useful in some applications, a major limitation in the use of these field instruments is that they provide a single numerical value for fluorescence intensity, which is subsequently converted to concentration based on a calibration equation. As a result, they cannot discriminate between the dye and other materials that may exhibit some fluorescence in the same excitation and emission wavelength ranges as the target dye. Additionally, as they are optical instruments, they cannot distinguish between a tracer dye and a decrease in water clarity. Finally, using multiple fluorescent dyes in a study may require compound-specific fluorometer sondes to be deployed at all sampling locations. In contrast, a spectrofluorophotometer operated under a synchronous-scan protocol (the approach recommended for laboratory tracer dye analysis) draws a complete fluorescence peak. The ability to obtain and examine the shape of the entire fluorescence peak simplifies and improves the unique identification and quantification of target dyes from background fluorescence or from other fluorescent compounds.

Figure 7 shows emission fluorescence profiles for fluorescein, brewed coffee, and water in which broccoli was cooked. A field instrument designed to give a single value for the fluorescence intensity of fluorescein would also give an elevated numerical value for groundwater containing brewed coffee or broccoli water. While brewed coffee and broccoli water are rarely encountered in groundwater samples, the graph illustrates the inherent problem presented by other common fluorescent compounds that may be present in water being tested for dye. If fluorescein, brewed coffee, and broccoli water were all present in the concentrations shown in Figure 7, the height of the combined emission peak on the field unit would be approximately 46 arbitrary fluorescent units with fluorescein only contributing 41 percent of the total fluorescence. There is a large array of natural and artificial fluorescent compounds present in water encountered in the field and especially at waste sites, and their concentrations can vary widely both spatially and temporally.

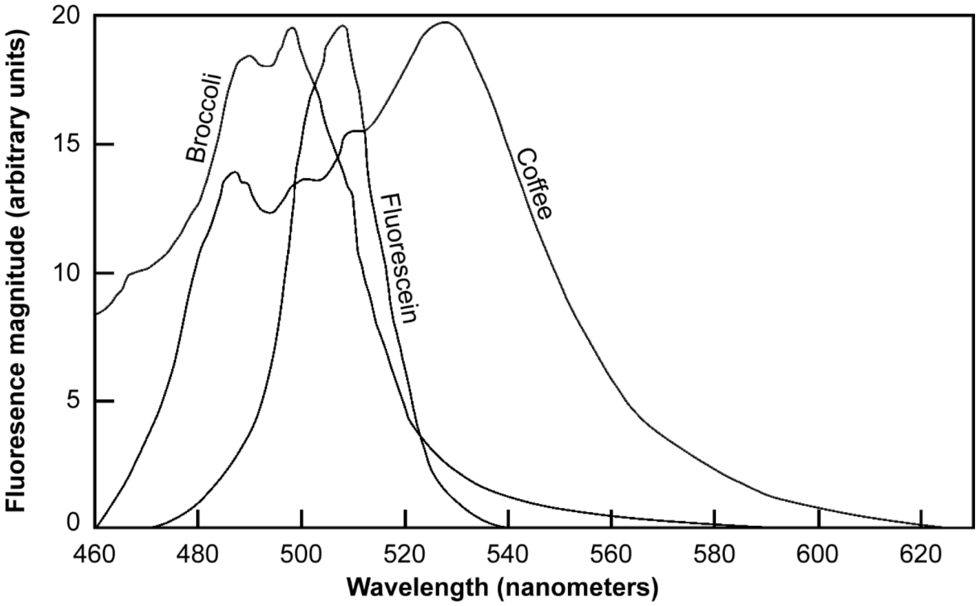


Figure 7 - Emission fluorescence profiles for fluorescein, brewed coffee, and broccoli water.

To illustrate why this is the case in terms of organic chemicals—of which fluorescent dyes are examples—fluorescence arises from electron excitation in p orbitals present in the rings of aromatic compounds (aromatic compounds are ones in which prescribed numbers of electrons are free to move between multiple atoms in a molecule rather than being constrained to a bond connecting only two atoms). Benzene is a simple example of an aromatic organic chemical, and it is known to fluoresce. More complex compounds common to groundwater, either natural (e.g., fulvic and humic acids) or from industrial sources (e.g., creosotes and related multi-ring compounds) also fluoresce, presenting many potential confounding sources of fluorescence that could be misinterpreted with field instruments.

Field fluorometers that record fluorescence intensity at frequent preset intervals can be used for short distance traces (typically 200 ft [61 m] or less) such as those to determine aquifer properties. In such traces, large volumes of water labeled with a constant concentration of fluorescein of 40 ppm or some similar value is introduced at a constant rate until the dye concentration (as measured by the fluorometer) at a monitoring point has reached and is maintaining a relatively constant concentration. The amount of dye used should produce fluorescence peaks that exceed maximum background fluorescence by at least one and preferably two orders of magnitude.

Field fluorometers can be used for longer distance traces if water samples and/or activated carbon samplers are also used to verify the presence and concentrations of tracer dyes. If field fluorometers are set for maximum sensitivity and used in streams or springs, they commonly record short-duration spikes resulting from precipitation events that flush turbid runoff water and other fluorescent materials into the water being sampled. The combination of turbid water and other fluorescent materials can lead the unwary into incorrectly concluding that dye has been detected.

To illustrate potential problems inherent in the use of field fluorometers, the OUL collected samples of the duff (decomposing organic matter) beneath an oak/hickory forest and beneath Eastern red cedar (*Juniperus virginiana*) trees. The samples were placed in jars, weighed, and water weighing five times the weight of the duff was added to each jar and allowed to stand at 74 °F (23.3 °C). The water was from a karst aquifer and had a pH of 7.9. After 24 hours, the water from each jar was poured off, passed through filter paper, and analyzed on a spectrofluorophotometer operated under the synchronous-scan protocol routinely used by the OUL for dye analysis in water samples. The color of the water was yellow to very pale brown (10YR 7/6 and 10YR 7/4; Munsell Color Company, 1975). While neither sample displayed fluorescence peaks in the acceptable wavelength ranges of the five tracer dyes, the decrease in water clarity would be equal to fluorescein concentrations of 0.59 ppb in the water from the oak/hickory forest duff and 1.61 ppb in water from the Eastern red cedar duff. Thus, field fluorometer readings would suggest detection of dye and be misleading.

The same test procedure was used for four samples of plant material: fescue (a common pasture grass), Eastern red cedar foliage and berries (*Juniperus virginiana*), white ash leaves (*Fraxinus americana*), and marijuana leaves and flowers. The leaves were crushed before being soaked in water for 24 hours. The fescue and cedar foliage/berries showed no fluorescence peaks. The white ash leaves displayed a significant fluorescence peak at 474.7 nm, while the marijuana leaves and flowers had a significant fluorescence peak at 538.2 nm. Eosine dye in water samples under the OUL protocol has peak fluorescence in the range of 532.5 to 537.0 nm. Again, this indicates that field fluorometer readings would be misleading.

The key message is that field fluorometers can produce misleading dye detection information due to a combination of fluorescence of materials other than dye and differences in water clarity. If field fluorometers are used, a prudent strategy is to send some split samples to a laboratory for analysis using a spectrofluorophotometer operated under a synchronous-scan protocol. In our experience, the field instruments produce both false positive and false negative concentrations at low dye concentrations, but they are reasonably consistent with laboratory results if the dye concentrations are at least an order of magnitude larger than the reported detection limit of the field instrument and if water samples all have equal clarity. Investigations where sampling relies on field fluorometers routinely require the use of substantially more dye than studies relying on laboratory instruments. Studies placing primary sampling reliance on activated carbon samplers and instrumental analysis of resulting samples require the use of much smaller amounts of dye for credible results.

2.8.2 Stability in the Environment

In general, the fluorescent dyes discussed in this book are adequately stable in natural water environments. A good example of the stability of dyes in aerobic groundwater comes from an experiment conducted at OUL field facilities where rhodamine WT dye has been present in an unused shallow well for over 18 years. The experiment was designed to provide a long-term evaluation of rhodamine WT stability and to determine the rate at which it would degrade (if at all) in a well-oxygenated dolomite aquifer without detectable man-made contaminants. All the periodic sampling conducted over the 18 years resulted in peak emission wavelengths that remained within the acceptable range of rhodamine WT with very similar base to height ratios, suggesting that little if any degradation of the dye was occurring. Leibundgut and others (2009) reported a study showing that eosine remained stable in groundwater for over 24 years.

Environmental factors and the characteristics of individual dyes must be considered in selecting appropriate dyes. Important environmental factors include, but are not limited to, redox conditions, pH, substrate, chemical composition of the water, and exposure to sunlight. Some dyes come with a higher likelihood of degradation than others—that is, deaminoalkylation of rhodamine-group dyes and reductive debromination of eosine. Important environmental factors and dye-specific degradation processes are described in more detail in the following subsections. In the experience of the OUL, fluorescein is more stable under a wide range of environmental conditions than any of the other four dyes.

2.8.3 Influence of Redox Condition

The redox condition of the water being traced, particularly in highly oxidizing or reducing environments, is important to consider, as it may irreversibly impact the fluorescence characteristics of a dye. In the case of an oxidizing condition (i.e., positive redox potential, also called Eh), the dyes are subject to fluorescence degradation. Based on OUL experience, fluorescein is more stable under oxidizing conditions than eosine, and eosine is more stable than rhodamine WT.

Most municipal water supplies in the USA are chlorinated and maintain a target residual chlorine concentration in the water of about 1 mg/L at the points where water is delivered to customers. If chlorinated water is used in the necessary cleaning of activated carbon samplers prior to analysis, some of the adsorped tracer dye is destroyed by oxidation prior to analysis. This will result in a biased low or false negative result. To demonstrate this, OUL conducted a bench test to assess the potential impact of chlorine residuals on dye concentrations. The test consisted of analyzing paired-activated carbon samplers from groundwater traces conducted in the field. One set of samplers was washed with OUL reagent water (untreated). The second set was washed and then allowed to sit in a beaker with water containing 4 mg/L sodium hypochlorite (treated) for 15 minutes. The charcoal samples were then eluted, analyzed, and compared; the results are shown in Table 4.

Table 4 - Dye loss from carbon samplers treated with a 4 mg/L sodium hypochlorite solution for 15 minutes.

Parameter	Fluorescein	Eosine	Rhodamine WT
Number of sample pairs	23	25	16
Mean dye loss*	45%	65%	63%
Samples where all dye was lost	1	4	2
Samples where no dye was lost	1	0	0
Mean untreated dye concentrations (ppb)	67.8	59.8	55.0
Minimum untreated dye concentration tested (ppb)	2.67	0.29	2.57

*Dye loss percentage = untreated sample concentration minus treated sample concentration divided by untreated sample concentration

Reducing conditions can also impact the stability of individual dyes, and in some cases degrade their fluorescence characteristics. This is illustrated by a OUL bench test of selected dyes in the presence of zero-valent iron (ZVI). Solutions of 100 ppb eosine, fluorescein, rhodamine WT, and sulforhodamine B dye mixtures were prepared. The mixtures were divided into two bottles each containing 100 ml of the dye solution, with one bottle being the control and the other being the treated sample. Five grams of ZVI were added to each of the four treated sample bottles. The bottles were sealed, and water samples were periodically withdrawn for dye analysis using a spectrofluorophotometer operated under a synchronous-scan protocol.

Table 5 shows the percentage of detectable dye in dye solutions to which ZVI had been added, and Figure 8 shows the results graphically.

Table 5 - Percent of detectable dye in dye solutions to which ZVI was added.

Hours after ZVI added	Eosine	Fluorescein	Rhodamine WT	Sulforhodamine B
1	88	100	88	87
3	81	93	80	81
24	35	80	69	10
48	10	76	70	3
72	4	---	---	---
96	2	---	---	---
168	2	72	68	2

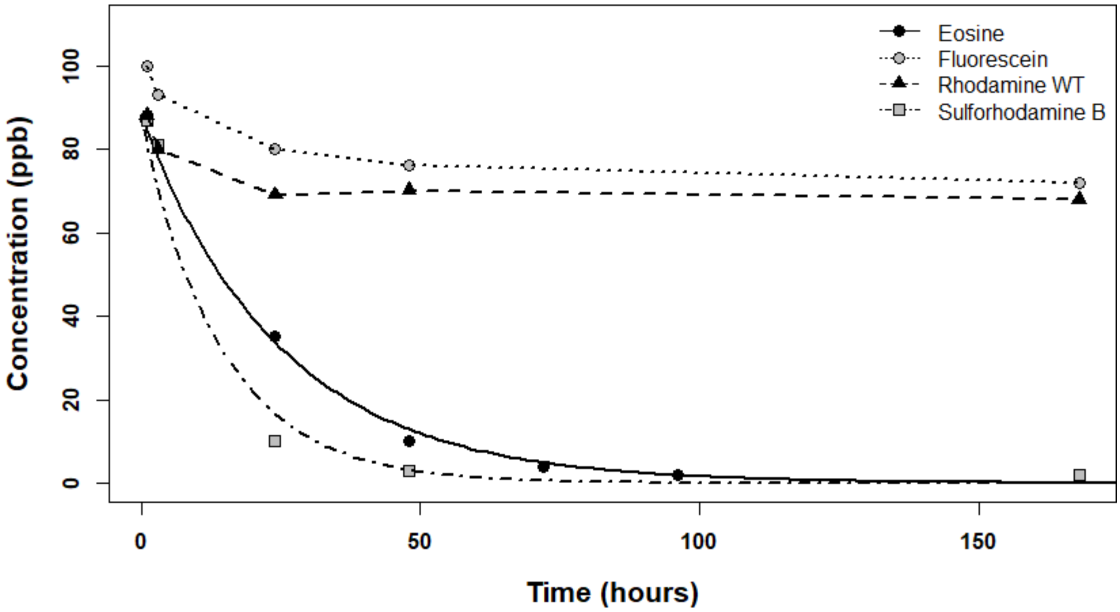


Figure 8 - Percent of detectable dye in solutions with zero-valent iron (ZVI).

The results in Table 5 show that ZVI has the capacity to degrade or destroy eosine and sulforhodamine B dyes as a function of contact time. Decreases in fluorescein and rhodamine WT probably indicate that fluorescein and rhodamine WT are experiencing about 30-percent adsorption, which would lead to an expected retardation factor of about 1.5 if these dyes were transported under conditions like those in the bench test.

The emission fluorescence peaks of three of the four tested dyes were not altered appreciably by 168 hours of exposure to ZVI. In contrast, the emission fluorescence peaks for eosine in the presence of ZVI, shown in

Table 6, declined by 10.8 nm after 168 hours of exposure. The acceptable wavelength range for eosine in untreated water samples was 532.5 to 537.0 nm.

Table 6 - Emission fluorescence peaks for eosine dye mixture exposed to zero-valent iron (ZVI).

Exposure time (hours)	Emission fluorescence peak (nm)
1	534.1
3	534.1
24	533.5
48	531.7
72	527.6
96	525.2
168	523.3

The OUL has encountered decreases in emission fluorescence peaks for eosine in both water and carbon sampler elutants from locations where the water has apparently encountered reducing conditions. Eosine is a brominated fluorescein molecule (as shown in Figure 5). Under reducing conditions, eosine is subject to reductive debromination where the bromine ions are apparently cleaved from the molecular structure. As eosine becomes sequentially debrominated, the peak fluorescence emission wavelengths become shorter and closer to that of fluorescein. If eosine were fully debrominated, the resulting molecule would be fluorescein. The OUL has never encountered a case where eosine being used in a groundwater trace was fully debrominated.

Reductive debromination of eosine could result in this dye being useful in assessing the effectiveness of ZVI reactive barriers. Eosine and water could be introduced on the upgradient side of the barrier and analyzed for in samples from wells on the downgradient side of the barrier. If eosine were detected and/or if it had emission fluorescence peak wavelengths in the normal range for eosine or only slightly shorter, then one could infer that the barrier was ineffective along the flow system followed by the eosine-tagged water. Such a tracer study could be coupled with a trace using fluorescein to mark the arrival time of that dye in case the eosine was removed or destroyed.

Rhodamine dyes are subject to molecular changes that cause their emission fluorescence peak wavelengths to become shorter. This process is called deaminoalkylation and occurs when amino groups are cleaved from the molecular structure of the fluorescent dye. Deaminoalkylation likely impacts all rhodamine-based dyes, but it appears to affect rhodamine B more than the other dyes. Rhodamine B is a cationic dye and is not suitable for most groundwater-tracing work. All practitioners implementing dye-tracing studies with rhodamine WT and sulforhodamine B should be aware that deaminoalkylation could shorten the peak emission wavelengths of their dyes, as discussed in Box 9⤵.

2.8.4 Tracing in Non-Neutral pH Water

Variations in pH can have significant impacts on the fluorescence intensity of groundwater-tracing dyes. The maximum fluorescence intensity for all five dyes discussed in this book occurs at pH of about 9.5. Figure 9 illustrates the effects of pH on the fluorescence intensities of eosine, fluorescein, pyranine, and rhodamine WT. Sulforhodamine B is not shown because, for practical purposes, it maintains its maximum fluorescence at pH values between 3 and 11 (Kass, 1998).

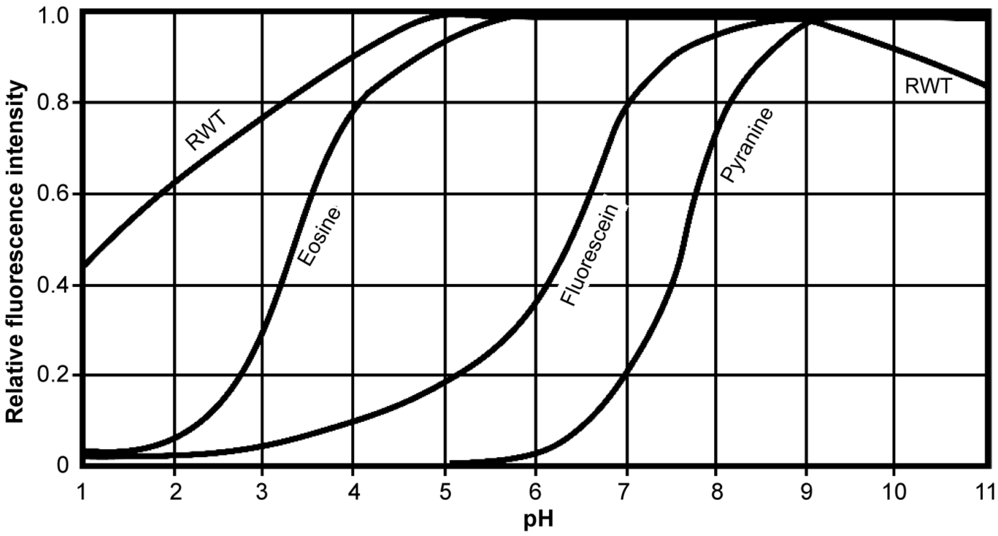


Figure 9 - Relative fluorescence intensity of dyes as a function of pH; RWT=rhodamine WT (Modified from Kass, 1998, p. 57).

Table 7 provides threshold pH values below which fluorescence is significantly decreased and mostly eliminated for each dye. Dyes can be used at pH values between the two values shown in Table 7 if the influence of pH on fluorescence is adequately considered.

Table 7 - Influence of pH on fluorescence intensity.

Dye	Fluorescence substantially decreased at pH less than:	Fluorescence mostly eliminated at pH less than:
Eosine	4.0	2.5
Fluorescein	6.5	5.5
Pyranine	9.5	6.5
Rhodamine WT	5.0	2.5
Sulforhodamine B	2.8	2.0

If groundwater tracer studies using fluorescent dyes are conducted in water with a pH value outside the range of most natural water (pH 6.5 to 8.0), we recommend that laboratory bench tests using the water in question be conducted as an initial phase of the project. A practical example of how bench testing can be important to the success of a groundwater-tracing project is provided by testing of water from an inactive metal mine in California (USA), as discussed in Box 10⤵.

2.8.5 Temperature

Fluorescence intensity varies inversely with temperature. The inverse relationship between temperature and fluorescence intensity is dye specific and generally takes the form shown in Equation (1).

$$F = F_o e^{-n(T-T_o)} \tag{1}$$

where:

F = fluorescence intensity at temperature T in °C (photons per unit area and time)

F_o = fluorescence intensity at T_o (assumed to be 0 °C) (photons per unit area and time)

n = constant for a given dye ($°C^{-1}$)

A list of the exponents for each of the five dyes, originally reported by Smart and Laidlaw (1977), is shown in Table 8. Figure 10 provides a graphical representation of temperature effects on fluorescent intensities.

Table 8 - Temperature exponents for tracer dyes. From Smart and Laidlaw (1977).

Dye	Temperature exponent, n in equation 1, $°C^{-1}$
Fluorescein	0.0036
Eosine	-0.00036
Rhodamine WT	0.027
Sulforhodamine B	0.029
Pyranine	0.0019

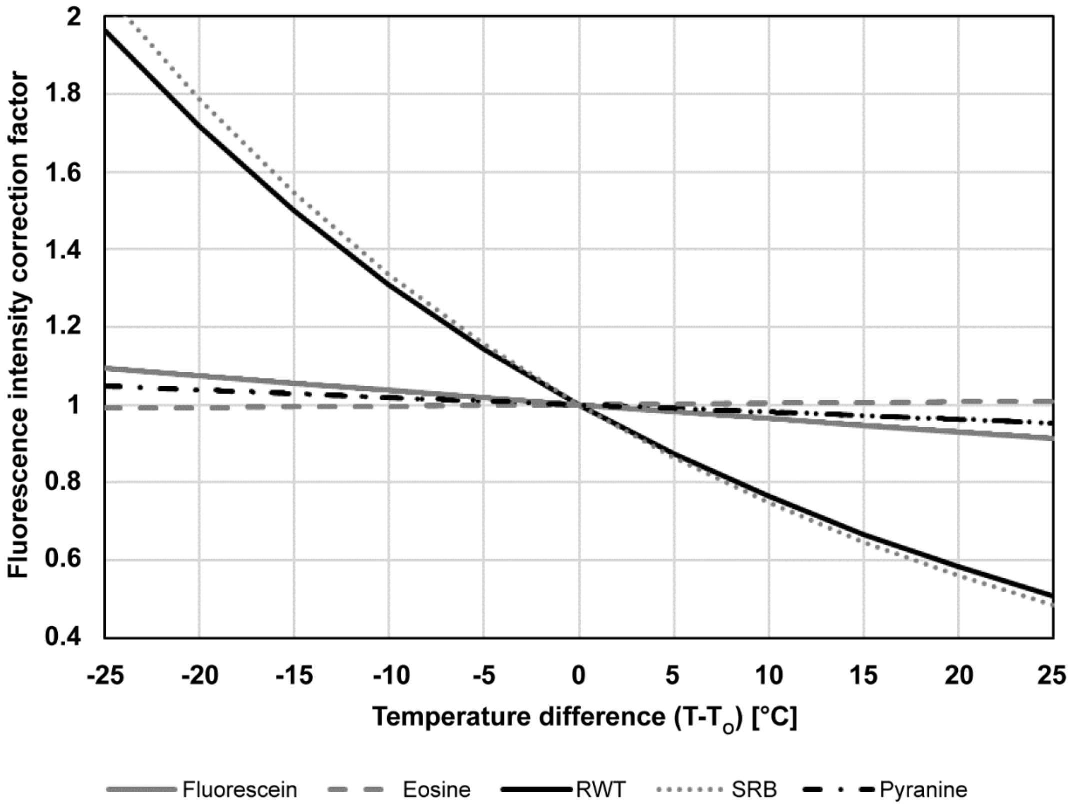


Figure 10 - Temperature correction curves for tracer dyes.

The influence of temperature on fluorescence intensity in practical tracing work is a minor issue. One laboratory that conducts tracer dye analysis specifies that they conduct all analysis at 30 °C. This is a strange protocol, because fluorescence intensity decreases with increasing temperatures for four of the five dyes considered in this book. The more common laboratory protocol is to store and analyze samples at approximately 4 °C. Used carbon samplers can be frozen for longer-term storage.

Fluorescein has been used successfully in tracing thermal groundwater. It has also been added to wastes that must be incinerated at high temperatures. After a waste burn, water is sprinkled on the ash and if any fluorescein color is seen, the ash is reburned.

2.8.6 Sorption to Earth and Organic Materials

An ideal groundwater-tracing dye would be entirely conservative—that is, it would experience no sorption at all. No groundwater-tracing dye is completely conservative and therefore it is important to understand the sorption and transformation properties of each individual tracer. Sorption retards the movement of dyes through groundwater systems. Although the five tracer dyes discussed in this book are anionic compounds, all are subject to at least some adsorption onto earth materials.

Several variables control dye sorption, including pH, temperature, water quality, degree of water agitation, sediment concentration, sediment type, dye concentration, and dye type. One of the most important variables is dye concentration. Smart and Laidlaw

(1977) reported a substantial decrease in the percentage of dye lost to adsorping materials with increasing initial dye concentration (Table 9). A practical implication of this is that more dye is required for similar tracing results in turbid water than in clear water. Additionally, more dye is required when the turbidity is primarily due to organic matter than when it is due to inorganic sediments. Shaking dyes in water with varying concentrations of sediment was the method used in developing Table 9. The percentage of dye adsorption on sediments increases more slowly than the sediment concentration. Additionally, the percentage of dye adsorption is dependent on sediment concentration in addition to dye concentration.

Table 9 - Comparison of tracer dye sorption onto mineral and organic materials. Values are percent of the dye remaining in solution from a 100 µg/L initial solution. Adapted from Smart and Laidlaw (1977). Eosine was not tested.

Material	Sediment concentration gm/L	Fluorescein	Pyranine	Rhodamine WT	Sulfo-rhodamine B
MINERAL					
Kaolinite	2	98	95	89	88
	20	93	95	67	51
Bentonite	2	98	100	92	98
	20	87	98	79	--
Limestone	2	98	96	93	97
	20	94	85	66	76
Orthoquartzite	2	98	100	98	--
	20	98	87	90	--
ORGANIC					
Sawdust	2	86	70	81	92
	20	11	30	42	--
Humus	2	83	76	82	92
	20	17	31	11	63
Heather	2	41	74	81	--
	20	0	18	18	--

An implication for tracer studies is that if a dye trace is replicated with twice as much dye, the resulting concentrations of detected dye will increase by more than twice. Mass balance calculations of dye traces to springs have routinely shown that the largest percentages of detected dye occur when large masses of dye are introduced. A case in point was a trace by Glenn and others (2013) that introduced 154.5 kg (340 lb) of fluorescein dye mixture with a 77-percent dye equivalent and accounted for 64 percent of the dye discharging from springs up to 932 m (3,057 ft) distant.

Practical examples illustrating the importance of dye concentration on sorption have been demonstrated through replicate traces in a karst aquifer conducted by OUL using twice as much dye. The resulting dye concentrations at sampling stations were substantially more than double the concentration of the initial trace. This is consistent with the observation that dye traces to karst springs, where a high percentage of the dye introduced is detected, typically involved unusually large amounts of dye. Larger than usual amounts of dye were commonly associated with traces where the primary sampling reliance was on water samples rather than activated carbon samplers. This apparent non-linear relationship between dye concentration and dye recovery results from the adsorption sites being overwhelmed by greater dye concentrations, allowing more of the dye to move through the system unimpeded. Additionally, dye–recovery percentages tend to be lower in karst aquifers at low flow rates than at high flows. The apparent reason for this is more and longer contact between the dyed water and substrates under low flow rates than under high flows allows for more sorption.

The data in Table 9 indicate that organic materials sorb much more dye than do inorganic sediments. Smart and Laidlaw (1977) commented that adsorption of anionic dyes is inhibited by repulsion from the negatively charged surface of organic matter. Greater adsorption is expected at low pH values, and the large concentrations of organic matter used in the adsorption tests lowered pH values in the tested water. Smart and Laidlaw noted that similar findings have been reported for the adsorption of organic pesticides on soils.

Fluorescein, eosine, and pyranine all have good resistance to adsorption onto inorganic material; in most cases, fluorescein has the smallest affinity for these surfaces. Rhodamine WT and, to an even greater degree, sulforhodamine B have greater affinities for inorganic materials; this is particularly noticeable when there is a larger area on which such contact can take place. For this reason, dyed water moving along preferential flow routes loses less dye to adsorption than when the flow routes are more dispersed. As an illustration, much less dye is lost to adsorption in deep clay-rich residuum with well-developed and integrated macropore drainage than in similar textured materials with less well-developed macropores.

The OUL conducted studies to compare rates of fluorescein and rhodamine WT dye sorptive losses to three different classes of materials routinely encountered in karst groundwater tracing. The materials were 1) surface soils collected directly beneath the leaf and humus layer of a hardwood forest, 2) silty clay loam sediments from a cave passage, and 3) pebbles from a cave stream. In these comparisons we use the symbol “>” to indicate that sorption onto the first material was greater than onto the second material and ”>>” to indicate that sorption onto the first material was much greater than onto the second. Rates of dye loss were as follows.

Fluorescein loss rates to surface soil >> cave stream pebbles > cave sediment.

Rhodamine WT loss rates to surface soil >> cave sediments > cave stream pebbles.

Extent of dye loss to surface soil was greater than with any other substrate. This is attributed to a combination of greater sorption and more biological decomposition of the dyes or deactivation of their fluorescence in surface soil. The loss of dye to cave stream pebbles is attributed to biological decomposition of the dyes or deactivation of their fluorescence. This attribution is based on experimental data showing that stream pebbles that had been sterilized by heating did not remove dyes from the test solutions (OUL bench test, unpublished). Cave sediments sorb rhodamine WT more readily than fluorescein. The rate of rhodamine WT dye loss to cave stream pebbles was greater than the rate of loss of fluorescein. The results of these substrate tests were partially verified by a groundwater trace from a sinking surface stream into a cave stream. The trace used similar quantities of both fluorescein and rhodamine WT dyes, and the straight-line underground distance was 1.22 km (4,000 ft). The percent of introduced fluorescein dye detected in both activated carbon samplers and in water samples was greater than the percent of rhodamine WT dye detected.

Lastly, when it comes to adsorption, it is important to understand the composition of rhodamine WT. This dye consists of equal amounts of two isomers, one of which has a much lower tendency to sorb than the other. This results in dual transport of RWT with half of it moving through the system as a relatively conservative tracer and the other half appreciably detained by adsorption. The movement of fluorescein and rhodamine WT through a soil column is shown in Figure 11, which is derived from Sabatini and Austin (1991). V/Vo in the illustration is the number of pore volumes of water passed through the soil column.

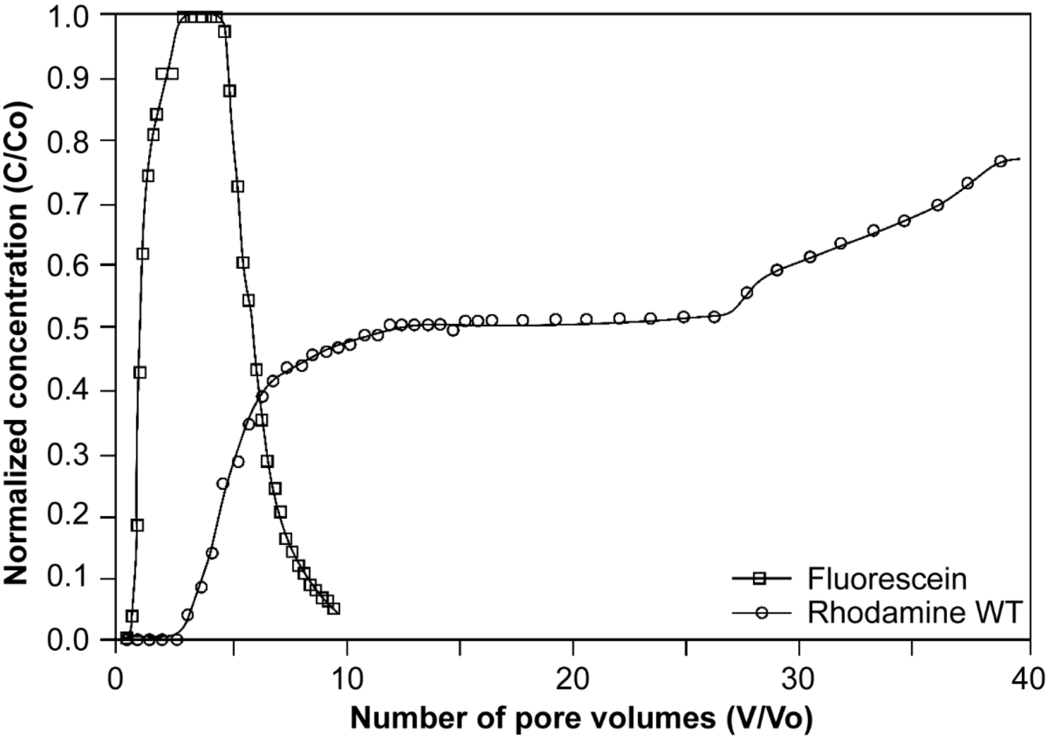


Figure 11 - Breakthrough curves for fluorescein and rhodamine WT through a soil column. Data from Sabatini and Austin (1991, Figure 6).

Generalizations for the tendency of different dyes to sorp based on OUL experience are as follows.

Tendency to sorb to inorganic material: SRB > RWT > Eos > Fl = Py.

Tendency to sorb to organic material: Eos > RWT = Fl > SRB > Py.

All five of the dyes experience some adsorption onto earth and organic materials. The percent of dye adsorbed onto sediment and organic material decreases with increasing initial dye concentration. As a result,

- increasing the amount of dye introduced will disproportionally increase the amount of dye detected in samples; and
- introducing dye as a short duration pulse followed by flush water will yield more dye at detection locations than introducing the dye mixed with the same volume of water with no subsequent flushing.

2.8.7 Retardation Factors

Intimately related to sorption is the concept of retardation factors. Retardation factors describe the magnitude by which contaminants are retarded (slowed) relative to water. The degree to which dyes or other compounds are retarded, commonly expressed as a retardation factor, is a function of chemical and aquifer properties. For porous media, Equation (2) is commonly used to estimate retardation factors.

$$\text{Retardation factor} = 1 + \left(\frac{\rho_b}{\theta}\right) K_d \qquad (2)$$

where (parameter dimensions are dark green font with mass as M, length as L, time as T):

p_b = dry bulk density of the soil (ML^{-3})

θ = total porosity (dimensionless)

K_d = distribution coefficient for the solute within the aquifer matrix (L^3M^{-1})

Understanding the retardation of various groundwater tracer dyes is critical to properly understanding the results of a given study. In most groundwater-tracing applications, a conservative tracer—that is, a tracer that moves with the water, having a retardation factor of 1—would be ideal. In other cases, a groundwater tracer with a known retardation factor might be useful for answering specific questions.

The tracer dyes are not perfectly conservative, but neither are most groundwater contaminants of concern. Retardation factors are not firm values but instead vary due to the nature of the geologic media and other factors. Table 10 provides reported retardation factors for the five fluorescent-tracer dyes and for some chemicals commonly found in contaminated groundwater. These values have been taken from the literature to highlight representative retardation factors under saturated conditions. These values were developed using various substrate types and therefore cannot be applied equally to all sites. The values illustrate that dyes are reasonable surrogates for common groundwater contaminants.

Table 10 - Reported retardation factors for five dyes and some common groundwater contaminants.

Constituent	Representative Retardation Factor (R)	Source
1,4 Dioxane	1.1	USEPA (2016)
MTBE	1.1	USEPA (2016)
Pyranine	1.11	Kass (1998)
Fluorescein	1.13	Kass (1998)
Benzene	1.3	USEPA (2016)
Eosine	1.45	Kass (1998)
PFOA	1.5	Concawe (2016)
Rhodamine WT	1.5*	Ptak and Schmid (1996)
Sulforhodamine B	1.6**	Kass (1998)
TCE	2.0	USEPA (2016)
PFOS	6.0	Concawe (2016)

* Representative retardation factor determined from an effective retardation factor of 1.34 compared to fluorescein transport as determined in the “Horkheimer Insel” field site. This factor would apply only to the more mobile of the two isomers in rhodamine WT.

** Representative retardation factor determined from an effective retardation factor of 1.4 compared to fluorescein transport as determined in the Merdingen Test Field.

2.8.8 Destruction by Sunlight

Sunlight degrades all five dyes, but the degradation rates for rhodamine WT and sulforhodamine B are much lower than for the other three dyes. As a result, rhodamine WT and sulforhodamine B are potentially good dyes for tracer tests involving water likely to be exposed to sunlight for extended periods of time. This is illustrated in Box 11↴. Pyranine, eosine, and fluorescein are all subject to relatively rapid degradation caused by the ultraviolet light in sunlight.

Smart and Laidlaw (1977) summarized degradation rates for fluorescein and pyranine. They reported that pyranine is degraded slightly faster than fluorescein. Based on four measurements of degradation rates for fluorescein under sunny conditions and three measurements under cloudy conditions, the mean degradation rate under sunny conditions is about seven times greater than the rate under cloudy conditions. Kass (1998) reports that the degradation rates for eosine are about twice those of fluorescein.

Some tracer studies require introducing dye into surface water and sampling for it in groundwater. In some cases, surface stream segments a few kilometers long need to be dyed to test for leakage into groundwater. In other cases, dyes introduced into groundwater may discharge through seeps or springs into surface streams and sampling must be conducted in surface water some distance downstream of the suspected discharge point(s). The OUL has conducted many traces where these conditions exist and has routinely, and successfully, used fluorescein, eosine, and occasionally pyranine in such studies. OUL dye introductions into surface streams are frequently made shortly before sunset. Sunlight penetration into water decreases with increases in water depth, and often, sunlight does not penetrate more than a meter (3.28 ft) below the surface. Other natural water is well shaded by vegetation. In some cases, plant growth or tannic compounds in the water limit light penetration into surface water and thus limit dye degradation. The net effect of these conditions is that sunlight degradation of tracer dyes can be overcome by the timing of dye introductions or by using more dye than would otherwise be needed. In most cases, sunlight degradation of dyes does not limit the dyes that can be used in tracing programs.

2.8.9 Mass Balance Calculations

Thousands of dye traces have been conducted on karst springs. If flow rates of the springs receiving the water are recorded, and sufficient water samples have been analyzed, the percent of introduced dye that has discharged from the spring or springs during a tracer study can be determined by performing mass balance calculations. Aley (2017) reported the percent of introduced dye that discharged from springs in the USA during 15 traces with straight-line distances of 0.30 to 28.18 km (0.19 to 17.47 mi). The detected percentages ranged from 0.01 percent to 98 percent; the median value was 4.9 percent. Hauwert and others (2004) made similar calculations for 20 groundwater traces over distances ranging from 3.2 to 30.5 km (1.98 to 18.91 mi) in the Barton Springs portion of the Edwards Aquifer. The detected percentages ranged from 0 to 77 percent and the median was 4.2 percent, as shown is Figure 12.

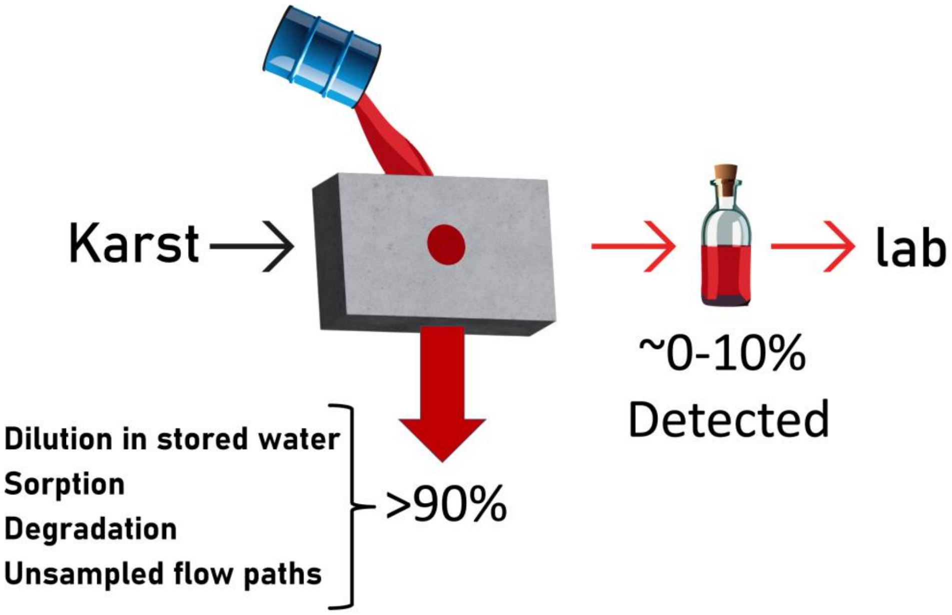


Figure 12 - Most karst traces result in detections of only a small fraction of the amount of dye introduced. Managing expectations is important.

The largest mass recoveries occur where large masses of dye were introduced for a trace of a kilometer (0.6 mi) or less and/or along a simple flow system with a well-documented flow path. Field (1999) introduced 7.0 kg (15.4 lb) of rhodamine WT dye mixture into a solutional karst conduit for a traced distance of 300 m (984 ft) to a spring with a flow volume of approximately 0.02 m^3/sec (0.7 ft^3/s), and this yielded a 96.6- to 98-percent dye detection. The plot of dye concentration with respect to time showed no indication of adsorption of the isomer in rhodamine WT that comprises 50 percent of the mixture and typically has a large retardation factor. In volcanic rocks and alluvium, a trace by Glenn and others (2013) introduced 154.5 kg (340 lb) of fluorescein dye mixture. It traveled 932 m and 821 m (3,057 ft and 2,693 ft) to two groups of springs and yielded a 64-percent dye detection. Sampling may not have tested all spring discharge points.

The epikarst is the weathered upper portion of the bedrock in a soluble rock terrane. Its thickness varies widely, but a common value is about 10 m (33 ft) (Ford & Williams, 1989). At waste sites in karst, a substantial portion of the interred wastes are detained within the epikarst. Aley (1997) divided epikarst water conditions into three categories: 1) rapidly draining epikarst; 2) seasonally saturated epikarst; and 3) perennially saturated epikarst. He reported that dye detection rates were typically 1 to 10 percent in the seasonally saturated epikarst and 0.1 to 1 percent in the perennially saturated epikarst.

2.8.10 Reasonable Cost

The dye cost for a fluorescent tracer study using technical-grade dyes is typically under 1,000 USD. For technical grade dyes used in groundwater tracing, the cost per kilogram of dye mixture is commonly less than USD 220/kg (USD 100/lb). Even when larger quantities of dye are required for a trace, the cost of the dye usually represents a small percentage of the overall project budget. Most of the costs involved in tracer tests are for field work involved in dye introduction and sampling, sample analysis, and reporting.

Attempts to minimize costs by using minimal amounts of dye and flush water can be shortsighted and risk project integrity, including false negatives. It is not unusual for traces to fail due to the introduction of insufficient dye quantities for the existing conditions. Technical publications routinely discuss tracing projects that succeed rather than those that fail, and many of the successful projects report higher dye-recovery values than are typical for most sites. As a result, those who are designing traces often overestimate dye concentrations likely to arrive at sampling locations. Concerns about not producing colored water at sensitive locations put additional pressure on those designing traces to minimize the quantities of dyes used and minimize the quantity of flush water introduced to move the dye into the aquifer. Finally, many tracing projects rely entirely on the analysis of water samples and do not include any sampling with activated carbon samplers. An important advantage of sampling for tracer dyes with both activated carbon samplers and water samples is that the carbon samplers substantially reduce the risk of study failures and missed dye-detection locations.

The quantity of dye needed for a trace varies with the type of dye. In most cases, fluorescein is the most effective and most detectable of the tracer dyes. Typically, a trace conducted with eosine will require at least 1.5 times more dye than if fluorescein were used. Traces conducted with rhodamine WT and sulforhodamine B typically require 4 or 5 times more dye than if fluorescein were used. These ratios are premised on the dye equivalent value for fluorescein, eosine, sulforhodamine B, and rhodamine WT dye equivalents being about 70 percent, 96 percent, 35 percent, and 20 percent respectively, as well as the overall detectability of the individual dyes. Pyranine requires a different analytical protocol from that used for the other four dyes. The OUL uses pyranine only in special cases where problems are anticipated with the use of the other four dyes.

Lastly, some laboratories that analyze for tracer dyes analyze for eosine, fluorescein, sulforhodamine B, and rhodamine WT in a single analytical scan and charge per sample. These laboratories will report on the presence and concentrations of all four dyes. If a laboratory using this approach is selected, there is no additional analysis charge for testing for more than one dye, and a second or third dye introduction using the same sampling points can be conducted at little additional cost.

2.9 Summary

1. This section focuses on important properties and characteristics of eosine, fluorescein, pyranine, rhodamine WT, and sulforhodamine B dyes. Traces can fail or yield incomplete results if the dye type and quantity selected are not appropriate for site conditions.
2. All dyes used in groundwater tracing come as mixtures that include diluents. The diluents are used to standardize the mixtures and, in some cases, aid in dissolving them in water. Common diluents are sodium sulfate, corn starch, and water. It is important to identify the dyes used by their Color Index Numbers and Names and by the percentages of the dye mixtures that are dye (i.e., dye equivalence). Technical-grade dye mixtures are fully adequate for groundwater tracing and are less expensive than those certified for other uses.
3. Table 2 lists detection limits for the five selected dyes under various conditions. The visual threshold concentration where the public will notice the dye in water is about 5 orders of magnitude greater than the instrumental detection limit. Water does not need to be visibly colored to have successful groundwater traces.
4. The five dyes discussed in this book have been extensively evaluated and are safe to use (Field et al., 1995). Three are also used to color drugs and cosmetics. When properly used in groundwater studies, they pose no risk to people, aquatic life, or the environment.
5. Regulation of dye tracing in the USA is minimal. In many states there are no regulatory controls applicable to tracer dyes when used in groundwater investigations. Some locales and states in the USA require notification to, and sometimes approval from, a local, regional, or state agency for planned dye traces. A good protocol is to inform appropriate organizations of planned dye-tracing investigations. This helps prevent interference between tracer studies.
6. In most cases dyes are very stable in the environment. Dyes can be degraded or destroyed by chlorination, and some dyes are rapidly degraded or destroyed by some groundwater remediation agents.
7. Eosine, fluorescein, and pyranine are all subject to fairly rapid degradation in sunlight; degradation rates are much slower for rhodamine WT and sulforhodamine B. Dyes can

be introduced shortly before sunset if traces need to be conducted from surface water into groundwater. Photodegradation of dyes is seldom a major problem and generally does not limit the type of dye used in tracing from surface water into groundwater.

8. Bench tests have shown that zero valent iron (ZVI) destroys eosine and sulforhodamine B dyes but that fluorescein and rhodamine WT can be used in the presence of ZVI.
9. Deaminoalkylation can degrade sulforhodamine B and rhodamine WT dyes and shorten peak emission wavelengths. This is likely to occur in reducing environments.
10. Water with pH values outside of the range for most natural water (pH 6.5 to 8.0) can impact dyes adversely. Bench tests to evaluate dye performance should be considered before starting tracer studies in atypical water, including acid mine drainage.
11. The fluorescence intensity of all five dyes except eosine decreases as water temperature increases. The temperature effect is greatest with rhodamine WT and sulforhodamine B.
12. All dyes experience some adsorption onto earth and organic materials. The percent of dye adsorption onto sediment and organic material decreases with increasing initial dye concentration. As a result,
    a) increasing the amount of dye introduced will disproportionally increase the amount of dye detected if sorption capacity is approached or exceeded; and
    b) introducing dye as a short-duration pulse followed by a given volume of flush water will yield more dye and higher dye concentrations at detection locations than introducing the dye mixed uniformly with the same given volume of water.
13. Rhodamine WT is composed of equal amounts of two isomers. One of the isomers is strongly adsorped onto earth materials and its arrival at sampling points will be delayed or may not occur during the study period. The other isomer is reasonably mobile in groundwater. This typically has the effect of the amount of dye used in a tracer test to 50% effectiveness.
14. Mass balance calculations for karst aquifers discharging from springs indicate median dye detections at discharging springs are about 4.5 percent of the amount of dye introduced. Detection percentages for tracer dyes in other hydrogeologic settings for similar travel distances are routinely smaller. Many traces have failed due to unrealistically large estimates of dye concentrations likely to reach sampling stations. A false negative due to inadequate design (e.g., dye application, monitoring locations/frequency, analytical detection limits) is a major risk for tracer tests.
15. Costs for dyes are reasonable. The amount of dye mixture needed for a trace is a function of dye type, dye equivalent in the dye mixture, and multiple site-specific and issue-specific considerations.

3 Sampling and Analysis for Tracer Dyes

3.1 Background Fluorescence

The five dyes discussed in this book are fluorescent compounds, and all sampling and analysis approaches utilize fluorescence characteristics to identify and quantify any dyes present. All water samples and elutants from activated carbon samplers will display some apparent fluorescence even if no dyes or other fluorescent materials are present. This apparent fluorescence, in combination with actual fluorescence (absent the dyes), is typically called *"background fluorescence*."

The intensity of background fluorescence must be subtracted from total fluorescence intensity to determine if a tracer dye has been detected in a water or elutant sample and to then calculate the concentration of dye in the sample. While this is a simple and reasonably accurate task for samples analyzed with a spectrofluorophotometer operated under a synchronous-scan protocol, it can be subject to significant errors for fluorometers that produce only a single value in the emission range of the dyes of interest. The reason for this difficulty is that the intensity of background fluorescence can vary widely among sampling sites and between samples at each sampling location and sampling event.

Figure 13 illustrates the layout of a basic fluorometer. In fluorescence analysis, light is beamed into a sample to excite molecules of fluorescent materials, and the emitted light intensity is measured at 90° to the light beam. This maximizes the percent of light reaching the detector that is from sample fluorescence. Monochromators—or in older instruments, filters—limit the wavelengths being utilized to excite molecules in the test solution and limit the wavelengths reaching the detector.

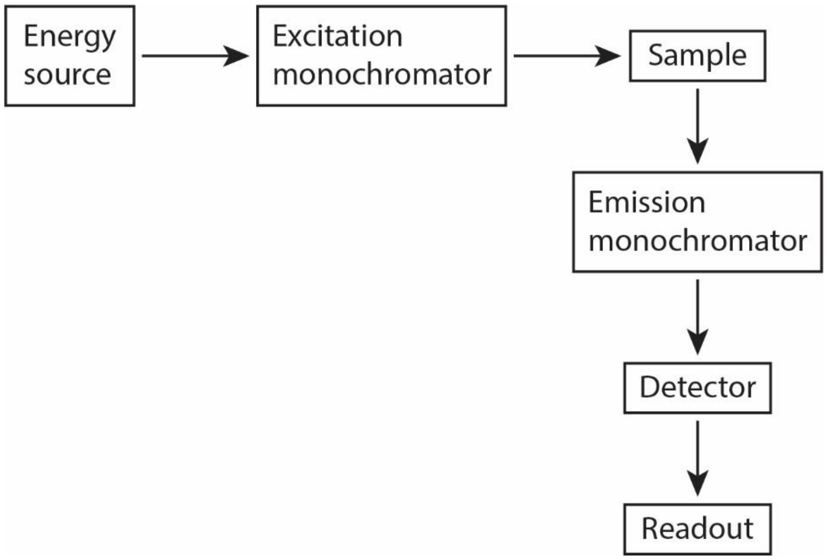


Figure 13 - Layout of a basic fluorometer.

The amount of light measured at 90° to the orientation of the excitation light beam is a combination of fluorescence and light scattering due to materials dissolved and suspended in the sample. In dye-tracing samples, these light-scattering materials include dissolved constituents plus suspended sediment, organic material, and colloidal particles. Except for medical-grade water, any water tested with a properly functioning and adequately sensitive fluorometer or spectrofluorophotometer will produce positive values that are commonly referred to as background fluorescence. The intensity of background fluorescence is due to the combination of fluorescence and light scattering, with light scattering often being responsible for most of the measured value. This is especially true for water samples from poorly developed monitoring wells or storm-related high flows from karst springs.

3.2 Sampling and Analysis Approaches

There are three basic sampling and analysis approaches that can be used for the tracer dyes discussed in this book. They are identified below, and each will be discussed in detail in this section.

1. Collection of water samples and fluorometric analysis with either field or laboratory instruments. If field instruments and fluorometers are used, then only fluorescein and rhodamine WT dyes should be used based on the state of technology in 2024.
2. Sampling with field instruments that record fluorescence intensity in the sampled water with one instrument and a separate probe for each dye. For this option, current (i.e., in 2024) instrumentation works only with fluorescein and rhodamine WT.
3. Sampling with activated carbon samplers that adsorp and retain tracer dyes. Laboratory methods are available to elute the five dyes discussed in this book from activated carbon. The recommended analytical method for dye analysis in both water samples and carbon elutant solutions is to use a spectrofluorophotometer under a synchronous-scan protocol. This method is described in detail later in this section under the heading “Analysis of Water and Carbon Samplers.” If only fluorescein is used, it is often visible in carbon sampler elutants.

Combined sampling methods are possible. This is often done by placing primary reliance on activated carbon samplers and secondary reliance on water samples collected each time activated carbon samplers are collected. Because carbon samplers are more likely than water samples to detect tracer dyes present in low concentrations, water samples are commonly analyzed only if dye is detected in associated carbon samplers.

Barry and others (2023) compared the three approaches in a Minnesota karst area. In that case, all approaches yielded useful information. However, this is not always the case at complex karst sites where tracing is conducted to identify springs and monitoring wells that receive dye from a particular location. An important distinction between springs and monitoring wells is that monitoring wells are commonly located anywhere in a network of solution openings, whereas springs occur at the downgradient point of an integrated network of solutionally-widened openings. In other words, springs occur where water converges; wells may not be similarly located. The result is that monitoring wells commonly receive much lower dye concentrations than springs, and studies that do not use an adequate amount of dye—and/or a sampling approach capable of detecting low dye concentrations—are unlikely to detect dye at all monitoring wells, even those that may be located within the flow path of the dye.

3.2.1 Water Samples

Water samples can either be collected as grab samples or with programmable automatic pumped samplers. Any sampling, but particularly automatic samplers, may expose samples to daylight, raising the issue of sample integrity due to photodegradation losses. While all five dyes are subject to some photodegradation, dissolved eosine, fluorescein, and pyranine are the most prone to this source of bias if exposed to sunlight. As a result, water samples collected in clear glass or plastic bottles must be stored in the dark until analysis. Photodegradation is slower in amber glass bottles than in clear glass but still occurs in the presence of sunlight or artificial light (Kass, 1998). If samples are kept in the dark, then brown and clear glass function equally well. A recommended protocol is to place all collected water samples in a cooler containing ice or a frozen gel pack as soon after collection as reasonable. If one is collecting water samples from an automatic sampler, the tray of bottles should be covered with a tarp as much as possible.

Some unique types of water can destroy dye in water samples, yet carbon samplers work adequately, as discussed in Box 12↴. Other unique types of water can destroy or elute dye from carbon samplers, yet water samples work adequately, as discussed in Box 13↴.

Automatic programmable water samplers, such as those routinely used for stormwater sampling and made by firms such as ISCO↗, are useful for collecting water samples at points where a detailed dye breakthrough curve is desired (Figure 14). Such instruments can collect samples at precise times or can be programmed to collect composite samples. Common instruments can collect a total of 24 individual samples. Samples may remain in the sampling instrument for a week or longer depending on the sampling interval used. This can raise concerns that dyes and their fluorescence intensities may deteriorate while stored in the sampling equipment. An approach for evaluating this possibility is to take only part of the most recently stored sample and leave the remainder in the instrument and collect it during the next visit. Both samples are analyzed to determine if there has been a measurable decrease in the dye concentration through time. In the experience of the OUL, dye deterioration while water is stored in automatic sampling equipment for a period of about a week is usually negligible but, in some cases, may depend on water quality. Preservatives should not be added to water samples that might contain tracer dyes.

Figure 14 - Photo of ISCO 3700 programmable water sampler. The bottom portion of the sampler, shown on the left side of the figure, contains 24 numbered bottles. The middle portion controls programming, and the top portion, shown on the right side of the image, provides a weatherproof cover. Water intake is through the white tube and connected tubing. A storage battery to power the unit is not shown.

In freezing weather, automatic water samplers are sometimes installed in small, heavily insulated enclosures with a heat lamp to prevent water from freezing. Heating options require caution. In one case, an electric blanket was wrapped around an automatic sampler and caused it to short out, resulting in a costly repair.

A subset of the samples submitted for analysis should typically be quality-control samples. Normal quality assurance/quality control (QA/QC) practice is to design a sampling program to have 10 percent of samples dedicated to field blanks and replicates (or split samples). This percentage may increase for programs involving small numbers of samples. The field blanks should include testing of sampling equipment and containers to ensure no tracer dyes are present in clean equipment. All types of plastic and glass bottles appear to function well for collecting dye samples. The OUL once encountered one of the rhodamine dyes in the rigid plastic used in Bakelite caps from glass specimen bottles.

Labels on collection bottles should be marked in black ink. Tracer dyes are present in some of the colored felt tip pens such as Sharpies and other permanent markers. Highlighter pens should not be in the possession of people sampling for tracer studies. Cords connected to bailers or other sampling equipment should be white; colored and black cords may contain tracer dyes that could leach into the water or onto carbon samplers during use. While it is reasonably difficult to contaminate or cross contaminate samples, field personnel should be cautioned about such risks and the care required because detection limits for tracer dyes are very low—that is, in the parts per trillion range.

As discussed in Section 2, the fluorescence intensity of eosine, fluorescein, and pyranine in water varies with pH and fluorescence intensity is used to quantify dye concentrations. As a result, water samples that may contain these dyes should be pH adjusted prior to analysis to maximize the fluorescence intensity of these dyes and to standardize dye-concentration determinations. Kass (1998) discusses this in substantial detail, and the pH ranges that will yield maximum fluorescence intensity for the dyes are discussed in Section 2.

3.2.2 Fluorescence Measuring Field Instruments

In the experience of the OUL, measurements of small concentrations of fluorescein and rhodamine WT in water samples with field fluorescence units yield both false positive and false negative results. The false determinations are greatest when concentrations are within an order of magnitude of the reported detection limit for the field instrument. False positives are common when the instrument is used at a waste site where manmade compounds are likely present. Air bubbles are a problem with field instruments because they produce erratic false positives. Both false positives and false negatives can occur at the same monitoring point in different samples. Differences between results from commercial field instruments and laboratory analysis results are largely due to variations in background fluorescence intensity and the presence of other fluorescence compounds in the water being sampled. The incidence of false results at low dye concentrations can be reduced by more extensive background sampling to better characterize fluorescence variability and by using more dye than would be required for analysis with laboratory-grade instrumentation. A good protocol for studies using field instruments is to submit some field-tested samples to a qualified laboratory for analysis.

Some field units can detect both fluorescein and rhodamine WT using sensors that do not operate simultaneously. Our search of available instruments indicates that a single instrument cannot record both dyes at the same time. Both handheld and submersible field units are available. The ability to monitor both dyes may encourage some workers to use both dyes concurrently. A risk of this practice is that relatively large concentrations of one of these dyes can appear to be a small concentration of the other dye on fluorometers. In addition, with these instruments the presence of eosine in the water could falsely indicate the presence of some fluorescein, and the presence of sulforhodamine B could indicate the presence of some rhodamine WT. As a result, care must be taken when using field instruments if there is a possibility that other interfering fluorescent compounds might be present. Costs in 2022 for base models of handheld and submersible field units with data loggers are in the range of 2,000 USD to 4,500 USD.

A benefit of a field unit is its ability to quickly obtain preliminary results that can guide further sampling or other activities. A correction factor can be applied to data from a fluorometer if some split samples are sent to a qualified laboratory that uses a spectrofluorophotometer operated under a synchronous-scan protocol.

Field fluorometers measure total fluorescence and light scattering, not just dye fluorescence alone. They commonly have wide excitation and emission wavelength settings, and as a result they are not able to credibly discriminate between a tracer dye—either fluorescein or rhodamine WT—and the many other fluorescent compounds that may be present in the water, thus the reported concentration may vary substantially as a result of precipitation or runoff events. Recording field fluorometers are most useful for short duration and short distance traces—generally 200 ft (61 m) or less. They have proven useful for longer distances in karst and fractured rock aquifers. They can prove useful where details on the timing and shape of the dye breakthrough curve are required. These instruments function best when the fluorescence intensity of dye at the sampling point is expected to be at least two orders of magnitude greater than the maximum background fluorescence intensity of the water being sampled. They can be used for longer duration and longer distance traces if those sampling locations are also monitored for the specific tracer dye being used with water or carbon samplers that receive laboratory analysis.

3.2.3 Activated Carbon Sampling

The discovery that groundwater sampling for fluorescein dye could be conducted with activated carbon was reported in a one-page article by Dunn (1957) in an obscure speleological publication that qualifies today as grey literature. The paper described how the dye could be eluted from the carbon with a 5-percent solution of potassium hydroxide in ethyl alcohol and would then be visible in the resulting solution. Dunn reported that the adsorption of fluorescein from water onto carbon was nearly irreversible and that using this method would permit dye tracing with much smaller quantities of dye than conventionally used. All this was conveyed on only a single page!

Sampling in streams with standard OUL carbon samplers placed in water moving at different velocities indicates that dye adsorption in activated carbon samplers reaches its maximum adsorption rate when water velocities at the carbon sampler exceed about 1 m/min (3.3 ft/min). Above that velocity threshold the carbon continues to collect dye at the same rate. This is because the carbon is not filtering dye out of the water, rather it is adsorping dye onto its surface and the rate of adsorption is limited. If the process were filtration, then the greater the volume of water passing through the sampler the greater would be the amount captured in the sampler. The controlling factor is the amount of dye in the flowing water that is in direct contact with activated carbon surfaces. This also explains why carbon samplers placed in flowing water will routinely adsorp more dye than samplers placed in monitoring wells with similar concentrations of dyes; flow in wells is typically below the 1 m/min threshold.

There is no standardized design for activated carbon samplers. The basic approach is to construct samplers that contain a relatively small amount of activated carbon that provides good contact between carbon particles and the water being tested. Some workers have constructed samplers consisting of tubes filled with activated carbon. However, unless a water supply is run directly down the length of the tubing, this design would not be expected to provide maximum contact between the tested water and carbon particles.

More effective samplers are flexible packets made of fiberglass screening or possibly a fabric such as *milk filter socks* that contain loosely packed activated carbon particles. Milk sock fabric is single-ply polyester characterized by micro-sized pores that filter sediment out of raw milk without decreasing the flow rate. It is used in the dairy industry, but of greatest interest to dye tracing is its ability to prevent unwanted sediment from entering the sampler, though it could clog with sediment.

OUL carbon samplers are made with fiberglass screening such as that used on windows. It is readily available at hardware stores. As with all materials used in the sampling and analysis for tracer dyes, each roll of screening should be tested before use to ensure it contains none of the tracer dyes. Some screening is impregnated with insecticides, especially in malaria-prone areas. Advertisements indicate that the insecticide-treated screening can still kill mosquitos even after washing from several rainstorms. Treated screen wire should not be used for carbon samplers.

A colleague in Minnesota has long used milk filter socks to enclose activated carbon for carbon samplers. Advantages of using this material include excluding sediment from reaching the activated carbon and thus not requiring a processing step of washing carbon samplers to remove sediment. Colloidal material would still be expected to pass through the milk filter sock material. Another possible advantage would be the ability to use smaller activated carbon particles than is possible with fiberglass screening. This would increase the amount of surface area on the activated carbon relative to the weight of the carbon. Milk socks that work well for manufacturing carbon samplers are Ken Ag Breakproof 45 Socks, which are used in piping of milking systems; there are multiple suppliers listed on the internet.

A likely disadvantage of milk filter sock fabric as compared to fiberglass screening is that milk filter socks are designed to capture sediment. During the time a sampler is in place, sediment could clog openings in the fabric and reduce the rate of water movement into and through the sample packet. This would reduce the amount of contact between activated carbon and dyed water and would be expected to limit the adsorption of dye. Regardless of whether carbon samplers are made from fiberglass screening, milk filter socks, or some other highly permeable material, loosely packed carbon in samplers is essential. Loosely packed carbon promotes more contact between the water being tested and carbon particles than tightly packed carbon, and as a result it will routinely adsorp more dye.

To our knowledge, nobody has conducted a study to indicate whether milk filter sock material or fiberglass screening provides the better material for carbon samplers. That would be a worthwhile investigation. Since the authors of this book have direct experience with carbon samplers made with fiberglass screening, further discussions about carbon samplers will be based on samplers made with this material.

Carbon samplers made of fiberglass screening can be heat sealed using commonly available heat-sealing equipment. Alternately, the packets can be stapled on three sides. OUL carbon samplers are made from fiberglass screen squares that are 11.4 cm (4.5 inches) on a side. The squares are folded to make a rectangular packet approximately 11.4 cm (4.5 in) long by 5.7 cm (2.25 in) wide. They are heat sealed on the long side and one end, loosely filled with 4.25 grams of activated carbon, and then heat sealed on the remaining end to make the completed sampler. The OUL makes small cylindrical samplers for use in wells with inside diameters less than 2 in (5.08 cm). They contain the same amount of activated carbon as the rectangular samplers, but the carbon particles are packed closer together. When these samplers are placed side by side in flowing water with the rectangular packets, OUL data indicate that they adsorp somewhat less dye than the rectangular packets. Even smaller carbon samplers are constructed for 2.54 cm (1 in) diameter piezometers. Figure 15 shows the three types of activated carbon samplers manufactured and routinely used by the OUL.

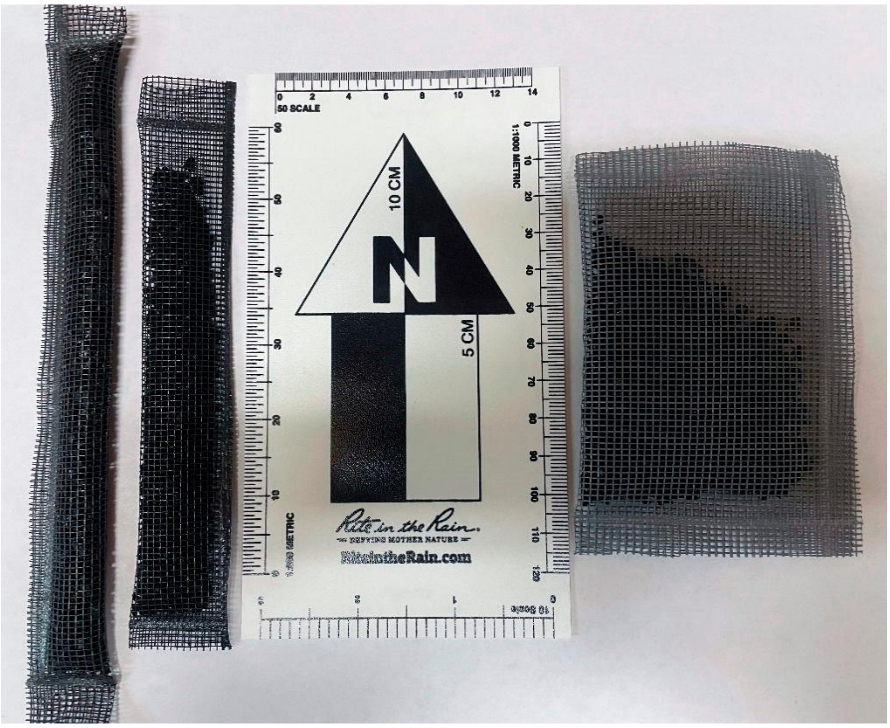


Figure 15 - Activated carbon samplers used by the OUL. The sampler on the left is used for 1-inch-diameter wells, the middle sampler for wells less than 2 inches in diameter, and the sampler on the right is used for wells 2 or more inches (5 or more cm) in diameter and in almost all other sampling locations.

Carbon samplers used in wells are typically attached to disposable bailers with dye-free, plastic-tie wire. It can be purchased in garden supply stores and is used to tie plants to stakes or fences. It should be tested before use to ensure it does not leach interfering fluorescent materials to the carbon. The carbon in the larger packets is typically divided into roughly equal amounts at both ends of the packet and tied in the middle to anchors; the samplers then have a bowtie shape. Installations in springs and surface water are anchored with plastic-tie wire or other materials to something firm in the channel.

The activated carbon used by the OUL is Calgon 207C coconut shell carbon 6 to 12-mesh (mesh size indicates the dimensions of square openings that the carbon particles have been sieved through, 6-mesh openings are 3.35 mm; 12-mesh openings are 1.52 mm). Based on manufacturer's data, the 4.25 grams of carbon in each OUL sampler has over 0.4 hectares (1 acre) of surface area. Sampling by the OUL indicates that approximately 3 percent of the carbon purchased will fall out of the samplers after manufacturing. The abundance of smaller particles of carbon increases near the bottom of the containers received from the carbon suppliers. These smaller particles are removed by using fiberglass screening to sift out and discard the smaller particles. Fiberglass screening is sufficiently sturdy that carbon samplers are seldom damaged when in place in flowing water moving at velocities up to 0.5 m/s (1.5 ft/s).

Activated carbon made from coal has been tested by the OUL and found to work less well for dye-adsorption purposes than the coconut shell carbon. For the activated carbons tested, the coconut shell carbon was slightly harder than the coal carbon and thus less likely to be lost to abrasion, and the coal carbon contained 14 percent ash whereas the coconut shell carbon contained only 3 percent fine material. The OUL has bench tested other mesh sizes of activated carbon and found them to work less well in adsorping dyes than the coconut shell carbon the OUL uses. However, there is a vast array of activated carbon on the market, and it is possible that other highly effective adsorpants are, or may become, available.

The size of carbon samplers, the amount of activated carbon they contain, and the amount of solution used to elute dyes from the carbon, are based on several considerations. Based on bench testing with the eluent solution the OUL uses, it was found that 60 minutes was effective for eluting combinations of eosine, fluorescein, rhodamine WT, and sulforhodamine B dyes from carbon samplers. The OUL found that a minimum of 10 ml of elutant sample was needed. Some solutions need to be centrifuged, and the volume placed in a cuvette for analysis is 3.5 ml; use of 10 ml allows for a second analysis and a decrease in sample volume if the sample were centrifuged. To be certain of obtaining at least 10 ml of elutant, it was decided to use 15 ml of eluting solution on each tested carbon sampler. Laboratory glassware exists that makes it easy to accurately pour 15 ml from a 250-ml flask into disposable beakers containing activated carbon from samplers that have been washed in strong jets of chlorine-free water to remove as much debris as possible. Bench testing indicated that maximum dye concentration was attained in 15 ml of eluting solution when samplers contained approximately 4.25 grams of activated carbon, and this became the basis for the quantity of carbon used in each OUL sampler. Knowledge of the approach used by the OUL can aid others in designing or evaluating other approaches.

It is important to maximize contact between dyed water and individual granules of activated carbon. In the opinion of the OUL, the dimensions of the fiberglass packets are adequate to loosely hold 4.25 grams of activated carbon. As discussed previously, tightly packed carbon samplers adsorp less dye than loosely packed samplers, and too much eluting solution dilutes the concentration of dyes in the resulting elutant. The relative amounts of activated carbon and volume of eluent used is specific to the type of carbon the OUL uses. A re-evaluation of the amounts of activated carbon in samplers, the size of the samplers, and the volume of eluent used for each sample is recommended for those who use different materials.

If there is very slow replacement of water in direct contact with the activated carbon during sampling, as is the case in many monitoring wells, the amount of dye adsorped on activated carbon samplers will be less than if the exchange rate of water in the well is more rapid. In some cases, it is desirable to sample for tracer dyes in slow recovery wells with both carbon samplers and grab samples of water, especially if laboratory detection limits are lower for water samples than for carbon sampler elutants. Longer deployment times of the carbon packets in slow water exchange wells is another option to obtain detectable levels of dye in some cases.

Carbon samplers placed in springs and streams can be lost to storm events or damaged by crayfish, beavers, muskrats, or other animals. As a result, we recommend placing at least two independently anchored carbon samplers close to each other at springs and surface streams. In some cases, placing a third carbon sampler where it is unlikely to be lost to high flows is a good protocol even if the sampler is not placed in a location where it is exposed to representative flow conditions. Such *standby samplers* are analyzed only if the main samplers placed in preferred locations are lost or damaged. When carbon samplers are collected and new samplers deployed, field personnel should be instructed to indicate which sampler appears to have been placed in the most representative location for flow conditions. OUL practice involves identifying the optimally placed sampler by folding it double when placed in the collection bag. An alternate approach is to put a staple in the corner of the sampler that is to be analyzed. The laboratory is informed of these practices so they select the proper sample for analysis.

We recommend using only one carbon sampler at a time in monitoring wells. If two carbon samplers are placed in a monitoring well with low rates of water exchange, the two samplers could compete for the available dye in the standing well water, depleting the dye mass and leading to a negative bias in the analysis. If duplicate samplers are needed for QA/QC purposes, they should be from spring or stream stations rather than from monitoring wells.

Screened wells are designed so that water exchange with the surrounding geologic unit occurs only within the screened interval. As a result, carbon samplers in screened wells are routinely placed in the middle of the screened interval. If the screened interval is longer than approximately 6 m (20 ft) and flow is predominantly horizontal, competition for dye across the screened zone is likely to be insignificant and consideration should be given to placing two or more samplers in the screened interval at different elevations. Open-hole monitoring wells should generally be sampled halfway between the bottom of the well and the mean water level. Carbon samplers can be placed at multiple locations in deep open-hole wells with placement depths dependent on interpretations from the drilling logs. If multiple carbon samplers are used, the credibility of individual samplers is enhanced if the entire line of samplers is rapidly pulled from the well and placed on a clean disposable plastic sheet before collecting used samplers and replacing them with new samplers. The rapid removal of the string of samplers is important to minimize the likelihood of a sampler adsorbing dye from shallower intervals as it is removed from the well.

Monitoring wells are commonly sampled for tracer dyes by anchoring the carbon sampler to the top of a weighted disposable bailer at least 45.7 cm (18 in) long. A white nylon cord runs from the bailer to the top of the well; colored cords sometimes contain fluorescent dyes and should not be used. Cords approximately 3.2 mm (0.125 in) in diameter are ideal. Nylon fishing lines should be avoided because they can snag and break.

Again, duplicate samplers are not recommended for use in monitoring wells with short screens, as they have the potential to interfere with each other and decrease the amount of dye adsorped on each sampler thus creating a negative bias in the data. In contrast, we recommend at least two independently anchored carbon samplers be placed at spring and stream sampling points in case a sampler is lost or damaged. Duplicate samplers also permit analysis of the second sampler and calculation of relative percent difference (RPD) values. RPD values are a common measure of variability in laboratory analysis work. RPD is calculated as the difference in concentration of a sample and its duplicate divided by the mean of the two values; then multiplied by 100 to express it as a percentage.

3.2.4 Assessment of Tracer Dye Adsorption and Effectiveness of Carbon Samplers

The OUL conducted a bench test to address three basic questions about the use of activated carbon in tracer studies. The questions were:

1. How effectively do the standard OUL carbon samplers adsorp each of the four commonly used tracer dyes from non-moving water over a period of 1 week? Those dyes are eosine, fluorescein, rhodamine WT, and sulforhodamine B. The 1-week period is a common duration for carbon samplers to be in place during tracer studies, and non-moving or slowly moving water is common in monitoring wells.
2. How effective is the OUL eluting solution in eluting each of the four dyes from carbon samplers using the standard OUL protocol?

3. Are the rates of adsorption and elution of each of the dyes reasonably consistent over the wide range of dye concentrations likely to be encountered in tracer studies?

The evaluation used 24 1-quart capacity (0.95 L) glass jars, each filled with 810 ml of dyed water. Dye concentrations in the water were nominally 10, 100, 1,000, and 10,000 ppb for fluorescein and eosine dye mixtures and 100, 1,000, 10,000, and 100,000 ppb for rhodamine WT and sulforhodamine B mixtures. These dye mixtures were approximately 70-percent dye equivalent for fluorescein, 96 percent for eosine, 35 percent for sulforhodamine B, and 22 percent for rhodamine WT. Since emission fluorescence peaks for fluorescein and rhodamine WT are substantially separated from each other, the number of samples in the study was minimized by including both fluorescein and rhodamine WT in the sample jars but with the latter at concentrations 10 times those of fluorescein. The same approach was used for jars containing eosine and sulforhodamine B with the sulforhodamine B concentrations being 10 times larger than those for eosine. Serial dilutions were used to prepare lower-dye-concentration mixtures. Each dye mixture was prepared and analyzed in triplicate.

The glass jars were 8.9 cm (3.5 in) in diameter. A sampler containing 4.25 grams of activated carbon was suspended in the middle of each jar, and jars were sealed and allowed to stand undisturbed for seven days at a temperature of approximately 20 °C (68 °F). Samples were then prepared and analyzed using standard OUL protocols.

Figure 16 shows graphs of residual dye concentrations in the water of the test bottles after a carbon sampler had been suspended in each bottle for 1 week versus the initial dye concentration measured in water of each bottle. The slope of the linear regression for each dye provides the average percentage of total dye that was not removed by the carbon samplers from the test water if the decimal point were moved two digits to the right. Figure 17 shows graphs of mean dye concentrations eluted from the carbon samplers that had been placed in the bottles for a week to adsorp dye versus the initial dye concentration in the test water. The slope of the linear regression for each dye provides the average accumulation factor of the carbon samplers. The accumulation factor in this context is defined as the ratio of dye concentration measured in the carbon sampler elutant to that measured in the initial water sample. The elutant is highly effective at removing dye that has accumulated in the carbon thus the concentration in the elutant is substantially higher than the initial concentration in the bottle.

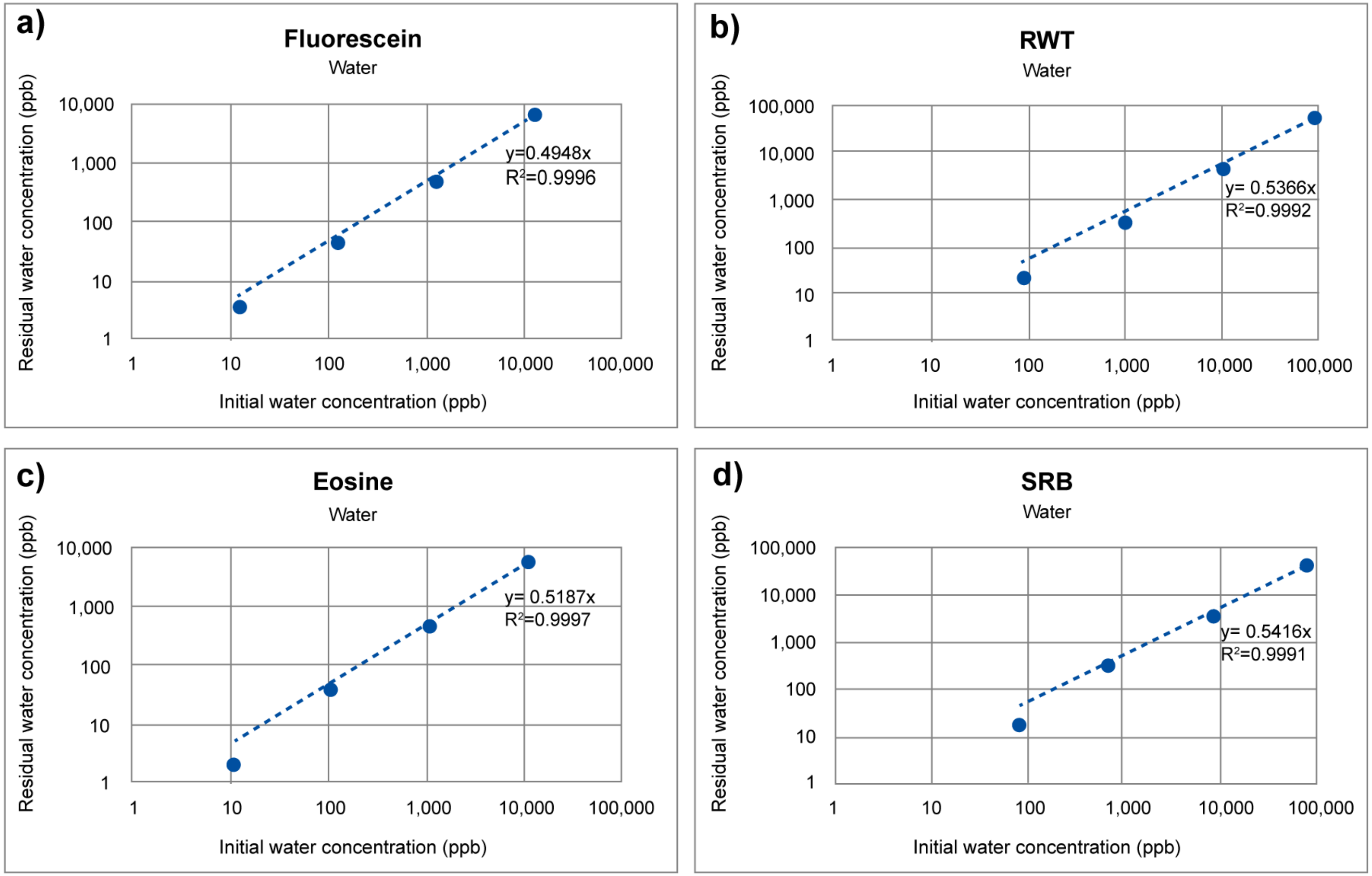


Figure 16 - Mean residual dye concentration in water as a function of initial water concentrations. The slope of the linear regression indicates the fraction of dye that was not removed by the carbon samplers from the test water.

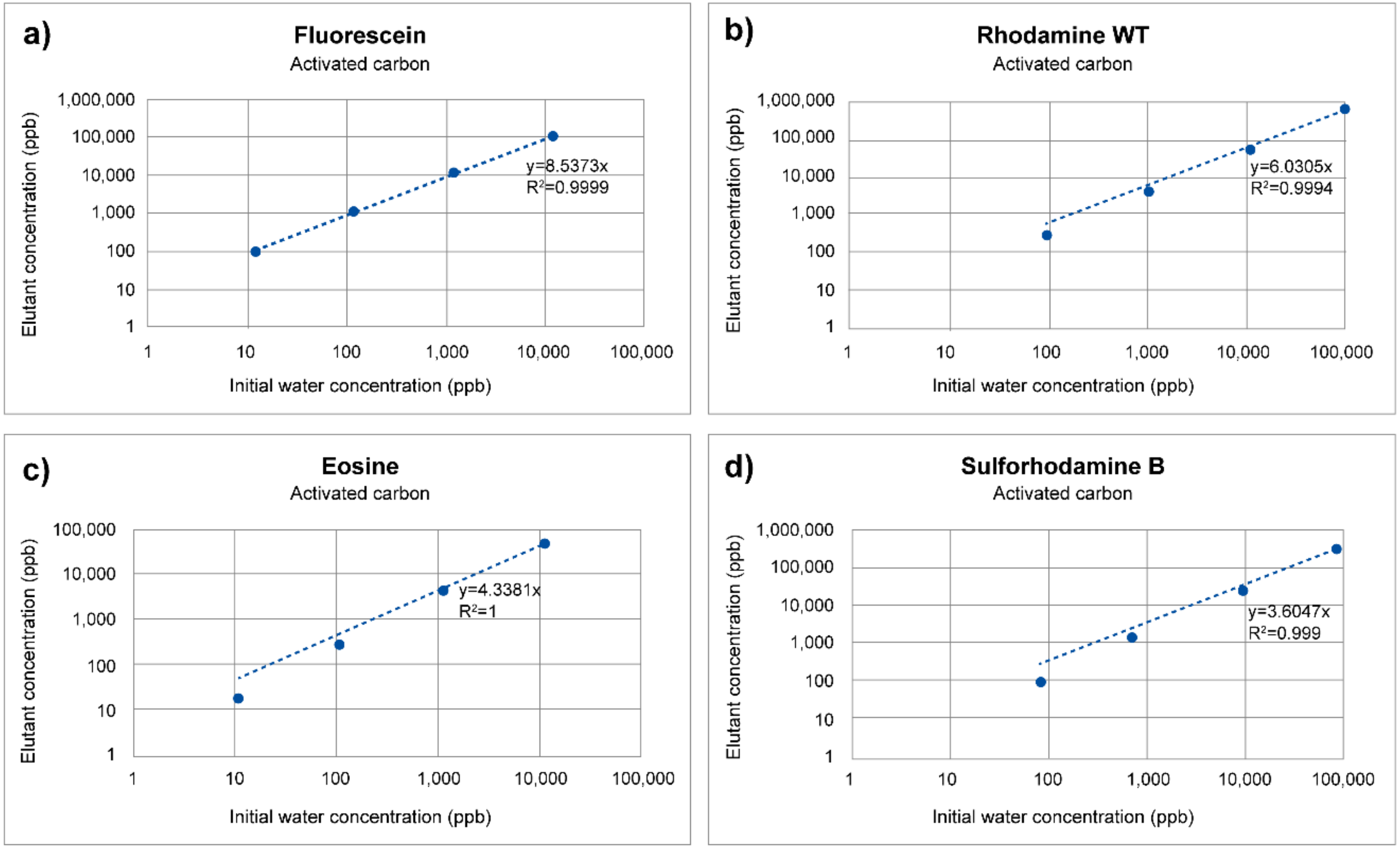


Figure 17 - Mean elutant concentrations versus initial water concentrations in the bench test. The slope of the linear regression indicates the accumulation factor, which is the ratio of dye concentration measured in the carbon sampler elutant to that measured in the initial water sample.

Table 11 combines results from Figure 16 and Figure 17 to show the mean percentage of dye adsorped by a carbon sampler in 1 week in the 810 ml of water in the test jars. The table also shows the mean accumulation factor of the carbon samplers in 15 ml of eluting solution.

Table 11 - Mean percent of dye in 810 ml of water that was adsorped by carbon sampler in place for 1 week and mean accumulation factor of adsorped dye subsequently eluted from carbon sampler.

Dye type	**Mean percent of dye adsorped by carbon samplers in 1 week** (**Figure 16**) $\left(\%\boldsymbol{Adsorbed} = \left[\mathbf{1} - \frac{\boldsymbol{Residual\ Dye\ Conc.}^{*}}{\boldsymbol{Initial\ Dye\ Conc.}}\right]\mathbf{100}\right)$	**Mean carbon sampler Accumulation Factor** (**Figure 17**) $\left(\boldsymbol{AF} = \frac{\boldsymbol{Elutant\ Conc.}}{\boldsymbol{Initial\ Water\ Conc.}}\right)$
Fluorescein	51%	8.54
Rhodamine WT	46%	6.03
Eosine	48%	4.34
Sulforhodamine B	46%	3.60
Mean of means	**48%**	**5.63**

*Conc. = Concentration

The bench test was simplistic and involved only a limited number of samples. Additional work as part of a more exhaustive study is needed, as is an evaluation of other eluting solutions. However, the developed data indicate that activated carbon samplers provide accumulated dye concentrations that are correlated to the mean dye concentrations in the water being sampled and that the response of dye accumulation to mean dye concentration is a power function, that is, it is linear when plotted on a log–log scale, over the concentrations tested.

Another observation from the bench test is that carbon samplers suspended for a week in the middle of a column of dyed water 8.9 cm (3.5 in) in diameter and 11.4 cm (4.5 in) in height yielded larger dye concentrations in the carbon sampler elutants than in the water of the test bottles. The bench test is reasonably analogous to conditions in monitoring wells with slow water-replacement rates and one or more tracer dyes. The results indicate that carbon samplers left in place for periods of at least 7 days provide credible monitoring in wells, even those with very slow rates of water exchange.

3.3 Desirable Features of Activated Carbon Sampling

Not all tracer studies can be conducted with activated carbon samplers. However, when tracer studies rely primarily on activated carbon samplers or use a combination of carbon samplers and water samples, the study has several important advantages over studies dependent solely on water samples. There are six important benefits of using activated carbon samplers and each will be discussed. The benefits of using activated carbon samplers are that they:

- provide continuous and cumulative sampling;
- decrease the amount of dye needed for successful traces;
- decrease risk of visibly colored water;
- improve determination of first arrival times and identify all receptors;

- lower costs of tracing projects; and they
- facilitate long-distance and large-area traces.

3.3.1 Activated Carbon Samplers Provide Continuous and Cumulative Sampling

One of the most important characteristics of carbon samplers is that they sample continuously and accumulate the dyes discussed in this book. Unlike grab samples of water, carbon samplers are less likely to miss short-duration pulses of dye at sampling points. This is critically important in many tracer investigations. By accumulating dye, carbon samplers left in place for an adequate period can yield positive dye detections even if the dye concentration in the water is below the detection limit in water samples. This is especially important in traces to springs with large flow rates and in groundwater systems with large volumes of water where the tracer dye may be heavily diluted.

Aley (2017) compared dye-analysis results from traces to springs and monitoring wells for sampling periods where both carbon and water samples were analyzed. Water samples were analyzed for the beginning and the end of each carbon-sampling period. For each sampling period, the dye concentration in the carbon sampler elutant was divided by the mean concentration in the associated water samples. If dye was not detectable in one or both water samples, the detection limit for that dye in water was used as the missing dye-concentration value. In this situation, the term "*accumulation factor*" was applied to the dye concentration in the carbon sampler elutant divided by the mean concentration in the associated water samples.

A common sampling interval in dye traces is seven days, plus or minus one day. Table 12, derived from data in Aley (2017), shows accumulation factors for springs and wells sampled for periods of 6 to 8 days and indicates detection limits. Accumulation factors were always greater than one in both springs and monitoring wells, and the accumulation factors were routinely greater at springs than in monitoring wells. This is explained by less water exchange in monitoring wells than in springs and the fact that samplers in wells routinely experience dye depletion in the water immediately in contact with carbon particles in the samplers because the water is not rapidly replaced as the dye is sorped to the carbon. The results in Table 12 are from OUL-directed studies, and differences in the accumulation factors among dyes are, at least in part, due to fluorescein routinely being used for longer distance and potentially more-difficult traces and rhodamine WT being used for shorter and potentially less-difficult traces. Aley (2017, page 44) reported that the median accumulation factor for carbon samplers placed in monitoring wells for periods of 6 to 8 days was 22 for fluorescein, 11 for rhodamine WT, and 5 for eosine. These values were based on a total of 383 sampling periods. The median accumulation factor for carbon samplers in place in springs for a period of 6 to 8 days was 102 for fluorescein, 255 for eosine, and 506 for rhodamine WT. These values were based on a total of 281 sampling periods.

Table 12 - Accumulation factors (AF) for carbon samplers in place for 6 to 8 days. There were insufficient samples for assessments of sulforhodamine B and pyranine dyes. These numbers vary from those listed in Table 11, because they were calculated using field data from a variety of study areas (data from Aley, 2017).

Dye fixture	Periods	Mean AF	Median AF
MONITORING WELL			
Eosine	93	38	5
Fluorescein	154	379	22
Rhodamine WT	136	28	11
Total	383		
Weighted mean/median		**166**	**14**
SPRING			
Eosine	106	415	255
Fluorescein	70	195	102
Rhodamine WT	105	658	506
Total	281		
Weighted mean/median		**445**	**311**

The mean and median accumulation factors for carbon samplers in springs are greater than those for wells because there is more rapid waterflow through samplers in springs than in wells. This results in less dye depletion in water in immediate contact with carbon particles in springs than in wells. There is a larger mean accumulation factor for carbon samplers in monitoring wells than in jars of water (values for jars are shown in Table 11) because there is more rapid waterflow through samplers in wells than in the jars used in the bench test. The weighted-mean dye concentration for carbon samplers in springs is only 30 percent greater than the median value, while it is nearly 12 times greater in monitoring wells. This is likely explained by a few wells having rapid water exchange and thus producing large accumulation factor values. Mean accumulation factors for springs are somewhat larger than median values because some carbon samplers were likely placed in water with velocities less than 1 m/min (3 ft/min); per discussion in this Section 3.2.3, *Activated Carbon Sampling*.

The Idaho Department of Water Resources, in cooperation with Idaho Power Company, has conducted multiple groundwater traces in lava flows of the Eastern Snake Plain Aquifer. Many of the traces used private wells as dye-sampling locations. An approach used to sample many of these wells has been to place activated carbon samplers in the reservoir tanks of toilets and leave the samplers in place for periods of up to several months. Depending on the frequency with which toilets are flushed, it is reasonable to expect that many of these sampling points are more effective in accumulating tracer dyes—that is, they experience larger flow-through volumes—than many monitoring wells. Sampling at toilets that receive the most use is appropriate. Drains on toilet tanks can be partially opened to increase water flow rates and provide better sampling for tracer dyes. A word of caution: cleaning and disinfection products are occasionally used in toilet reservoirs that color the water in the bowl. Some of these may be adsorped onto activated carbon samplers in toilet reservoir tanks.

A commonly expressed concern with carbon samplers used at waste sites is that the adsorptive surfaces on the carbon might be substantially reduced by adsorping other compounds present at the site and thus rendered incapable of effectively adsorping tracer dyes. The OUL has successfully detected tracer dyes in monitoring wells that had petroleum hydrocarbons floating on the top of the water column and other wells with free-product TCE in the bottoms of the wells. Placement of carbon samplers avoided free-product zones, and sampling intervals in such wells were never longer than about 1 week. A recommended approach for dye tracing at sites with large concentrations of contaminants likely to adsorp to activated carbon is to increase the dye quantity and leave samplers in place no longer than about 1 week. However, the authors know of successful tracer studies that have been conducted at waste sites where dye sampling occurred quarterly in monitoring wells and relied on activated carbon samplers. An excellent, easily understandable discussion of activated carbon in a readily accessible journal is provided by Lehr (1991). While it does not deal specifically with activated carbon samplers in tracer studies, it is recommended reading for those using these samplers in tracer studies.

Based on manufacturer data for the activated carbon used by the OUL, each 4.25-gm carbon sampler has in excess of 0.4 hectares (1 acre) of surface area capable of adsorping dyes and other materials that may be present in the water being sampled. Unless dye concentrations are much greater than needed for most dye traces, this large sorption capacity permits the carbon samplers to sample continuously for periods of at least 7 days and frequently longer. At hazardous waste sites carbon samplers routinely perform well for at least 7 days and for at least 14 days in monitoring wells that are not heavily contaminated. Carbon samplers perform well for durations of a month in flowing water from springs with few compounds that could be adsorped on the carbon. No maximum recommended exposure times for carbon samplers have been established, as discussed in Box 14↴.

3.3.2 Decrease the Amount of Dye Needed

Carbon samplers can be placed in water being pumped from wells. They will routinely accumulate substantially more dye than is typical for carbon samplers in monitoring wells. Recovery wells at waste sites or production wells can be sampled effectively if a flow of about 4 L (1 gallon) per minute is diverted from the main discharge and passed through a chamber or container with a carbon sampler. Flow from the chamber can be returned to the main discharge line (essential for recovery wells at waste sites), discharged to waste, or used to irrigate nearby vegetation. Figure 18 shows a simple pumping well sampler. Both ends of the sampler are connected to hoses.

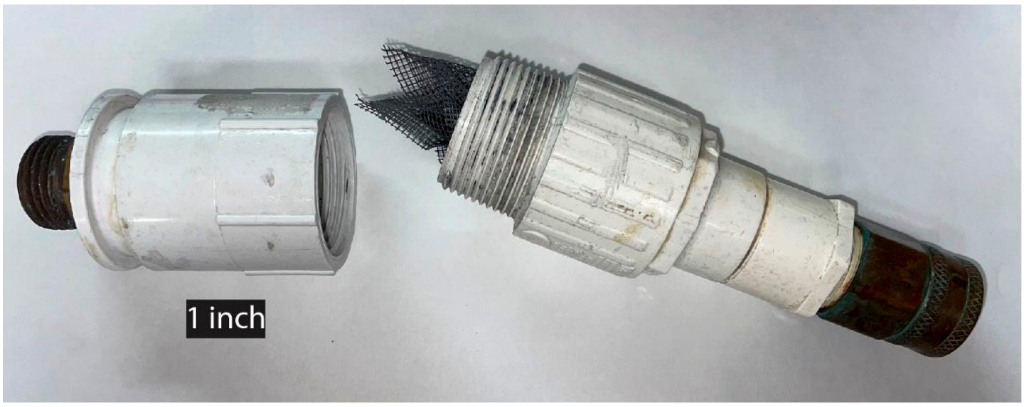


Figure 18 - Photo of carbon sampler holder with sampler; one inch=2.54 cm.

3.3.3 Decrease the Risk of Visibly Colored Water

By decreasing the amount of dye needed for successful traces, carbon samplers decrease the risk of visibly colored water. While the City of Chicago turns the Chicago River green with fluorescein every year in celebration of St. Patrick’s Day, dye-tracing investigations are sometimes opposed by people concerned about the possibility that water in nearby water supply wells, springs, or creeks may be colored. Although the dyes discussed in this book do not create problems in the amounts used in professionally directed investigations supervised by experienced people, it is rarely desirable to produce colored water visible to the naked eye at groundwater discharge points. Placing primary sampling reliance on activated carbon samplers is an effective way of minimizing the amount of dye needed for many tracing projects. Another effective action to consider is using eosine, which is less visually detectable than the other four dyes discussed in this book, as discussed in Box 15⤵ and Box 16⤵.

3.3.4 Improve Determination of First Arrival Time and Identify All Receptors

Carbon samplers accumulate dyes whereas water samples only reflect the dye concentration at the time the water sample is collected. The difference in data interpretation between carbon samplers and water samples is particularly important in studies where 1) the travel time for first dye arrival is essential and 2) identifying all monitoring points reached by the introduced dye is important. While both sampling approaches can accomplish this, the carbon samplers are less likely to miss an early first arrival of small amounts of dye that travel along a preferential flow route and arrive at a sampling point earlier than the larger mass of the dye. The precision of time to first arrival is a function of the frequency of sampling intervals. If well-constrained time to first arrival is an objective of the study, a variable sampling schedule is recommended. A commonly used sampling schedule is Day 1, 3, 5, 7, 10, 14, then weekly (dye introduction = Day 0).

In general, water samples are less likely than carbon samplers to detect small concentrations of dye at sampling points. The basic reason for such failures to detect dyes in water samples is that carbon samplers accumulate dyes and water samples do not. Field fluorometers require that substantially more dye be introduced for credible detections than is necessary for water samples with laboratory analysis, as discussed in Box 17⤵.

Aley (2017) compared sampling periods where both carbon samplers and water samples were analyzed and dye was detected in one or both kinds of samples. Data were presented for eosine, fluorescein, rhodamine WT, and sulforhodamine B dyes. Based on 1,002 sampling periods at springs with a mean sampling period of 15 days, dye was detected in 44.3 percent of the water samples and 98.9 percent of the carbon samplers. This indicates that dye concentrations in water were too small to be detected 55.7 percent of time, but that dye accumulated in carbon samplers and was therefore detectable with much greater frequency. If dye was detected in water samples for a sampling period, then it was also detectable in the associated carbon sampler 98.9 percent of the time.

In the case of monitoring wells, based on 939 sampling periods with a mean sampling period of 22 days, dye was detected in 80.9 percent of the water samples and 95.7 percent of the carbon samplers. All traces evaluated were designed for primary sampling reliance on activated carbon samplers and secondary reliance on grab samples of water. As a result, they used substantially less dye than would have been recommended for traces where sampling reliance was solely based on water samples, but also used more dye than if sampling had not planned to collect water samples, as discussed in Box 18⤵.

3.3.5 Lower Cost of Tracing Projects

In many cases the main purpose of a groundwater trace is to identify the location, or locations, that receive dye down gradient from a source area as well as the approximate time of first dye arrival at those locations. In such cases, placing primary sampling reliance on carbon samplers is an appropriate strategy that provides the needed information for the least effort and expense (Exercise 4⤵). Carbon samplers continuously monitor for dye and adsorped dye accumulates in the carbon samplers, facilitating identification of detection sites. The carbon samplers do not miss short-duration dye pulses and sampling schedule can be varied to better constrain time of first arrival. In contrast, sampling focused primarily on grab samples of water or on measurements with field fluorometric units typically require more dye, more site visits, and more analytical costs to ensure dye detections at all locations reached by the dyed water.

At waste sites with contaminants that can adsorp onto carbon samplers, one must expect the number of available activation sites on the carbon to decrease as the amount of time the samplers are in place increases. Even when this occurs, carbon samplers routinely work well, but should not be left in place for too long in order to prevent overloading the sampler. At waste sites, the OUL usually recommends recovery and replacement of carbon samplers approximately once a week. Sometimes logistics and available funding may require the frequency to be once every two or three weeks.

A risk of leaving carbon samplers in place for two weeks or longer is that they may be lost or damaged; their loss can create a significant gap in the resulting data set. Samplers in springs and streams can be lost to flood events. Beavers will incorporate carbon samplers in their dams where they are difficult to find and recover, and the results may be compromised. Crayfish and muskrats are suspected of damaging samplers in some locations. Children and fishermen sometimes find carbon samplers and, not knowing what they are, throw them up on the bank or into a trash can. Regardless of how carbon samplers are lost, their loss has the potential to damage the integrity of a trace by creating gaps in the data. The longer the gap, the greater the potential damage to the study. This risk is decreased if two or more independently anchored samplers are placed and recovered at each sampling visit; typically, only one sample is analyzed. The risk is also decreased by more frequent sampling visits, although this is often difficult and expensive in remote locations.

Carbon samplers can be left in place for periods of a few months in remote locations where there are limited interfering substances that are likely to be adsorped on them. In remote cold-weather locations, the OUL has sometimes placed carbon samplers prior to snowfall and recovered them (and adsorped tracer dyes) after spring runoff from snowmelt. A risk associated with this approach is that large flows during snowmelt periods can damage or dislodge samplers.

The US Environmental Protection Agency (Field, 2002) published a monograph and computer program for breakthrough curve analysis for tracer tests in karst aquifers and other hydrologic systems. It is designed for tracer tests totally reliant on grab samples of water and, as a result, requires the use of substantially greater quantities of tracer dyes and number of analyzed samples than traces reliant on activated carbon samplers. The Field (2002) document was used for guidance in a groundwater-tracing study at Lahaina, on Maui, Hawaii (Glenn et al., 2013) where 154.5 kg (340 lb) of fluorescein dye mixture with a 77-percent dye equivalent was introduced for traced distances of 821 m and 932 m (2,693 ft and 3,057 ft) to two groups of springs. That Hawaii program relied on grab samples of water. While a massive amount of data may impress an agency or a court, the critical information of where the treated wastewater discharged and the shape of the dye breakthrough curve could have been less expensively developed if sampling had placed primary reliance on activated carbon samplers. Most practical projects lack large budgets, as discussed in Box 19⤵.

3.3.6 Adequacy of Activated Carbon Sampling

A limitation to using carbon samplers in tracer studies is that the dye concentrations in sampler elutants cannot be directly converted to concentrations in water. This is because carbon samplers accumulate dyes rather than yield a dye concentration at a given point in time and for a well-defined volume. An obvious solution, if one needs actual concentrations at known times, is to collect both carbon and water samples. Having data from both kinds of samples strengthens the results of tracer studies and typically does not substantially increase project costs. However, as has been demonstrated by some of the case studies, more dye will need to be introduced if credible sampling results are to be gained from water samples, and even more dye for recording field instruments making fluorescence intensity measurements of water.

In some investigations, custody of samples may be an important issue. If a carbon sampler is in a locked well or similar structure, investigators have maintained good custody of the sampler. However, if a carbon sampler is placed in a surface stream or spring where continuous surveillance is not possible, then a good chain of custody has not been maintained. This can be important if legal actions are taken where the tracing work is a part of the evidence. The senior author of this book has experienced this in civil cases in both state and federal courts. Collecting and analyzing water samples each time carbon samplers were collected provided data that allowed him to conclude that the results from both types of samples were sufficiently consistent to conclude there had been no tampering with the carbon samplers. While this approach has been accepted by courts in the past, it may or may not always be adequate.

As discussed earlier in this book, relative percent difference (RPD) is a statistical measure of the accuracy of duplicate measurements. RPD equals the difference between two measurements divided by the mean of those measurements. Aley (2019) reports that mean RPD values for dye concentrations in water samples typically range between 1.7 percent and 6.0 percent and vary somewhat among the dyes. In contrast, mean RPD values for dye concentrations in carbon sampler elutants typically vary from 26 percent to 49 percent. Reasons for the higher RPD values for carbon sampler elutants are related to variability in the behavior of the carbon and differences in the positioning of carbon samplers in the water being tested.

A bench test in which carbon samplers were suspended in glass jars containing different concentrations of tracer dyes is discussed in Section 3.2.4. Mean RPD values for all dyes and dye concentrations was 14 percent, a relatively low value for carbon samplers; individual RPD values increased as dye concentrations in the test water decreased. These smaller-than-typical RPD values suggest that the larger RPD values typical of dye in carbon sampler elutants are largely due to factors in the field that impact the sorption of dye onto the carbon.

It has been suggested that concerns raised by relatively large RPD values from carbon samplers could be reduced by analyzing two carbon samplers for each sampling point for each sampling period. Either the mean value or the larger of the two values could then be used. The rationale for using the larger of the two values is that this approach would minimize the impact of a sampler that had yielded a dye concentration smaller than representative conditions. These approaches were evaluated using data from a Nevada case history, as discussed in Box 20↴.

Data from a groundwater-tracing investigation near Southeast Spring in Arkansas, USA, where both fluorescein and rhodamine WT dyes were introduced concurrently at the same location and detected at a spring provides another comparison of sampling based on both grab samples of water and activated carbon samplers, as discussed in Box 21⤵.

3.4 Handling of Collected Samples

Different laboratories have slightly different protocols for handling collected samples. The standard approach the OUL uses is to collect approximately 30 ml of water in new 50-ml capped centrifuge tubes made of research-grade polypropylene copolymer. Information written on the outside of the tube includes station number and name plus date and time of collection. *Sampling information is not written on the caps.* Activated carbon samplers are shaken to remove free water and then placed in sterile plastic bags, such as those made by Whirl-Pak, and sealed. Information on the outside of the collection bags includes station number and name, and date and time of collection. Preprinted labels can be used on the centrifuge tubes and on the bags so that only time of collection needs to be added in the field. All ink on containers must be black, as some colored inks contain dyes used in tracing work. Black inks may be a combination of several colored inks and might include some dyes used in tracing work, although the OUL has never encountered a problem with black ink in Sharpie felt-tip pens.

Custody sheets are completed to accompany all samples. They include the same information written on sample containers plus, in the case of carbon samplers, dates and times when the samplers were deployed. For the reason described above, only black pens are recommended for use in completing custody sheets.

As described in Section 2, all samples are kept in the dark after collection and placed in a cooler with ice or a gel-type refrigerant as soon as reasonable. Disposable gloves are worn. No preservatives are added to samples.

3.5 Sample Preparation

3.5.1 Water Samples

A major benefit of fluorescent tracer dyes is their high detectability when analyzed by instruments that measure fluorescence intensity. The relationship of fluorescence intensity to dye concentration, for the five tracer dyes discussed in this book, is linear over a range of three to four orders of magnitude. However, the fluorescence intensity of dyes in water varies with pH so it is necessary to adjust water samples to pH values that maximize the fluorescence intensity of the dyes that may be present in the sample. Table 13 shows the pH ranges where each of the five dyes has maximum fluorescence intensity (Kass, 1998); related information is shown in Figure 9 and Table 7.

Table 13 - The pH ranges for tracer dyes that maximize fluorescence intensity.

Dye	pH ranges that maximize fluorescence intensity
Eosine	5.4 to 11.0
Fluorescein	9.0 to 11.0
Pyranine	9.0 to 11.0
Rhodamine WT	5.0 to 9.0. Intensity declines to 85% of maximum at pH 11.0
Sulforhodamine B	3.0 to 11.0

The OUL adjusts the pH of water samples by placing uncapped samples in a high-ammonia environment for a minimum of two hours. This approach does not alter the volume of the sample. The target pH is 9.5 or greater and is verified by putting a drop of a representative sample on pH paper. Reagent water is placed in the same ammonia environment, and if the dye concentration in a sample is off-scale and requires dilution for quantification, the diluting water used is the pH-adjusted reagent water. Samples that are analyzed only for rhodamine WT or sulforhodamine B do not require pH adjustment.

Turbidity in water samples interferes with fluorometric analysis by increasing light scattering and the magnitude of the apparent background. Samples can be centrifuged to clarify the water, and the dyes will remain in the clarified water. Turbidity in water samples due to the presence of some remediation agents, such as molasses or emulsified vegetable oil, can be overcome if the analysis is by a spectrofluorophotometer operated under a synchronous-scan protocol. In some cases, samples must receive a 10-fold dilution to adequately reduce turbidity. Turbidity can be a major problem for detecting dyes and quantifying them when field fluorometers are used.

3.5.2 Carbon Samplers

Carbon samplers should be washed in strong jets of chlorine-free water to remove as much foreign material as possible prior to elution. The duration of packet washing depends on the condition of the sampler. Very clean samplers may require less than a minute of washing, while dirtier samplers may require two or three minutes. Effective cleansing cannot be accomplished simply by washing in a conventional laboratory sink, even if it is equipped with a spray unit. Washing of carbon samplers does not appear to elute detectable concentrations of dye, and the benefits of cleaner samples for analysis offset any dye lost to washing.

Municipal water supplies are not suitable for cleaning carbon samplers for two reasons. First, the municipal supply may contain fluorescent materials that could be adsorped on the carbon sampler. Second, almost all municipal water supplies in the USA are chlorinated, and distribution systems maintain a chlorine residual that can oxidize some, or potentially all, of the dye in a carbon sampler. As an illustration, based on website information for 2021, the average annual concentration of free chlorine in the finished water distribution system for Bowling Green, Kentucky, was 1.16 mg/l (Bowling Green Municipal Utilities–Water, 2021). Based on a limited number of carbon samplers containing fluorescein dye that were split with a laboratory in Bowling Green, Kentucky, the greatest percentage of dye losses likely attributable to washing samplers with chlorinated water occurred in samplers with the lowest dye concentrations. Section 2.8.3 presented data on a study of dye destruction in carbon samplers exposed to chlorinated water.

Section 3.2.3 discussed the use of milk filter sock material for manufacturing carbon samplers. Workers using the milk filter sock material do not wash their samplers, as the fabric excludes sediment. This could be an advantage for laboratories not having ready access to chlorine-free wash water.

After washing, some workers dry the carbon prior to eluting the samplers while others simply elute the wet carbon. The OUL is not aware of test data suggesting that drying carbon before elution results in higher dye yields after elution, and it does raise a risk of cross contamination of samples. As eluant solutions include water, the amount of water in samplers should be taken into consideration if they are not dried prior to analysis. The OUL approach is to empty the washed carbon into a disposable beaker that can be capped; the carbon is then eluted with 15 ml of the OUL's standard elution solution.

There are several recipes for eluant solutions. All of them routinely include one or two strong bases (typically sodium hydroxide, potassium hydroxide, or ammonium hydroxide) in a water-and-alcohol solution. Alcohols that are commonly used include isopropyl, ethyl, and 1-propanol. The OUL routinely analyzes for eosine, fluorescein, rhodamine WT, and sulforhodamine B dyes in a single analytical scan and uses an eluting solution that adequately elutes all four of these dyes from a single carbon sampler. The OUL occasionally samples for pyranine, and the standard OUL eluant solution also works adequately for eluting pyranine from activated carbon. The OUL eluant solution is a mixture of 5-percent aqua ammonia and 95-percent isopropyl alcohol solution and sufficient potassium hydroxide to saturate the solution. The aqua ammonia solution is 29-percent ammonia. All percentages are by volume. The isopropyl alcohol solution is 70-percent alcohol and 30-percent water by weight. The potassium hydroxide is added until a super-saturated layer is visible in the bottom of the container. This super-saturated layer is not used for elution.

The elution period used by the OUL is one hour at room temperature. This 1-hour period is based on bench testing that showed a 1-hour elution period maximized rhodamine WT elution, and that after 1 hour the concentration of rhodamine WT in the elutant began to decline. After a 2-hour elution period, the rhodamine WT concentration in the eluting solution was approximately 93 percent of the concentration after one hour. Testing also showed that most of the fluorescein was eluted within one hour. The amount of fluorescein eluted with a 30-minute elution period was approximately 88 percent of the concentration after one hour; the concentration of fluorescein eluted after two hours was approximately three percent greater than the amount eluted after one hour. Given these findings, and other laboratory considerations, the OUL established a 1-hour elution period.

An eluent solution developed by Smart and Brown (1973)—sometimes known as the Smart Solution—was found to be the best eluent for rhodamine WT. It consists of

10-percent ammonium hydroxide in 50-percent aqueous 1-propanol. The OUL knows of no studies showing the relative effectiveness of the Smart Solution in eluting other dyes. 1-propanol appears to be the most effective alcohol for the elution of eosine. Potassium hydroxide is also an effective base for eluting fluorescein and eosine; the OUL has not tested sodium hydroxide. The OUL routinely traces with multiple dyes and has found that the OUL eluent with a 1-hour eluting period is effective in eluting all dyes discussed in this book; it may or may not be the best general eluent, as there are many potential mixtures.

There are multiple differences among laboratories in the preparation and elution of activated carbon samplers. Important differences are listed here, with the OUL protocols given for comparison purposes.

- Wash water for cleaning carbon samplers is chlorinated or not. OUL wash water is calcium magnesium carbonate groundwater that has never been chlorinated.
- Carbon samplers are washed and then dried prior to elution or are eluted wet following washing. The OUL elutes samples when they are wet.
- Duration of the elution period. The OUL uses one hour. At least one other laboratory uses 30 minutes.
- Composition of the eluting solution. The OUL eluting solution is a mixture of 5-percent aqua ammonia and 95-percent isopropyl alcohol solution and sufficient potassium hydroxide to saturate the solution. The aqua ammonia solution is 29-percent ammonia. All percentages are by volume. The isopropyl alcohol solution is 70-percent alcohol and 30-percent water by volume. The potassium hydroxide is added until a super-saturated layer is visible in the bottom of the container. The super-saturated layer is not used for elution.
- Ratio of quantity of eluting solution to quantity of carbon. The OUL ratio is 15 ml of eluting solution added to 4.25 grams (dry weight) of activated carbon that is wet from being washed to remove detritus.

The net result of these differences is that analysis results vary among laboratories doing the analytical work. To maintain data consistency, it is recommended that the same laboratory be used for all analysis during a tracing study.

3.6 Analysis of Water and Carbon Samplers

Water samples with large concentrations of dye can be visually analyzed for the presence of the tracer dyes if the observer is not color blind. Table 2 in Section 2 lists approximate visual thresholds for detecting tracer dyes; the table presumes that only one type of dye is present in the water. The detectability of dyes is substantially improved if water samples are examined in a dark room by beaming a focused light into a clear glass bottle containing the liquid sample and the sample is viewed at 90° to the orientation of the light beam. A focused flashlight beam is adequate for the light source, and a black velvet background behind the bottle aids in the testing.

Most of the early, long-distance groundwater tracing to large springs in Missouri, USA, by Aley (1978) used fluorescein dye and activated carbon samplers. Samplers were eluted with a solution of 70-percent isopropyl alcohol saturated with potassium hydroxide. The analysis of the elutants was visual and the relative concentration of dye in the sample was based on the amount of elution time required to be able to confidently conclude that fluorescein was present. In some cases, dye was visible within a few minutes, but elution time before concluding that a sample was negative was 10 days. This visual approach yielded lower detection concentrations than filter fluorometers available during the 1960s and early 1970s due to large amounts of organic matter adsorped onto the carbon that interfered with the tracer signal. It is still a viable approach for some tracing work such as many of the traces from on-site sewage systems to surface water.

To visually identify fluorescein in carbon samplers, wash the sampler in a strong jet of dye-free water (chlorinated tap water can be used, although it may destroy a small amount of the dye). Next, open the sampler and put the carbon in a capped, clear glass jar about 5.7 cm (2.25 in) in diameter. Jars used for baby food are ideal. Cover the carbon with about 7.6 mm (0.3 in) of eluting solution, cap the bottle, and allow the contents to stand undisturbed in a dark place. If fluorescein is present, it can be seen as a green layer with the distinctive fluorescein color lying on top of the carbon. The best way to see the dye is by shining a narrow beam of light horizontally into the bottle and viewing the bottle at 90° to the orientation of the light beam. This testing is best done in a dark room with a black background such as black velvet behind the bottle. If visual methods are used, sending some carbon samplers to a laboratory that specializes in analysis of fluorescent tracer dyes for confirmation is recommended. Visual analysis of carbon sampler elutants is not recommended for dyes other than fluorescein or for samples that may include two or more dyes.

Both water and carbon sampler elutants can be analyzed with fluorometers or spectrofluorophotometers. Fluorometers are the simpler instruments. Using filters or electronics, they can measure the fluorescent intensity of a sample at or near the peak emission wavelength of the dye in water. Digital units are available for measuring fluorescein and/or rhodamine WT in water. Analysis of water with a known concentration of either fluorescein or rhodamine WT is used as a standard. Analysis of samples compares the fluorescence intensity of the sample with the standard to calculate the dye concentration. Fluorescence intensity is a combination of any dye that may be present plus other fluorescent materials that are present and fluorescing. A major limitation of fluorometers is that they provide a single value and do not distinguish the fluorescence intensity due to dye that may arise from other fluorescent materials. Apparent fluorescence intensity is increased in turbid samples. Fluorometers can be useful in the field because they provide real-time insight into whether a dye they are capable of detecting is likely present in the water. However, their limitations preclude them from serving as reliable laboratory instruments.

With sufficient funds, one can buy an airplane, but owning an airplane is not proof of the skills necessary to fly it competently. The same applies to spectrofluorophotometers suitable for detecting tracer dyes. These are research-grade instruments with research-grade complexities. Many settings can be adjusted to optimize analysis results. Their use for detecting dyes in groundwater traces is a niche use for these instruments. As a result, one cannot simply purchase the instrument, read the instructions, and begin competent analysis of samples from groundwater traces. As is the case with most analytical equipment, experience, skill, and great care are needed in the interpretation of the resulting analytical graphs. As a caveat, the spectrofluorophotometer technology has continued to improve, and the software tools for analyzing and interpreting the data obtained from analytical tools also continues to improve. Periodic reviews of methods are warranted.

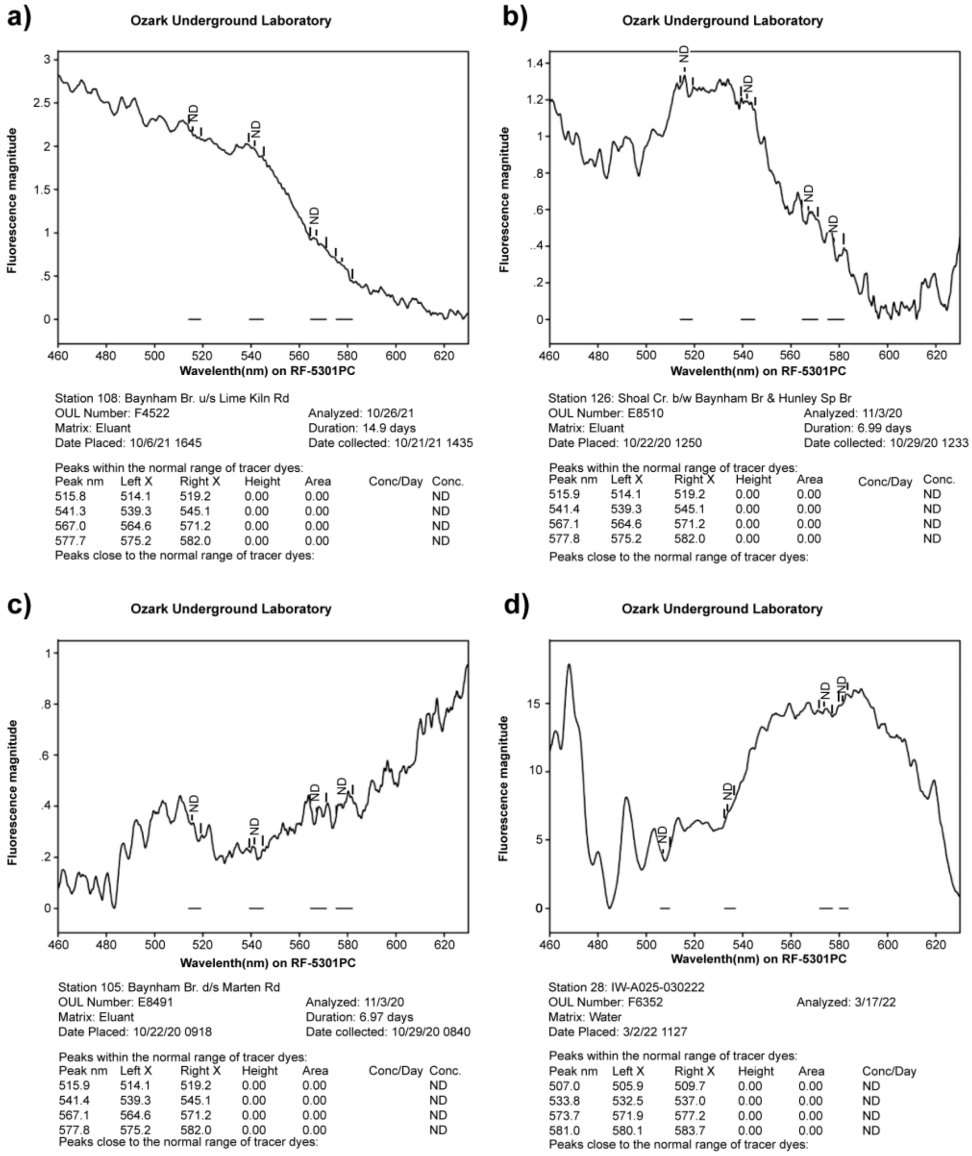
Spectrofluorophotometers are capable of multiple analytical methods, but what is most valuable for detecting and quantifying tracer dyes is the ability to synchronously scan samples. Under the synchronous-scan approach, the instrument measures and records fluorescence intensity over a wavelength range that includes the excitation and emission wavelengths for the target dye or dyes. The operator sets the beginning and ending excitation wavelengths and the beginning and ending emission wavelengths; the difference between the two is set as the approximate difference between the peak excitation wavelength and the peak emission wavelength of the dye in question. Fortunately, the differences between peak excitation and peak emission wavelengths are similar for eosine, fluorescein, rhodamine WT, and sulforhodamine B. This permits sample analysis for these four dyes with a single analytical run. Table 14 shows peak excitation and emission wavelengths for the five tracer dyes in water and the wavelength separation difference between these values (delta lambda) for each of the dyes.

Table 14 - Peak excitation, peak emission, and wavelength separations (delta lambda) for five dyes in water (data from Kass, 1998, page 80).

Dye	Peak excitation (nm)	Peak emission (nm)	Wavelength separation (nm)
Eosine	516	538	22
Fluorescein	491	512	21
Pyranine	455	512	57
Rhodamine WT	554	580	26
Sulforhodamine B	564	583	19

The OUL uses 17 nanometers (nm) as the bandwidth separation for the analysis of the four dyes other than pyranine in both water samples and carbon sampler elutants. The OUL standard excitation scan is from 443 to 613 nm; the standard emission scan is from 460 to 630 nm. The emission fluorescence from the scan is plotted on a graph. While the 17 nm bandwidth separation is narrower than suggested by values in Table 14, testing at the OUL with the excitation and emission slits used indicated that this setting yielded the best results for the mix of four dyes that are routinely analyzed.

Figure 19 and Figure 20 present a total of eight emission fluorescence graphs using the OUL spectrofluorophotometer operated under a synchronous-scan protocol. This is an optical analysis, and anything that reduces the ability of the sample to transmit light increases the magnitude of what is generally called the fluorescence background. The graphs are provided to help readers better visualize analytical outputs and the variable nature of background fluorescence profiles. Seven of the eight graphs are from the same study area and illustrate the variability encountered in fluorescence background conditions even with relatively clear water conditions. Samples from hazardous waste sites commonly show greater fluorescence variability.



a)

Station 108: Baynham Br. u/s Lime Kiln Rd
OUL Number: F4522 Analyzed: 10/26/21
Matrix: Eluant Duration: 14.9 days
Date Placed: 10/6/21 1645 Date collected: 10/21/21 1435

Peaks within the normal range of tracer dyes:

Peak nm	Left X	Right X	Height	Area	Conc/Day	Conc.
515.8	514.1	519.2	0.00	0.00		ND
541.3	539.3	545.1	0.00	0.00		ND
567.0	564.6	571.2	0.00	0.00		ND
577.7	575.2	582.0	0.00	0.00		ND

Peaks close to the normal range of tracer dyes:

b)

Station 126: Shoal Cr. b/w Baynham Br & Hunley Sp Br
OUL Number: E8510 Analyzed: 11/3/20
Matrix: Eluant Duration: 6.99 days
Date Placed: 10/22/20 1250 Date collected: 10/29/20 1233

Peaks within the normal range of tracer dyes:

Peak nm	Left X	Right X	Height	Area	Conc/Day	Conc.
515.9	514.1	519.2	0.00	0.00		ND
541.4	539.3	545.1	0.00	0.00		ND
567.1	564.6	571.2	0.00	0.00		ND
577.8	575.2	582.0	0.00	0.00		ND

Peaks close to the normal range of tracer dyes:

c)

Station 105: Baynham Br. d/s Marten Rd
OUL Number: E8491 Analyzed: 11/3/20
Matrix: Eluant Duration: 6.97 days
Date Placed: 10/22/20 0918 Date collected: 10/29/20 0840

Peaks within the normal range of tracer dyes:

Peak nm	Left X	Right X	Height	Area	Conc/Day	Conc.
515.9	514.1	519.2	0.00	0.00		ND
541.4	539.3	545.1	0.00	0.00		ND
567.1	564.6	571.2	0.00	0.00		ND
577.8	575.2	582.0	0.00	0.00		ND

Peaks close to the normal range of tracer dyes:

d)

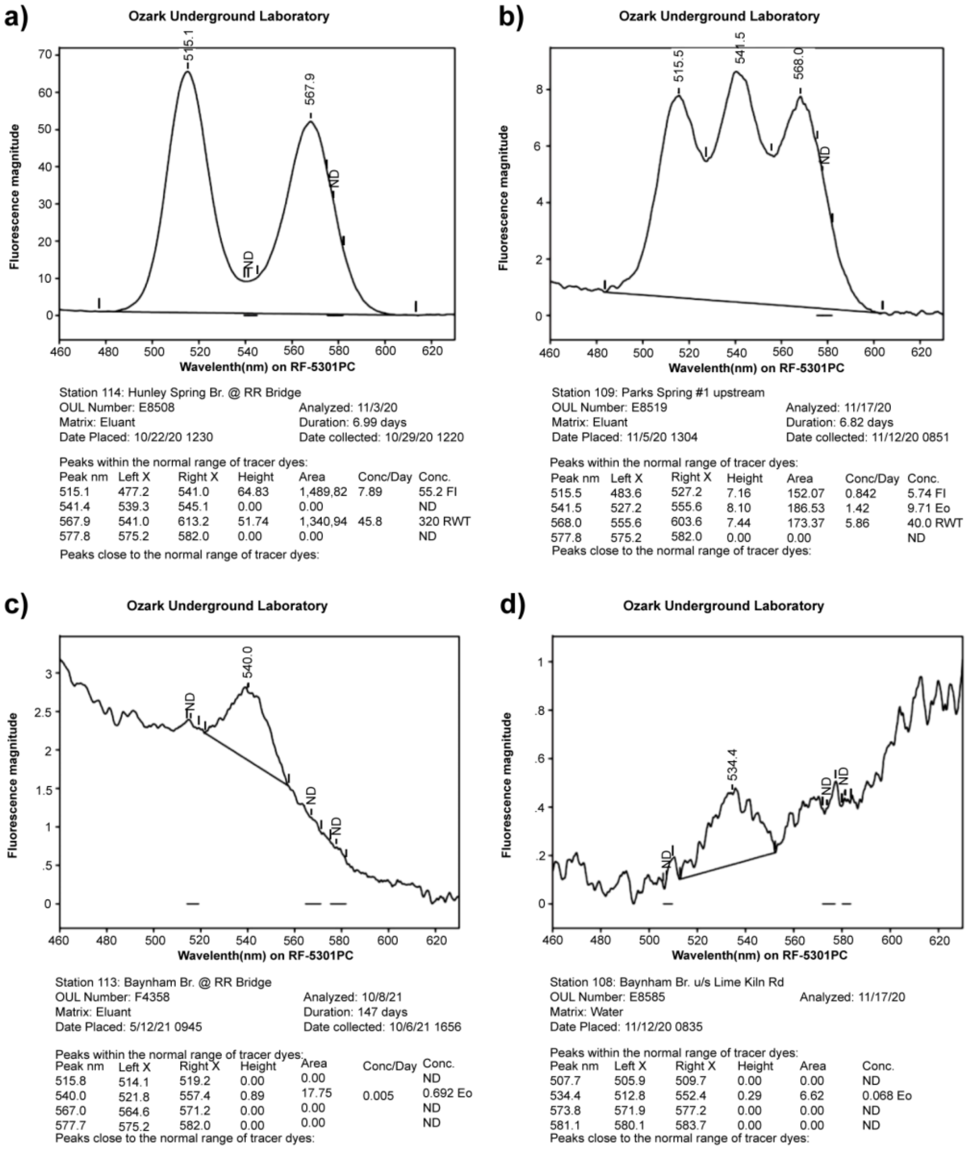
Station 28: IW-A025-030222
OUL Number: F6352 Analyzed: 3/17/22
Matrix: Water
Date Placed: 3/2/22 1127

Peaks within the normal range of tracer dyes:

Peak nm	Left X	Right X	Height	Area	Conc/Day
507.0	505.9	509.7	0.00	0.00	ND
533.8	532.5	537.0	0.00	0.00	ND
573.7	571.9	577.2	0.00	0.00	ND
581.0	580.1	583.7	0.00	0.00	ND

Peaks close to the normal range of tracer dyes:

Figure 19 - Emission fluorescence graphs of carbon sampler elutants and a water sample showing background fluorescence with no dyes present, with short horizontal bars showing the normal acceptable emission wavelength ranges in increasing order for fluorescein, eosine, rhodamine WT, and sulforhodamine B **(**acceptable wavelength ranges for each dye are different for elutant and water samples. a) A common background fluorescence pattern where fluorescence intensity decreases as emission wavelengths increase in a sample from a spring fed surface stream. b) A more irregular background fluorescence pattern from a shorter deployment period in a surface stream. c) Fluorescence background increasing with increasing emission wavelengths from a surface stream with intermittent flow. d) Background fluorescence from a monitoring well at a hazardous waste site, with fluorescence intensity scale approximately 6 to 20 times greater than on the other three samples. Additional discussion is provided in the text.



a)

Station 114: Hunley Spring Br. @ RR Bridge
OUL Number: E8508 Analyzed: 11/3/20
Matrix: Eluant Duration: 6.99 days
Date Placed: 10/22/20 1230 Date collected: 10/29/20 1220

Peaks within the normal range of tracer dyes:

Peak nm	Left X	Right X	Height	Area	Conc/Day	Conc.
515.1	477.2	541.0	64.83	1,489,82	7.89	55.2 Fl
541.4	539.3	545.1	0.00	0.00		ND
567.9	541.0	613.2	51.74	1,340,94	45.8	320 RWT
577.8	575.2	582.0	0.00	0.00		ND

Peaks close to the normal range of tracer dyes:

b)

Station 109: Parks Spring #1 upstream
OUL Number: E8519 Analyzed: 11/17/20
Matrix: Eluant Duration: 6.82 days
Date Placed: 11/5/20 1304 Date collected: 11/12/20 0851

Peaks within the normal range of tracer dyes:

Peak nm	Left X	Right X	Height	Area	Conc/Day	Conc.
515.5	483.6	527.2	7.16	152.07	0.842	5.74 Fl
541.5	527.2	555.6	8.10	186.53	1.42	9.71 Eo
568.0	555.6	603.6	7.44	173.37	5.86	40.0 RWT
577.8	575.2	582.0	0.00	0.00		ND

Peaks close to the normal range of tracer dyes:

c)

Station 113: Baynham Br. @ RR Bridge
OUL Number: F4358 Analyzed: 10/8/21
Matrix: Eluant Duration: 147 days
Date Placed: 5/12/21 0945 Date collected: 10/6/21 1656

Peaks within the normal range of tracer dyes:

Peak nm	Left X	Right X	Height	Area	Conc/Day	Conc.
515.8	514.1	519.2	0.00	0.00		ND
540.0	521.8	557.4	0.89	17.75	0.005	0.692 Eo
567.0	564.6	571.2	0.00	0.00		ND
577.7	575.2	582.0	0.00	0.00		ND

Peaks close to the normal range of tracer dyes:

d)

Station 108: Baynham Br. u/s Lime Kiln Rd
OUL Number: E8585 Analyzed: 11/17/20
Matrix: Water
Date Placed: 11/12/20 0835

Peaks within the normal range of tracer dyes:

Peak nm	Left X	Right X	Height	Area	Conc.
507.7	505.9	509.7	0.00	0.00	ND
534.4	512.8	552.4	0.29	6.62	0.068 Eo
573.8	571.9	577.2	0.00	0.00	ND
581.1	580.1	583.7	0.00	0.00	ND

Peaks close to the normal range of tracer dyes:

Figure 20 - Emission fluorescence graphs for carbon sampler elutants and a water sample containing tracer dyes, with short horizontal bars showing the normal acceptable emission wavelength ranges for dyes that were not detected. Concentrations are reported in units of parts per billion (ppb): a) fluorescein and rhodamine WT dye in carbon sampler elutant; b) fluorescein, eosine, and rhodamine WT dyes carbon sampler elutant; c) eosine from a sampler in place for 147 days where background fluorescence is nearly twice that of the peak that represents the eosine dye mixture; d) eosine dye in a water sample. Additional discussion is provided in the text.

Figure 19 presents synchronous emission fluorescence scans for four samples showing only background fluorescence with no dyes present. The horizontal scale shows the emission fluorescence wavelengths in nanometers, and the vertical scale shows fluorescence magnitude in arbitrary fluorescence units. The scales vary among the graphs, as they are drawn where the highest point on a graph is at 90 percent of the height of the graph. The short horizontal bars near the bottom of the graph show the normal acceptable emission wavelength ranges of the four dyes, in carbon sampler elutant, that are analyzed under this protocol. The bar from 514.1 to 519.2 nm is fluorescein, the bar from 539.3 to

545.1 is eosine, the bar from 564.6 to 571.2 nm is rhodamine WT, and the bar from 575.2 to 582.0 is sulforhodamine B.

Figure 19a (OUL F4522) shows a common background fluorescence pattern in a carbon sampler elutant where the fluorescence intensity decreases as emission wavelengths increase. The sample is from a spring-fed surface stream.

Figure 19b (OUL E8510) shows a more irregular background fluorescence pattern. This carbon sampler was in place in a surface stream for about half as long as Figure 19a, yet fluorescence magnitude is similar in the two samples.

Figure 19c (OUL E8491) shows fluorescence background increasing with increasing emission wavelengths. This sampler was in place in a surface stream with intermittent flow.

Figure 19d (OUL F6352) is a water sample from a monitoring well at a hazardous waste site. The fluorescence intensity scale is approximately 6 to 20 times greater than on the previous three samples, in part due to the wider slit openings for water samples than for carbon samplers.

Figure 20a shows both fluorescein and rhodamine WT dye mixtures in a carbon sampler elutant. The area within the peak is proportional to the dye concentration; the fluorescein concentration in this sample is 55.2 ppb and the emission fluorescence peak is at 515.1 nm—within the emission fluorescence range typical for fluorescein in elutant samples under the OUL protocol. The rhodamine WT mixture concentration in the elutant sample is 320 ppb and the emission wavelength peak is at 567.9 nm—within the emission fluorescence range typical of rhodamine WT in elutant samples under the OUL protocol. While the concentration of rhodamine WT is nearly 6 times greater than that for fluorescein, the area of the fluorescein peak is larger (1,469.82 arbitrary fluorescence units) than the area of the rhodamine WT peak (1,340.94 arbitrary fluorescence units). This is because the fluorescein mixture is more fluorescent than the rhodamine WT mixture.

Figure 20b shows fluorescein, eosine, and rhodamine WT dyes in a carbon sampler elutant. The emission peak at 515.5 nm is fluorescein; that at 541.5 nm is eosine, and that at 568.1 nm is rhodamine WT. The concentration of each dye mixture is shown in the table below the graph.

Figure 20c shows an eosine peak in the elutant from a carbon sampler in place for 147 days. The straight line (baseline) on the graph separates fluorescence due to eosine dye from that due to fluorescence background. The height of background fluorescence is nearly twice that of the peak that represents the eosine dye mixture. If this sample were analyzed on a fluorometer that produced only a single value at about the fluorescence peak of the dye, it would not be possible to credibly determine the presence and concentration of eosine.

Figure 20d shows a water sample containing eosine dye. The eosine concentration in this sample is approximately 10 percent of that in Figure 20c, yet the physical size of the two peaks is similar. This occurs because the excitation and emission slit openings are set narrower for carbon samplers than for water samples in order to maximize the ability to discriminate between dye and other fluorescent materials in carbon sampler elutants.

Pyranine in water or in carbon sampler elutants requires a different protocol because of the large difference between peak excitation and emission wavelengths. OUL analysis of pyranine in water or carbon sampler elutants uses a 35-nm wavelength separation with the excitation scan set from 360 to 600 nm and the emission scan set from 395 to 635 nm. Instrument settings for this dye are the same for water samples and carbon sampler elutants.

Tests involving two dyes require data interpretation capabilities that permit the peaks from each dye to be separated and quantified. Commercial laboratories may have software capable of performing these tasks; PeakFit is one such software package. In some cases, the analytical instrument software may have similar features. The OUL uses proprietary software to achieve these goals, performing preliminary baseline determinations and peak area determinations for comparison with daily standards in the process.

A particular dye may be excited and emit light at more than one pair of wavelengths. The selected set of excitation and emission wavelengths commonly used in dye analysis work typically produces the most useful emission fluorescence peaks. The peak emission fluorescence wavelength is a function of the dye, the matrix in which the dye is present, and the instrument and analytical protocol being used. For example, the peak emission fluorescence for fluorescein in water samples under the OUL's protocol is from 505.9 to 509.7 nm; in the elutant it is from 514.1 to 519.2 nm. These values are from positive dye detections resulting from field groundwater traces at multiple locations in the USA. The range is the mean, plus and minus two standard deviations. Values can change slightly as instruments and optics age and will vary with differences in excitation and emission slit widths. Slit widths control the amount of light that reaches the sample (excitation slit) and is received from the sample (emission slit). The emission fluorescence peak for dye in water is different from the same dye in carbon sampler elutants. In the case of fluorometers, the setting of peak emission wavelengths is based on dye concentrations in medical grade water.

The detection limits the OUL uses for eosine, fluorescein, rhodamine WT, and sulforhodamine B are lower for water samples than for carbon sampler elutants. This is because the excitation and emission slit settings used in the analytical instrument for carbon sampler elutants are both narrower than those used for water samples. The rationale for this difference is that carbon sampler elutants routinely contain greater concentrations of fluorescent materials than water samples. The narrower the excitation and emission slits, the more readily one can discriminate between a tracer dye and other fluorescent material that may be present. The OUL uses very narrow slit settings because much of our tracing work is at waste sites where other fluorescent compounds may be present and credible discrimination between dyes and other compounds is essential. OUL slit settings and detection limits for the five tracer dyes are shown in Table 15. The detection limits are based on the dye concentration needed to produce a signal-to-noise ratio of 3:1.

Table 15 - Slit settings and detection limits used by the OUL in synchronous-scan analysis using a spectrofluorophotometer.

	Water samples			**Elutant samples**		
Dye	**Excitation slit (nm)**	**Emission slit (nm)**	**Detection limit (ppb)**	**Excitation slit (nm)**	**Emission slit (nm)**	**Detection limit (ppb)**
Eosine	5	3	0.015	3	1.5	0.050
Fluorescein	5	3	0.002	3	1.5	0.025
Pyranine	5	3	0.010	5	3.0	0.015
Rhodamine WT	5	3	0.015	3	1.5	0.170
Sulforhodamine B	5	3	0.008	3	1.5	0.080

Laboratory analysis of water and carbon sampler elutants with spectrofluorophotometers operated under a synchronous-scan protocol is currently the best approach for identifying tracer dyes, separating them from fluorescent background, and determining their concentrations. There are significant differences in sample preparation and analytical protocols among laboratories that conduct tracer dye analysis using spectrofluorometers and synchronous-scan protocols. These differences are at least partly due to differences in the types of tracer studies routinely conducted by the laboratories.

The authors of this book recommend that those planning to send dye-tracing samples to a laboratory for analysis consider the following factors in deciding on an appropriate facility:

1. Does the laboratory have a written document outlining their sample preparation and procedures for analysis plus clearly defined and reasonable criteria for positive detections of tracer dyes?
2. Does the laboratory have qualified and experienced staff capable of credibly evaluating samples that are not clearly positive or negative?
3. Does the laboratory adjust the pH of some or all water samples to pH 9.5 or greater prior to analysis? This is especially important for the quantitative measurements of eosine, fluorescein, and pyranine.
4. Washing of carbon samplers prior to elution is an important step. Does the laboratory use reasonable volumes (average of at least 5 liters per sampler) of unchlorinated wash water at high pressure to clean samplers? This is especially important for samplers likely to contain small dye concentrations.
5. Is the ratio of eluent volume to the weight of activated carbon being eluted reasonable? Too much eluent dilutes the dye in the resulting solution. The ratio the OUL has found to maximize recovery concentrations of the dyes discussed in this book is 15 ml of eluent to 4.25 grams of activated carbon (dry weight). Lower ratios work well in laboratory bench testing but do not always produce elutant solutions transparent

enough for proper analysis. Slightly different ratios may apply for different eluting solutions.

6. Does the laboratory have established acceptable wavelength ranges for positive dye results? Appropriate ranges should be based on samples from field studies rather than laboratory spikes.

In addition to addressing these questions, a good policy is for the laboratory to also provide the tracer dyes so that laboratory standards are consistent with the dye mixtures being used. It is also advisable for the laboratory to provide activated carbon samplers (if they are to be used) and sample containers shown by quality assurance testing to be free of dye or fluorescently similar materials.

3.7 Summary

1. What is commonly called *fluorescence background* includes both fluorescence and light scattered by suspended and dissolved materials in the water sample.
2. The five tracer dyes found to be very stable in most water samples and in activated carbon samplers are eosine, fluorescein, pyranine, rhodamine WT, and sulforhodamine B. The OUL's recommended maximum hold time for water samples is 30 days and for activated carbon samplers, 60 days; both durations begin after collection.
3. Handheld and submersible recording fluorometers suitable for field use exist, but at the time of this writing most are available only for fluorescein and rhodamine WT dyes. The instruments measure total fluorescence, not fluorescence due to the particular dye. Except for short-distance or short-duration traces, laboratory analysis of water samples is recommended to verify that estimates of dye concentrations obtained from fluorometers are reasonable.
4. Activated carbon samplers are discussed in substantial detail because many practitioners do not adequately appreciate their utility in groundwater-tracing work. Basing primary sampling reliance for a tracer study on carbon samplers rather than water samples has the following benefits.
    a) Not missing short-duration or intermittent dye pulses. The carbon samplers are continuous, accumulating samplers whereas water samples are point-in-time samples.
    b) Less frequent sample collection and fewer samples requiring analysis.
    c) Traces can be conducted with smaller amounts of dye, decreasing the risk of visibly colored water at groundwater discharge points.
    d) Unless large amounts of dye are used, carbon samplers are less likely than water samples to fail to detect dye at sampling points, as carbon samplers concentrate tracer dyes from the water. This advantage also applies to the detection of tracers at locations reached by only small amounts of dye.
    e) Providing more accurate data on time of first dye arrival at sampling points.

    f) Permitting long-distance and/or large-area groundwater traces using limited amounts of dye.

5. Water samples need to be pH adjusted to about 9.5 or greater prior to analysis to maximize the fluorescence intensity and standardize concentrations of eosine, fluorescein, and pyranine dyes.
6. Activated carbon samplers need to be washed with strong jets of dye-free water prior to analysis. If the water supply is chlorinated, the chlorine residual in the water can destroy some of the dye adsorped on the carbon. The washing process with chlorine-residual-free water does not appreciably elute dyes from the carbon.
7. Mass balance calculations for dye traces discharging from karst springs indicate that the median percentage of introduced dye detected is about 4.5 percent. In general, the percent of introduced dye detected increases as the amount of dye introduced increases and as the distance between the introduction point and detection point decreases.
8. Based on precision, accuracy, ease of use, and cost, the best method for tracer dye analysis in water samples and carbon sampler elutants is using a spectrofluorophotometer operated under a synchronous-scan protocol. Laboratories with this instrumentation, and the experience to properly operate it, can provide analytical services for groundwater-tracing studies. Several factors that should be considered in deciding on an appropriate laboratory for providing this dye analysis work are identified in Section 3.6.

4 Designing Traces: General Considerations

4.1 Study Plans

Preparing a study plan prior to initiating a dye-tracer study should precede even simple studies. Typical components of plans for tracer studies are identified in the general outline for a dye-tracing study plan in Figure 21 and Table 16. Section 4.2 provides more detailed discussions of important study design considerations. The organization of Section 4.2 follows the general outline of a typical study plan and includes comments and recommendations based on the authors' experiences and case histories to illustrate particular points. One of our reviewers noted the military adage *"No battle plan survives contact with the enemy!"* attributed to Helmuth von Moltke and commented that it applies with a vengeance to dye traces where the *"enemies"* are unanticipated complications both natural and human. Study plans must have sufficient flexibility to permit adaptation to unanticipated complications.

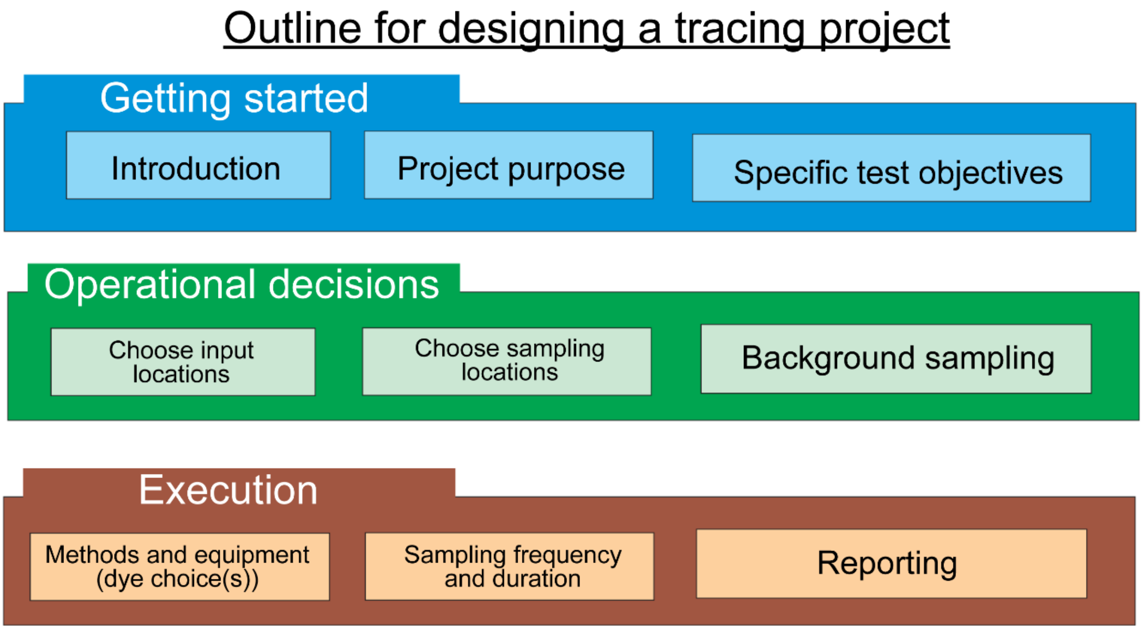


Figure 21 - General considerations for the design of a dye-tracing study. These considerations should be settled before beginning even a small-scale tracing study. Each consideration is described in detail in Table 16.

Table 16 - General outline for a dye-tracing study plan.

Table 16: General Outline for a Dye-Tracing Study Plan	
GETTING STARTED	
Getting Started: Introduction	• Authors of the plan and their affiliations. • Date of plan. • Location where work will be conducted. • When work is planned to be conducted. • People and entities that will be involved in doing the work and individual responsibilities. • Project manager and his affiliation. Identify client or funding source. • Briefly discuss any previous dye traces at the site. • Case-specific factors. • Quality assurance and quality control. • Health and safety considerations.
Getting Started: Purpose of tracing project	• Explain in a clear and concise statement why the work is being done. • If there are multiple purposes, state all of them.
Getting Started: Specific test objectives	• Explain what you expect to determine and how it will be useful. • Multiple objectives are nice, but do not list too many. • Make sure objectives are attainable with the planned work. • In the final report, you should be able to restate the objectives and demonstrate that they were adequately met. Consider this in identifying the study objectives.
Getting Started: Data quality objectives	• Be specific, but do not specify a higher quality of data than the project requires. It is better to exceed expectations than to fall short.
OPERATIONAL DECISIONS	
Operational Decisions: Identification of dye introduction points	• If you are planning on sample analysis with a spectrofluorophotometer operated under a synchronous-scan protocol you can do up to three (and sometimes four) separate dye introductions concurrently. Theoretically, you could work with more dyes in the samples, but under field conditions and varying dye concentrations, this is seldom advisable, as fluorescence peaks can interfere with each other. • Recognize that analysis approaches other than spectrofluorophotometers operated under synchronous-scan protocols limit the number of concurrent traces that can be done without producing confusing results. • If you need to do work to identify and evaluate potential dye introduction points, state that and briefly explain what that work will entail. • Show the dye introduction points on a map. It helps to also show planned dye-monitoring points on the same map. You can refer to the map in the report section on planned monitoring points. • Provide rationale(s) for selecting dye introduction points. • State how you plan to introduce the dye(s) and flush water at each dye introduction point. Also indicate the amount of flush water to be used and give the rationale. Indicate planned source for water. • Identify the tentatively proposed dye type and quantity for each dye introduction point. • Indicate that the dye types and amounts for each point may change in response to results of background sampling or some other finding. State that the project manager will have the authority to make modifications in dye types and amounts based on background sampling or some other finding without any additional approval. Plan for the maximum amount of each dye that you believe may be needed for each dye introduction point; it is easier to use less dye than to gain approval for more dye after submitting the initial tracer plan. Explain rationales for your choices. • Allow for a few additional sampling stations to be identified during field work.

Table 16: General Outline for a Dye-Tracing Study Plan	
	• If using activated carbon samplers at a well that has a long open hole, consider sampling at two or more depths. Carbon samplers can be anchored at multiple points on a long, weighted line. Well logs can help determine depths. If needed for budget constraints, consider combining several sample depths during analysis. • If private water supply wells are sampled, the ideal approach is to allow them to flow continuously through carbon samplers at a rate of about 4 L (1 gal) per minute. If this is not acceptable to well owners, then timers, such as those used in cities to control irrigation times, can be used to regularly pump the well. Farmer and Blew (2021) in rural Idaho successfully placed carbon samplers in the reservoir tanks of domestic toilets to detect tracer dyes. This approach is often effective. If this approach is used, residents need to not place any materials—such as toilet-bowl-coloring and cleaning agents—in the supply tanks. If acceptable to owner, block the tank drain partly open so some water runs continuously. This increases the amount of water sampled. • For each dye proposed for use, include its safety data sheets in an appendix to the study plan. • Cite authoritative reference(s) on safety of dye to people and the environment. A good reference for this is Field and others (1995). A copy can be obtained from one of the authors (Aley) at the OUL. • Additional case-specific factors.
Operational Decisions: Identification of dye sampling points.	• Identify sampling points with both a number and name. Unless required by policy to use a convention that requires station number to end with the date of collection, use names and station numbers that will not change during the study. This should allow an analytical laboratory to sort sample results by sampling station using commonly available software. • Show all sampling points on a map or maps. In many cases, the map showing dye introduction points can also show sampling points. • List GPS coordinates for each station or indicate that you will confirm them during field operations. • If you are sampling in a stream, identify upstream control points in the event a fluorescent dye or other fluorescent material flows into your study area during the study. • Routine sampling of a few control points at wells upgradient of dye introduction points is often advisable. Indicate they are control points. • Give rationale for the selection of sampling points. • Springs, wetlands, and drainage ditches with intermittent groundwater contributions may be important sampling points; do not ignore them.
Operational Decisions: Background sampling	• A minimum of two consecutive rounds of background sampling at most or all sampling points is generally recommended prior to dye introduction. Two consecutive samples from each sampling point provides a measure of variability in samples at each location. • The ideal approach is to conduct background sampling the same way as post-dye introduction sampling. The sampling is best done with activated carbon samplers, but grab samples of water can also be used. Collection of both carbon samplers and water samples is an ideal approach even if most water samples are not analyzed. More than two rounds of background sampling is advisable at sites where residual tracer dyes may be present from previous studies or where fluorescent contaminants are suspected of being present. • Background carbon samplers should be left in place for a minimum of 3 days; approximately 7 days is better, especially if this will be the typical sampling interval after dye is introduced. When the first group of carbon samplers is collected, new samplers are placed at each sampling point and

Table 16: General Outline for a Dye-Tracing Study Plan	
	collected samplers are submitted to a laboratory for rapid turnaround analysis. • The final decisions on dye types and amounts should be made after analysis of the first round of sampling. • The second round of background samples is typically collected shortly before introducing tracer dyes. If there are many sampling points, this second round of sampling should occur on the day before dye introduction. If there are only a few points, the second round of background samples can be collected shortly before dye introduction(s). When the second round of samplers is collected, new samplers are placed for studies where activated carbon sampling is planned. Avoid a time gap between background sampling and routine sampling. • Most background sampling does not detect any dye or fluorescent compounds with emission wavelength peaks in or near the wavelength range of one of the tracer dyes. If a fluorescent compound is in or near the wavelength range of one of the dyes that might be used is detected, it can be quantified for background purposes as if it were the dye. The OUL often stipulates that positive dye detections for an introduced dye must have concentrations at a sampling point at least an order of magnitude larger than the largest concentration of dye (or any other fluorescent compound in or near the emission wavelength range of the dye) in any background sample from that station.
EXECUTION	
Execution: Procedures for sampling and analysis	• To reduce the risk of transferring dye from one well to another on sampling equipment, consideration should be given to suspending routine monitoring of wells that will be sampled during a tracer study. • Monitoring and sampling methods should be identified and explained. • If field fluorometers are used, explain how (or if) water samples will be pH adjusted prior to taking instrument readings. • If water samples are collected, explain how the collections are made and how the samples are analyzed. • If activated carbon samplers are collected, explain how they are anchored in place, how they are collected, and how they are analyzed. • Provide information on criteria for positive dye detections, detection or reporting limits, and similar information. • If water and/or carbon samplers are sent to a laboratory for analysis, identify the laboratory and include a copy of their procedures and criteria document as an appendix to the study plan. • Samples sent to a tracer dye analysis laboratory should identify the sampling point for each sample. An appropriate laboratory will have extensive experience in evaluating fluorescence intensity graphs and comparing them with background sampling results on a station-by-station basis as an integral part of the service they provide. Failing to identify the sampling stations for samples eliminates this important work and decreases the quality of the study. We recognize that this approach is different from that often used for submitting samples to laboratories for chemical analysis. • If primary sampling reliance is placed on activated carbon samplers, state this in the study plan. The same applies if primary reliance is placed on grab samples of water. • Describe how samples are handled prior to analysis. • Describe how samples are analyzed. • Carbon samplers in place for different deployment periods can be compared by dividing total dye concentrations by the number of days the sampler was deployed.

Table 16: General Outline for a Dye-Tracing Study Plan	
Execution: Sampling frequency and duration	• Identify sampling frequency. In many dye-tracing studies, primary sampling reliance is placed on activated carbon samplers with samplers collected and new samplers placed about once per week. In some studies, samplers are collected more frequently during the first 2 weeks of the study after dye introduction. A common approach is to collect samples 1, 2, 4, 7, 10, and 14 days after dye introduction and then once per week after that. Grab samples of water are often collected at each station when carbon samplers are collected. • If modeling has been conducted and provides estimated time of travel in the aquifer, one can plan to end the sampling when the model suggests that dye should have reached most or all sampling stations if they are in the flow path for the dye. A safety factor of 25 percent or 50 percent should be added to the estimated travel time. • Avoid open-ended study statements such as “Sampling will continue until the dye is detected.” • The experience of the person designing the trace in similar settings often provides the best estimate for the duration of sampling. The better the rationale, the more likely the tracer study will gain approval. An arbitrary termination time for sampling can be established. Common ones for short distance traces are 6 or 8 weeks of weekly sampling. Approximately 3 months of weekly sampling represents 13 rounds of samples; for many studies, this is an adequate study duration. Sampling for some tracer studies lasts more than a year. • Recognize that some study durations may need to be controlled by the total amount of precipitation received at the site. • Consider including a statement that the duration of the study can be increased or decreased based on the data collected. • Another approach is to state that sampling will end when dye concentrations are declining at most or all stations where it has been detected and that there have been no new detection sites within the last 3 (or 2) weeks. Include a statement that a new detection site near a site that previously received tracer dyes will be viewed as not representing a new detection site.
Execution: Reporting	• Identify the nature and timing of anticipated reports.

4.2 Important Study Design Considerations Requiring Particular Care

4.2.1 Purpose and Objectives of the Study

The design of the tracing program must be capable of accomplishing the identified purposes and objectives. It is important to identify specific objectives. Common objectives are determining if there are hydrologic connections between the dye introduction point and other identified points and determining travel times for groundwater flow from the dye introduction point to the detection sites. There are at least five different travel times that may be important for these types of traces; they include the following (Figure 22).

1) Travel time for the first dye arrival at sampling points.
2) Time of peak dye concentration at sampling points.
3) Time when approximately 50 percent of the *detected* dye mass, of a recorded breakthrough curve, has reached important sampling points. Mass balances show that most of the dye introduced does not reach sampling points within the

study period so percentages need to be expressed in terms of detected mass. At springs, these data should be multiplied by flow rates, if they are available, to quantify total mass recovery.

4) Time at which dye is no longer detectable at sampling point.
5) Time when there are no new dye detections and when dye concentrations at all sites where dye has been detected are decreasing.

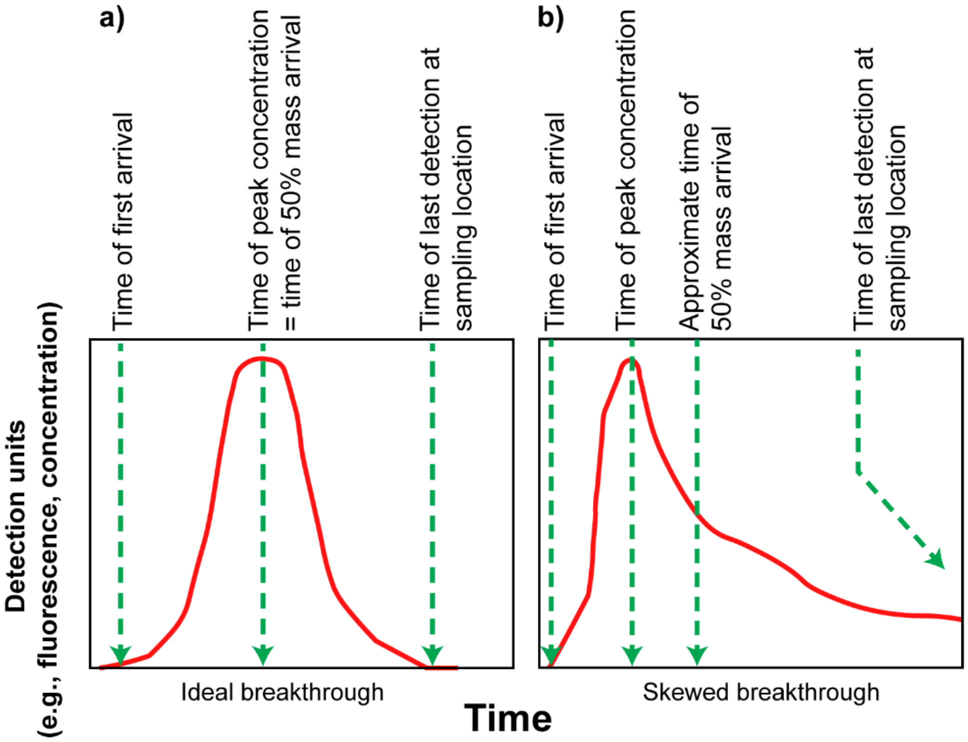


Figure 22 - Schematic representations of tracer breakthrough curves at sampling points. a) Idealized breakthrough with complete record of tracer arrival; this seldom if ever occurs in nature. b) Skewed breakthrough with incomplete record of tracer arrival. Skewed breakthrough curves with or without a complete return to background (zero on the x-axis) are most common, but nonetheless convey highly useful information concerning tracer appearance at the sampling point, and first arrival time. In porous media applications, the arrival time of 50 percent of the detected mass can be viewed as representative of average groundwater velocity in mobile pore space (i.e., the higher permeability zones). Later arrivals are governed by slower advection zones and forward/back diffusion interactions with lower permeable materials.

Which of the five identified travel times are important for a study depends on the purposes and objectives of the investigation. The travel time for first dye arrival is often the most useful for water-quality concerns, easiest to recognize as data are accumulated, and the least expensive to obtain. It documents tracer appearance at a sampling point establishing a connection between the source and sampling locations. Time of first dye arrival was a critical value in the Green Forest sewage trace discussed in Box 11, as arrival time at drinking water supplies was shown to be shorter than likely survival times for many waterborne pathogens. Time of peak concentration and, in cases where a complete breakthrough curve is recorded, a time when approximately 50 percent of the detected dye mass has arrived at a sampling point is important for in situ remediation planning. This is an estimated 50 percent of the detected dye mass, and is almost never 50 percent of the introduced mass of dye.

Determining the time at which dye is no longer detectable at sampling points provides an estimate of residence time in the aquifer. This comes from a complete breakthrough curve and is therefore expensive to obtain. Fortunately, it is seldom necessary for solving the problem of interest. Also, it is not rarely practical to continue sampling and analyzing until dye is no longer detected. As an example, rhodamine WT dye was introduced into a karst aquifer near Frederick, Maryland, in 1995 and remained detectable for 18 years in carbon samplers placed in flows from several springs (White et al., 2015). Eighteen years of continuous sampling would not be practical for most projects. In the Maryland case, other dyes were introduced at different points in the same aquifer, and they traveled underground for similar distances in a few weeks. Differences in travel rates are attributed to a combination of differences in the flow paths and retardation of the rhodamine WT dye, as shown in Box 18. Retardation of dyes is discussed in Section 2.8.7, and rhodamine WT is composed of equal amounts of two isomers, one of which has a large retardation factor.

Study plans for tracer studies should always have a planned time for sampling to be terminated. The time when there are no new dye detections and dye concentrations at all sites where dye has been detected are decreasing is a common approach for defining the time at which sampling for a tracer study can be terminated. If this approach is used, the number of sampling rounds during which no new dye detections occur should be specified in the design plan; the OUL commonly uses two or three rounds of sampling.

Recently, one additional travel time has become important in artificial groundwater recharge work in California, USA. In this work, one of the key metrics in obtaining credits required in the permitting process of groundwater recharge projects that use a tracing test is the time at which 10 percent of the peak concentration arrives at a sampling location.

If dye sampling places primary reliance on carbon samplers, the concentration in the carbon sampler elutant can be divided by the number of days the carbon sampler was placed, to develop a standardized breakthrough curve. The time at which 50 percent of the detected mass at a sampling point can be determined from the standardized breakthrough curve. Box 6 provides an example of this for a rhodamine WT dye trace to Town Well #1, Walkersville, Maryland, USA. In that case the time to arrival of 50 percent of the detected mass was approximately 5 days.

Retardation coefficients of common dyes are discussed in Section 2.8.7. If time of travel is an important consideration, the likely retardation of tracer dyes in comparison to retardation of contaminants that may be present at the site should be considered when estimating contaminant travel times (Exercise 5↴).

4.2.2 Identification of Dye Introduction Points

Dye introduction points may be natural features, such as streams, or in karst areas sinkholes, losing streams segments, and some cave entrances or cave streams. Man-made features that can be used for dye introductions include stormwater detention or groundwater recharge basins; borings; backhoe pits; and, in some cases, monitoring wells if the use of these wells as dye introduction points will not interfere with the purposes for which the wells were constructed. If monitoring wells are used for dye introduction, it should be recognized that residual dye may remain in the well for months or years. This can limit or preclude the subsequent use of these wells as monitoring points if the same type of dye is used at a new dye introduction point. Long persistence times are especially common for low-yield wells. If monitoring wells are used as dye introduction points, it is important to determine if they can accept water at rates adequate for the tracer study. To the extent reasonable, such testing should be conducted prior to completion of the study plan or at least prior to the start of a dye-tracer study.

In some areas, residents have excavated shallow wells called "*dug wells*" (Figure 23). These wells were often constructed in locations where groundwater discharged during wet periods. Dug wells have sometimes been used as dye introduction points, but they are often undesirable for this purpose. The typical dug well is located at a point where water discharges from, rather than enters, groundwater. Unless it is clear that groundwater flow continues downgradient of the well, dug wells are generally poor dye introduction points. However, if existing dug wells are present, they may be good monitoring points during dye traces.

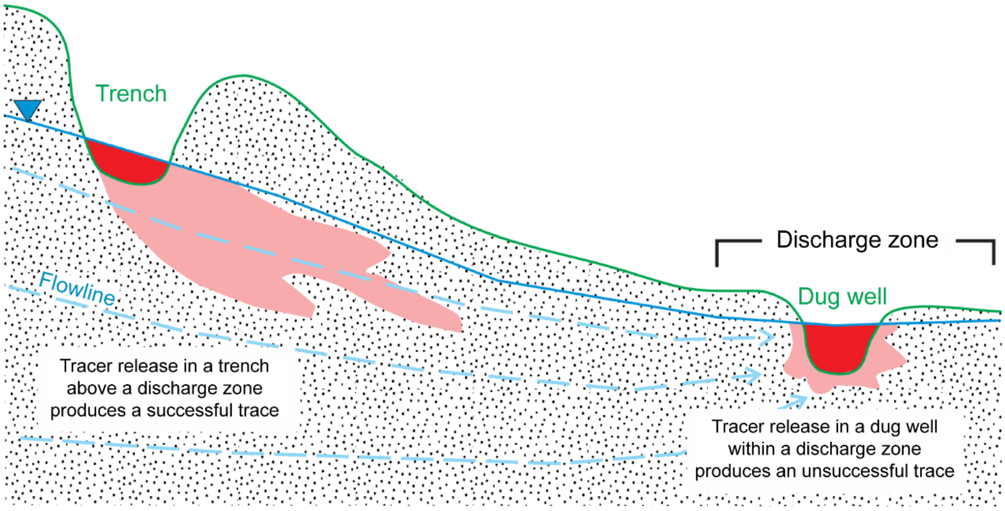


Figure 23 - The appropriate use of *dug wells*, or trenches, in tracing studies. Avoid introducing the tracer dyes in discharge zones.

Borings and backhoe pits can be located at points ideal for the purposes of the dye study. Backhoe trenches are especially useful as dye introduction points because their size provides a greater likelihood of encountering preferential flow routes through soil and residuum than do borings. Typical dye introduction trenches are 10 to 15 m (33 to 50 ft) long, as wide as the backhoe bucket, and as deep as possible with the available equipment (or to bedrock)—whichever is less. In cases with shallow groundwater, the bottom of the trench may intersect the water table. Trenches can be lengthened if they do not initially intersect areas that will accept water at an acceptable rate, as discussed in Exercise 6↴.

Perforated plastic pipe, such as used in septic fields, can be installed horizontally in the bottom of a trench with an upright piece of nonperforated pipe at both ends. The nonperforated pipes typically extend to a meter (3 ft) or so above the ground surface. Alternately, the tops of the vertical pipes can be installed flush with the ground surface. If trenches are deep, the pipes can be assembled on the surface and lowered into the trenches without any need for personnel to enter the trenches. After construction, the trenches are backfilled. Dye introduction trenches with perforated pipe are especially useful when rates of water acceptance are low, as they easily permit the addition of more flush water. Once the dye introduction has been completed, the pipe system can be abandoned in place or, more commonly, filled with grout or excavated and removed.

When wells, borings, or backhoe pits are planned as dye introduction points, they should first be tested with clean water to demonstrate they will accept water at rates adequate for the study. With trenches, a piece of lumber anchored to the bottom of the trench with marks every half meter (or each foot) can be used as a staff gauge to measure the rate of water decline in the trench and allow calculation of the rate of water acceptance. Figure 24 shows an example from a dye introduction trench in Alaska. Testing of planned dye introduction points prior to introducing water and dye should be specified in the study plan, and minimum water-acceptance rates should be identified. The OUL has had successful traces from trenches with water acceptance rates as low as about 15 liters (4 gallons) per minute and from wells or borings at rates of at least 11 liters (3 gallons) per minute.

Figure 24 - Fluorescein introduction into a trench in alluvium, showing safety rope, staff gauge, hose leading from a meter measuring flow rate, and blue diffuser in bottom of trench. The diffuser is designed to minimize erosion of the sides of the trench (thus reducing sediment in the infiltrating water). The trench bottom is level and 9.1 m (30 ft) long, 1.1 m (45 in) wide, and 3.4 m (11 ft) deep on the uphill side. The trench accepted 18,900 liters (5,000 gal) of water and fluorescein dye at a mean rate of 92 L (25 gal) per minute.

Leaking wastewater lagoons and storage basins can be effectively traced to wells or nearby springs or streams using tracer dyes. Dye mixed with water can be poured into the impounded water from points near suspected leakage zones, near points where water is discharged into the impoundment, or along the shoreline Box 22↴.

On small lakes where localized leakage zones are suspected, investigators sometimes work from a boat and add small amounts of dye to the lake's surface and watch for it to drift toward a localized area. If a localized area is identified, then a mixture of dye and water can be poured into the water over or near the suspected leakage point. Rhodamine WT mixtures commonly contain approximately 20-percent dye and have a specific gravity of about 1.2 (Turner Designs, 1995). When poured onto the surface of a lake, the dye mixture sinks rapidly and can readily flow to submerged leakage points. One can make solutions of powdered dye in water that have similar specific gravity values by mixing 200 grams of powdered dye mixture with 800 ml of water. Denser solutions can be made if desired. The utility of dye solutions denser than water is illustrated by the case history in Box 23↴. Another example of tracing work involving possible leakage through an earth-fill dam is provided in Box 24↴.

Dye tracing at industrial sites often involves testing for improper connections between sanitary or industrial sewers and stormwater sewers. Old manufacturing plants that have experienced many infrastructure modifications—often with lost or poor records—and military facilities that have experienced many similar changes are common sites where this type of tracing is needed. Dyes are introduced into drains or manholes on sanitary or industrial sewer lines and sampling is conducted at various points in other pipes and/or in monitoring wells. Sampling with activated carbon samplers is ideal for traces focused on finding leaking sewer segments or improper sewer connections, as it requires minimal sampling and minimal interruption of site activities.

With a good analytical protocol, it is often possible to make three dye introductions with three different dyes concurrently and separate and quantify each of the dyes. This is especially valuable at wastes sites but is also important in other types of investigations. Laboratory testing may suggest that more dyes could be used concurrently, but dye degradation that modifies the emission wavelengths of some dyes and major differences in the size of emission peaks makes the concurrent use of more than three dyes generally unwise. Each introduction of a different dye is made at a different location, so multiple traces provide more information about the hydrology of the area being investigated than would a single trace. Some dye analysis laboratories charge per sample rather than for the number of dyes that may be present. Especially with these laboratories, the marginal cost of doing multiple traces versus a single trace is small, making multiple (concurrent) traces financially attractive. In developing study plans, it is strongly recommended that the desirability of using two or more dyes be evaluated, especially if they would mostly use the same sampling points.

4.2.3 How Dyes Will be Introduced

Dye traces need both dye and water. In general, the more water used with a trace the more effective the resulting trace. Introducing more water than might occasionally enter groundwater at a particular point is generally not advisable. At waste sites in karst, introducing too much water or introducing it too rapidly could potentially result in unnatural contaminant migration. At industrial sites, adequate water volumes are usually available at or near potential dye introduction points. In other cases, water must be hauled or piped to the selected dye introduction point. Determining the volume of water needed to flush introduced dye into the groundwater system is a critical part of tracer test planning.

Introducing the tracer dye as a high concentration slug followed by flush water makes the most effective use of the dye because it reduces losses to short-term adsorption and requires less dye than if the dye is mixed with a similar total volume of water and introduced at a relatively constant rate. The slug approach also provides better time-of-travel information than that provided by the large-diluted volume approach. The slug approach also limits the amount of equipment that needs to be cleaned after introducing dye for a trace. In contrast, if a trace is designed to determine some specific aquifer characteristics over a relatively short travel distance, the dye may need to be mixed with a substantial volume of water and then introduced at a constant rate. In addition, in at least one jurisdiction, a regulation limits the maximum concentration of dye that can be introduced into groundwater. Other than these two specific situations, the best general approach is to introduce the dye as a concentrated pulse and follow it with flush water.

Dye introductions function best if the introduced dye is moved into the aquifer with as much flush water as is reasonable for the study. At a minimum for introductions into wells, this amount of water should be three to five times the volume of water in the saturated portion of the well bore. This is the same volume of water typically extracted from monitoring wells prior to collecting water samples that may contain volatile compounds. In our experience, this approach is generally acceptable to regulators who may be concerned about introducing flush water.

In general, traces from wells function best if more than the minimal amount of water is added after introducing the tracer dyes. A common specification the OUL uses for the volume of flush water to be added following a dye introduction is that it be as much water as can reasonably be introduced during an 8-hour workday. In some cases, it may be appropriate to establish a minimum allowable distance from land surface to the water level in the well during the addition of flush water. Great care must be taken to not overfill a dye introduction well with water and create a spill of dyed water.

For most tracing projects, dye introductions into wells can be done simply by pouring a concentrated liquid dye mixture into the top of the well and then flushing it with clean water. Rhodamine WT is commonly available as a liquid with a dye equivalent of about 20 percent; mixtures with a lower dye equivalent are also on the market. The specific gravity of the 20-percent dye equivalent mixture is approximately 1.2 (Turner Designs, 1995) so it will sink rapidly in water. Powdered mixtures of eosine, fluorescein, and sulforhodamine B are commonly mixed with water prior to use. Mixing at a ratio of 200 grams of powdered dye to 800 ml of water will also result in a dye solution with a specific gravity of about 1.2. If dense dye solutions are poured into the top of a well, they sink rapidly and color the water column through much and sometimes all of the well. In some screened wells, the screen does not extend to the bottom of the well. If a dense dye solution is poured into the top of the well some of it is likely to become trapped below the bottom of the screened interval. In some cases, this trapped dye can be mixed with water higher in the well by pumping air or water through a hose that extends to the bottom of the well. Alternately, the entire dye and flush water introduction can be piped to the bottom of the well and this will ensure that concentrated dye is not retained in the well below the screened interval.

Pouring dye into a well can create foam that will rise in the well above the liquid level. Aley (2019) reports that the tendencies of the five dyes to create noticeable foam at concentrations of 100 ppm is as follows.

Water = Fluorescein = Pyranine < Eosine << Sulforhodamine B << Rhodamine WT

The senior author once poured several kilograms of a rhodamine WT mixture into flowing water at the top of a 15-m (49-ft) waterfall and created an impressive mass of red foam several meters high at the base of the waterfall. This fortunately occurred in a remote area and no foam was visible the following day. The important point here is that agitation of dye mixtures creates varying amounts of foam. Careful introduction of dye mixtures will prevent problems.

In some cases, it may be desirable to introduce dye at a specific depth in a well. This requires that the dye be *tremmied*—conveyed in a pipe or hose—to that depth, and it may also require the use of an inflatable packer below the desired dye introduction interval. To the extent reasonable, these approaches should be avoided as they increase the complexity of the tracing project and the equipment must either be cleaned or discarded.

Dyes can be introduced into drains and toilets to conduct traces from sewers. Cracks in the upper portions of buried sewers due to heavy vehicles passing over soft ground conditions are unfortunately common. To test for such breaks, it is best to introduce dyes into sanitary and industrial sewers during periods of high flow rates. If a facility closes for weekends, an alternate approach is to insert an inflatable plug at a downstream manhole and fill the upstream portion of the sewer with dyed water. This approach, which also requires recirculation of the dyed water, was used successfully by the OUL to trace leakage from an industrial sewer at a military facility in Pennsylvania, USA to areas of contaminated groundwater.

In many cases, water must be hauled or piped to the selected dye introduction point. Although stock ponds can be used as water supplies, potable water is desirable for such use and in some cases may be required by regulatory entities or by good professional practices. Chlorine residuals in the potable water will oxidize a trivial amount of the dye, so this is of insignificant concern. In one case where the OUL was introducing water for a dye trace to the habitat for an endangered aquatic species, chlorinated water from a fire hydrant was passed through activated carbon to remove most of the chlorine. This may have been unnecessary, as untreated water from the hydrant was periodically discharged by the water utility to the same portion of the habitat when flushing their lines. Nevertheless, the use of the activated carbon satisfied the regulatory entity and allowed the tracer study to go forward.

In some cases, and especially for the purpose of identifying natural flow patterns in karst areas, the absence of surface flow can sometimes be overcome by placing dyes in dry stream channels or in dry road culverts where the dyes will be taken into solution and transported into groundwater by the first stormflow. Such dye placements are called "*dry sets*." It is often a good practice in placing dry sets to use a powder form of dye and place it in a container where the dye and the container can be recovered if the dye has not been introduced by a runoff event within a reasonable amount of time.

A good protocol in cases where dry sets are used is to place activated carbon samplers at one or more points downstream of the dry set to a) verify that the dye was taken into solution by passing water and b) indicate how far downstream the dye persisted in surface runoff. The case history in Box 25↴ provides an example of a groundwater trace that used dry sets as an appropriate dye introduction method.

Powder dyes are remarkably mobile in air. Never use or mix powder dyes inside a building or near sampling points. If powder dyes are to be introduced into wells, sewers, or any indoor location they should first be mixed with water. The OUL commonly packs up to 8 lb (3.64 kg) of powdered eosine, fluorescein, or sulforhodamine B in a 20 L Nalgene carboy. The carboy fits snugly into a cooler for shipment. At the mixing site the carboy is placed upright, the cap removed, and a funnel is inserted in the opening. If the carboy contains 8 lb of powder dye, then about 4 gal (15.1 L) of water is poured into the carboy through the funnel. Smaller amounts of dye use proportionally less water. The funnel prevents dye powder from wafting out of the top opening. After filling the carboy with water, it is capped and the funnel is double-bagged and discarded. The carboy is then shaken and rolled on grassy ground to mix the dye; rough surfaces could damage the carboy. If the dye mixing is done at least an hour before use, there are seldom any lumps of powdered dye in the mixture and any foam has dissipated. After use, the carboy is rinsed at least once to remove residual dye, it is returned to the cooler, the cooler is securely taped shut, and the equipment is returned to the OUL for cleaning and reuse.

There is almost always minor dye spillage during dye introductions. Spilled dye can be oxidized by spraying it with household bleach that contains at least 5.25-percent sodium hypochlorite. To facilitate cleanup after dye introductions, the OUL routinely has the following materials available:

- 1 gal (3.785 L) of bleach and a plastic spray bottle;
- 3 large rolls of paper towels;
- 40 large plastic trash bags;
- a large bag of cat litter (in the event of larger spills);
- a transmission fluid funnel to place in the top of monitoring wells (these funnels have long spouts that help keep the funnel in place during pouring);
- a roll of duct tape;
- a plastic apron and multiple disposable gloves; as well as
- safety glasses and any other normal safety equipment required by client or the nature of the site. A dust mask is appropriate if dye is applied as a powder.

4.2.4 Selection of Dyes and Dye Quantities

Many factors go into the selection of the dye or dyes to be used in a tracing program. Information in Section 2 should help the reader make good selections. The following general principals provide further help, as discussed in Exercise 7↴.

Fluorescein is commonly the best choice of the dyes for use in groundwater-tracing projects. While fluorescein is subject to photodegradation, this should not preclude it from use where it will be in surface water for a period of only a few hours. Very small concentrations of fluorescein, commonly derived from vehicle coolants, are often present in urban water and stormwater runoff but seldom create problems if adequate background sampling has been conducted. Fluorescein is often encountered at industrial sites where there are cooling towers.

Pyranine requires a separate analytical run whereas most laboratories can analyze for fluorescein, eosine, rhodamine WT, and sulforhodamine B with a single analytical run. If the laboratory used for analysis makes a single charge for analysis for eosine, fluorescein, rhodamine WT, and sulforhodamine B, it will be less expensive to select all dyes from this group rather than including pyranine in the group of dyes used.

Rhodamine WT is generally available only as a liquid with a dye equivalent value of about 20 percent. It is sometimes found at bargain prices, but the dye equivalent in those mixtures is typically substantially less than 20 percent. The other four dyes are commonly available as powders, and eosine and fluorescein can also be purchased as a liquid. Liquid dye mixtures routinely have lower dye equivalent values than the powder form dyes. Some companies sell fluorescein and eosine in tablet form; these are designed for use by plumbers and are not recommended for use in groundwater-tracing investigations. Other rhodamine dyes with fluorescence peaks near those of rhodamine WT are common in hydraulic fluids. Equipment with leaking hydraulic lines is common at heavy industries and mines. Potential interference with rhodamine WT can be detected and quantified during background sampling.

Most professionally directed tracer studies will use one of the analytical laboratories that specialize in the detection of fluorescent tracer dyes. Some of these laboratories buy dyes in bulk and provide them to clients. Purchasing dyes through the laboratory that will conduct the analytical work for a study is a good protocol, as their standards will be made from the same dye mixtures being provided.

Although experience is often the best guide for determining how much dye is needed for successful traces, the following general principles relate to the amount of dye needed for a particular trace (Exercise 8⤵):

- A kilogram of one dye does not equal a kilogram of another dye in terms of detection characteristics. Based on the as-sold mixtures routinely used by the OUL, a trace that can be done with 1 kg of fluorescein will require at least 1.5 kg of eosine or pyranine, about 4 kg of rhodamine WT, and about 5 kg of sulforhodamine B. The ratios vary with the nature of the earth materials involved and with sampling and analytical protocols.
- Tracing in karst typically requires less dye than tracing in fractured rocks and tracing in fractured rocks requires less dye than tracing in alluvium and glacial outwash.
- The more rapid the groundwater movement the less dye will be required for detections at similar travel distances. Rapid water movement provides less opportunity for sorption than does slower water movement.

- Introducing dye as a slug followed by flush water is usually the most effective approach and provides the most accurate time of travel information.
- Rhodamine WT is a mixture of equal amounts of two isomers, one of which has a large retardation factor (Sabatini & Austin, 1991; Vasudevan et al., 2001). In most groundwater situations only half of the introduced rhodamine WT mixture is effectively transported by groundwater.
- While eosine, fluorescein, and pyranine are all photodegraded by exposure to sunlight, they can still be used effectively in surface water that recharges groundwater. Dyes introduced at dusk have several hours before being exposed to sunlight. Dyes introduced beneath ice cover are also protected from sunlight destruction. Dyes introduced into water with limited sunlight penetration due to tannic compounds or turbidity are not subject to appreciable sunlight destruction.
- Traces dependent upon dye detections with field instruments measuring fluorescence intensity require the use of more dye than traces relying on water samples and laboratory analysis. Traces placing primary sampling reliance on activated carbon samplers and laboratory analysis will routinely require the use of much less dye than the amount needed for alternate methods of sampling and analysis.
- In many cases a good sampling protocol includes collection of grab samples of water each time carbon samplers are collected. If dye is detected in a carbon sampler, then the associated water samples for the beginning and end of the period that the carbon sampler was in place can be analyzed to determine mean dye concentrations in the water—presuming that dye concentrations in water samples are large enough to be detectable.
- Carbon samplers are continuously collecting and accumulating all five of the tracer dyes. Resulting dye concentrations in carbon sampler elutants are commonly at least one to two orders of magnitude larger than dye concentrations in water samples. The magnitude of dye accumulation is greater for carbon samplers that have been in springs and streams than for those from most monitoring wells. This is because the volume of dyed water passing through a carbon sampler in a well is less than in springs and streams so the well samplers adsorp less dye than samples in springs and streams. Wells that are continuously pumped and the water is passed through carbon samplers result in dye accumulations similar to those attained in streams and springs.
- Primary sampling reliance on carbon samplers may not be appropriate for some tracer studies designed to measure aquifer properties. For example, the effects of sorption are more easily shown in breakthrough curves for water samples than for cumulative samples from carbon packets.

4.2.5 Identification of Sampling Points

Sampling points are chosen based on the purposes and objectives of the tracer study and the location(s) of the dye introduction points. In some studies, the focus is on identifying points that provide groundwater to specific sampling points. An example is an underground mine receiving undesirable quantities of water in a localized area, and the objective is to determine if a surface lake is a source of the water. In other cases, the focus of the study may be determining the flow routes and travel rates for water moving away from a particular location. An example is a trace to determine the downgradient points affected by a solid waste management unit and to determine travel times to monitoring wells where the dye is detected.

If monitoring wells already exist, an ideal monitoring approach is to routinely sample all wells within the area of interest. In addition, it is desirable to have some wells outside the area where dye detections are expected to occur to serve as controls. It is appropriate to use potentiometric head maps to predict flow patterns, but not to place undue confidence in such maps for identifying all the wells that should be sampled. Most groundwater-tracing work is done in aquifers with heterogeneity; flow-direction predictions from potentiometric maps are conventionally premised on homogeneous conditions. In addition, there may be important differences in potentiometric heads between the time of mapping and the time the tracer study is conducted.

Groundwater velocities, based on modeling, are available for some sites where tracing work may be planned. The modeling results may or may not be accurate. Section 1 linked to Box 6 which presents a case history from a karst site in Walkersville, Maryland, where dye was introduced at a point where a groundwater model indicated groundwater would require 5 years to reach a production well. Actual travel time for first dye arrival was 17.5 hours. Even in non-karst settings there can be dramatic differences between modeled and actual travel times. Avoid the temptation to save money by not sampling some of the more distant points until several weeks or months after dye introductions. Such a strategy risks losing time of first dye arrival data if dye is detected in the delayed first samples from these locations.

4.2.6 Routine Sampling

If samples are sent to a laboratory capable of tracer dye analysis, it is advisable to identify samples by station name and number rather than to omit this information and send them with only an anonymous identification number. A good tracer laboratory will have the experience and skill needed for interpreting the analytical graphs and comparing current and previous graphs for each station. Not using this expertise is likely to result in a poorer quality investigation and an increased risk of incorrect interpretations.

4.2.7 Sampling Frequency and Duration

Sampling frequency and duration is largely a function of the objectives of the study, the nature of the site and associated aquifer(s), distances to be traced, logistics, and funding.

Sampling Frequency

Sampling once per week for the anticipated duration of a tracer study is commonly a reasonable approach. An activity that occurs once per week is easier to put into work schedules than activities that occur sporadically. In the experience of the OUL, carbon samplers work well for a week even at sites where non-aqueous phase liquids are present. Carbon samplers in wells at these sites should not be in contact with free product. Passing samplers rapidly through a zone of free product does not destroy the capability of the sampler to subsequently adsorp dyes.

If time of first arrival is important, and if it might occur within the first 2 weeks after a dye introduction, a common protocol is to sample 1, 2, 4, 7, 10, and 14 days after dye introduction and then subsequently every 7 days until the end of the planned study period. In addition, the study plan should allow for departures from this regular schedule for logistical reasons. Since activated carbon packets are continuous and accumulating samplers, dye concentrations in elutants can be normalized by dividing the measured concentrations by the number of days the sampler was in place. Dye detections are subsequently reported as concentration per day.

Sampling Duration

At waste sites, a common approach for identifying an end of the sampling phase of a groundwater trace is to specify that sampling will end when no new dye detections have occurred at any sampling locations during the last two (or perhaps three) back-to-back rounds of sampling, and that dye concentrations were decreasing at most or all sampling points where dyes have been detected. Modeling or previous tracing at the site may indicate that some other ending time for sampling is appropriate.

Arbitrary sampling durations such as 6, 8, or 13 weeks are often used for groundwater traces, especially at waste sites where potential travel distances are 100 m (328 ft) or less. Dye traces of septic fields in Washington State typically end 2 weeks after dye introduction. The rationale for this is that most fecal coliform bacteria survive outside of their hosts for less than two weeks, and determinations of failing sewage systems are based on a combination of dye-tracing results and fecal bacteria abundance. Other sampling durations can end once other predetermined events occur.

There can be substantial time between sample collection and reporting of the analytical results. A significant investment has been made in a tracer study and much of this value can be lost if a study is terminated too early, even if permitted in the study plan. Clients should be aware that sampling will not end until the project manager is reasonably certain that adequate data have been (or will be) obtained. This can create budgeting problems. This can be overcome by the project manager recommending that the project budget allow for two or three rounds of additional sampling and analytical costs beyond the planned end of the field work, in the event this proves necessary. One can also increase the interval between sampling events toward the end of the study or terminate sampling at some locations based on accumulated data.

As counterintuitive as it sounds, it is risky to assume that dyes will arrive at locations further from the dye introduction points later than at closer locations and that the start of sampling at the more distant points can be deferred for a several rounds of sampling. This is especially true at karst sites where both monitoring wells and springs are sampled. In these settings, dyes often appear at one or more distant springs before being detected at wells located closer to the dye introduction point. Springs are better connected with the conduits transporting dyed water than are monitoring wells, which may or may not have good hydrologic connections with conduits transporting dyed water. Analogous situations may occur in any aquifer that contains preferential flow routes; buried utility lines bedded in gravel within the seasonal high-water table at waste sites are an example.

4.2.8 Background Sampling

Four of the five dyes highlighted in this book, excepting rhodamine WT, are primarily used for a wide range of other industrial and commercial purposes. As a result, background levels of the tracer dyes may be present in water being sampled during a tracer study. A common problem occurs with fluorescein, which is the most common colorant used in vehicle coolants. Fluorescein can commonly be detected in runoff water from large parking lots and major highways. At industrial sites, fluorescein is often present in blowdown from cooling towers. Eosine is often the colorant in nontoxic vehicle coolants, and rhodamine dyes provide the pink color in hydraulic fluids. Plumbers sometimes use fluorescein, sulforhodamine B, or rhodamine WT for testing connections to sewers. Fluorescein and eosine are common dyes in felt-tip pens and highlighters, and if these common items are used on sample labels, they could contaminate a sample.

There are many natural and manmade fluorescent compounds and some of them have fluorescence peaks in or near the excitation or emission wavelength ranges of some of the dyes used in groundwater tracing. This potential interference is greatly reduced if sample analysis is conducted using a spectrofluorophotometer operated under a synchronous-scan protocol. Fluorometers do not have this capability. Even with a synchronous-scan protocol, some compounds still produce fluorescence peaks near those of some of the tracer dyes. For example, pentachlorophenol and diesel, previously used in wood treating, are readily adsorped on carbon samplers and produce emission fluorescence peak wavelengths slightly longer than fluorescein in carbon sampler elutants. This was a useful reconnaissance tool for identifying groundwater discharge points around wood treating plants that used pentachlorophenol before this use of the chemical was banned.

Background sampling for the presence of tracer dyes or other compounds with fluorescence characteristics like one of the dyes is a critically important part of tracer studies. In the absence of adequate background sampling, fluorescence peaks consistent with the tracer dye used will likely be interpreted as being the dye that was introduced. False positives, if they occur, are detrimental to everyone.

In general, at least two rounds of background sampling should be conducted at all planned sampling locations prior to dye introduction. If there is little or no indication of existing dye or potentially interfering fluorescent compounds at any sampling locations after the first round of background sampling, then dye usually can be introduced immediately following the collection of the second round of background samples even though these samples have not yet been analyzed. Two rounds of background samples are collected from each sampling location to provide a measure of variability. Two rounds of background may not be feasible, or logical, for traces associated with emergency responses or traces in remote areas. More than two rounds of background sampling may be desirable in areas where previous tracing has been conducted or where there are major political or legal issues involved.

Background sampling is best conducted with activated carbon samplers. The carbon samplers are continuous and accumulating samplers and are well suited to detecting fluorescent compounds in the water being sampled. We recommend that water samples also be collected during background sampling and analyzed if any fluorescent peaks in or near the emission wavelength range of tracer dyes are detected. Water samples may be the only type of background sample that can be collected from wells that have pumps installed in them, at sites in remote locations, or for traces done in response to emergency situations.

If an investigation plans to place substantial sampling reliance on water samples, then background sampling should use both carbon samplers and water samples. Although rare, it sometimes occurs that there are fluorescence peaks near the emission fluorescence range of tracer dyes found in water samples but not in elutants from carbon samplers. The explanation for this is that not all fluorescent materials can be adsorped on carbon samplers and then eluted.

A study plan will usually identify one or more dyes likely to be used for a particular project. The plan should recognize that changes in dye types and quantities may be needed because of the background sampling. If there are fluorescent peaks in or near the range of a tracer dye, then a somewhat larger amount of that dye can be used or the use of an alternate dye can be considered. A good plan will allow flexibility in the types and quantities of dyes that are introduced. It is highly desirable to not have any time gap in sampling between the background sampling period and dye introductions. In recognition of this, changes in dye types and their quantities based on findings during background sampling should be the prerogative of the project manager without additional approvals. This should be stated in the study plan.

If background sampling detects a fluorescence peak in or near the acceptable emission fluorescence range for a dye, then the concentration of dye capable of producing an equal sized peak can be calculated. If that dye is subsequently used, then the OUL protocol is to require that at least one sample meets all criteria for the presence of that dye and the dye concentration is at least an order of magnitude larger than the maximum detection at that sampling station during the background sampling. Most background samples with fluorescence peaks at or near the acceptable emission fluorescence wavelength range for tracer dyes do not contain those dyes and do not have peak shapes characteristic of those dyes. As a result, the reports should make it clear that expressions as equivalent dye concentrations are carried out only for background characterization purposes, as discussed in Box 26↴.

4.3 Mass Balance Calculations

Accurate mass balance calculations require reasonably accurate flow rate measurements and dye concentration data from water samples collected with sufficient frequency. Aley (2017) summarized mass balance data for dye traces to karst springs. Dye concentration data were from water samples and these concentrations were multiplied by flow rates to determine the total mass recovery at individual sampling locations. The percent of introduced dye mass detected, at a given station, was determined by dividing the total mass recovered by the total mass introduced. Straight-line distances traced ranged from 0.3 to 28.18 km (0.2 to 17.5 mi); the median distance was 1.46 km (0.9 mi). The percent of introduced dye detected ranged from 0.01 percent to 98 percent; the median value was 4.9 percent. In both the Aley (2017) and the Hauwert and others (2004) reports, daily flow rate data were available for most of the traces. The total number of traces was 15 from six different USA states and a wide range of climates. Five traces were done with fluorescein, five with eosine, and five with rhodamine WT.

Hauwert and others (2004) calculated dye recovery percentages for 20 traces in the Barton Springs portion of the Edwards Aquifer, Texas, USA. These involved straight line distances of 3.2 to 30.5 km (2.0 to 18.9 mi). The percentage of introduced dye detected ranged from 0 to 77 percent with a median value of about 4.2 percent.

The traces with the highest percent recoveries were made directly into karst conduits and traced to nearby springs. Some of these used unusually large quantities of dye. Larger quantities of dye were typically used for longer distance traces. Shorter distance traces commonly yielded larger percentage dye recoveries than longer distance traces.

Glenn and others (2013) reported on a massive quantity of dye introduced at Lahaina, Maui, Hawaii. They introduced 154.5 kg (340 lb) of fluorescein dye with a 77-percent dye equivalent into sewage effluent discharging into disposal wells 3 and 4. These wells routinely discharged about 12.1 million liters (3.2 million gallons) per day. Sampling was dependent on water samples. Groundwater flow was through jointed basalt and alluvium. The dye discharged from springs in shallow water of the Pacific Ocean. The North Springs Group was 831 m (2,726 ft) from the dye introduction well and the South Springs Group was 932 m (3,057 ft) from the well. First dye arrival at the North Springs Group was 86 days after dye introduction and 109 days at the South Springs Group. Maximum measured dye concentrations at the North Springs Group were 22.5 ppb and at the South Springs Group were 35 ppb. All dye analyses were performed on water samples, and the estimated percent of introduced dye that discharged was 64 percent. The estimated length of time from injection until fluorescein dye concentrations would decrease to below the method detection limit was estimated to be 2,435 days at North Springs Group and 2,001 days at South Springs Group. The US Supreme Court on April 23, 2020, ruled in *County of Maui, Hawaii v. Hawaii Wildlife Fund, et al.* (2020), that this discharge was the functional equivalent of a direct discharge to navigable water without an appropriate permit from the US Environmental Protection Agency.

The mass balance results from karst springs illustrate that the percent of dye detected from a groundwater trace will commonly be only a small fraction of the amount of dye introduced. The 64 percent of introduced dye detected in the Maui trace is due to the very large quantity of dye introduced, the relatively short travel distances, and probably a small number of highly favored flow pathways. The typical percent of dye detected from traces in alluvium and glacial outwash is almost certainly smaller than for karst systems due to greater contact between earth materials and dyed water. The calculation of the amount of dye needed for a trace should not assume that most of the dye will reach sampling points in the aquifer. Unfortunately, this has been a common presumption that led to underestimates of dye mass needed in the design of many false-negative traces.

The mass balance data show that most of the dye introduced for a trace is not accounted for. Much of the mass may be bound to sediment, diffused into the aquifer matrix, or degraded. Using the example of a karst spring, if one detects 5 percent of the mass of dye introduced into groundwater through a sinkhole at the spring, it is not correct to conclude that the sinkhole contributes 5 percent of its inflow to the spring. It is possible that much more than 5 percent of the water that flows into the sinkhole discharges at the spring, but that much of the dye introduced at the sinkhole was lost to sorption, diffusion, or degradation. This is especially likely if sampling of all potentially relevant springs and wells shows they are free of detectable dye.

Tracer dyes are very good for showing where the water goes and how quickly the first dye arrives at individual sampling points. This is valuable information. Equally important, and with full recognition that a negative cannot be proven, the dyes give us information on locations not reached by dyed water during the study period and under the conditions existing during the study. In contrast, without the introduction of much larger quantities of dye than necessary for activated carbon sampler testing, sole reliance on water samples in dye tracing must be expected to frequently miss discovering some of the flow pathways.

4.4 Aquifer Characterization and Remedial System Design

Tracer studies are a powerful and cost-effective tool for the characterization of heterogeneous aquifers, including parameters of importance for contaminant transport and remedial system design. Injected tracers circumvent the need to rely on bulk average estimates of aquifer properties, because they are traveling through the system just as many potential contaminants would. Common applications of tracer studies for aquifer characterization and remedial system designs are aimed at estimating:

- injection volumes of remediation agents needed to achieve a target injectate distribution within the subsurface (volume-radius relationships);
- mobile porosity;
- groundwater velocities and solute transport rates; and
- groundwater flow directions.

Useful case studies highlighting tracer studies focused on aquifer characterization and remediation are presented in *Tracers in Hydrology* (Leibundgut et al., 2009) and *Remediation Hydraulics* (Payne et al., 2008).

Tracer studies designed to determine groundwater velocity, solute transport rates, and groundwater flow direction can be designed to meet budgets or desired level of detail/resolution. The key for studies where groundwater velocity and solute fluxes are of interest is to ensure an adequate sampling schedule is incorporated into the study design to ensure crucial aspects of tracer breakthrough are not missed. Sampling durations may need to be shorter than those estimated using more traditional approaches, as preferential transport has been shown to exist in aquifers thought to be nearly homogeneous. Water samples (as opposed to carbon samplers) are best suited for accurate determination of tracer breakthrough as they provide point-in-time concentration measurements. The differences and advantages that distinguish carbon samplers from grab water samples are discussed in detail in Section 3. Despite the advantage of carbon samplers being able to detect first arrival times with greater sensitivity than grab water samples, reliance on carbon samplers alone may limit the level of quantitative analyses possible with the data.

Tracer studies designed to determine the injection volume of remediation fluids needed to reach target locations and to estimate mobile porosity are nuanced and interrelated. The tracer test involves introducing dye at an injection well and measuring the volume injected until samples at surrounding wells (called dose-response wells) indicate injection has continued for the duration of the average dye-travel time to those wells. The injected volume when average travel time has been reached ($\mathrm{Vol}_{\mathrm{Inj50}}$) is used to estimate the mobile porosity that was filled by that volume of injected water as shown in Equations (3) and (4). The average travel time is taken as the time required for the observed dye concentration to reach 50 percent of the maximum dye concentration at the dose-response wells surrounding the injection well. Tracer studies of this nature are specialized and may not be well suited for longer distance traces.

$$Vol_{Inj50} = \pi\, r^2\, h\, \theta_m \tag{3}$$

$$\theta_m = \frac{Vol_{Inj50}}{\pi\, r^2\, h} \tag{4}$$

where (parameter dimensions are dark green font with length as L):

Vol_{Inj50} = volume injected by the time the observed concentration reaches 50 percent of the maximum concentration at dose-response wells (L^3)

r = radial distance to the dose-response wells (L)

h = thickness of the injected interval often assumed to be equal to the screened interval of the injection well (L)

θ_m = mobile porosity (dimensionless)

A detailed description of a study design for determining injection volumes and estimating mobile porosity can be found in *Chapter 12 of Remediation Hydraulics* (Payne et al., 2008). Briefly, when designing a tracer study for an injection volume/mobile porosity tracer test, selection of the radial injection well (IW_x) and the dose-response wells (DR_x) is key to a successful test. Dose-response wells, as shown in Figure 25, are monitoring wells located radially (up-, down-, and side-gradient to the injection well, although the configuration may vary from study to study). These are not wells that are typically installed for a classic tracer study. Concentration in the dose-response wells is monitored through time. Once a complete breakthrough has been obtained for each dose-response well, the time at which 50 percent of the maximum concentration occurs and the total injection volume for that time can be determined. As such, it is critical to maintain records of injection volume as a function of time throughout the study. The mobile porosity can then be calculated using Equation (4). Assuming the mobile porosity is similar throughout the site, the estimated mobile porosity can be used to determine the volume of remediation fluid needed to provide a given coverage/distribution of the remedial fluid can be estimated. It is important to recognize that the t_{50} will vary as a function of the heterogeneity and anisotropy fields as well as with variation of ambient flow direction. Studies of this nature can also provide data important to understanding heterogeneity and anisotropy.

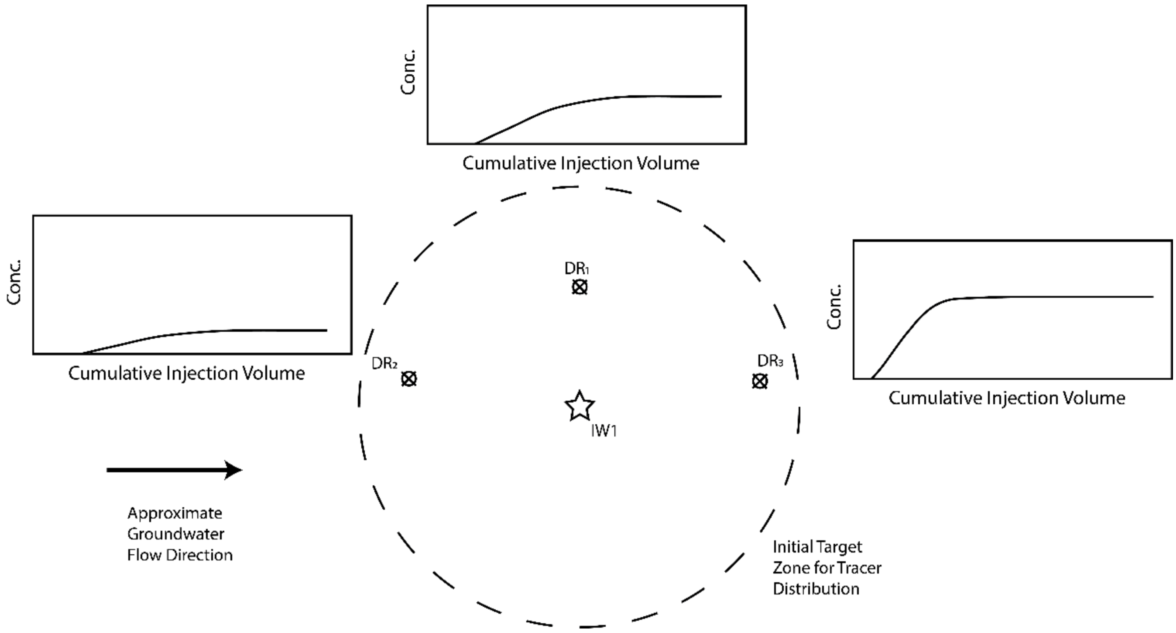


Figure 25 - Conceptual design for mobile porosity and injection volume and distribution tracer studies. The dye introduction location is labeled IW_X and the sampling locations are labeled DR_X (modified from Suthersan et al., 2014).

4.5 Well Development and Purging

Many drilling activities use fluids such as air, water, or mud to facilitate drilling and to flush the cuttings from the drill rods. Some of these fluids migrate into the subsurface and must be extracted upon completion of the borehole or well. Rather than purging an arbitrary volume of water prior to sampling, fluorescent tracers can be added to the drilling fluid as a quantitative approach for determining when an adequate purge has been achieved. This method has been described in detail by McCaughey and others (2016). Briefly, once the tracer and initial concentration have been selected, the proper mass of tracer dye can be added to each drill fluid tank. Once the drill water is prepared, a sample of the drill water is collected to prepare visual standards. These visual standards—commonly the drill water, 90 percent freshwater, 95 percent freshwater, 97.5 percent freshwater, and 99 percent freshwater—are used to determine when the borehole has been adequately purged. Periodic grab samples of purge water can be compared to the visual standards in the field as an initial qualitative screening tool. If the grab water samples collected during purging are turbid, allowing the samples to settle in a dark environment will allow more accurate visual comparison to the standards. Field fluorometers are also useful if samples are not too turbid. Once the purge goals have been achieved, groundwater sampling can be initiated. In addition to the field screening data, laboratory analysis of the periodic grab samples can be conducted to verify the field screening results in a quantitative fashion. Fluorescein dye is typically the best choice for this purpose as it is highly visible and is less likely than rhodamine WT to be masked by turbid water. Since fluorescein is subject to relatively rapid degradation in sunlight, dye standards and drilling fluids containing dyes should be kept in the dark as much as reasonably possible. If multiple tests will be conducted where overlap in the radius of drilling fluids could occur, it is important to account for any residual dye that may be present in background fluids to ensure the accuracy of subsequent testing. Although, as most of these tests are conducted visually, developing previous wells to the extent that the visual detection of dye is no longer possible is likely to eliminate any potential for background interference in subsequent testing. Finally, if fluorescent dyes are used to assess removal of drilling fluids, it should be recognized that this use may limit or prevent the use of the same dye in subsequent tracing work in potentially associated portions of the aquifer.

4.6 Summary

1. A study plan should precede even simple tracer studies. Table 16 provides a general outline for dye-tracer study plans.
2. It is important to identify purposes and objectives of tracer studies and to ensure that the design of the investigation will be capable of fulfilling those purposes and objectives.
3. Study plans should always have a specified time for sampling to terminate. Several strategies are offered for establishing the end of the sampling period.
4. Three different dyes can be used concurrently with a different dye introduced at three different points if dye-analysis work is based on laboratory analysis for tracer dyes using a spectrofluorophotometer operated under a synchronous-scan protocol.
5. Dye introduction points may include karst features such as losing streams and sinkholes. Backhoe trenches, borings, and monitoring wells are often used for dye introductions at waste sites. In some cases, shallow hand-dug wells may be used as dye introduction points, but they are typically located at groundwater discharge points rather than at points where water enters groundwater. In some cases, dyes can be placed as *dry sets*, where they will be introduced into an aquifer in recharge water from storm runoff or snow-melt events.
6. Tracer studies can be effective in assessing leakage from wastewater detention basins and in determining time of travel to receiving points. Tracer dyes are useful in assessing leakage and time of travel through dams, barrier walls, slurry trenches, and similar structures.
7. Dye tracing at industrial sites often involves testing for improper connections between sanitary or industrial sewers and stormwater sewers. Tracer dyes are an effective way to test for such connections, and using activated carbon samplers are the recommended strategy for sampling in this type of work.
8. Dye traces need both dye and water. Introducing tracer dyes as pulses followed by flush water makes the most effective use of the dye, reduces losses to short-term adsorption, and requires less dye than if the dyes are mixed with a similar volume of water and introduced at a relatively constant rate and concentration. Introducing a substantial

quantity of water containing dye is needed for some investigations focused on determining aquifer properties.

9. Identification of a suitable dye or dyes for use in a proposed tracing investigation, and determining dye quantities needed, requires consideration of several factors. Among the most important are the type of sampling that will be conducted and results of background sampling. Smaller amounts of dye are needed if primary sampling reliance is based on activated carbon samplers and laboratory analysis rather than water samples or, worst of all, field fluorometers.
10. Avoid the temptation to save money by not sampling some of the more distant points until several weeks or months after dye introductions.
11. Tracing dyes can help optimize efficiency and coverage of injection-based remediation systems.
12. Tracing dyes, and especially fluorescein, can be helpful in assessing the adequacy of well development.

5 Strategies for Some Common Types of Traces

5.1 Introduction

This section discusses seven categories of common groundwater-tracing projects, outlines common issues, and identifies dye-tracing strategies that have worked well. The seven types of projects are delineated here and illustrated in Figure 26.

1. Conservation issues including recharge area delineations and vulnerability assessments for species of conservation concern. These projects occur primarily in karst landscapes and occasionally in fractured rock aquifers.
2. Reservoir sites and leaking impoundment investigations.
3. Public water supplies including establishing hydrologic connections and determining time of travel. Recent traces for groundwater replenishment reuse projects in California are a good example of studies to determine underground retention times. Alabama may require dye tracing if a contaminated site is located within a complex hydrogeologic setting (i.e., karst and fractured flow environments) to identify all direct migratory pathways from the site to potential surface water bodies or public water supply wells in the area.
4. Active or planned mines including tracing associated with zone of influence delineations around limestone quarries; identification of sources for water flowing into mines; evaluation of wastewater disposal options; evaluation of waste rock disposal areas; identification of off-site springs and streams that might be impacted by mining activities; and mine drainage planning.
5. Closed or abandoned mines.
6. Industrial sites.
7. Waste sites.

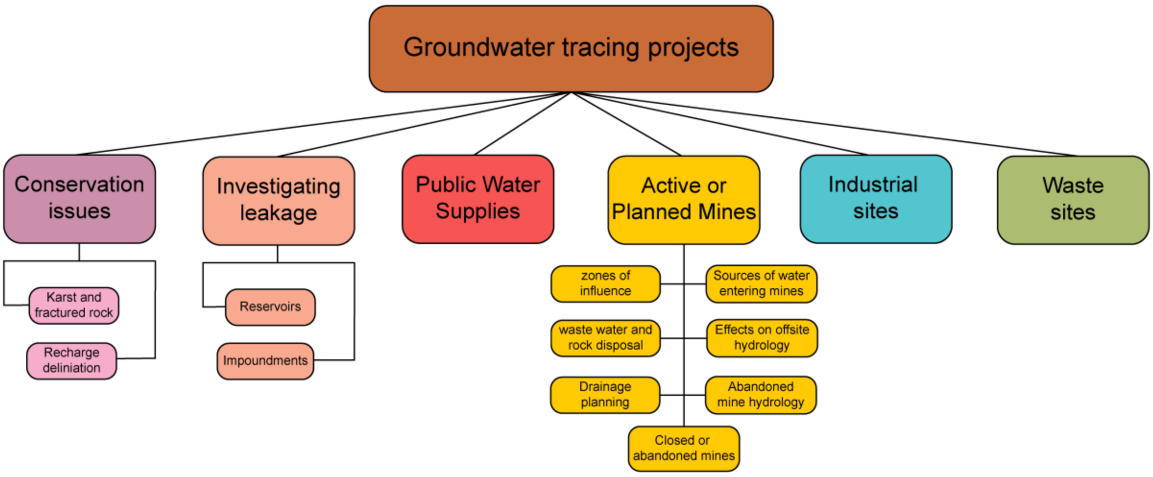


Figure 26 - The seven major categories of groundwater tracing projects, with common subcategories, discussed in this section.

5.2 Recharge Area Delineations and Vulnerability Assessments

Springs draining karst and fractured rock aquifers often have significant environmental and economic values. In addition, some caves associated with karst springs provide habitat for species of conservation concern, including a number that are state or federally protected as threatened or endangered species. If water quality and quantity for important springs and caves is to be protected, then delineating the land areas contributing water to those features is essential. Dye tracing is commonly the most credible and cost-effective tool for conducting recharge-area delineations, especially if groundwater travel times from dye introduction points to detection sites range from a few days to a few months. Thousands of groundwater traces using tracer dyes have demonstrated that rapid groundwater travel rates along preferential flow paths are common features associated with most springs in karst and fractured rock settings.

5.2.1 Study Designs

Recharge area delineations need dye introduction points likely to be well connected with preferential groundwater flow paths. Karst features, such as sinkholes and losing stream segments, are often suitable dye introduction points. In contrast, random borings, or unused wells, typically are not favorable locations, although there are exceptions. Shallow wells, often dug by hand, were abundant in the past in some rural areas. Wells were typically located at groundwater discharge points and, when they still exist, are generally poor dye introduction points. As an example, the senior author introduced 57 grams (2 oz) of a 20-percent rhodamine WT dye mixture into a dug well as part of an investigation of the stability of this dye in groundwater. As of the date of this writing, dye has remained in this well for 18 years and the concentration has not declined markedly during this period. This indicates that the water in the well is stagnant and not hydraulically well connected to the aquifer even though it is within 100 m (328 ft) of a major losing stream segment that provides rapid groundwater flow to local springs. If this well had been used as a dye introduction point for a recharge area delineation study, the trace would have failed.

It is important to select dye introduction points that will provide as much information on the recharge area as possible. For example, a trace from a losing stream with a topographic basin of at least a few square kilometers will yield more information valuable for management than a dye introduction in a sinkhole with only a small topographic basin, as discussed in Box 27⤵ and Exercise 9⤵.

The larger the mean flow rate of a spring, the more traces are needed to credibly delineate its recharge area. Many small recharge areas can be reasonably well delineated with only two to four well-placed dye introductions. In contrast, recharge areas that share water with multiple springs routinely require more traces to produce adequate recharge area delineations.

Dye introduction points that yield detections at springs other than the target spring are often as important in determining recharge area boundaries as are traces that discharge from the target spring. Some dye introductions need to be made at locations where they will help evaluate impacts of current or proposed land uses on groundwater quality. An example of such a trace focused on a proposed landfill site. This trace at a proposed landfill site also illustrates the importance of sampling for dyes in adjoining topographic basins in karst areas, as discussed in Box 28⤵. Dye tracing in the area around Silver Springs, Florida, USA, showed much slower groundwater flow rates than those encountered in the case history presented in Box 28, as discussed in Box 29⤵.

5.2.2 Flush Water for Dye Introductions

Groundwater tracing for recharge area delineations requires both dye and water. The water is needed for three purposes:

1. to moisten dry surfaces prior to dye introduction to reduce loss of dye solutions to surface absorption and adsorption;
2. to flush dye into groundwater;
3. for transporting dye and water to a spring or springs.

Ideal dye introduction points are likely to be well connected with preferential groundwater flow paths and to have water supplies readily available. An example is a spring that produces surface flow for only a short distance before the water re-enters groundwater. In this case, the dye is introduced into the surface flow channel a short distance upstream of the sink point.

In karst areas, surface streams with perennial flow often lose a portion of their flow to groundwater supplying one or more springs. Flow-rate measurements can sometimes identify stream segments that are losing water and help identify good dye introduction points. In other cases, dye is simply added to the surface stream and springs are sampled to determine if there are hydrologic connections. If a dye subject to appreciable sunlight destruction is used, the dye introduction can be made shortly before dark to minimize dye losses. Tracing to detect possible leakage from surface streams into groundwater and springs is usually best conducted under low flow conditions when streams are most likely to be losing flow to the subsurface conduits.

In some cases, water can be piped or hauled to selected dye introduction points. Water sources can be wells or sometimes ponds. In some cases, the water used will be municipal water and will contain residual chlorine from chlorination at a typical concentration of about 1 mg/L. If the trace might show a direct connection with an important aquatic habitat, the residual chlorine may be an issue of concern. In most cases this is not a problem, because the residual chlorine will be lost by the time the water reaches groundwater. Chlorine concentrations can also be reduced by treating the water. The use of water from farm or woodland ponds is generally acceptable, although in some cases there may be other water-quality concerns.

Traces can fail if an insufficient volume of flush water is used. The OUL typically uses at least 3,800 L (1,000 gal) for a dye introduction and commonly uses as much water as can reasonably be delivered within a workday. Cost may be a significant consideration. In remote areas with winter snowpacks, dye introductions can be made during heavy spring runoff or dry sets can be established before snowfall so that snowmelt will take the dye into solution and introduce it into groundwater. This type of introduction is not well suited to determine precise travel times.

At the other end of the flush water volume spectrum, the OUL has made substantial use of dry sets when working in remote areas or in places where water supplies for dye introductions are only occasionally present. Box 25 describes a trace using dry sets that were placed in highway culverts. Recommendations for dry sets are listed here.

- Powder dyes usually work better than liquid dyes.
- Ensure that the dye is placed where it will readily be taken into solution and transported.

- Place the dye in some type of container so it can be recovered in the event an adequate runoff event does not occur within an acceptable amount of time. To the extent reasonable, minimize the chance for people or animals to disturb the dye.
- Establish one or more sampling stations with activated carbon samplers at points downstream of the dry set. These locations will provide evidence that the dye in the dry set was taken into solution and will indicate how far downstream the dye was detectable. This provides information on the actual location of the dye introduction.
- If feasible, periodically check on conditions at the dry set until the dye is dissolved.

5.2.3 Selection of Sampling Stations

Thorough field work is required before introducing dyes for a recharge area delineation project to ensure that all potential dye discharge locations are sampled. It is important to know where a trace is detected even if it is not at the site that is the target of the study. Furthermore, if dye discharges from an unmonitored site, it will not be prudent to make another introduction at a different site with the same dye. Finally, detections at nontarget locations are valuable in drawing recharge area boundaries.

Many karst springs share portions of their recharge areas with other springs. For example, Indian Spring near Springfield, Missouri, USA, shares portions of its recharge area with at least nine other springs. Some of these springs are in a different river basin from Indian Spring. Under low flow conditions, the recharge area for Indian Spring is less than 1.6 km^2 (1.0 mi^2). Under high flow conditions, the recharge area is 20.48 km^2 (12.72 mi^2). This amount of essential information for Indian Spring would not be known without a comprehensive sampling program, as discussed in Exercise 10↴.

5.2.4 Vulnerability Assessments

Vulnerability assessments are commonly made for lands contributing water to caves and springs providing habitats for aquatic species that are federally listed as threatened or endangered. This work recognizes that not all lands in a recharge area pose equal potential threats to water quality (or sometimes water quantity) in a cave or spring.

Vulnerability mapping is a qualitative assessment of the relative risks posed to the target cave or spring by both natural and land-use conditions on lands in the recharge area.

Three to five vulnerability classes are commonly used in such mapping. For example, in the case of three classifications, the divisions could be labeled low, moderate, and high vulnerability lands. While a more detailed discussion of vulnerability mapping is beyond the scope of this book, it is a logical next step after recharge area delineations. Vulnerability mapping has been applied to most delineated recharge areas for caves and springs that provide habitat for threatened and endangered species—commonly referred to as "*listed species*"—in Missouri, Arkansas, Oklahoma, and Illinois of the USA. Time of travel and other information from groundwater traces is useful in determining the vulnerability classifications for lands in the recharge areas.

Figure 27 shows vulnerability mapping for three adjacent groundwater basins in an Oklahoma karst area providing habitat for a listed aquatic species. Four groundwater traces were conducted in conjunction with these delineations. High Vulnerability lands are losing stream valleys with rapid groundwater recharge and include areas around cave entrances. Moderate Vulnerability lands are upland areas used for grazing, and Low Vulnerability lands are steep, forested hillsides with little routine use. Land-use practices relevant to each land classification are recommended. Point sources of potential contamination are indicated by a letter and number.

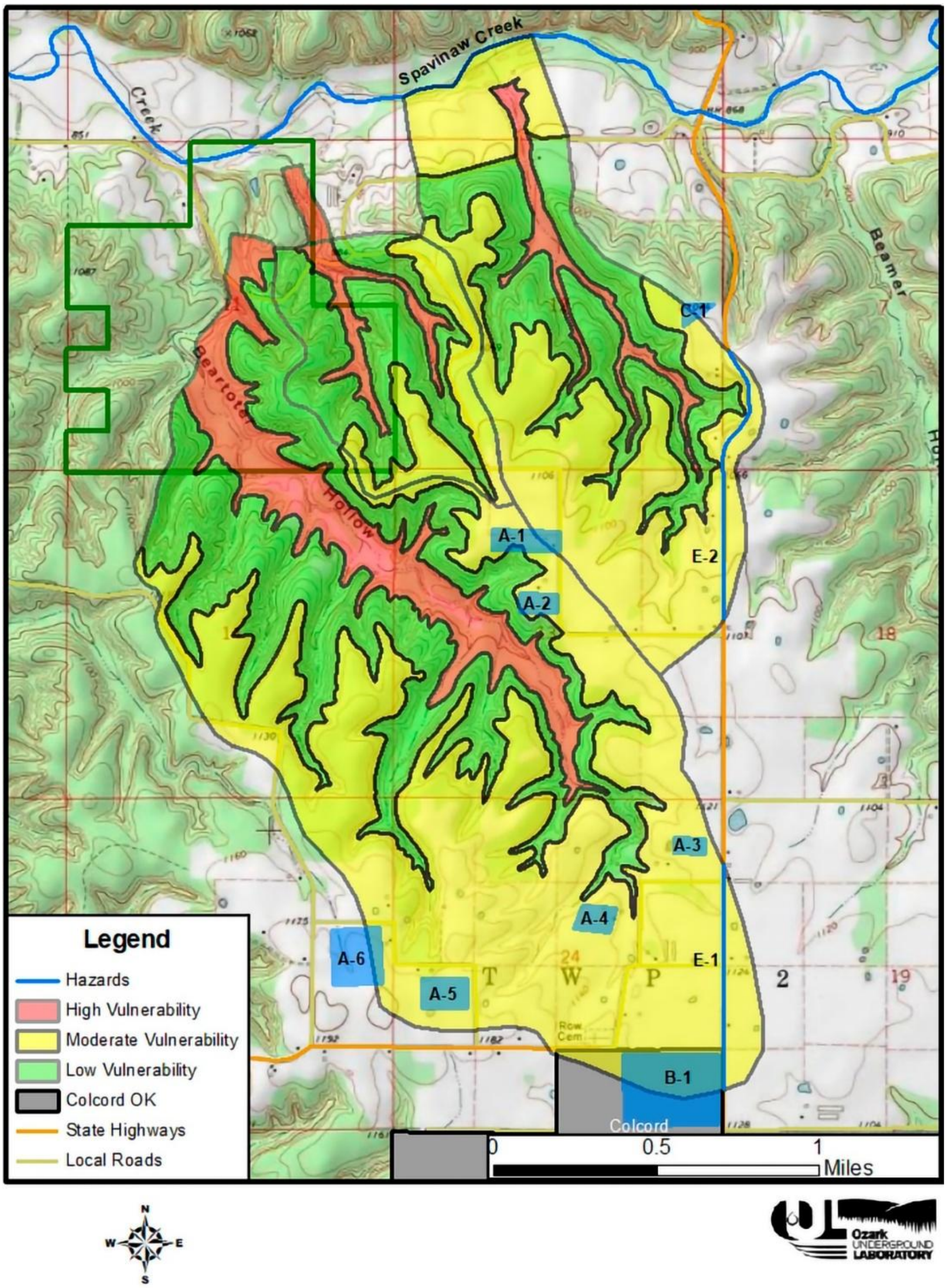


Figure 27 - Vulnerability mapping of three adjacent groundwater basins. Points with the letter "A" are agricultural point sources, the letter "B" indicates a sewage disposal facility, the letter "C" indicates a vehicle salvage yard, and the letter "E" indicates a state highway segment. The hazardous highway segments are blue and occur on the east side of the area. No hazards of type D occurred in the area.

5.3 Reservoir Sites and Leaking Impoundment Investigations

Impoundments that lose appreciable volumes of water into groundwater are common features in soluble-rock and fractured-rock landscapes. Groundwater tracing with fluorescent tracer dyes can be a useful tool for identifying leakage zones, providing time-of-travel information for leakage zones, and assessing site suitability for impoundments.

Leaking impoundments such as sewage lagoons and stormwater detention basins in karst often induce catastrophic sinkhole development (Aley et al., 1972). Such sinkholes can rapidly drain impoundments and introduce poor-quality water into karst groundwater. When this occurs, dye can be introduced into the newly formed sinkhole(s) and traced to springs and local wells. Sampling at private wells can be conducted effectively by allowing the well to be pumped continuously for the duration of the study at a rate of about 4 L (1 gal) per minute through an activated carbon sampler, as was shown in Figure 15. Carbon samplers are commonly left in place for periods of a week and sampling continues for a period appropriate for the study. New carbon samplers are deployed each time used samplers are collected. This approach is cost-effective and more likely to detect all impacted wells than is the approach of periodically collecting grab samples of water. A good protocol is to collect a grab sample of water each time carbon samplers are collected and analyze them for tracer dyes if dye is detected in an associated carbon sampler. The carbon samplers are the best approach for determining if dye reached the sampling point. The water sample provides dye concentrations in the water at the time the sample was collected.

Box 23 and Box 24 discuss traces designed to address leakage and possible leakage from reservoirs and resulting time-of-travel information. Traces associated with earth-fill dams have also been successfully conducted by introducing dyes followed by flush water into piezometers installed along the centerline of dams. These traces have involved placing tanks of water adjacent to dye introduction piezometers and installing equipment so that a constant head is maintained in the piezometer for a specified period (such as a week). These traces have been conducted to assess whether seepage zones downstream of dams represented reservoir leakage and, if they do, the groundwater travel rates involved. Still other traces have been successfully conducted to determine which of several possible areas within an impoundment are responsible for observed springs downstream of the dam. In most cases, primary sampling reliance has been based on activated carbon samplers and secondary reliance on grab samples of water. Dye tracing can also be a useful tool for evaluating proposed reservoirs, as discussed in Box 30↴.

5.4 Public Water Supplies

Groundwater tracing using fluorescent dyes is useful and cost-effective for studies involving public water supply wells, particularly when evaluating connections to surface water and determining groundwater travel time.

In California, tracer tests are used to determine credits for groundwater replenishment reuse projects, which involve injecting recycled water into the aquifer for beneficial reuse. Projects must meet pathogen removal requirements to ensure protection of public health, and groundwater tracing allows project management to demonstrate underground retention time and earn virus-log removal credits related to subsurface travel time from injection to extraction. "*Intrinsic*" groundwater tracing, which estimates travel and retention times using computer modeling, earns only 0.67 virus log-removal credits per month of underground retention. In contrast, a fluorescent dye-tracer test is considered an extrinsic tracer study and earns 1.0 virus log-removal credits per month of underground retention.

In Alabama, dye tracing has been successfully conducted in karst environments to test whether new public water supply wells are under the influence of surface water infiltration. A positive dye trace, combined with high bacteria counts, has resulted in the closure of proposed public water supply wells to ensure public safety.

5.5 Active or Planned Mines

Groundwater tracing has been successfully used to address relevant issues at active or planned mines. Common issues have included the following, and each will be described in subsequent subsections:

1. zone of influence (ZOI) delineations;
2. identification of water sources for water inflowing to underground workings;
3. evaluation of wastewater disposal options;

4. evaluation of waste rock disposal areas;
5. identification of springs and streams that might be impacted by mining activities;
6. mine drainage planning.

5.5.1 Zone of Influence Delineations

Quarries in limestone and marble often extract substantial volumes of water to permit mining of the rock. In many cases, quarry depths increase with time, and this consequently increases the rates of water extraction associated with the mining. Some states in the US (for example, Maryland) have regulations requiring that the ZOI for limestone and marble quarries be delineated, and this applies both to active and planned quarries. The ZOI is the land area surrounding a quarry where groundwater levels decline (or are expected to decline) as a result of quarry dewatering of the aquifer. Within the ZOI the quarry has an enhanced financial responsibility for the loss of groundwater supplies and property damage due to land subsidence and sinkhole collapse.

As an illustration of how much land surrounding a quarry might be impacted by groundwater extraction in karst, Waltham and others (2005, pages 170–171) describe a limestone quarry and deeper limestone mine in Hershey, Pennsylvania, USA. This quarry was extracting groundwater at a rate of 0.45 m^3/sec (15.9 ft^3/sec) and lowered the regional potentiometric surface by greater than 3 m (9.8 ft) over an area of 30 km^2 (11.5 mi^2). The senior author of this book did groundwater tracing at two quarries in Alabama that extracted similar volumes of water. This aquifer dewatering resulted in the formation of hundreds of sinkholes on lands up to 2 km (1.25 miles) from the edge of the quarry pits.

The extraction of appreciable volumes of groundwater by quarries is routinely associated with decreases in water available to wells, springs, and surface streams on surrounding properties. In some settings, the lowering of groundwater levels on surrounding lands results in land subsidence and/or sinkhole collapse. When groundwater is removed, unconsolidated materials overlying solutional cavities lose the buoyant support previously provided by water, and sinkhole collapses occur frequently.

Dye tracing is a commonly used technique for verifying connections between sites where sinkholes have damaged off-site property and quarries. Figure 28 shows a sinkhole that formed in a Frederick, Maryland, USA, street on May 7, 2022, near a deep limestone quarry. It is relatively easy to introduce a tracer dye into a sinkhole, such as shown in this figure, followed by at least 3,785 L (1,000 gal) of flush water. Sampling for the dye can be conducted in the pumping pool for the quarry or at the point where quarry pumping is discharged to a surface watercourse. The typical approach is to conduct at least two rounds of background sampling at all sampling points prior to introducing dye and sample using both carbon samplers and grab samples of water collected each time carbon samplers are collected and replaced. Control stations should be established at locations where fluorescent dyes or similar fluorescent materials might be present to address the possibility that dye detected in quarry water might have a source other than the sinkhole being tested. The duration of sampling should be based on local conditions and on previous tracing work (if any) in the area (Benson & Yuhr, 2016). While first dye arrival often occurs within a few days or weeks, the senior author has encountered a first dye arrival travel time at a quarry of approximately 80 days after dye introduction into a sinkhole. That sinkhole was continuously conveying flow from a surface stream into groundwater and was approximately 1.6 km (1 mi) from the quarry. The quarry pumping rate during this period was approximately 0.44 m^3/sec (15.5 ft^3/s or 6,950 gal/min). In court testimony, the formation of approximately 200 sinkholes was attributed to groundwater extraction from this Alabama quarry and even larger numbers of sinkholes have been attributed to dewatering by other quarries. Dye tracing in conjunction with surface stream flow measurements are cost-effective tools useful for ZOI delineations for new quarries or when quarries propose to go deeper and increase water-extraction rates.

Figure 28 - Sinkhole formed in a street in Frederick, Maryland, USA, on May 7, 2022. Dye and flush water were introduced into this collapse for a successful trace.

5.5.2 Identification of Water Sources for Inflowing Water

Substantial or very localized increases in the flow rates of water entering mines is often a significant issue of concern, especially at underground mines. Catastrophic increases in flow rates at underground coal mines in China, where coal units border karstified limestone, have resulted in many miner fatalities. Tracer dyes can be used to determine if newly encountered water is derived from surface ponds, lakes, or streams, or from older underground workings. In most cases the inflowing water does not have pH values so low that it seriously impacts the performance of tracer dyes. However, determining the pH of the incoming water is an important early action. Another important early action is field work to identify water sources that could contribute to newly encountered inflowing water. Basic water chemistry may provide some helpful insight. The type and amount of dye needed for traces of inflowing water is largely a function of the volume of water to be colored, travel distance, and estimated travel time. Fluorescein is generally the most effective dye for this type of work, but eosine and rhodamine WT also work well if multiple potential water sources need to be tested. Primary sampling reliance on carbon samplers with grab samples of water usually works well.

Mines are often in remote areas where it may take several days to get samples for dye analysis to a laboratory. If fluorescein dye and carbon samplers are available, duplicate carbon samplers can be visually tested in the field for the presence of fluorescein by eluting the carbon sampler with a saturated potassium hydroxide solution in 70-percent isopropyl alcohol (ethyl alcohol will also work). The charcoal is placed in a narrow clear glass bottle and covered to a depth of about 12 mm (0.5 inch) with the eluting solution. A super-saturated solution develops in the bottom of the mixing container and is not used for elution. The eluting solution is allowed to sit undisturbed on the charcoal; if fluorescein is present it will appear as the characteristic color in the liquid on top of the charcoal. If a large concentration of fluorescein is present, the dye will appear in the solution within a few minutes. Smaller dye concentrations may not appear until two or three days after treating the carbon with the eluting solution. The dye is best seen if a focused light beam—such as a flashlight—is shined horizontally through the glass bottle and viewed at 90° to the direction of the light beam. This is best done in a dark room. Laboratory analysis of a duplicate sampler is recommended, but rapid results from a field test are sometimes very valuable.

5.5.3 Evaluation of Wastewater Disposal Options

The US Supreme Court decision in the *County of Maui, Hawaii v. Hawaii Wildlife Fund et al.* (2020) case is likely to impact mine sites that routinely discharge water encountered in mining to groundwater or plan to do so. The court ruling applies to any discharge *that is the functional equivalent of a direct discharge* from the point source into navigable water. Interpretation of the phrase in italics is in the domain of both attorneys and hydrogeologists; good luck to us all. If such a discharge exists, or is proposed, a permit under the Clean Water Act is required. The court decision noted seven factors that may prove relevant, depending on the circumstances of a particular case. These include:

- transit time;
- distance traveled;
- nature of the material through which the pollutant travels;
- extent to which the pollutant is diluted or chemically changed as it travels;
- amount of pollutant entering the navigable water relative to the amount of pollutant that leaves the point source;
- manner by or area in which the pollutant enters the navigable water;
- degree to which the pollution—at that point—has maintained its specific identity.

The court stated that time and distance would be the most important factors in most cases but not necessarily in every case (Exercise 11↴).

Groundwater tracing with fluorescent dyes addresses several of the factors identified by the Court's decision. This approach is especially useful in addressing travel time. If dye tracing is used in an investigation designed to help determine if a permit under the Clean Water Act is required for a wastewater discharge from a mining site, then the trace should, to the extent reasonable, be designed to address the seven identified factors. Dye tracing can also be useful in evaluating wastewater disposal options beyond the issue of what permits may or may not be required.

Closed or abandoned coal and metal mines can discharge poor-quality water that enters groundwater before reaching a surface stream. The OUL has conducted many dye traces to determine if (or where) the mine discharge water enters surface streams or, in some cases, off-site wells. These are generally not complicated traces, but one must give careful attention to the pH of the water in selecting dye types and quantities. If the potential receiving stream has a large flow rate, one can effectively sample for the presence of dye introduced into mine water by placing carbon samplers in the edge of the stream at regular intervals along the mine side of the stream—for example, every 30 m (100 ft)—along the stream segment where the mine water is expected to discharge. It is advisable to establish one or two sampling stations upstream of any possible dye detection location as a control.

5.5.4 Evaluation of Waste Rock Disposal Areas

Waste rock from mining activities, commonly referred to as tailings, or sometimes, *chat*, commonly contains some of the minerals that were the target of the mining plus other associated materials that, when exposed to oxygen or water, are likely to produce—or help produce—contaminants of concern. Nitrates derived from explosives can be a significant constituent. Costs associated with haul distances and other factors frequently necessitate that disposal areas be near the mine, and this greatly limits potential waste-disposal locations. The net result is that many hydrologically undesirable waste sites have been used in the past and many are still in use today. Truly good disposal sites for current and future use seldom exist. Engineering can improve waste-disposal sites but, especially given the long-term nature of the wastes, is commonly incapable of preventing off-site migration of contaminants into surface and groundwater.

Dye tracing is useful in determining discharge points for contaminated groundwater from waste rock disposal areas and tailings ponds. In most cases, pH values are not so acidic that they limit the use of tracer dyes. Tracer studies can be conducted prior to waste rock deposition to determine if the monitoring well network is adequate to detect leakage and off-site migration of contaminants. Tracing can also identify off-site water sources likely to be impacted by waste rock disposal areas so they can be monitored or alternative water supplies can be developed, as discussed in Box 31↴.

5.5.5 Identification of Off-Site Springs and Streams that might be Impacted

Ore processing and tailings ponds are common potential sources of groundwater contamination. Inactive or abandoned mines, such as the small mine shown in Figure 29, can be significant sources of acid mine drainage and heavy metal contamination. Groundwater tracing using fluorescein, eosine, and rhodamine WT with activated carbon sampling was successfully conducted by the OUL in the area shown in the figure.

Figure 29 - Acid mine drainage from a small gold mine in southern Peru. Illegal mining operations often use mercury for gold recovery, and dangerous concentrations of it are commonly found in streams such as the one shown in this photo. Also note the black tailings along the stream from this and other mines.

5.5.6 Mine Drainage Planning

Natural rates of groundwater movement between monitoring wells can be determined by introducing tracer dyes into some wells and sampling for them in other monitoring wells.

5.6 Closed or Abandoned Mines

Typical issues include acid mine drainage or drainage of water with undesirable concentrations of multiple constituents. In coal mining areas of Appalachia in the US, various coal seams have been mined at locations close to one another by multiple mining companies, and valid questions commonly arise as to which mine is responsible for off-site problems in wells and/or springs. Dye tracing has been used to address mine drainage issues and to assign responsibility for off-site problems to specific mines, as discussed in Box 32↴, Box 33↴, Box 34↴, and Box 35↴.

Another common problem associated with closed or abandoned mines is treatment of acid-mine drainage. In some cases, surface water or water from other mines contributes to the volume of water that must be treated. Tracer dyes can be useful in identifying surface points where water is entering groundwater and contributing to the discharge volume from mines, but in the experience of the OUL the results appear to be strongly influenced by water chemistry and especially pH. Bench tests using site water is recommended, and very low dye detection concentrations are common. This is typically the result of low pH and excessive adsorption of dyes onto rock surfaces in the mines due to large surface areas of exposed rock faces and mineral precipitation that occurs on those surfaces.

5.7 Industrial Sites

A common issue at older industrial sites is the possibility of improper connections between or among industrial sewer lines, sanitary sewer lines, and stormwater lines. An example of an improper connection is a sanitary sewer line connected to a stormwater line. It is relatively easy to design traces to test for these possibilities. A tracer dye is typically introduced into the line suspected of leaking and activated carbon samplers are placed at appropriate points in the line suspected of receiving the leaked liquids. If there is an improper connection between the various types of lines, the results are typically obtained within a few hours to a few days. Leakage to groundwater that passes between two sewer lines may require several weeks to show a connection. It is often prudent to sample the sewer used for the dye introduction at a few downstream locations to verify that at least some of the dyed water reaches these locations and that the tested points are on the same sewer line downstream of the dye introduction point.

Background sampling prior to conducting tracer studies is recommended at industrial sites, as fluorescent compounds with characteristics similar to tracer dyes—or actual tracer dyes—may already be present. Some industrial activities may sporadically use or spill tracer dyes or materials with fluorescent characteristics similar to those of the tracer dyes. For example, fluorescein is commonly present in cooling tower blowdown, and many hydraulic fluids have a pink color due to one of the rhodamine dyes. Background sampling lasting long enough to capture such use of fluorescent compounds is essential. Laboratory analysis of the carbon samplers by an experienced laboratory that uses a synchronous-scan spectrofluorophotometer for analysis is recommended when tracing at industrial sites.

Liquids leaking from sewer lines or stormwater detention basins sometimes infiltrate into nearby buildings. Tracer dyes are a cost-effective method for identifying such connections, especially if sampling is conducted with activated carbon samplers and appropriate laboratory analysis.

5.8 Waste Sites

Some important study design considerations commonly appropriate to dye-tracing work at waste sites are included in the list provided in Sections 5.8.1 and 5.8.2. The key items in these design considerations are the solution to Exercise 12↴.

5.8.1 Background Sampling

Background sampling is essential for good-quality tracer studies at hazardous waste sites. Background fluorescence in or near the normal emission wavelength ranges of the tracer dyes occurs at some sites, and some tracer dyes may already be present at some sites. For example, sanitary landfills commonly contain fluorescein initially used in household products or in vehicle coolants. If adequate background sampling is not conducted, then fluorescence peaks consistent with tracer dyes being used will be interpreted as positive dye detections even if they do not represent the dye introduced for a trace.

a) A minimum of two rounds of background sampling at all planned sampling locations is recommended. More rounds of sampling are advisable if tracer dyes have previously been used at the site or if conditions warrant more-comprehensive background sampling.

b) Background sampling reliant on carbon samplers is more effective than grab samples of water, as the carbon samplers provide an integrated sample of background conditions rather than a point-in-time sample.

c) If a fluorescence peak in or near the emission wavelength range of one of the tracer dyes is detected in the carbon sampler elutant, the associated water sample should also be analyzed and the results evaluated.

d) An ideal approach is to leave background carbon samplers in place for the same duration as planned for collecting and replacing carbon samplers after dye introduction. If this is not possible, the recommended minimum duration for a background carbon sampler to be left in place is approximately 3 days.

5.8.2 Dye Introductions and Sampling

1. Dye introduction points that are specifically located and designed for dye introduction are usually preferable to using monitoring wells for dye introduction. While monitoring wells can be used, their locations and designs are often not ideal for introducing dye. Additionally, use of a monitoring well for a dye introduction point may reduce its utility for present or future monitoring. Visible concentrations of dye will commonly persist for months, and sometimes years, in the immediate vicinity of a monitoring well used as a dye introduction point. This can occur for multiple reasons, including heterogeneity in the aquifer, the well screen being poorly placed with respect to permeable zones in the aquifer, and poor well development or fouling of the well.
2. In some cases, larger-diameter (four- or six-inch diameter) injection wells that can accommodate large volumes of injection fluid) work well.
3. Backhoe trenches work well as dye introduction points. Their design and use are discussed in Section 4.2.2.
4. A boring to the top of bedrock with 1 to 2 m (3 to 6 ft) of gravel in the bottom and a temporary casing above the gravel often works well as a dye introduction point. At the end of the tracing work, such borings should be properly abandoned.
5. Before introducing dye, proposed dye introduction points should be tested with clean water to ensure they will accept a reasonable amount of water. The minimum acceptable rate should be specified in the study plan. Based on OUL experience, the acceptance rate should be at least 3 gpm (12.6 L/min).
6. Ideally, tracer studies should identify all sampling points reached by the introduced tracer dyes. This is often not possible due to multiple factors. The sampling approach that requires the use of the smallest amount of dye and is most likely to not miss dye detection sites is sampling with activated carbon samplers. In addition, it is good protocol to collect water samples each time carbon samplers are collected

and new samplers are placed. Water samples are typically analyzed for tracer dyes only if dye is detected in the associated carbon samplers. This approach requires that samples be analyzed by an experienced laboratory using a spectrofluorophotometer operated under a synchronous-scan protocol. Consideration should be given to analyzing both water samples and carbon samplers in monitoring wells that yield very little water, as under these conditions the carbon samplers are likely to accumulate less dye than in more productive wells.

7. Except for traces where the design and objectives specifically need dye to be introduced at a constant concentration, the best general approach for introducing tracer dyes into groundwater is as a high-concentration slug followed by flush water. Traces conducted this way require less dye and yield better time-of-travel information than traces where the dye is diluted in the mass of water introduced. The purpose of the flush water is to transport dye from the dye introduction point into the aquifer, and minimum desirable quantities will vary among dye introduction points.
8. Dye introductions into boring or wells are usually made by pouring the concentrated dye solution into the top of the well and following it with flush water. When used to support remediation agent design, it may be best to inject a large volume of dye mixture at a constant concentration. This provides a more uniform distribution and is more representative of remediation agent behavior. If the screened interval does not extend to the bottom of a well, some water with large concentrations of dye will be trapped in the well below the screened interval. This problem can be overcome in several ways. One method is to pipe flush water to the bottom of the well to provide good mixing of dye and water in the well bore.
9. A recommended minimum volume of water to add to a boring or well after dye introduction is three to five times the volume in the saturated portion of the casing. In many cases, a more reasonable volume of water to add to a boring or well after dye introduction is as much as can reasonably be added during a normal workday. Specifying the volume this way rather than as a finite volume in a study plan helps prevent extra hours (or days) spent at a dye introduction point that did not accept water as rapidly as anticipated and as specified in the study plan.

10. Mass balance calculations for dye traces to karst springs indicate that the median percent of introduced dye detected is approximately 4.5 percent (Aley, 2017). Unless excessive amounts of dye are used, a smaller detection percent should be expected in the geologic settings at most waste sites. Many traces have failed due to unrealistically low estimates of adsorption and dispersion of dyes in aquifers and by not recognizing that half of the rhodamine WT dye mixture is an isomer with a large retardation factor.
11. If tracing is a part of the introduction of a remediation agent into the aquifer, the tracer dyes will usually work best if introduced as a slug prior to the introduction of the remediation agent rather than mixed with the agent.
12. While many compounds may be present at waste sites, most of them do not degrade the tracer dyes discussed in this book. Positive dye traces have occurred in monitoring wells containing free product petroleum or TCE when sampling is limited to the portion of the well where only the dissolved phase of these compounds exists. At study sites and sampling locations where free-phase NAPL is present, tracer dyes may experience partitioning with the NAPL. As a result, partitioning processes may need to be considered in the interpretation of tracer study results in the presence of NAPL. However, if an adequate mass of dye is introduced, partitioning processes are unlikely to significantly impact the results of the study.
13. Many contaminants in groundwater will adsorp onto activated carbon and reduce the ability of the carbon to adsorp tracer dyes. The greater the concentration of such contaminants, the greater the reduction in adsorption of tracer dyes. On-site contamination does not appear to substantially limit effective tracing programs if the carbon samplers are in place for no longer than about a week, even in heavily contaminated water. Carbon samplers can be left for two-week periods at less-contaminated sites and in some cases appear to have functioned well when left in place for periods of up to about a month and perhaps longer. Leaving samplers in place for too long will degrade the quality of the tracer study and may bring negative results into question.

14. At sites heavily contaminated with petroleum products, the use of 50 percent to 100 percent more dye than would otherwise be used is appropriate if primary sampling reliance is placed on activated carbon samplers.
15. The recommended sampling interval for tracer studies at most waste sites is approximately seven days, at least during the first two or three months after dye introduction. Longer intervals may be acceptable after the first two or three months. Shorter sampling intervals can be used during the first 7 to 14 days after dye introduction if the first arrival time for dye might occur at some sampling points within a week or two of dye introduction.
16. An effective method for sampling wells and borings is to attach a single carbon sampler to the top of a disposable bailer and suspend the sampler in the middle of the screened interval. If the well is not screened, then sample in the middle of the saturated zone or in the zone where the most water is expected to move into the well. The disposable bailer permits easy collection of water samples. When a carbon sampler is recovered, the water in the bailer should be either poured back into the well or disposed of in accordance with site protocols, whichever approach governs at the site. The bailer is then lowered back in the well to obtain a fresh sample. After the water sample has been collected, a new carbon sampler is attached to the top of the bailer and it is lowered back into the well. Always make certain that the bailer and carbon sampler are below the water level in the well.
17. Open-hole wells with a long-saturated zone can be sampled at different levels by attaching single carbon samplers at each selected depth.
18. Dye-concentration data from carbon samplers left in place for different periods of time can be normalized for comparison by dividing the dye concentrations, by the number of days the samplers were in place. In most cases, this is a reasonable approximation of mean dye concentrations.
19. Dye-concentration results from successive samplers can be summed to estimate the total amount of dye detected at each sampling station.

5.9 Summary

This section summarizes common issues and resulting strategies related to the seven categories of common groundwater-tracing projects. The categories are: conservation issues; leaking reservoirs and impoundments; public water supplies; active or planned mines; closed or abandoned mines; industrial sites; and waste sites.

1. Conservation issues are typically related to the recharge area of springs draining karst and fractured rock aquifers. These springs often have significant environmental and economic value, thus delineating the land areas contributing water to these springs—and sometimes associated caves—is essential for managing and protecting their water quality. Dye tracing with primary sampling reliance on activated carbon samplers is commonly the most credible and cost-effective tool for conducting recharge area delineations. The following are important considerations:
    a) Dye introduction points need to be well connected with preferential groundwater flow paths. Because shallow wells are often constructed at groundwater discharge points, they are commonly not good dye introduction points.
    b) Dye introduction points that result in dye detections at springs other than the target spring are often very important in recharge area delineations.
    c) In karst areas it is important to sample springs in adjacent topographic basins, as was illustrated by findings associated with Mitch Hill Spring, Arkansas, USA, (Box 28).
    d) While not common, groundwater flow paths, and occasionally groundwater basin divides, can change seasonally in karst areas.
    e) There are dramatic differences among karst groundwater basins. There are conduit-dominated spring systems (Big Spring, Missouri, USA, (Boxes 3, 14, and 17) and Mitch Hill Spring, Arkansas, USA) and more dispersive flow systems (Silver Springs, Florida, USA, Box 29). Regardless of the type of aquifer, failed tracer studies are often related to inadequately characterized site conditions.
    f) There is often pressure to minimize the amount of dye introduced into groundwater due to concerns about colored water at receptors or off-site properties. Sampling with activated carbon samplers is an excellent tool for conducting credible traces while using minimal amounts of dye. This applies not only to recharge area delineations but also to reservoir sites, mines, and waste sites.

   g) Vulnerability mapping is a qualitative assessment of the relative risks posed to the target cave or spring by both natural and land-use conditions on lands in recharge areas. It is a common next step for water-quality protection after recharge areas have been delineated.

2. Dye tracing can be a valuable tool for assessing potential reservoir sites for leakage that will bypass the planned dam or discharge through groundwater to an adjacent stream basin. This is especially true in karst and fractured rock landscapes. Box 30 provides an example of the usefulness of dye tracing in demonstrating massive natural leakage from a planned water supply reservoir.
3. Dye tracing can be used to test whether new public water supply wells are under the influence of surface water infiltration. Dye tracing can also be to determine the underground retention time for groundwater replenishment reuse projects in California, USA. Underground retention time validated by a tracer test, such as a fluorescent dye-tracer test, is credited with a 1-log virus reduction, as opposed to 0.67-log virus reduction per month.
4. Dye tracing is relevant to many common mining issues (Categories 4 and 5) as illustrated by the following examples.
   a) Some limestone quarries continuously extract large volumes of groundwater to mine rock. The area impacted by this aquifer dewatering (called the "*zone of influence*," or ZOI, of the quarry) can be very large and the dewatering reduces both surface and groundwater supplies. In some limestone areas, quarry dewatering results in sinkhole collapses and other property damage within the ZOI. Dye tracing is an effective tool in delineating ZOIs and in determining if there are direct connections between newly formed sinkholes and water extraction at quarries or underground mines.
   b) Substantial, or very localized, increases in the flow rates of water entering mines is often of significant concern, especially at underground mines. Tracer dyes introduced into surface water sources or into inactive older underground workings with sampling at various points in the mine works well for identifying the source of the water, and fluorescein is generally the most useful of the tracer dyes for this purpose.

   c) Mines and other activities in the USA that have discharged wastewater to groundwater in the past may need to obtain an NPDES permit under a US Supreme Court ruling in 2020. Dye tracing is uniquely suited to obtaining some of the data that the Court indicated is important in determining if a discharge to groundwater is the functional equivalent of a direct discharge from the point source into surface water.
   d) Waste rock from mining activities commonly contains some of the minerals that were the target of the mining, plus other associated materials, that when exposed to oxygen or water are likely to produce (or help produce) contaminants of concern. Box 31 discusses aquifer vulnerability mapping for planned waste rock dumps where dye tracing played an important role in the investigations.
   e) Dye tracing can be useful in mine-drainage planning, as indicated in Box 32.
   f) Dye tracing is a useful tool at many closed or abandoned mines. Low-pH water can significantly affect dyes. Bench tests using water from the specific mine is often useful and should be conducted prior to tracer studies.
5. Dye tracing is useful at industrial sites to test for improper connections between or among industrial sewer lines, sanitary sewers, and stormwater pipes. A connection of a sanitary line to a stormwater sewer is a common example of an improper connection. Tracing with fluorescent dyes is also useful when the connections between various types of wastewater lines may leak to groundwater.
6. Important study design considerations for tracer studies at waste sites, are identified and elaborated on in this section as summarized here.
   a) The importance of comprehensive background sampling with some recommendations on how it should be conducted.
   b) The importance of sampling approaches that identify all sampling points reached by the introduced tracer dyes. Sampling using only field instruments or water samples routinely fails to detect some to many sites where activated carbon samplers would have produced positive dye detections. The risk of false-negative results increases with traces that use insufficient amounts of dye and/or introduce it premixed with the entire volume of flush water.

c) Except for traces where the design and objectives specifically need dye to be introduced at a constant concentration, the best general approach is to introduce dye as a high-concentration slug followed by flush water.

d) Many traces have failed because of unrealistically low estimates of adsorption and dispersion of dyes within aquifers. Mass balance calculations for karst aquifers discharging to springs indicate a median dye detection of approximately 4.5 percent of the dye introduced (Aley, 2017). Most aquifers at waste sites provide greater adsorption and dispersion than karst aquifers.

e) If tracing is part of the introduction of remediation agents into the aquifer, it is generally best to introduce the dye as a slug followed by the remediation agent(s).

6 Exercises

Exercise 1

If colored water at a potential dye receptor site is a significant concern, list three actions that can be taken to minimize the possibility of visual detection by the public without compromising the trace.

Solution to Exercise 1↓

Return to where text linked to Exercise 1↑

Exercise 2

What is the concentration difference between the visual detection limit for the public for the five tracer dyes and the instrumental detection limit using a laboratory spectrofluorophotometer?

Solution to Exercise 2↓

Return to where text linked to Exercise 2↑

Exercise 3

Why is it important to know and report the percentage of diluent in the dye mixture used for a trace?

Solution to Exercise 3↓

Return to where text linked to Exercise 3↑

Exercise 4

Budget constraints often limit the breadth and duration of tracer studies. Explain how the use of carbon samplers rather than grab samples of water can yield equal or better tracing results with a similar budget.

Solution to Exercise 4↓

Return to where text linked to Exercise 4↑

Exercise 5

What are travel times may be important to determine in tracer tests?

Solution to Exercise 5↴

Return to where text linked to Exercise 5↥

Exercise 6

What are some advantages of using shallow trenches for dye introduction rather than new or existing boreholes?

Solution to Exercise 6↴

Return to where text linked to Exercise 6↥

Exercise 7

What are some primary factors that must be considered when selecting the dye type(s) and quantity for a given tracer study?

Solution to Exercise 7↴

Return to where text linked to Exercise 7↥

Exercise 8

Groundwater traces can fail to yield positive detections at some or all the sampling points to which the dye-tagged water flows due to the use of an inadequate amount of dye. List several factors related to the dye selected for use that can be included and explained in the study plan that can limit this risk.

Solution to Exercise 8↴

Return to where text linked to Exercise 8↥

Exercise 9

Why are losing stream segments and sinkholes preferrable to borings and wells for dye introduction locations in recharge area delineation studies?

Solution to Exercise 9↴

Return to where text linked to Exercise 9↥

Exercise 10

Is there a general rule of thumb in karst terranes for the size of the recharge area relative to the flow rate of the feature of interest?

Solution to Exercise 10↴

Return to where text linked to Exercise 10⮥

Exercise 11

What are the seven factors the US Supreme Court has identified in the *County of Maui, Hawaii v. Hawaii Wildlife Fund, et al.* (2020) decision as pertinent to obtaining an NPDES permit for discharge to groundwater?

Solution to Exercise 11↴

Return to where text linked to Exercise 11⮥

Exercise 12

What are key design considerations for tracer studies at waste sites?

Solution to Exercise 12↴

Return to where text linked to Exercise 12⮥

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8 Boxes

Box 1 - Case History 1: Trace from a gas station tank pit to a well, Arkansas, USA

A family owned a small resort near a large reservoir in northern Arkansas, USA. Their water supply was a well drilled into the cavernous dolomite karst that typified the region. Wells are often located where they will likely not interfere with other buildings or activities. That was the case with this well. It was drilled in a corner of the property near the property line and near a state highway where it would be out of the way. That was a bad idea. Especially in karst areas, wells need to be protected from land uses that could degrade groundwater quality, and (as the owners learned) a well in the corner of their property was not easily protected.

A few years after the resort went into operation, a small store with gasoline pumps was constructed on the neighboring property. This occurred in the era when most gasoline storage tanks in the United States were buried in unlined pits. A better approach for protecting groundwater, especially in karst areas, is locating tanks on the surface in concrete containment basins capable of holding the entire capacity of the tank(s) should they leak or rupture. Containment basins have drains that allow accumulated precipitation to be discharged from the basins. A common flaw in this approach is that people do not always close the drain after use.

This particular gasoline station used buried tanks. The property owner, who was also the county sheriff, probably wanted the tanks where they were out of the way. He selected a corner of his property and buried the tanks. The tank pit was within 13.7 m (45 ft) of the resort's well. The steel tanks, like thousands of others in the United States, leaked or experienced one or more overflows. Gasoline appeared in the resort's well and made the water unusable.

It does not take much gasoline to render water unpotable; the taste and odor threshold for gasolines of that era was approximately 0.005 mg/L (Bouwer, 1978). This concentration can be conceptualized as roughly equivalent to five droplets of gasoline in an Olympic size swimming pool. Once gasoline enters a well it is routinely persistent, and the well is usually lost as a drinking water supply. Gasoline also persists in the piping and fixtures connected to the well water, and that was a major problem in this case. The loss of the well and the connected water system was financially devastating to the resort.

The thickness of soil and residuum overlying the dolomite bedrock was approximately 18.3 m (60 ft). Unfortunately, as was often the case in Arkansas, the well was only cased to about 21.3 m (70 ft) below ground surface. The bottom of the casing barely penetrated into the bedrock. That was typical for wells drilled in northern Arkansas during the 1950s through 1980s. Such shallow casing was effective at keeping sediment derived from the adjacent soil and residuum out of the well, but it would not (and in this case did not) protect the well water from contamination. The typical depth to water in the well was about 43 m (140 ft).

An existing monitoring well that bottomed in the tank pit was designed to detect tank leakage if it occurred. It was positive for Total Petroleum Hydrocarbons (TPHs). The presence of gasoline in the monitoring well and in the nearby resort well, combined with the proximity of the tank pit to the well, should have convinced the state's pollution control agency of a hydrologic connection. Either the agency had an unusually high bar for evidence or the influence of county sheriffs in the rural American south was stronger than hydraulic gradients. Either way, the connection was not regarded as proven.

Consultants were retained. A local engineering company conducted laboratory permeability testing of the clay-rich residuum, and based on low permeability values, it concluded that gasoline could not have moved from the tank pit to the well. They did not recognize that *in situ* permeability of soils in karst regions are commonly three to four orders of magnitude higher than remolded soils in laboratory permeability testing (Sauter, 1991). The relevant state agency funded a remedial investigation conducted by a consulting company with offices scattered around the United States. That company made four soil borings (none of which reached water). They concluded (like the local firm) that the soil and residuum had low permeability, and that gasoline from the tank pit could not have reached the well. They suggested that the source might be another gas station more than a kilometer (0.62 mi) distant. That hypothetical pollution source was not owned by the sheriff.

The OUL was retained by an attorney for the well owners and conducted a dye trace. A court order allowed the OUL to introduce 0.9 kg (2 lb) of a fluorescein dye mixture to the tank pit. Fluorescein is also commonly known as uranine. Dyes used in groundwater tracing all come as mixtures containing dye and a standardizing agent known as a diluent. The amount of dye in dye mixtures varies widely, so it is important to report the amount of dye in the mixture used (called the dye equivalent). Dye equivalent values are the percent of dye in a dye mixture on a weight basis. The fluorescein mixture used for this trace contained approximately 75 percent dye and 25 percent diluent.

The dye mixture was a powder and was mixed with approximately 7.6 L (2 gal) of water and then poured into the tank pit through the monitoring well. The dye solution was followed by approximately 375 L (100 gallons) of water. More water was desirable for flushing the dye through the vadose zone. It would not have disturbed the tank but was not acceptable to the state agency, thus only 375 L was used to flush the dye into the system.

A simple study plan was developed for the investigation; study plans for tracer studies are discussed in Section 3. Background sampling, always an important step, was conducted prior to introducing the dye. This sampling demonstrated that fluorescein or other fluorescent materials that could produce emission fluorescence peaks in or near the acceptable emission wavelength range of fluorescein were not present in ambient groundwater at the well. The well was pumped continuously at a rate of about 4 L/min (1 gal/min) for the duration of the study. OUL experience indicated that this was a good approach for sampling wells for tracer dyes. Sampling for the dye used both grab samples of water and activated carbon samplers; both approaches are discussed in Section 3. Dye first reached the well within 20 hours of its introduction into the tank pit, and the peak dye concentration occurred about nine days after dye introduction.

Discussion. The case went to trial in an ancient county courthouse on the town square. The jury awarded the resort owners about USD 150,000. They were happy, the sheriff was not, and the testifying expert who did the dye trace carefully complied with all laws in the county until there was a change in sheriffs. The tracing work was simple and easy, but it directly addressed the question of whether water contaminated with gasoline could move from the tank pit to the well. The dye tracing was both the lowest cost and most appropriate tool for addressing the issue at hand and required only simple field work and an analytical laboratory capable of analyzing activated carbon and water samples for fluorescent tracer dyes. Figure Box 1-1 illustrates this simple and appropriate tracer test.

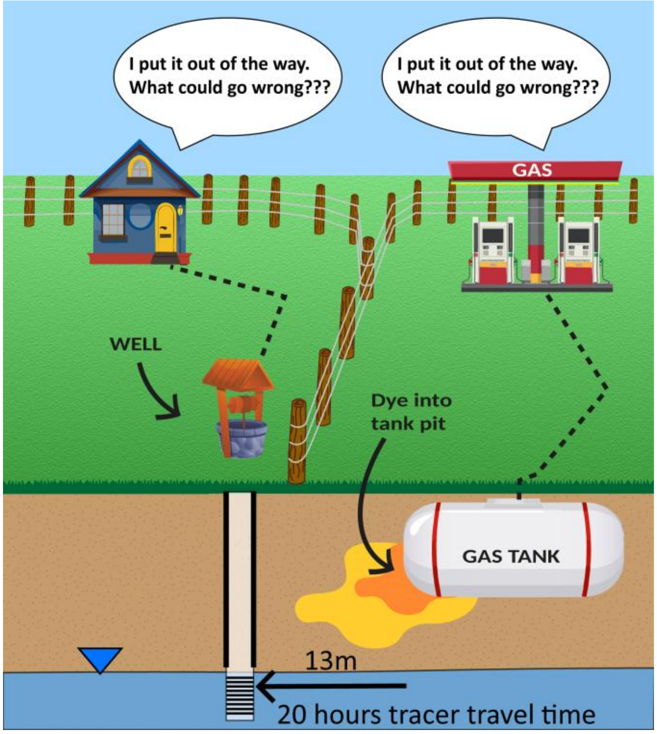

Figure Box 1-1 - What could go wrong? Travel time for first arrival of dye at the well was less than 20 hours.

Back to main text for Box 1↥

Box 2 - Case History 2: Traces from on-site sewage systems to marine shellfish beds, Washington State, USA

A major impetus for the tracing work in Washington state was the presence of valuable shellfish beds in the shallow water of Puget Sound and the human health implications of consuming shellfish contaminated with pathogens. Thurston County, Washington, has nearly 161 km (100 mi) of shoreline on Puget Sound and lakes; most of these shorelines are populated. Hofstad and Davis (1995) described a standardized tracing method for Thurston County where 170 ml of fluorescein dye solution containing eight grams of fluorescein dye was introduced into each tested on-site sewage system. Sampling was conducted in bulkhead drains and used activated carbon samplers which adsorp and retain passing fluorescein dye. Bulkhead drains are pipe segments that extend through concrete walls (bulkheads) that have been constructed along many miles of shoreline to separate backyards of homes from the tidal zone. The bulkhead drains allow water levels on the two sides of the bulkheads to equalize as tides rise and fall.

Fecal coliform testing was conducted at all dye sampling locations. A failing sewage system was defined as yielding one or more dye detections in the activated carbon samplers plus fecal coliform counts of more than 200 colonies per 100 ml at the bulkhead sampling point. A dye detection identified which residence was responsible for the inadequately treated sewage; sampling lasted for approximately two weeks after dye introduction. Based on 1,600 dye introductions, Hofstad and Davis (1995) estimated that 12 to 14 percent of on-site sewage systems serving shoreline homes in Thurston County were failing. This rate was approximately twice that from previous work that used dyes, but that did not use activated carbon samplers for detecting the dye.

Discussion. As a result of these findings, sampling with activated carbon samplers has been conducted at thousands of sites in multiple counties bordering Puget Sound. In some cases, eosine, and rhodamine WT dyes have also been used, but fluorescein is preferred because it is detectable with carbon samplers and laboratory analysis may not be required in many cases. If it is possible that two or more houses may contribute water to a particular sampling point, then multiple dyes are typically used with one type of dye for each house so that all houses can be concurrently tested.

Most of the traces around Puget Sound used a dye mixture that contained eight grams of fluorescein dye. The travel distances were less than 40 m (130 ft), and they were designed to yield positive dye detections only when sewage systems were clearly contaminating marine water with fecal bacteria. Dye was introduced as a short-duration pulse into household sewers. With failing treatment systems, the dye was routinely detectable at sampling points within two weeks. The approach has received broad public support.

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Box 3 - Case History 3: Long distance traces to Big Spring, Missouri, USA

Big Spring is the largest groundwater contributor to the Ozark National Scenic Riverways, a unit of the US National Park system. Aley (1978) conducted eight successful traces to Big Spring with each dye mixture containing 2.3 kg (5 lb) or less of fluorescein dye. The mean straight-line underground travel distance for these traces was 36.9 km (22.9 mi) and the greatest distance was 63.6 km (39.5 mi). The mean flow rate of Big Spring is 13.32 m^3/sec (470 ft^3/sec) and most of the traces were conducted during periods when the spring flow rate was within about 20 percent of the mean flow rate. The photograph on the cover of this book is of Big Spring when the flow rate was 9.58 m^3/sec (338 ft^3/sec). The time of first dye arrival at Big Spring was usually within two weeks of dye introduction. The rapid arrival indicates that most of the travel was through solutionally widened openings in the dolomite karst. The dye introductions were made as pulses in water with average flow rate of less than 100 L/min (25 gpm) at the points of dye introduction.

Discussion. The traces to Big Spring used very small amounts of dye for very large volumes of water and long travel distances. This was possible because sampling was conducted with activated carbon samplers, as discussed in greater detail in Section 3. Inexpensive groundwater tracing such as the traces to Big Spring are fully adequate for addressing many questions. In the case of the traces to Big Spring they were designed to delineate recharge areas for several major springs and to derive estimates of travel rates to these springs from losing stream segments. Information from the tracing studies provided a technical foundation for choosing the approach to land management in areas contributing water to the high-value springs of the region.

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Box 4 - Case History 4: Tracing of sewage effluent from disposal wells to springs on the floor of the Pacific Ocean, Hawaii, USA

A dye trace of treated sewage effluent at Lahaina, Hawaii, USA (Glenn et al., 2013), used a powdered fluorescein dye mixture that weighed 154.5 kg (340 lb) with a dye equivalency of approximately 77 percent. The dye was mixed with water and introduced over a 19-hour period with a mean flow rate of approximately 9.5 million L (2.5 million gal) per day. First arrival for the dye at the North Seep Group of submarine discharges was 86 days for a straight-line distance of 821 m (2,693 ft), and 109 days for a straight-line distance of 932 m (3,057 ft). The calculated average time of travel was more than one year. The authors of the study concluded that approximately 64 percent of the introduced dye discharged from the springs. Groundwater travel was through fractured volcanics and alluvium. A second dye introduction using 81.8 kg (180 lb) of sulforhodamine B with an approximate dye equivalency of 25 percent was not detected using the sampling and analytical methods of the study, which did not include activated carbon samplers.

Discussion. The groundwater trace in Maui illustrates the value of groundwater tracing with fluorescent tracer dyes in the United States. The trace results were an integral part of the US Supreme Court decision of April 23, 2020, that pollutant discharges to groundwater require federal permits, just like surface water discharges, if they are the *functional equivalent of direct discharges to surface water*. The phrase in italics is key to the decision of the court. In the Maui case, the court determined that dye tracing demonstrated that the treated sewage discharged into disposal wells traveled through the alluvial and fractured rock aquifer and was the functional equivalent of a direct discharge to the Pacific Ocean. In determining whether a discharge to groundwater required a permit, the court suggested several factors that might prove relevant to the circumstances of a particular case. They included:

1. Transit time.
2. Distance traveled.
3. The nature of the material through which the pollutant travels.
4. The extent to which the pollutant is diluted or chemically changed as it travels.

5. The amount of pollutant entering the navigable water relative to the amount of the pollutant that leaves the point source.
6. The manner by, or area in, which the pollutant enters the navigable water.
7. The degree to which the pollution (at that point) has maintained its specific identity.

The court noted that time and distance will be the most important factors in most cases, but not necessarily every case.

Prior to the Maui decision wastewater discharges to groundwater were excluded from requirements to obtain National Pollution Discharge Elimination System (NPDES) permits. In the opinion of the authors of this book, it is likely that thousands of wastewater discharges to groundwater in the United States will need to be evaluated to determine if they require NPDES permits and that dye tracing is likely to be a common part of many such evaluations. The elaborate and expensive tracing at Maui may have been important in the court's decision. However, in the opinion of the authors of this book, much simpler and far less expensive traces could have adequately developed scientifically acceptable data on the same factors that the court identified. We wrote this book to be helpful to people involved in similar issues, either in the conduct of tracer studies or in recognizing their value.

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Box 5 - Case History 5: Groundwater traces in East Snake Plain Aquifer, Idaho, USA

From 2008 to2024, the Idaho Department of Water Resources and Idaho Power Company have conducted at least 24 groundwater traces in the East Snake Plain Aquifer in Idaho using fluorescent tracer dyes. The aquifer is hosted in fractured, shield-volcano, basaltic, lava flows that cover 10,000 mi^2 (26,000 km^2) and provide water for extensive irrigated agriculture on the overlying lands. The aquifer also discharges to the Snake River through many springs, many of which have flow rates approaching 300 ft^3/s (8.5 m^3/sec). Some of these are used for electric power generation. The studies have largely relied on fluorescein dye and have used a wide range of sampling and analytical methods. Activated carbon samplers placed to sample water from irrigation and domestic water supply wells have been a key sampling approach and are critical for the detection of dye from long-distance traces. In many cases, successful dye detections have resulted from carbon samplers that were placed in the reservoir tanks of residential toilets that are served by water wells and left for varying durations of up to several months. The rate of water exchange in toilet reservoir tanks often exceeds that in some monitoring wells such as those common at waste sites.

Two major traces have been conducted from groundwater recharge basins, and the longest groundwater distance traced to date in the Idaho studies has been over 49.9 km (31 mi). For this trace 56.4 kg (124 lb) of powder-form fluorescein dye mixture with an approximate dye equivalent of 75 percent was introduced into a 48-hectare (120 acre) groundwater recharge basin. Water depth in the basin was approximately 4.6 m (15 ft), and the basin floor was thin soil and exposed basalt. Several open-file reports are available on the Idaho Department of Water Resources website. Those by Farmer and Blew (2021), Farmer and others (2014), and Farmer and Owsley (2009) are good examples. Blew and Farmer (2015) published a paper on high velocity flow and small transverse dispersion based on their tracing work in the fractured basalt.

Discussion. Previous to the work by Farmer, Blew, and their associates, dye tracing in the East Snake River Plain Aquifer on the scale described above had not been done. Data from the Idaho tracing work is providing valuable estimates of effective hydraulic conductivity; net groundwater direction and velocity; and lateral, longitudinal, and vertical dispersion (Figure Box 5-1). Dye-tracing results are consistent with US Geological Survey aquifer tests and analytical models. Dye-tracing results are more accurate than previous the results of numerical groundwater models along the traced flow paths. The information is valuable in assessing where, and how rapidly, the introduced water reaches springs and production wells as well as for guiding water management practices. For this work, the seepage velocity was calculated using the time from dye introduction to peak dye concentration at the sampling station. The data presented in Figure Box 5-1 indicate that the seepage velocity decreases with increasing travel distance; this correlates with decreasing groundwater gradients further away from the springs. Many of the traces with the more rapid travel rates are near springs where the gradient is steeper. Travel rates shown by dye traces have routinely been more rapid than indicated by modeling or long held assumptions based on historical conceptual models. The senior author visited most of the key tracer site locations on the Eastern Snake River Plain in 2018 and evaluated the dye-tracing program. He found that both the field work and data analysis was being conducted properly by experienced hydrogeologists and has enhanced the credibility and public acceptance of this very important work.

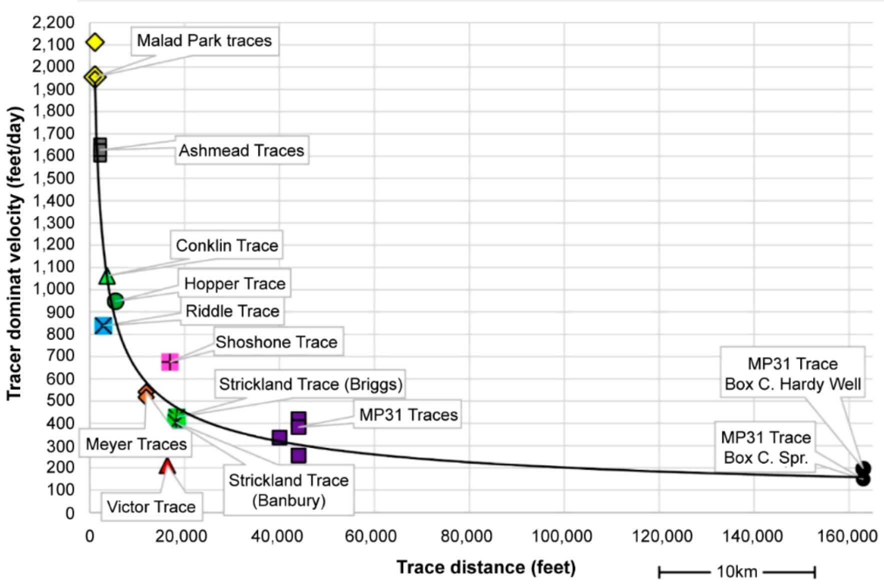

Figure Box 5-1 - Seepage velocity and trace distance relationships. The seepage velocity is calculated using the time to peak dye concentration; 1 meter=3.28 feet (illustration courtesy of Neal Farmer, Idaho Department of Water Resources).

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Box 6 - Case History 6: Trace from drainage ditch to municipal well, Walkersville, Maryland, USA

In the early 1990s, Walkersville, Maryland, USA, had three active municipal wells in the Grove Limestone; these wells supplied water to about 7,500 people. Much of the town is underlain by karst with springs and sinkholes; small sinkhole collapses occasionally form in town. As part of the United States Environmental Protection Agency's (EPA) wellhead protection program the agency used models to delineate 5- and 10-year time of travel (TOT) lines around the three town wells and provided the town with maps showing these lines. The burgess of the town (the equivalent of a town mayor) was concerned that the delineated TOTs had substantially under-estimated groundwater travel rates. Using an EPA grant and town funds Walkersville retained the OUL to conduct groundwater tracing in the community to assess the reasonableness of the TOT delineations provided by EPA.

One of the dye introductions made by the OUL in 1992 was into a shallow drainage ditch near the town library at a point on EPA's 5-year TOT line for Town Well #1. A small, recently formed sinkhole about a meter in diameter and half a meter deep, was found in the ditch during field work. This feature was selected as the dye introduction point, but it was filled with soil by town workers before the date of dye introduction. Ignoring this change in surface conditions, water from a fire hydrant was discharged into the ditch at a rate of approximately 490 L/min (130 gpm) and flowed for about 75 m (245 ft) to the location of the former sinkhole. When water reached the former sinkhole the fill dirt collapsed and 454 g (1 lb) of rhodamine WT dye mixture with a dye equivalent of approximately 20 percent plus approximately 19,000 L (5,000 gal) of water went into the small sinkhole and disappeared into the underlying limestone. The dye introduction was made at 13:55 hours on August 19, 1992. Modest rainfall events routinely yielded flow volumes to the ditch that equaled or exceeded 19,000 L (5,000 gal) so the tracer study was conducted under near-natural hydrologic conditions.

The introduced dye was first detected in Town Well #1 within 17.5 hours. This well is approximately 700 m (2,650 ft) from the dye introduction point. Dye also reached the other two municipal wells in less than a week. Based on the first arrival time at Town Well #1, the EPA's five-year TOT boundary, determined from modeling without field verification, was in error by a factor of 2,500. This is an unacceptably large error for modeling intended to provide water users protection from spills or discharges within wellhead protection zones.

Discussion. Given the distances involved in the trace at Walkersville, the amount of dye introduced was very small. Using a small amount of dye ensured that the town's water supply would not be visibly colored. However, using such a small amount of dye increased the chance that dye would not be detectable in collected water samples. Sampling the wells with activated carbon samplers in addition to water samples ensured that, if there were a hydrologic connection between the dye introduction point and the well during the study period, dye would at least be detected in the carbon samplers. Activated carbon samplers continuously accumulate dyes from the water, ensuring "gaps" between water samples do not miss short pulses of tracer. They are discussed in detail in Section 3. The concentration of dye eluted from the carbon sampler is routinely one to two orders of magnitude greater than the mean dye concentration in water samples (Aley, 2017). Since carbon samplers accumulate dye, the dye concentrations from consecutive samplers can be summed and plotted to show the total accumulated concentration of dye at a sampling location (as shown in Figures Box 6-1 and 6-2 for data from Town Well #1). Dye was continuously detectable in water samples from Town Well #1 for about 15 days, but it was detectable in carbon samplers for at least 100 days. This occurred because the carbon samplers accumulate dye from water even when dye concentrations are below the minimum detection concentration in water samples.

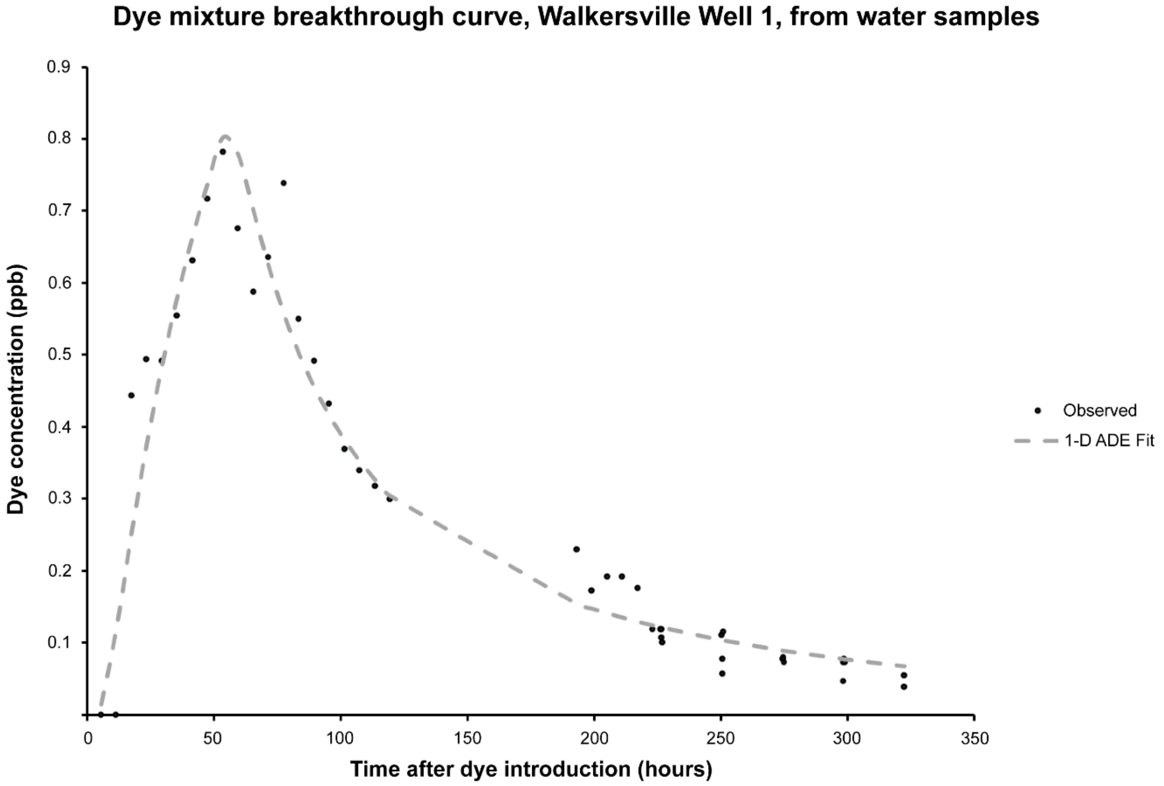

Figure Box 6-1 - Dye break-through curve of water samples from Town Well #1. Measured dye mixture concentrations through time are shown by the solid black circles. A one-dimensional advection-dispersion equation (ADE) was fit to the raw concentration measurements, shown in the dashed gray line, to illustrate an analytical method for analysis of break-through curves.

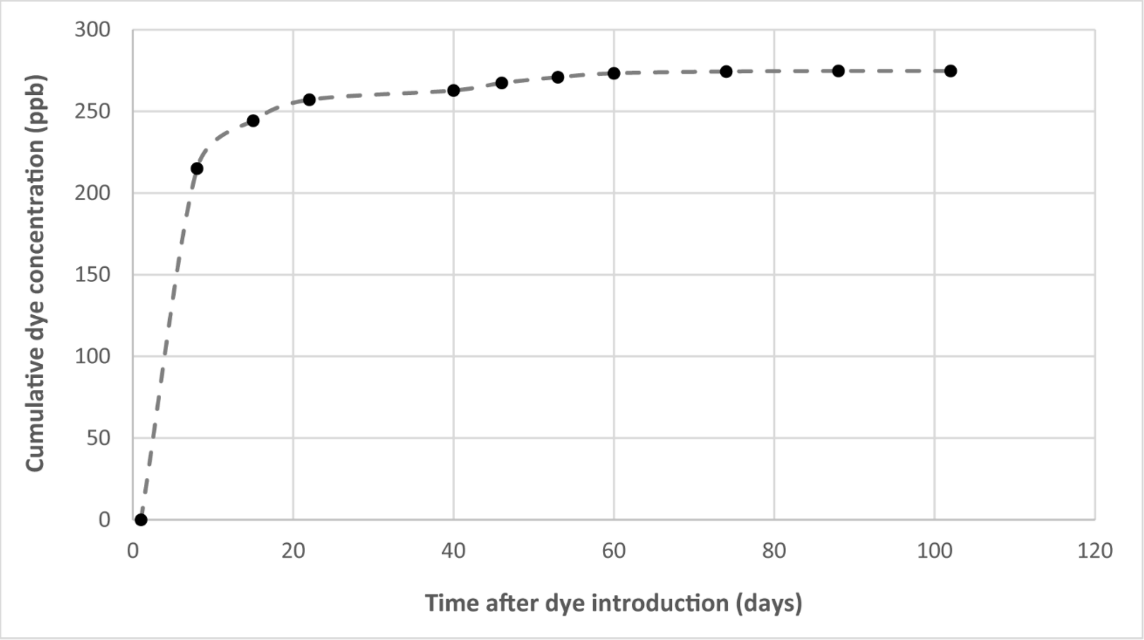

Figure Box 6-2 - Cumulative plot of dye mixture concentrations from carbon samplers, Town Well #1.

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Box 7 - Case History 7: Post-spill trace from ruptured sewer trunk to municipal wells, Walkersville, Maryland, USA

In 1999, seven years after the dye trace from near the town library, workers were constructing a sewer line for a new subdivision in Walkersville and blasted on a Friday afternoon to remove a limestone pinnacle. The blast fractured a nearby regional raw sewage line, but this was not known until the following Monday morning when raw sewage was discovered in the basement of a town bank. An estimated 3.4 million L (900,000 gal) of raw sewage was lost from the sewer line. Very little of the sewage appeared on the surface near the break, and the break might have gone undetected for many days had sewage not appeared in the bank's basement. Using EPA's 5- and 10-year time of travel (TOT) lines the estimated time of travel from the vicinity of the sewage line break to the town wells was expected to be approximately eight years.

Fortunately, town officials remembered the groundwater-tracing report that projected travel time from the vicinity of the sewer break to the town's municipal wells at approximately a week. The town went into emergency mode. Police and the fire departments, sirens and loudspeakers blaring, warned the public of the massive sewage spill and urged people to not use the water or, if it was used, to boil it before use. The health department required all 30+ restaurants in town to close. Intensive bacteriological testing of water from the municipal wells was started, and fecal coliform counts began to rise in these wells within five days of the spill. Eleven days after the blasting, fecal coliform in two municipal wells reached 5,000 colonies per 100 ml, and the peak concentration—30,000 colonies per 100 ml—occurred at one municipal well 14 days after the spill.

Seven days after the pipeline rupture the OUL introduced 4.5 kg (10 lb) of fluorescein dye mixture with a dye equivalent of approximately 50 percent plus 30,800 L (8,100 gal) of water at the re-excavated point of the spill. The flush water was introduced at a mean flow rate of 415 L/min (110 gpm) (Figure Box 7-1). A sewer spill of 3.4 million L (900,000 gal) spread over three days equals a mean leakage rate of 790 L/ min (210 gpm). Reasons for conducting this trace included concern that pathogens, including *Cryptosporidium* sp., would have a longer survival time in groundwater than fecal coliform and that dye would better reflect the longer-term presence of sewage in town wells than would fecal coliform bacteria. This proved to be the case.

Figure Box 7-1 - Dye and flush water introduction at the re-excavated site of the pipeline rupture. The introduction of dye was followed by 8,100 gallons (30,800 L) of potable water delivered through a hose from a fire hydrant.

Fecal coliform colony numbers returned to the normally low numbers typically found in the wells within 30 days of the spill. The subdivision developer responsible for the sewer line break urged town officials to quickly resume use of the wells (and reduce his costs). However, fluorescein dye was detectable in activated carbon samplers until 118 days after dye introduction, and the town used the duration of detectable amounts of dye in town wells as an appropriate metric to indicate when the wells could be returned to use. Prior to that time, the three municipal wells were heavily pumped to help remediate the aquifer and the extracted water was piped to the town's sewage treatment plant. The pumping was apparently beneficial, as fluorescein dye was still detectable in activated carbon samplers from an unused city well that had not been heavily pumped for 319 days after dye introduction.

Discussion. Dyes at the concentrations reaching the wells do not present health or environmental problems. However, client or public concern about potentially colored water is a common issue. It is seldom an actual problem, as sampling methods and analytical detection limits allow successful groundwater tracing with peak concentrations several orders of magnitude below visual detection thresholds. The visual detection limit for a rhodamine WT mixture with a 20-percent dye equivalent, in water, under field conditions, and with an experienced observer is 125 μg/L (Aley, 2019); the instrumental detection limit is approximately four orders of magnitude lower at 0.015 μg/L.

In the 1992 tracer study 454 gm (1 lb) of rhodamine WT dye mixture with a 20-percent dye equivalent was used; in 1999 4.5 kg (10 lb) of fluorescein mixture with a 50-percent dye equivalent was used. The net result was that 25 times more dye was used for the 1999 trace than for the trace conducted in 1992. In the first study the town was concerned that the amount of dye used would visually color the water, so the sampling approach was designed to minimize that risk. The maximum measured concentration of the rhodamine WT mixture was 0.782 µg/L; adjusting for the rhodamine WT in the mixture the maximum concentration of rhodamine WT detected at Town Well #1 was 0.156 µg/L.

In the 1999 dye trace water samples were collected periodically at all three of the normally operating town wells. The maximum measured fluorescein dye mixture concentration in water from any of the wells was 0.538 µg/L from Town Well #3 on July 23, 1999. Adjusting for the 75 percent dye in the mixture, the maximum fluorescein concentration in a water sample was 0.404 µg/L. Field and others (1995) recommend that tracer dye concentrations not exceed 1 to 2 mg/L persisting for a period more than 24 hours at the point of groundwater withdrawal or discharge. That conservative recommendation is based on preventing visible color in water rather than on any indication of health or environmental harm. Both dye traces resulted in maximum dye concentrations in town wells substantially below the recommended maximum dye concentration.

In the trace after the sewage spill, 4.5 kg (10 lb) of fluorescein dye mixture was used, as colored water was the least of the town's concerns. One challenge in this trace was to identify all wells to which the dye might move, and dye was detected in several private wells. Equally important was the need to use sufficient dye to demonstrate how long pathogens with long underground survival times could persist in the vicinity of the town wells. *Cryptosporidium* was found in the raw sewage and became a significant issue of concern. Both considerations justified using a larger mass of dye for the second trace, and fluorescein dye was a good choice because it is the most detectable of the fluorescent tracer dyes.

In the Walkersville case, dye tracing was the most credible and least expensive approach for determining if EPA's TOT lines were credible. The tracing clearly demonstrated that EPA's TOT lines grossly under-estimated groundwater travel rates. Furthermore, the 1992–1993 tracing work showed the town that all three of their municipal wells could rapidly be impacted by a major spill. When such a spill occurred within the wellhead protection zone for the three town wells the town recognized the emergency and responded rapidly. Actions taken by the town included extracting large volumes of contaminated groundwater and directing it to the town's sewage treatment plant and construction of an emergency water line across the Monocacy River from the City of Frederick to Walkersville. The dye trace from the spill site clearly demonstrated that the rapid and massive response taken by the town was essential. Figure Box 7-2 illustrates groundwater travel rates between the sewage spill site and the three town wells.

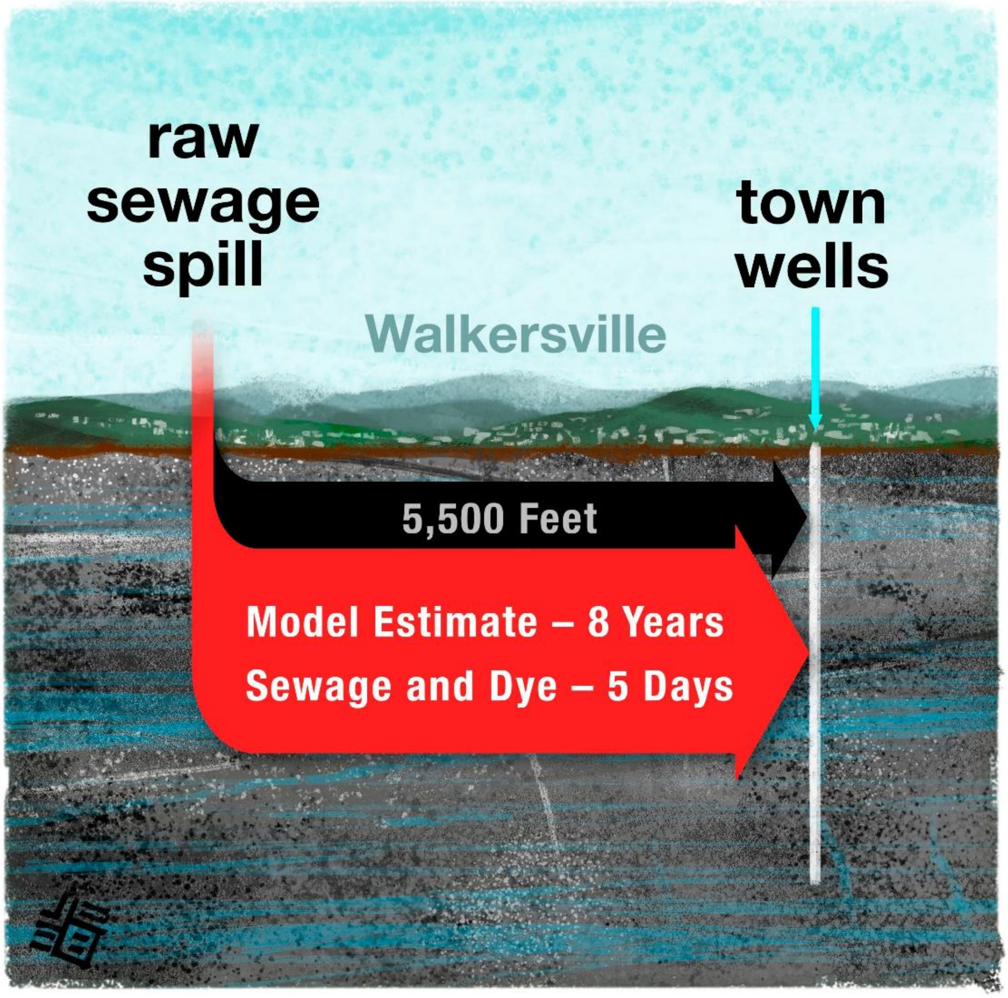

Figure Box 7-2 - An unverified model put the town at risk; 5,500 ft =1,677 m. A useful quote to support the use of dye tracing is “Dye does not lie.”

Dye tracing from the spill site to the town wells helped Walkersville recover most of their costs from the parties responsible for the sewer line damage. Tracing results were also valuable in showing that sewage persisted in water from the town wells for about four months, rather than for less than one month after the spill as fecal coliform data alone suggested. This protected the public from longer-lived pathogens, one of which (*Cryptosporidium* sp.) was known to be present in sewage from the regional sewage line.

Neither of the traces at Walkersville required difficult field work, materials, or analytical services that were not readily available in the United States. There are several US firms that assist clients in designing groundwater traces using fluorescent tracer dyes, who supply appropriate dyes in appropriate quantities, and provide analytical services. While it is good to have an experienced person directing critical or high visibility projects such as the 1999 trace at Walkersville, the work was not complicated, and most users of this book should be able to conduct similar work successfully.

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Box 8 - Case History 8: Traces from monitoring wells to production well in glacial outwash, South Dakota, USA

Sioux Falls, South Dakota, USA, derives municipal water from the Airport Well Field which is largely recharged by the nearby Big Sioux River. The well field is in glacial outwash which, in this area, is predominantly sand and fine gravel. Production Well 34-A is about 42.7 m (140 ft) from the river. Dye tracing to determine time-of-travel for groundwater moving from the river to this well was conducted as part of a wellhead protection strategy (Barari et al., 1993).

Well 34-A was pumped until the drawdown curve reached both monitoring wells planned for dye introduction, and pumping continued throughout the tracing period. Dye could have been introduced into the river, but for convenience and to minimize the amount of dye used, it was introduced as a liquid followed by five well volumes of flush water into each of the two dye introduction wells. Fluorescein dye was introduced into two monitoring wells located approximately 12.2 m (40 ft) and 42.7 m (140 ft) from production well 34-A.

For the first trace, 113 g (0.25 lb) of fluorescein dye mixture was added to the monitoring well nearest the production well; the dye mixture contained approximately 75 percent dye and 25 percent diluent. Based on laboratory analysis of carbon samplers, dye was first detected at the production well 12 hours after introduction. Measurements of dye concentrations in water with equipment available at the site indicated a first arrival time of 18 hours after dye introduction. Dye from the introduction nearest to production well 34-A peaked 1.3 days after dye introduction (peak concentration 132 μg/L) and had mostly passed through the well after six days.

The second dye introduction was into the monitoring well 42.7 m (140 ft) from the production well and used 341 g (0.75 lb) of fluorescein dye mixture. Dye was introduced three days after the first dye introduction. Based on activated carbon samplers, the dye first arrived at the production well seven to nine days after dye introduction and the dye concentration peaked at about 17 days.

Discussion. Dye analysis included on-site water measurements with a fluorometer and laboratory analysis of both water samples and activated carbon samplers. The field data on dye concentrations in water samples were useful in providing real-time information to guide sampling intervals. The laboratory analysis of water samples accurately separated background fluorescence from that due to fluorescein dye. Finally, as these dye introductions used very little dye, the carbon samplers ensured that important data would not be lost if dye concentrations in the production well were too low to be detected in water samples. The report by Barari and others (1993, pp. 4–33) stated, "The charcoal pack data were very valuable in determining time of first arrival of the dye because the concentrations were initially too low to be detected in the water samples." The resulting data were of practical value in operational planning for management of the well field in the event of a spill of a harmful compound into the Big Sioux River upstream of the airport wellfield.

The study at Sioux Falls, South Dakota, was one of five wellhead protection case studies conducted for the US Environmental Protection Agency. Dye tracing was a component of three of the five case studies which illustrates the utility of tracing in the wellhead protection program. The other two case studies using tracer dyes were in karst in Missouri and fractured bedrock in New Hampshire.

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Box 9 - Case History 9: Deaminoalkylation of sulforhodamine B in a trace at Ocala, Florida, USA

URS and others (2011) reported on four dye introductions by the OUL at Pontiac Sink within the recharge area for Silver Springs, Ocala, Florida, USA. Pontiac Sink is used to introduce urban stormwater runoff into the aquifer after that water has passed through a constructed wetland. To conduct this trace, dye and flush water bypassed the wetland. A total of 22.7 kg (50 lb) of sulforhodamine B mixture with a 35-percent dye equivalent followed by 651,000 L (172,000 gals) of chlorinated water from a municipal fire hydrant was introduced into the Ocala Limestone through the sinkhole. The dye was introduced as a slug lasting seven minutes followed by a continuous flow of flush water that lasted for approximately 21 hours. Subsequently, sulforhodamine B was detected at three production wells located 3.9 km (2.4 mi) to 7.1 km (4.4 mi) from the dye introduction point.

The mean emission peak wavelength for sulforhodamine B in carbon sampler elutants with the protocol and analytical equipment used was 578.1 nm with a standard deviation of 0.85 nm. The shortest emission fluorescence wavelengths of multiple samples from the three wells were 15.7, 16.5, and 18.5 nm. These are shorter than the normally obtained mean emission peak value. The emission peak wavelengths were over 18 to 21 standard deviations shorter than the normal emission peak wavelength of sulforhodamine B. This decrease in the emission peak wavelength was attributed to deaminoalkylation of the sulforhodamine B dye. All fluorescence peaks had shapes typical of sulforhodamine B dye except for their peak emission wavelengths.

Discussion. In the opinion of the OUL the large volumes of stormwater runoff routinely discharged to this sinkhole likely created a reducing environment that resulted in the deaminoalkylation of the sulforhodamine B dye.

The OUL has occasionally encountered samples with emission peaks for rhodamine WT in water and carbon sampler elutants that were shorter than the normal wavelength ranges for this dye. This has been attributed to deaminoalkylation of the rhodamine WT dye and is most commonly encountered in samples with small dye concentrations. In a Maryland tracing study, rhodamine WT was introduced at the site 18 years before it was detected at several groundwater sampling sites (White et al., 2015). The dye was detected in carbon sampler elutants; the normal acceptable wavelength range for this dye in elutants is 565.2 to 571.8 nm. A total of 31 of 105 samples positive for rhodamine WT had emission fluorescence peaks at or shorter than 563.2 nm, and 26 of the 105 samples had emission peaks within the normal acceptable wavelength range. The shortest emission wavelength measured in any sample was 560.0 nm. These results are like OUL experiences at other locations where some degradation of rhodamine WT apparently occurred as a result of deaminoalkylation. The OUL tries to not use rhodamine WT in groundwater that is likely to have reducing conditions.

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Box 10 - Case History 10: Impacts of acidic water from an abandoned metal mine on tracer dyes, California, USA

This section provides a practical example of how bench testing can be important to the success of a groundwater-tracing project. The water discharging from an abandoned metal mine typically ranged in pH from 2.9 to 4.3. Mine water may be mineralized with various elements and aqueous compounds that may interfere with the fluorescent dyes. To test for potential interference, solutions of 10 ppb and 100 ppb of each of the five dyes were made using water from the mine site that had a pH of 3.7. The water was sampled after two hours and again after 15 days for fluorescence intensity. All water samplers were adjusted to pH greater than eight immediately prior to analysis because low pH of water during analysis drastically reduces the fluorescence intensity of the dye. The results of this experiment showed pyranine was never detectable. The remaining dyes demonstrated reasonable resistance to degradation during the first two hours. However, both rhodamine WT and sulforhodamine B were not detectable after 15 days. Therefore, for this mine, fluorescein and eosine were the only two dyes stable enough to withstand 15 days in solution with fluorescein being more stable than eosine (Table Box 10-1).

Table Box 10-1 - Dye concentrations in mine water. Eos=eosine, Fl=fluorescein, Py=pyranine; RWT=rhodamine WT, and SRB=sulforhodamine B. ND=none detected.

Conditions	Eos	Fl	Py	RWT	SRB
Mine water with 100 ppb dye 2 hours after mixing. pH adjusted to >8 immediately before analysis	108	96	ND	112	197*
Mine water with 10 ppb dye 2 hours after mixing. pH adjusted to >8 immediately before analysis	10.4	6.9	ND	4.6	11.6
Mine water with 10 ppb dye 15 days after mixing. pH adjusted to >8 immediately before analysis	2.3	8.2	ND	ND	ND

*The elevated concentration of 100 ppb sulforhodamine B dye after two hours is attributed to the sample being accidentally spiked twice.

Subsequent sampling was conducted by spiking carbon samplers with known concentrations of each of the tracer dyes and then leaving them in place in flowing discharge water from the mine for approximately one week. Carbon samplers were then returned to the laboratory for analysis. The results of the work indicated that fluorescein would be the best tracer dye for use in this mine water.

Discussion. Aldous and Smart (1988) conducted tracing experiments over distances of 0.5 to 3.6 km (0.3 to 2.2 mi) in abandoned coal mine aquifers in the United Kingdom and found that sulforhodamine B worked well in two of the three traces. They reported on five tracing efforts by other authors using fluorescein for travel distances ranging from 0.1 to 1.7 km (0.06 to 1.0 mi). All but one of these fluorescein traces reportedly failed. Aldous and Smart (1988) conducted laboratory studies with dye adsorption onto ferric hydroxide, a mineral that is routinely precipitated on mine surfaces. They attributed much of the loss of fluorescein dye in their tests to adsorption on precipitated minerals. It was not clear if the failure to detect fluorescein in most of the cases was due in part to the low pH of the water during analysis, which drastically reduces the fluorescence intensity of the dye unless the water is adjusted to a pH of about 9.5 or greater to maximize fluorescence intensity. Aldous and Smart (1988) recommended that as much dye as reasonable should be used in attempts to trace coal mine water.

The OUL has conducted at least 40 traces of coal mine drainage in West Virginia and found that fluorescein has worked adequately in most cases, especially if the dye was captured on activated carbon samplers. Rhodamine WT has also worked in some coal mines. OUL tests with sulforhodamine B were conducted at one mine but it did not work as well as fluorescein. Poor performance or failures with tracer dyes in coal mine drainage appears to be especially prevalent at pH less than about 3.5 and in water that is depositing ferric hydroxides—called "Yellowboy" in coal country. In a coal mine in Utah the pH of mine drainage was near neutral, and fluorescein worked well.

Mining and crushing of rock and ore presents large amounts of fresh rock surfaces. Such fresh surfaces have more adsorption potential than natural rock surfaces, and this is a suspected partial explanation for low dye detections in many mine water traces. In a South American project, the OUL detected fluorescein in water samples from a drainage tunnel beneath an old tailings pond. Activated carbon samplers in the water were negative for tracer dyes when collected and analyzed after being in place for a week. The carbon samplers either did not adsorp fluorescein or else the water derived from the tailings ponds was capable of eluting dye from the carbon or, by some process, prevented dye from being adsorped on the carbon. Information on the quality of water below the tailings ponds was confidential and could not be obtained.

Although the testing described in Table Box 10-1 represents a single site-specific study, results are representative of many dye-trace studies conducted by OUL in acidic mine water.

The experiences of OUL indicate the general suitability of fluorescent dyes for tracing in acidic mine water is Fl > Eos > SRB > RWT. Pyranine is unsuitable for this use.

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Box 11 - Case History 11: Comparison of fluorescein and rhodamine wt performance in traces to water supply wells, Arkansas, USA

The OUL, in cooperation with the Arkansas Health Department and a local citizen group, introduced 4.55 kg (10 lb) of fluorescein dye mixture with a 50-percent dye equivalent and 12.95 kg (28.5 lb) of rhodamine WT dye mixture with a 20-percent dye equivalent into Dry Creek, which at that time was fed primarily by partially treated poultry processing wastewater. The flow rate of the stream was approximately 3,400 L/min (900 gal/min). Each dye introduction lasted approximately five minutes and both were completed at the same introduction point within a 10-minute period. The dyed water entered the channel of Dry Creek under summer conditions when there was no natural flow in the stream channel. All dyed water entered groundwater within approximately 915 m (3,000 ft) of the point of dye introduction. The study area is adjacent to Green Forest, Arkansas, and is underlain by the Boone Formation, which is largely comprises very cherty limestones.

Two dyes were used because it was expected that some of the dyed water would discharge from springs and be exposed to sunlight for periods of up to about one day before again entering groundwater and reaching some water supply wells. Since sunlight can destroy fluorescein more rapidly than rhodamine WT two dyes were used to ensure that all sampling points impacted by the wastewater discharge would be identified. Fluorescein was regarded as the better groundwater-tracing agent in this setting because of lower rates of adsorption onto earth and organic materials, and it was expected that it would be detected at more wells than would rhodamine WT.

Sampling for the dyes placed primary reliance on activated carbon samplers. Carbon samplers were collected, and new samplers placed once per week. There were two weeks of background sampling prior to any dye introduction. Sampling continued for four weeks after dye introduction. Four weeks was the maximum anticipated survival time for fecal coliform bacteria. A total of 87 sampling stations was established, of which 44 were domestic wells. During the study at least 50 percent of the wells were in use as domestic water supplies without any chlorination or other treatment. Some of these were dye detection sites.

One purpose of the groundwater-tracing study was to determine the areal extent of groundwater contamination attributable to poultry processing wastes from a nearby plant. Fluorescein was detected in carbon samplers from 18 domestic wells; rhodamine WT was detected at only four of those wells, and there were no wells where rhodamine WT was detected without fluorescein also being detected. First arrival of dye at most wells was during the period from 8 to 15 days after dye introduction. Dye concentrations were based on the dye mixtures used. So, the ratio of the introduced amount of rhodamine WT to fluorescein was 2.85:1 and this ratio was, ideally, expected in the samples collected. The mean ratio of rhodamine WT to fluorescein at three wells where both dyes were detected was 1.9:1. At one well the mean ratio of rhodamine WT to fluorescein was 33:1, suggesting that dyed water reaching this well had infiltrated from the nearby surface stream where a substantial part of the fluorescein in the water had been destroyed by exposure to sunlight.

Discussion. Adsorption characteristics of rhodamine WT prevented it from reaching many of the wells where fluorescein was detected. The tracer study demonstrated that the area impacted by the waste discharges into Dry Creek encompassed approximately 156 km^2 (60 mi^2). Rural water district lines were extended throughout this area to supply residents with potable water, and there was lengthy litigation over the property damage. Funding to increase the duration of sampling beyond four weeks was not available.

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Box 12 - Case History 12: Deterioration of fluorescein in water samples containing oil field brine, Texas, USA

All five dyes are reasonably stable in most water samples although the OUL once dealt with flow from a Texas, USA, spring contaminated with oil field brines where this was not the case. Fluorescein dye was used to trace from a brine injection well to a spring and the dye was readily detectable in activated carbon samplers at the spring. However, water samples from the spring, when analyzed within normal two-week hold times, were routinely negative for fluorescein even though dye concentrations in associated carbon samplers were sufficiently large that the dye should have been detectable in water samples. To test for the possibility that fluorescein was rapidly degrading in water samples, a sample was collected from the spring and analyzed in the laboratory within 20 hours of collection; fluorescein was detected in that sample. When the sample was reanalyzed 20 hours later fluorescein was no longer detectable.

Discussion. A lesson from this experience is that oil field brines and other waste mixtures may occasionally degrade or destroy dyes in samples. Photodegradation was not the process involved in this case and is only one of several processes that can degrade dyes—dye degradation processes are discussed in more detail in Section 2. A good general protocol is to analyze samples as soon after collection as reasonable. In some cases, a second analysis of some samples made a few days after the first analysis may be a useful quality assurance step.

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Box 13 - Case History 13: Failure of carbon samplers to detect fluorescein in sampling beneath tailing ponds, Peru

A study at a mine in Peru sampled water seeping into a drainage tunnel beneath tailings ponds. Tracer dyes were introduced into monitoring wells adjacent to the tailings ponds. Fluorescein dye was detectable in water samples but not in activated carbon samplers. The OUL suspected that the seepage water was either preventing the carbon from adsorping the dyes or was eluting dyes from the carbon as quickly as they were adsorped. The chemical nature of the seepage water was not determined.

Discussion. While the OUL has worked extensively with mine water and to a lesser extent with drainage from tailings ponds, in our experience this was the only case of carbon samplers not functioning well in flowing water. Tracer dyes, and especially fluorescein, generally work well in tracing mine water. Activated carbon samplers also generally work well. In OUL experience, coal mine drainage with pH values of 2 or 3 presents difficult tracing situations regardless of sampling methods. Bench testing of dyes and sampler performance in mine water can be valuable in making decisions about the amounts and types of tracer dyes to introduce and adds confidence to conclusions from traces where dyes are not detected at key sampling points.

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Box 14 - Case History 14: Results of ten long-distance groundwater traces to Big Spring, Missouri, USA

During the period 1966 to 1973, the senior author directed the Hurricane Creek Barometer Watershed study in Missouri for the US Forest Service. This was the national type-example study area for watershed management in karst areas on national forest lands.

Much of the precipitation that fell in the 294 km^2 (113 mi^2) topographic basin of Hurricane Creek, a surface tributary to the Eleven Point River, entered the karst groundwater system and discharged from Big Spring, a tributary to the Current River. A major facet of the Hurricane Creek studies was developing practical and cost-effective groundwater-tracing approaches. Table Box 14-1 summarizes the results from ten groundwater traces to Big Spring (Aley, 1978). The mean amount of fluorescein dye used for each of the 10 traces was 4.4 kg (9.7 lb); the approximate dye equivalent in the mixtures used for these traces was 50 percent. In all traces fluorescein dye was visually present in the eluting solutions.

Table Box 14-1 - Summary data from ten successful groundwater traces to Big Spring, Missouri, USA conducted from 1968 through 1972 (Aley, 1978).

Parameter	**Maximum**	**Minimum**	**Mean**
Straight line distance traced	63.6 km (39.5 mi)	24.2 km (15.0 mi)	39.6 km (24.6 mi)
Time between day of dye introduction and day of first dye arrival (days)	67	8.5	17.9
Mean flow rate of spring during tracing period	23.2 m^3/sec (820 ft^3/s)	7.9 m^3/sec (280 ft^3/s)	14.9 m^3/sec (527 ft^3/s)
Mean groundwater velocity for first dye arrival.	213 m/hr (1,688 ft/hr)	33.5 m/hr (110 ft/hr)	123.5 m/hr (405 ft/hr)
Mean groundwater gradient	0.004	0.001	0.0026
Fluorescein dye mixture used; dye equivalent in all mixtures 50%	6.8 kg (15 lb)	2.3 kg (5 lb)	4.5 kg (10 lb)

Discussion. Martel (1913), in tracing water to karst springs in France, recommended using 1 kg (2.2 lb) of fluorescein for every kilometer (0.62 mi) of travel path multiplied by the outflow of the spring in m^3/s (35.29 ft^3/s). The dye equivalent in the mixtures Martel used was not specified but was probably at least 50 percent. Dye detections in Martel's work were based on visual observations, so people were stationed at possible discharge sites to watch for the appearance of dye. Using Martel's equation and the Aley (1978) trace of 63.6 km (39.5 mi) to Big Spring which was flowing at a mean rate of 9.10 m^3/s (321 ft^3/s) during the period of dye detection, the amount of fluorescein needed with Martel's approach would have been approximately 579 kg (1,273 lb). The trace was done with 6.82 kg (15 lb) of dye mixture. The 85-fold difference in amounts demonstrates an advantage of using activated carbon samplers for dye detections rather than visual observation.

Table 12 of Section 3.3.1 shows that dye concentration in eluting solutions from carbon samplers at karst springs is routinely at least two orders of magnitude greater than mean dye concentration in the associated water. For monitoring wells, the dye concentration in eluting solutions is commonly at least an order of magnitude greater than mean concentration in the associated water. A major benefit of groundwater traces placing primary sampling reliance on carbon samplers is the ability to introduce smaller quantities of dye than would be necessary if primary sampling reliance were based on field fluorometers or water samples.

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Box 15 - Case History 15: Traces to Barton Springs, Texas, USA, that did not result in visually colored water

Barton Springs, in downtown Austin, Texas, US, is a very popular public swimming area in a city park (Figure Box 15-1). Numerous dye traces have been conducted to this spring by the Barton Springs/Edwards Aquifer District and the City of Austin (Hauwert et al., 2004) without creating any visible colored water or changing the color of white swimsuits.

Figure Box 15-1 - Barton Springs with swimmers **(**Photo by Alan Andrews).

Discussion. These traces placed primary sampling reliance on carbon samplers and secondary reliance on grab samples of water. All dye analysis was with a spectrofluorophotometer operated under a synchronous-scan protocol. Several traces yielded dye detection in public and private water supply wells, but dye concentrations were below visual detection limits.

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Box 16 - Case History 16: Trace to municipal well that resulted in visually colored water, Miami, Florida, USA

Poorly conceived tracing projects that introduce excessively large amounts of dye adversely affect public perception of tracer dye studies. An example occurred because of a US Geological Survey (USGS) trace to a municipal water supply well in the Miami-Dade County Northwest Well Field, Florida, USA, in June 2003. The trace and related work was conducted by Renken and others (2005). Benson and Yuhr (2016) discuss the excessive quantity of dye used and resulting problems and adverse media attention.

The USGS introduced 50 kg (110 lb) of rhodamine WT dye mixture into a well approximately 100 m (328 ft) from one of the high yield municipal wells in the Biscayne Limestone aquifer. The dye equivalent in the mixture used was not reported but was probably about 20 percent, as this is a typical value for rhodamine WT dye mixtures. During the study the production well was extracting groundwater for municipal use at a rate of approximately 0.49 m^3/sec (7,900 gpm).

Benson and Yuhr (2016), in commenting on the USGS work, indicated that dye was expected to reach the production well in two to three days. Instead, first dye arrival occurred approximately four hours after dye introduction and peak dye concentration at the production well occurred approximately six and a half hours after dye introduction in water with an easily detected pink color (Renken et al., 2005). The situation is characterized in Figure Box 16-1.

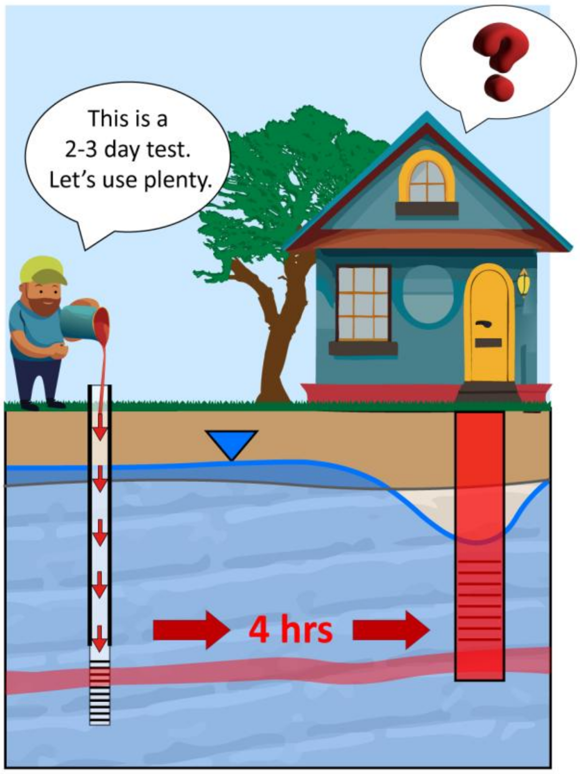

Figure Box 16-1 - Schematic of a common error made in design of a tracer test that leads to visible color.

Peak dye concentration in water discharging from the well was approximately 3,200 ppb; at this concentration the water had a distinct pink color. For comparison purposes, Aley (2019) reports that under field conditions a rhodamine WT mixture with 20-percent dye equivalent is visually detectable by an experienced person at 125 ppb and is noticeable by the public at 2,500 ppb. The OUL analytical detection limit for 20-percent dye equivalent mixture of rhodamine WT in water samples is 0.015 ppb.

Discussion. Pink water discharging from household taps created unwanted media attention including an article in the *Miami News Times* for June 5, 2003, titled "Beneath the Pink Underwear." There was concern that the dyed water would turn underwear pink—hence the title of the newspaper story. The underwear concern was not warranted, as rhodamine WT is not a direct dye and would not color fabrics; however, the concentrated mixture introduced for dye traces can stain equipment and other surfaces. Renken and others (2005) reported that the test was prematurely terminated at 12.75 hours after dye introduction. They failed to mention that the well was shut off by the Miami-Dade Water and Sewer Department due to concern about the pink water. The rhodamine WT dye concentration at the time pumping ended had declined to approximately 1,000 ppb. Standard chlorination conducted by water utilities readily destroys small concentrations of dye in municipal water supplies but was incapable of oxidizing the large concentrations of rhodamine WT dye being delivered to the water distribution system.

The Renken and others (2005) paper specifically states that rhodamine WT dye is a conservative tracer. It is not. As discussed in Section 2, rhodamine WT is composed of equal amounts of two isomers. One of the isomers is relatively conservative, but the other has a large retardation factor. Given that the study was supposed to determine time of travel, rhodamine WT was a poor choice for the project, even in channelized carbonate where adsorption may not be as significant as in other hydrogeologic settings. Fluorescein or eosine would have been preferable. The nine authors in the Renken and others (2005) paper missed papers in major journals clearly demonstrating that rhodamine WT was unsuited for the purposes of this trace. Papers by Sabatini and Austin (1991) and Sutton and others (2001) in the journals *Groundwater* and *Water Resources Research* respectively, are examples of relevant papers.

Excessively large amounts of dye are unnecessary for successful tracer tests designed to address time of travel. The use of excessive amounts of dye resulted in concentrations more than 5 orders of magnitude greater than the detection limit using good analytical instruments and was clearly unnecessary. This is especially so, as the tracing target was a public drinking water supply. Poorly designed and conducted tracing projects with inexperienced personnel make it more difficult for competent workers to conduct needed investigations involving sensitive water supplies. Studies that rely on activated carbon samplers require far less dye than studies dependent on water samples, and they are far less likely to create colored water problems. They are also far less expensive and routinely answer the relevant questions.

Figure Box 16-2 - Avoid using excessive amounts of dye, especially when tracing to sensitive locations.

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Box 17 - Case History 17: Long distance traces to multiple wells in a deep fractured rock aquifer, basin and range province, southwestern USA

Four dye introductions were made by the OUL into a deep fractured rock aquifer impacted by mobile contaminants. The dye mixtures introduced were 18.2 kg (40 lb) of eosine, 18.2 kg (40 lb) of fluorescein, 27.3 kg (60 lb) of rhodamine WT, and 11.4 kg (25 lb) of sulforhodamine B. Sampling points included monitoring wells and discharges from a few extraction wells. Dye sampling lasted for approximately 800 days after dye introductions, but not all locations were monitored over the entire period. A total of 124 wells (or separate depths in multi-port wells) were monitored for at least a portion of this time.

Dyes were detected in 12 wells. Two dyes were detected in only one of these wells. Water samples from 108 wells (or different levels in wells) were analyzed for tracer dyes and detectable dye concentrations were found in four (3.7 percent) of them. Excluding wells sampled for only a very short time, 76 wells were sampled with activated carbon samplers and dyes were detected in 11 (13.2 percent). If dye was detected in a carbon sampler, then the associated water samples were also analyzed. Dye was detected in water samples from three of the 11 wells where dye was detected in carbon samplers.

The time from dye introduction to first arrival at sampling points based on water samples was 16 percent, 25 percent, and 34 percent longer than the time for first arrival in activated carbon samplers at the same sampling points. Based on carbon samplers, the median groundwater travel rate for first dye arrival was 11.3 m (37 ft) per day; values ranged from 1.5 m (5 ft) per day to 146 m (479 ft) per day. The median straight-line distance between dye introduction and detection points was 2,718 m (8,915 ft) and the range was from 854 m (2,800 ft) to 4,561 m (14,960 ft).

Discussion. This large-scale trace shows the importance of sampling with activated carbon samplers even when relatively large amounts of dye are introduced. Water samples substantially under-estimated the number of sampling stations reached by tracer dyes. Furthermore, the mean straight-line distance for traces proven by water samples was 1,390 m (4,559 ft) whereas it was 2,526 m (8,286 ft) for activated carbon samplers. A key issue in this tracing project was whether dyes could move to the furthest down-gradient group of monitoring wells. The carbon samplers demonstrated that this occurred; the water samples were not able to credibly answer the question. Even with the use of carbon samplers a large amount of dye was introduced; substantially more dye would have been needed if primary sampling reliance had been placed on water samples and credible results were needed. A total of 75 kg (165 lb) of dyes were introduced, and it is unlikely that the client would have been willing to substantially increase this dye quantity simply to be able to detect dyes in water samples. The carbon samplers answered the relevant questions and indicated that dye would probably have been detected at more sampling stations if it had been possible to monitor all locations with carbon samplers.

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Box 18 - Case History 18: Results from Traces Eighteen Years Apart at a Waste Site, Maryland, USA

Two dye-tracing studies 18 years apart were conducted at the same hazardous waste site in a karst area in Maryland, US (White et al., 2015). The first study was in 1995 and involved dye introductions into shallow portions of the karst aquifer. A total of 3.18 kg (7 lb) of eosine dye mixture containing approximately 75-percent dye equivalent was introduced into each of two EDIPs—Epikarstic Dye Introduction Points; these are borings designed for tracer dye introduction that extend into the top of the epikarstic zone. All EDIPs for this investigation extended between 7 and 15 m (23 and 49 ft) below ground surface. A total of 2.27 kg (7.5 lb) of fluorescein dye mixture containing approximately 70-percent dye equivalent was introduced into each of two additional EDIPs, and 4.54 kg (10 lb) of rhodamine WT with a dye equivalent of approximately 20 percent was introduced into a shallow sinkhole. Each of the dye introductions was followed by 9,500 L (2,500 gal) of flush water.

The second dye-tracing study at the Maryland site was conducted in 2013 (White et al., 2015). In this case, an intermediate depth dye introduction of 10.4 kg (23 lb) of eosine dye mixture containing approximately 96-percent dye equivalent was introduced into a monitoring well screened from 42.7 to 47.2 m (140 to 155 ft) below ground surface. The target was a fracture at 45.7 m (150 ft) below ground surface. An associated deep well introduction used 6.8 kg (15 lb) of fluorescein dye with a dye equivalent of approximately 70 percent. The dye was introduced into a deep monitoring well screened from 95.4 to 100.0 m (313 to 328 ft) below ground surface; the target was a void encountered at 96.5 to 96.8 m (316.5 to 317.5 ft) below ground surface. Approximately 5,700 L (1,500 gal) of water was introduced into each well following dye introduction. Sampling was conducted for over 6 months at most sampling points and for some selected locations for nine months.

In the 1995 study, sampling was conducted at 30 wells and 10 springs. Eosine was detected in carbon samplers from 8 springs and 6 wells. The water samples also contained detectable eosine from 8 springs, but only 2 of the wells. Fluorescein was detected in carbon samplers from 6 wells and 6 springs, but in water samples from only 4 wells and all 5 springs. Rhodamine WT was not detected during the study period at any sampling points during the 17 weeks of sampling after dye introduction.

In the 2013 study sampling was conducted at 32 wells and 9 springs. Eosine was detected in carbon samplers from 11 wells and 6 springs, but in water samples from only 5 of the wells and 2 of the springs. Fluorescein was detected in carbon samplers from 2 of the wells and 7 of the springs and in water samples from both wells and 6 of the 7 springs.

Combining the results from the 1995 and 2013 studies, dyes introduced for the traces were detected in carbon samplers from 46 groundwater sampling stations but in water samples from only 26 of these locations. As a result, water samples detected dye at only 57 percent of the sampling points where dye was detected using carbon samplers. In addition, during the 2013 sampling, rhodamine WT dye introduced in 1995 was detected in carbon samplers from 8 springs and 1 well; rhodamine WT was not detected in any water samples from these locations.

Based on dye concentrations in water samples, White and others (2015) made estimates of the percent of dye introduced for the study that discharged from the springs. For the 1995 study, the detection percent was 0.2 percent for fluorescein and 0.9 percent for eosine. In the 2013 study, the percentages were 0.01 percent for fluorescein and 0.1 percent for eosine. No estimate was made for rhodamine WT.

Discussion. Primary sampling reliance was based on carbon samplers at the Maryland site because the client, the US Army, and their consultants recognized that adequate site characterization needed a good understanding of the complexity of the groundwater system at the site, and especially source areas for wells showing relatively low levels of contamination. Tracer studies were the ideal method for obtaining the needed information. Tracer studies placing primary reliance on carbon samplers—which would identify the largest number of flow paths—were recognized as the appropriate strategy. Additionally, the 2013 study was designed to evaluate how intermediate and deep portions of the aquifer related to the on-site monitoring wells. Carbon samplers provided the needed information and accomplished it at a lower cost than if much larger quantities of dye and more frequent sampling had been used to permit comprehensive identification of all detection sites based on water samples. Data interpretation showed that multiple flow paths in this karst aquifer transport water both laterally and vertically.

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Box 19 - Case History 19: Tracing poultry processing wastes to water supply wells, Arkansas, USA

The OUL conducted several groundwater traces at Green Forest, a town in the limestone karst of northern Arkansas, USA. At the time of the tracing work the town had a population of about 1,000 people. The town obtained funding to build a sewage treatment plant suitable for a small town without any significant industrial activity. The sewer plant discharged to Dry Creek, so-named because its flow routinely sinks into the karst aquifer within a kilometer (0.62 mi) downstream of the sewer plant. The affected karst aquifer supplies water to several hundred families living outside of town. Sewer plants attract industries in rural America, and a large chicken processing plant was constructed in Green Forest. Poultry wastes overloaded the town's sewer plant, and the incompletely treated effluent was often red with pieces of what appeared to be chicken entrails. A US EPA investigation found that 95 percent of the Biochemical Oxygen Demand (BOD) in the wastewater at the town's plant was from the poultry operation.

The OUL conducted several groundwater traces of sewage discharges in cooperation with the Arkansas Health Department and a local citizen organization. The final trace, conducted in 1987, was under relatively low flow conditions when the discharge from the sewer plant was approximately 15.75 million L (4.16 million gal) per day. All of this entered groundwater within approximately 1 km (0.62 mi) of the sewer plant.

The OUL introduced 4.5 kg (10 lb) of fluorescein dye mixture with a dye equivalent of approximately 50 percent and 13 kg (28.5 lb) of rhodamine WT mixture with a dye equivalent of approximately 20 percent. Both dyes were introduced at the same point and within a few minutes of each other at a point immediately downstream of the sewer plant discharge to Dry Creek. The reason for using both dyes was concern that some of the sinking water might return to the surface and be exposed to sunlight before sinking back into groundwater. If this were the case, then fluorescein might be destroyed by sunlight and then the OUL would not be able to identify water supplies impacted by water entering groundwater downstream of the second sinking zone. Since rhodamine WT is substantially more resistant to degradation in sunlight it was intended that the use of this dye would help ensure that all wells and springs receiving water from the sewer plant discharge would be identified.

A total of 87 sampling stations were established at springs, streams, and domestic wells. Sampling continued weekly for four weeks after dye introduction. The four-week sampling period was used because it is approximately the maximum survival time for fecal coliform in groundwater at local temperatures, and the health department was concerned with bacterial indicators in the sampled water supplies. The dye sampling was primarily conducted with activated carbon samplers with analysis using a spectrofluorophotometer operated under a synchronous-scan protocol. Most wells were sampled by allowing a continuous flow of approximately 3.79 L (1 gal) per min to pass through carbon samplers connected to hose bibs or hoses receiving water from wells. A few low yield wells were pumped intermittently. Springs were sampled with carbon samplers placed in the discharging water. Fluorescein was detected in 18 wells and 17 springs. Rhodamine WT was detected in the same 17 springs but in only 4 of the wells. The greatest distance from the dye introduction point to a dye detection site was 10.6 km (6.6 mi). The first arrival times for one or both dyes are shown in Table Box 19-1.

Table Box 19-1 – Time of first dye arrival at sampling points during the 1987 Green Forest trace.

First arrival time	**Wells**		**Springs**	
	Number	**Percent**	**Number**	**Percent**
8 days or less	2	11	10	59
8 to 15 days	14	78	4	24
15 to 22 days	2	11	3	17
22 to 29 days	0	0	0	0
Total	**18**	**100**	**17**	**100**

Discussion. The Green Forest tracing was conducted prior to a paper by Sabatini and Austin (1991) showing that rhodamine WT was composed of equal amounts of two isomers, and that one of these isomers had a large retardation factor. In effect, only half of the rhodamine WT introduced was likely to function as a reasonably conservative groundwater tracer. The fluorescence intensity of fluorescein is greater than that for rhodamine WT, and this partly accounts for the failure to detect rhodamine WT in most of the wells where fluorescein was found. Finally, the results indicate that even the more mobile isomer of rhodamine WT is a less conservative groundwater-tracing agent than fluorescein.

Karst springs in north Arkansas are more heavily associated with solutionally enlarged bedrock openings than are most wells, and most wells are more heavily associated with diffuse flow than is the case for most springs. This dichotomy is largely responsible for rhodamine WT and fluorescein working equally well in identifying springs receiving water from the sewer plant, but fluorescein being superior to rhodamine WT in identifying wells receiving such water.

The tracing work showed that an area of approximately 156 km^2 (60 mi^2) was impacted by the sewage discharges. Based on the tracing results, rural water district lines were extended to most, and possibly all, of the residences in the delineated area. A citizen suit under provisions of the Clean Water Act was heard in federal court for the Western District of Arkansas followed by appeals and other court actions; results were favorable to the plaintiffs. Sampling private water supply wells by allowing a continuous flow of water through carbon samplers undoubtedly resulted in more wells being identified as dye detection sites than would have been the case if wells had only been sampled with grab samples of water or with carbon samplers in toilet tank reservoirs.

The scientific credibility of the dye-tracing methods was challenged by the poultry company but was accepted by the Court and sustained by the Federal Appellate Court. The introduction of two tracer dyes was technically sound, but it created an opportunity for opposing attorneys to argue that the failure to detect both dyes at all positive dye detection locations indicated that the test was somehow flawed and that all tracing data should be ignored. The court did not accept this argument.

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Box 20 - Case History 20: Long duration sampling for dyes in a karst area, Nevada, USA

A groundwater-tracing study was conducted for the US National Park Service in a karst area within Great Basin National Park, Nevada, USA. Eosine, fluorescein, and rhodamine WT dyes were all introduced at different locations and sampling with activated carbon samplers and grab samples of water was conducted at 32 groundwater and surface water sampling points. One of the groundwater sampling points monitored flow from Model Cave. Grab samples of water and duplicate carbon samplers were routinely analyzed from this sampling point.

On September 27, 2011, (Day 0) at 1325 hours 2.72 kg (6 lb) of rhodamine WT dye mixture with a 20-percent dye equivalent was introduced into flowing water in the bottom of Wheeler's Deep Cave. Based on carbon samplers, the dye was first detected in water discharging from Model Cave at some time between 1610 hours on September 28 and 0920 hours on September 29, 2011. Dye was first detected in a water sample from Model Cave collected on September 29 at 0920 hours. The approximate straight-line distance between the dye introduction point and the discharge point at Model Cave is 756 m (2,480 ft), and the elevation difference is approximately 36.6 m (120 ft).

Dye concentrations in carbon sampler elutants were summed and the accumulated dye concentration plotted. Flow rate data were not available, but generally decreased with time as the dry season progressed. Figure Box 20-1 shows cumulative plots of rhodamine WT dye concentrations from Model Cave for the 191 days after dye introduction. Two independently anchored carbon samplers were in place at this spring, and both were collected and analyzed. Using these data, the senior author wished to determine if there was a need to analyze more than one carbon sampler from each sampling period. Figure Box 20-1a is a cumulative dye concentration graph based on dye concentrations in the first carbon sampler analyzed for each sampling period. Figure Box 20-1b is a cumulative dye concentration graph based on mean dye concentrations in carbon samplers when two samplers were analyzed for each sampling period. In a few cases only one sampler was analyzed; in that case the concentration in that sample was used as the mean concentration. Figure Box 20-1c is a cumulative dye concentration graph based on the larger dye concentration when both a sample and a duplicate were analyzed. Figure Box 20-1d is a plot of dye concentrations in water samples. Dye was not detectable in water samples after day 85 but was detectable in carbon samplers until the end of sampling.

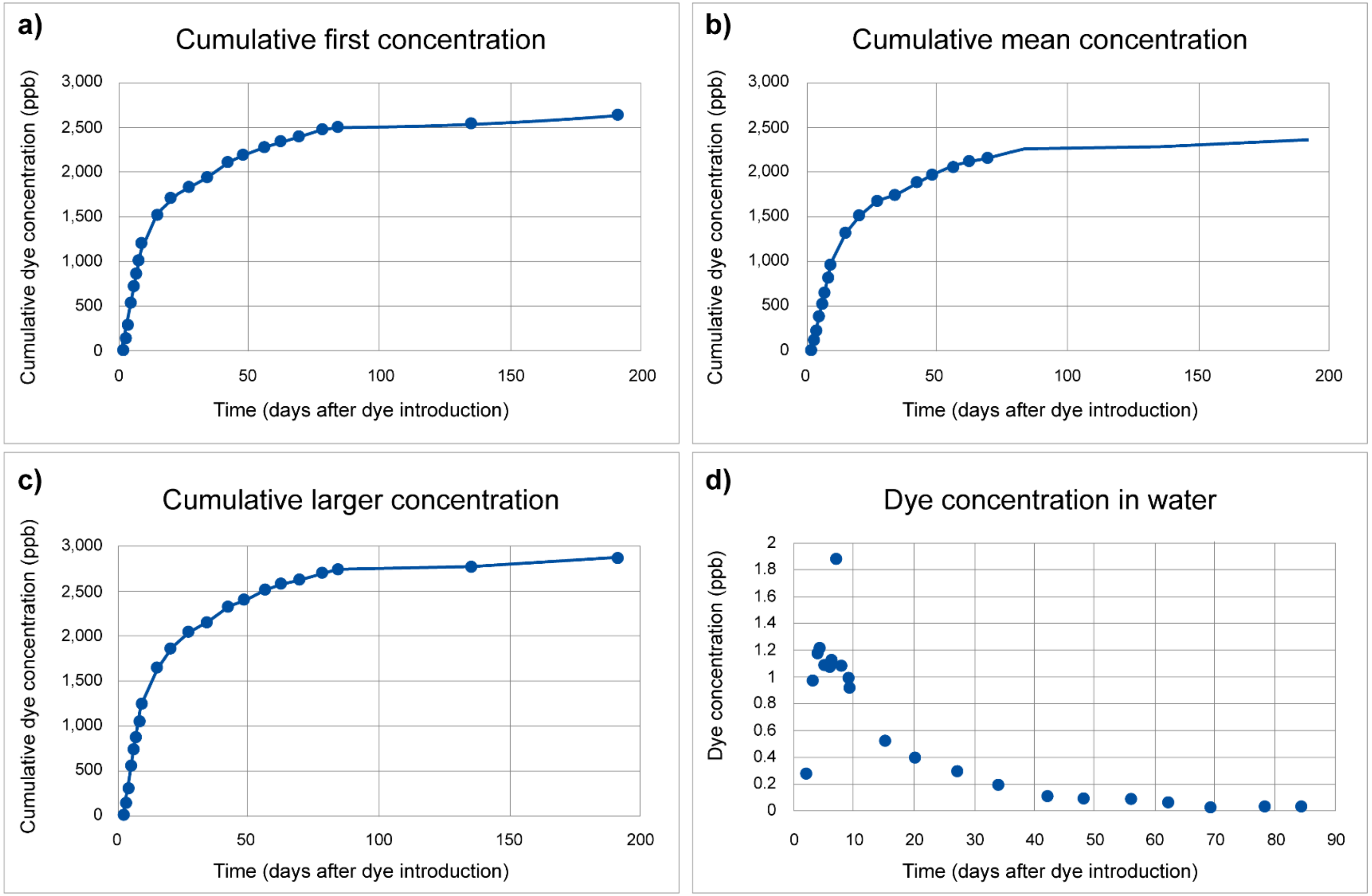

Figure Box 20-1 - Cumulative plots of rhodamine WT dye mixture concentrations in carbon sampler elutants from Model Cave streamflow compared with a breakthrough curve for dye concentrations in water samples: a) cumulative dye concentration in the first carbon sampler analyzed for each sampling period; b) cumulative mean dye concentration in carbon samplers when two samplers were analyzed for each sampling period; c) cumulative larger dye concentration when both a sample and a duplicate were analyzed; d) dye concentration in water samples. Dye was not detectable in water samples after day 85.

Cumulative dye concentration graphs can be used to estimate the times at which selected percentages of the detected dye passed a sampling location. It is common to use 25 percent, 50 percent, and 75 percent values. Dye concentrations for each sampling period must be multiplied by the mean flow rate during the sampling period to correctly determine the time at which the various portions of the mass of the dye have passed the sampling station. This has not been done for the data from Model Cave, but it is not necessary to evaluate whether analyzing two carbon samplers and using either the mean dye concentration or the larger of the two concentrations would improve data quality over simply analyzing one sampler.

Table Box 20-1 presents the dates when 25 percent, 50 percent, and 75 percent of all detected rhodamine WT dye passed the sampling point monitoring water discharging from Model Cave. It is based on the cumulative dye concentration graphs in Figure Box 20-1. This table only presents data from a single trace and only from a single monitoring point. A single monitoring point from a single trace was presented for simplicity, but the findings are consistent with past OUL experience. The data suggest that routine analysis of duplicate carbon samplers from sampling sites is probably not worth the additional analytical costs for most investigations. The analysis of two samplers can improve confidence in the results and might be warranted in some situations, especially if the number of samples is limited. The recommendation to only analyze a single activated carbon sampler from spring and stream sampling stations does not apply to the desirability of analyzing 5 percent, 10 percent, or some other percentage of duplicate samplers as part of a QA/QC protocol.

Table Box 20-1 - Day numbers when 25%, 50%, and 75% of all detected rhodamine WT dye discharged from Model Cave.

Method	25% of All Dye	50% of All Dye	75% of All Dye
First Carbon Sampler Analyzed	Day 6	Day 11	Day 37
Mean of Two Carbon Samplers	Day 7	Day 13	Day 35
Larger Value Carbon Sampler	Day 6	Day 12	Day 34

The Nevada tracing project included the introduction of three dyes at three different points and dye detections at multiple sampling points including flow from Model Cave and Rowland Spring. All dye introductions were made on September 27, 2011. The rhodamine WT introduction discussed thus far in this Box was introduced into the flow of a small cave stream—estimated flow rate 170 L/min (45 gpm)—in the bottom of Wheeler Deep Cave. The eosine dye introduction used 2.72 kg (6 lb) of a dye mixture that was approximately 96 percent dye and 4 percent diluent. It was introduced into the flow of a sinking stream—estimated flow rate 95 L/min (25 gpm)—in Pole Canyon. The fluorescein dye introduction used 2.27 kg (5 lb) of a dye mixture that was approximately 70 percent dye and 30 percent diluent. It was introduced into the flow of Baker Creek—estimated flow rate 0.26 m^3/sec (9 ft^3/sec). Approximately a third of the flow of Baker Creek entered groundwater within the study area. Figure Box 20-2 shows the locations of the three dye introduction points, the Model Cave discharge sampling point, and Rowland Spring.

Rowland Spring is in the Lehman Creek topographic basin and all dye introductions were made in the Baker Creek topographic basin.

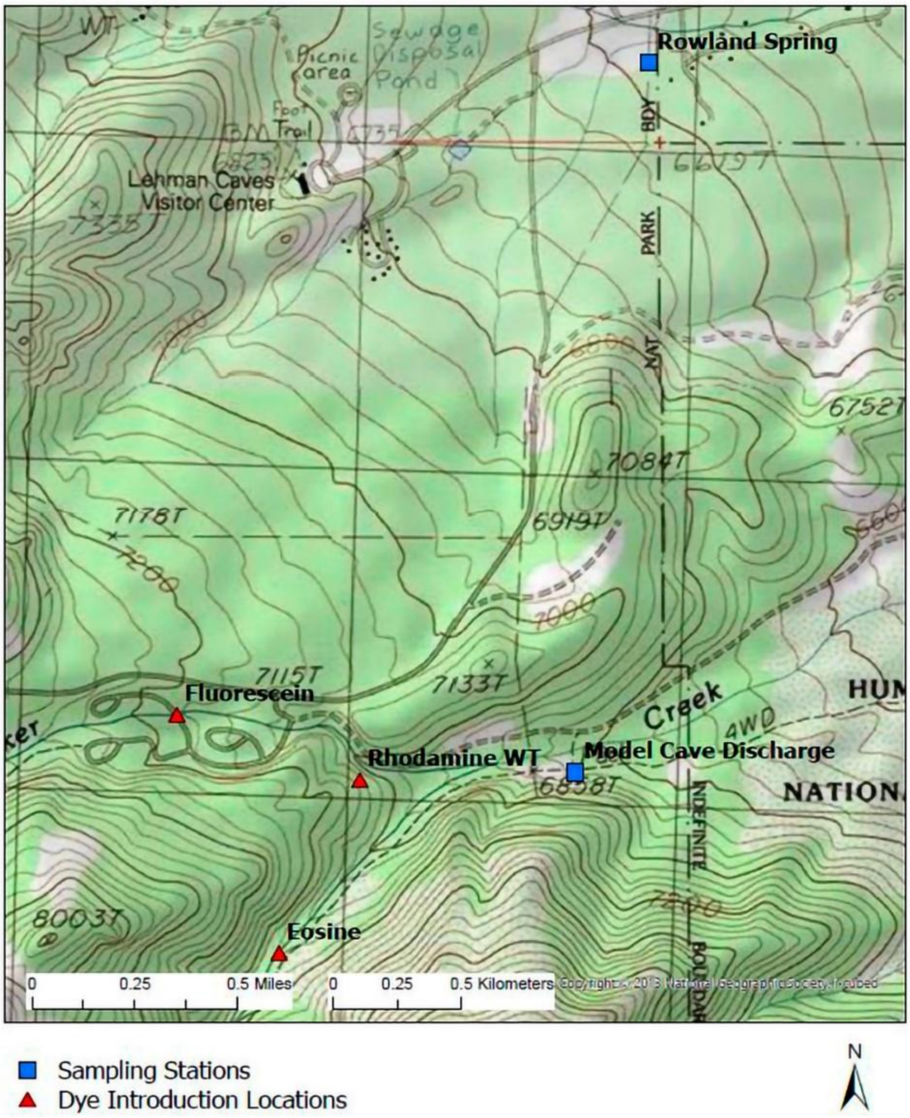

Figure Box 20-2 - Dye introduction points and key sampling locations near Lehman Caves.

Discussion. Figure Box 20-3 shows dye analysis results from Rowland Spring and Model Cave Discharge for all three dye traces. It compares cumulative dye concentrations from carbon samplers with dye concentrations in water samples. Water samples where no dye was detected are not plotted on the graphs. There are major differences in the dye recovery graphs from the eosine dye introduction in Pole Canyon and the other two dye introductions. Having two types of samples for traces can be valuable in understanding differences between different groundwater flow paths. Estimates of the total mass of each of the dyes detected at the two sampling locations are shown in Table Box 20-2. These estimates are based on dye concentrations in water samples and flow rate measurements.

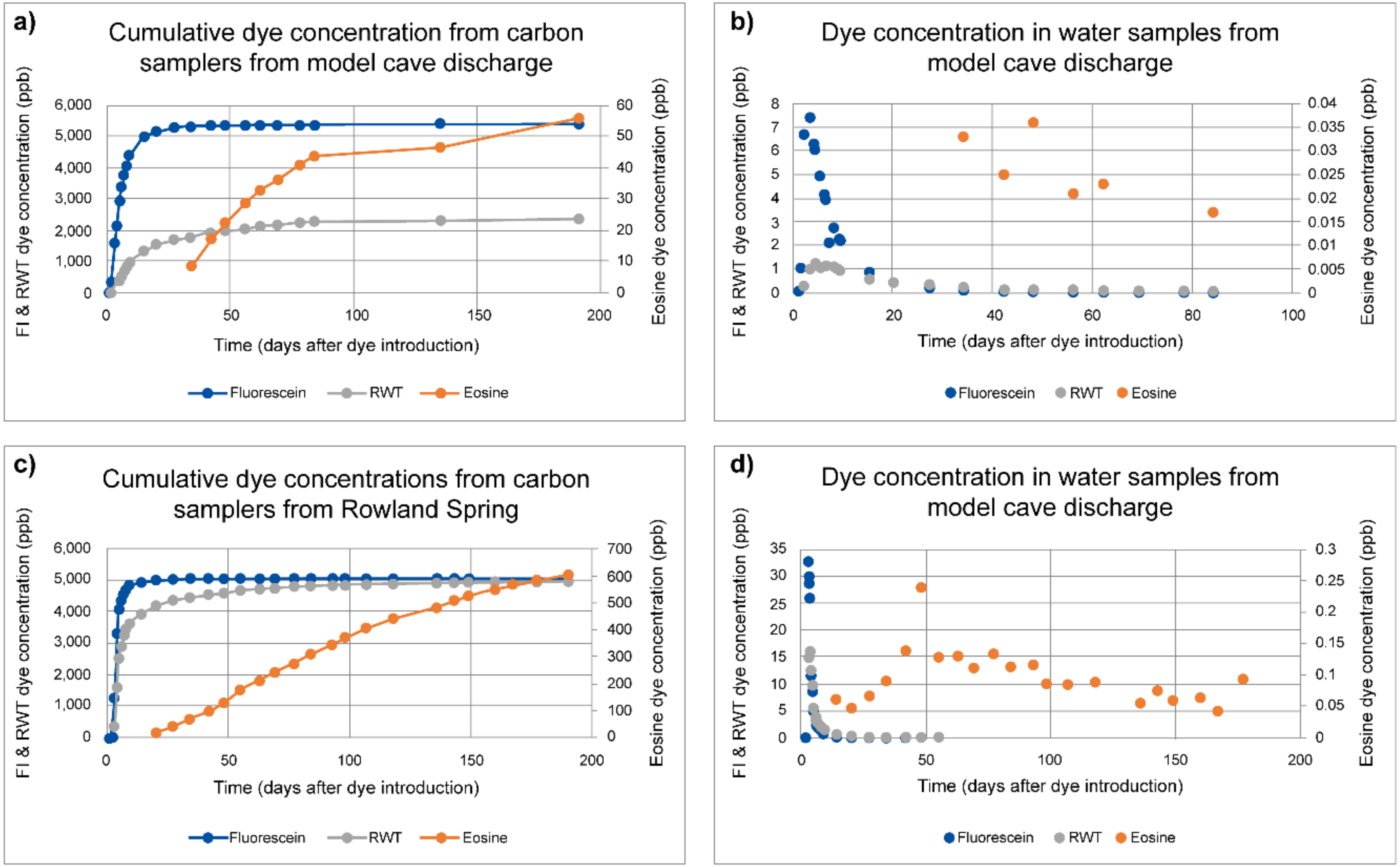

Figure Box 20-3 - Dye results in water and carbon samplers from Rowland Spring and Model Cave Discharge.

Table Box 20-2 - Percent of introduced mass of dye detected, in water samples, at sampling stations during the study period and approximate straight-line groundwater travel distances.

Trace	**Traces to Rowland Spring**		**Traces to Model Cave Discharge Point**	
Dye Introduction	Distance	% Mass Detected	Distance	% Mass Detected
Wheeler Deep Cave (Rhodamine WT)	3,780 m 12,400 ft	15.88%	760 m 2,480 ft	1.05%
Pole Canyon (Eosine)	3,260 m 10,700 ft	4.85%	1,220 m 4,000 ft	0.07%
Baker Creek (Fluorescein	2,130 m 7,000 ft	19.40%	610 m 2,000 ft	2.80%
Elevation differences and mean flow rates				
Dye Introduction	**Rowland Spring**		**Model Cave Discharge Point**	
Wheeler Deep Cave	116 m (380 ft)		37 m (120 ft)	
Pole Canyon	195 m (640 ft)		116 m (380 ft)	
Baker Creek	134 m (440 ft)		55 m (180 ft)	
Mean Flow Rate During Study	5,620 L/min 1,485 gal/min		680 L/min 180 gal/min	

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Box 21 - Case History 21: Results when two dyes were introduced at the same point and time, Arkansas, USA

This case history provides a comparison of the behavior of fluorescein and rhodamine WT dyes in a karst area. 4.5 kg (10 lb) of fluorescein dye mixture with a dye equivalent of approximately 70 percent and 12.9 kg (28.5 lb) of rhodamine WT mixture with a dye equivalent of approximately 20 percent were introduced within a few minutes of each other at the same point in a losing stream segment of a small headwater stream. There was no natural flow at the dye introduction point; so, water was hauled by tank truck. A total of 81,750 L (21,600 gal) of potable water was introduced into the stream channel over an eight-hour period and all flow entered the subsurface within 12 m (50 ft) of the dye introduction point. The large amount of dye was used because of the possibility that a discharge point for the dye would be at large karst springs up to 25 km (15.5 mi) from the dye introduction point. The volume of water used was similar to modest stormwater flow volumes.

Dye from the introduction was detected at Southeast Spring 320 m (1,050 ft) from the dye introduction point and 23 m (75 ft) lower in elevation. There were no other points where either of the dyes discharged from groundwater. The thickness of clay-rich residuum beneath the dye introduction point was approximately 10 m (33 ft). The mean flow rate of Southeast Spring during the study was approximately 2.5 L/sec (40 gpm).

Discussion. Dye concentrations in water samples indicated that 0.612 kg of fluorescein dye mixture discharged from Southeast Spring during the 58-day study period. This was 13.5 percent of the mass of fluorescein mixture introduced. Rhodamine WT mixture detections at the spring were 0.145 kg; this was 1.1 percent of the mass of rhodamine WT mixture introduced. This indicates that fluorescein is the more conservative tracer.

Figure Box 21-1 shows cumulative breakthrough curves for both dyes from both water and activated carbon samplers during the 58-day study period. The dyes were first detected at Southeast Spring four days after dye introduction. All curves, except the one for rhodamine WT measured in water samples, were similar and produced comparable estimated times at which 50 percent and 90 percent of all dye was detected. The close correspondence in the curve shapes for activated carbon samplers and fluorescein dye in water suggests that both sampling approaches provide similar estimates for the percentage of the total mass of dye detected at selected times during a study. The authors have no explanation for the appreciable departure between the curves for rhodamine WT in carbon samplers and in water samples.

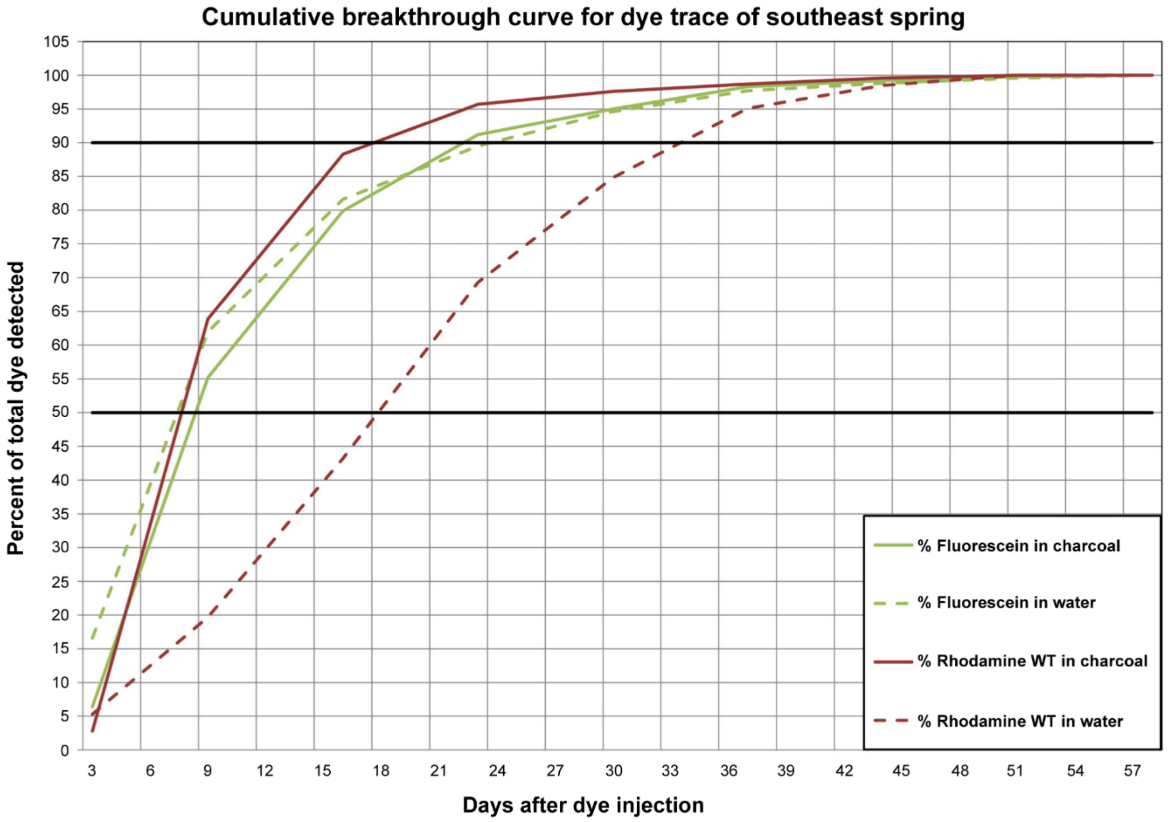

Figure Box 21-1 - Cumulative break-through curves at Southeast Spring.

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Box 22 - Case History 22: Groundwater trace from municipal sewage ponds to a river, Montana, USA

Fluorescein dye was used to prove that leakage was occurring from a group of three holding ponds containing partially treated municipal sewage. At the time of the dye introduction, the surface area of the holding ponds was approximately 6 hectares (15 acres), the volume of treated sewage in the ponds was estimated at approximately 133,000 m^3 (35 million gallons). The mean leakage rate was approximately 150 liters/minute, or 40 gallons/minute. The leakage rate was based on the facility's inflow and outflow records in combination with a water budget adjusted for precipitation and evaporation. All holding ponds had synthetic liners 1.524 mm (0.06 in) thick separating the treated sewage from an underlying alluvial aquifer. Drainage pipes beneath the liners were designed to prevent water levels in the aquifer from contacting the liner. In the event of liner failure, the drainage pipes conveyed aquifer water contaminated with sewage to a discharge point at an adjacent river.

During the tracing period, natural flow from the aquifer, augmented by leakage from the holding ponds, was estimated at approximately 375 L/min (100 gal/min). A total of 7.25 kg (15 lb) of fluorescein dye mixture with a dye equivalent of approximately 70 percent was dissolved in water and introduced into the holding ponds from the edges of two of the ponds and through an inflow structure at the third pond. Figure Box 22-1 shows one of the dye introductions being conducted near damaged gabions that were suspected of having punctured the liner. The gabions were filled with rocks.

Figure Box 22-1 - Beginning a fluorescein introduction into a sewage pond. The damaged gabions are shown to the right of the person introducing dye.

In this project, dye sampling placed primary reliance on activated carbon samplers. First dye arrival in carbon samplers occurred within six days of dye introduction and the mean concentration in carbon sampler elutants for samplers in place for the six days after dye introduction averaged 39.46 µg/L. The laboratory's detection limit for this fluorescein dye mixture in carbon sampler elutants was 0.025 µg/L. Sampling for fluorescein dye continued at the aquifer discharge point until 29 days after the date of dye introduction; dye was still readily detectable in carbon sampler elutants from samplers in place from 23 to 29 days after dye introduction. The rapid transport of dyed, partially treated, sewage to the river was facilitated by the drainage pipes under the liner.

Discussion. Tracing to identify leakage zones in a large body of water is difficult unless the dye can be introduced at a point near the leak, unless the leak is large. While dye was introduced at four points in the lagoons, the existence of damaged gabions was a useful indicator of likely leakage locations. The difficulty of the trace was increased by the large volume of water in the underlying aquifer, which served to further dilute the tracer dye. Even with these difficulties, dye was successfully traced from the lagoons to a discharge point at the adjacent river.

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Box 23 - Case History 23: Tracing to determine time-of-travel for leakage through an earth-fill dam, Arizona, USA

River Reservoir 3 is on the Little Colorado River in east central Arizona, USA, near the town of Greer. The dam was constructed in 1896 and was modified at least four times; no as-built plans were found. The earth-fill dam was about 21.3 m (70 ft) high and approximately 335 m (1,100 ft) long (Shannon et al., 2007). During the last half of March 2004, the water surface in the reservoir was higher than normal and water was discharging through the spillway. The sequence of events is detailed in Table Box 23-1. Day 0 was March 28, 2004. An artist's rendition of conditions during the brief tracing test is provided as Figure Box 23-1.

Table Box 23-1 - Sequence of events at River Reservoir 3, Arizona, USA. Day 0 was March 28, 2004.

Day	Event
0	Owners of the dam, who used the water for irrigation, reported that the left abutment drain was discharging more than 450 L/min (120 gal/min), a rate 10 times more than normal.
4	Sediment in the discharging water contained sand and some clay consistent with core materials in the dam (Shannon et al., 2007).
14	Monitoring of sediment discharges and reservoir water levels twice per day began.
16	Left drain on dam was discharging large pulses of sediment and the outlet gate in the dam was fully opened to drain the reservoir as rapidly as possible.
18	Apache County declared an emergency, evacuations were ordered, the sheriff moved a mobile command post to the site and began continuous surveillance.
22	Governor of Arizona declared a state of emergency.
31	OUL arrived on site facing pressure from dam owners and county sheriff to conclude there was no imminent threat of dam failure. The dam was still visually intact, discharge through the outlet gate had ended, and the water level in the reservoir was approximately 2.7 m (9 ft) below the spillway elevation. OUL suggested that a dye trace to determine the time of travel from the reservoir to the drains would provide valuable information for assessing the risk of sudden dam failure.
32	On April 29 (Day 32) 1.82 kg (4 lb) of fluorescein dye mixture with a 70% dye equivalent was dissolved in water in a carboy and produced a dye solution with a specific gravity of approximately 1.12. This solution was poured from a boat onto the surface of the reservoir water where it rapidly sank and created trails of strongly dyed water. Six dye introduction points were used in an area approximately 12 m (40 ft) by 18 m (60 ft) (Aley, 2004). There were two rows of dye introduction points with one row approximately 1.8 m (6 ft) from shore and the second row 3.7 m (12 ft) from shore and parallel to the first row; introductions were made during a three-minute period. The dye introduction area centered on the reservoir area nearest the left drain. The distance from the center of the dye introduction area to the weir box for the left drain (the dye monitoring point) was approximately 125 m (410 ft). Visible dye first appeared at the left drain weir box nine minutes after the first dye introduction. Based on seeing the highly visible dye, the sheriff immediately stated that he would keep his personnel at the dam and the representative of the dam owners said the outlet gate would be opened as soon as possible. OUL recommended that everyone move rapidly uphill. Visible dye first arrived at the right drain weir box 63 minutes after the start of the dye introductions. Additional quantitative sampling and six additional dye introductions were made to develop data useful for designing corrective actions.

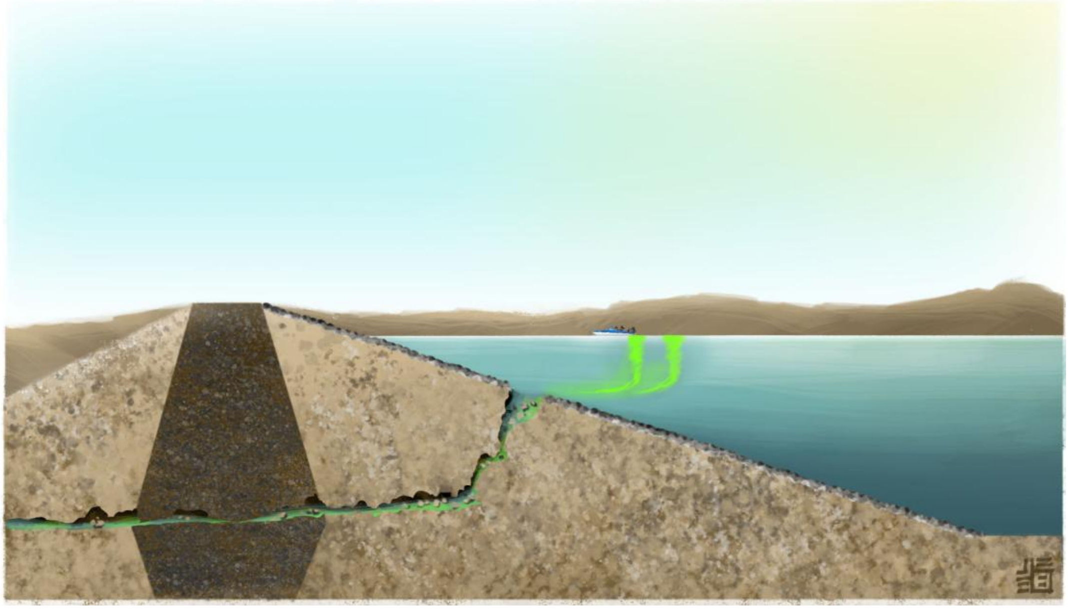

Figure Box 23-1 – Schematic of a nine-minute travel time for dyed water through the dam along soil piping channels at River Reservoir 3, Little Colorado River, Arizona, USA.

Discussion. Dye tracing is an excellent technique for determining travel times for water moving through, around, or beneath dams. Resulting data are easily understood by the public and officials. At other sites the OUL has introduced tracer dyes and water into piezometers within dams and determined travel rates to downstream seeps and springs. It is often necessary to position water tanks near the piezometers and introduce water at slow rates for periods of a few days to a week. Water must not overflow the top of the piezometer during such traces.

When water levels in the reservoir were lowered it was evident that a large amount of earth was missing from the upstream face of the dam in the vicinity of the left drain due to soil piping, and that the core of the dam had also been damaged. Shannon and others (2007) detailed the reconstruction of the dam.

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Box 24 - Case History 24: Dye tracing to test for leakage from an earth-fill dam, Texas, USA

The management of a large dam in Texas, USA, was concerned that there might be appreciable leakage beneath a discharge gate in their dam. A hard hat diver installed the base of a pipe in the area of concern beneath a gate, with the top of the pipe extending to approximately a meter (3 ft) above the surface of the reservoir. Working from a pontoon boat, reservoir personnel poured a dense dye solution into the pipe and flushed dye out of the pipe by adding twice the original injection volume of water as a chaser. Subsequent sampling did not detect significant amounts of dye that would have represented leakage from beneath the discharge gate. It was concluded that if there was any leakage from the tested area, it was minor.

Discussion. Barrier walls and slurry trenches are sometimes used to control pollutant migration (Spooner et al., 1985) or other water flow control purposes. Issues sometimes arise about possible leakage along the outside of pipes that pass through the barriers or flow beneath the barriers. Dye tracing is a cost-effective method for assessing possible leakage through or beneath such structures. A common approach used by the OUL is to construct a dye introduction boring on the up-gradient side of the barrier and three borings for dye sampling down-gradient of the barrier. The three borings on the down-gradient side are to reduce the chance that dye passes through or beneath the barrier but is not detected at a down-gradient boring. Where pipes or other features pass through the barriers the down-gradient borings bracket those features.

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Box 25 - Case History 25: Trace to determine time-of-travel for water from a highway to endangered species habitat, Missouri, USA

The only known population of the federally endangered Tumbling Creek cavesnail is in the underground stream that flows through Tumbling Creek Cave and in the inaccessible karst system lying between cave passages and Big Creek. The cave, located at the OUL, is used for research and educational purposes. The cave is a designated National Natural Landmark and is recognized as having the most diverse cave fauna of any American cave west of the Mississippi River. There are over five miles of state and federal highways within the recharge area for the cave. A major recognized threat to the continued existence of the cavesnail is spills on the highway or compounds used in periodic resurfacing of the highway.

The Missouri Department of Transportation (MoDOT) planned a chip seal resurfacing of part of US Highway 160 that included a segment within the delineated recharge area of the cave. MoDOT, in cooperation with the US Fish and Wildlife Service and OUL, wanted to determine if the resurfacing work, when conducted as planned, would introduce detectable concentrations of Total Petroleum Hydrocarbons (TPH) into the cave stream. MoDOT placed tight constraints on weather conditions during which the resurfacing could be done to minimize the chance of stormwater runoff if precipitation occurred when the resurfacing compounds were not fully cured.

There were ditches along the highway that conveyed highway runoff water to two culverts under the highway. The culverts were located on small intermittent stream channels known to sink into the groundwater system feeding the cave. A mass of 454 gm (1 lb) of powder-form fluorescein dye with a 70-percent dye equivalent was placed in each of the culverts in such a way that it would be taken into solution by the first runoff water.

An automatic pumped water sampler with 20 collection bottles was placed next to the cave stream and programmed to collect composite samples of approximately 0.9 L (1 quart) of water every eight hours with a quarter of each sample collected at 2, 4, 6, and 8 hours. A weir for measuring flow rate of the stream was located adjacent to the automatic water sampler. The plan was to use the automatic sampler to capture water that would contain runoff from the first precipitation to fall on the highway after the resurfacing. The OUL measured fluorescein concentrations in the collected water to determine the times of first dye arrival and of peak dye concentration arrival.

Resurfacing was conducted on November 7 and 8, 2006. Dye was placed in culverts on November 9. On November 13 there was 0.3 cm (0.12 in) of precipitation, but it did not produce runoff in the road ditches. On November 15 precipitation of 1.78 cm (0.70 in) occurred with the storm beginning in the early morning hours. First flow reaching the dye occurred at about 0500 hours and by 0745 hours all placed dye had been taken into solution. Two water samples from road ditches on November 15 at 0745 hours had TPH at concentrations below the detection limit of 100 µg/L. November 16, 2006, had precipitation of 2.82 cm (1.11 in).

Dye was first detected in the cave stream 5.09 km (16,700 ft) from the nearest dyed road culvert in a composite water sample collected 51 to 59 hours after the dye was introduced by runoff water on November 16. The dye concentration, based on as-sold weight of the dye mixture, was 0.082 µg/L. Peak dye concentration was 0.326 µg/L in a composite water sample from 59 to 67 hours after dye introduction. Water samples associated with the first dye arrival and the peak dye concentration were analyzed for TPH and concentrations were less than the detection limit of 100 µg/L. Figure Box 25-1 shows dye concentrations in the cave stream water and flow rates of the stream.

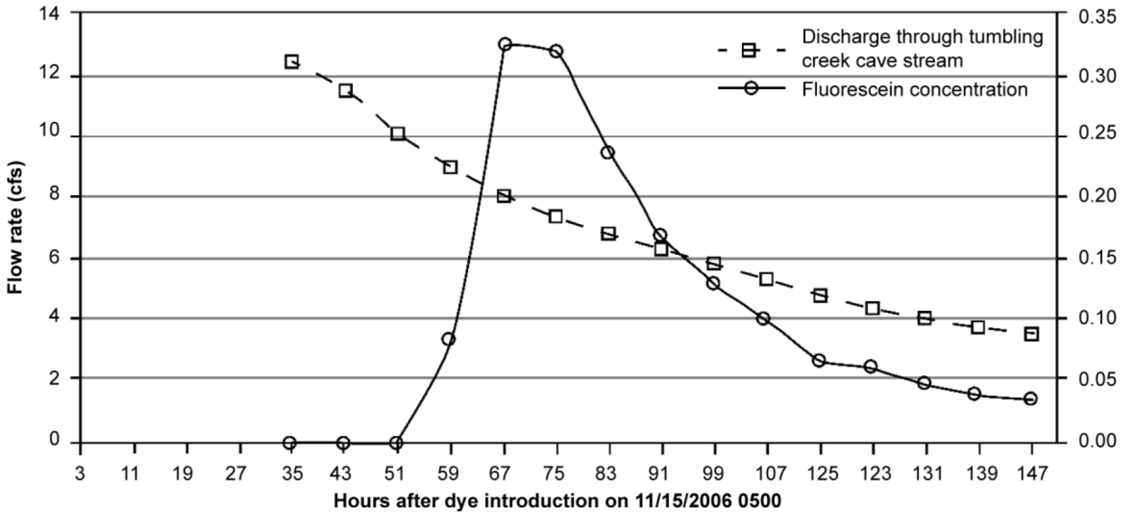

Figure Box 25-1 - Fluorescein concentrations and flow rates in Tumbling Creek Cave stream.

This case history shows how tracer dyes can provide useful time-of-travel information. In addition, the dye showed which water sample represented the first arrival of runoff water from the highway segment and which sample represented the peak runoff rate from the highway segment. The absence of detectable TPH in either sample indicated that the paving mixture was unlikely to represent a significant threat to aquatic life in the cave stream. A total of 6.1 percent of the introduced dye passed the sampling point during the 6 days after dye introduction.

Discussion. Road culverts are often good dye introduction points and are especially useful in tracer studies where highway runoff is a concern. There are often plunge pools at the downstream end of culverts and dry sets can be placed in the bottoms of such pools or inside the culverts.

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Box 26 - Case History 26: Background sampling at a waste site where multiple dyes had previously been used, Tennessee, USA

The OUL conducted a tracer test in Tennessee, USA, at a large waste site in karst where six different fluorescent dyes (including the five that are the subject of this book) had been introduced into groundwater three years before the OUL work began. It was suspected that other fluorescent compounds might also be present. Because of this history, the study plan specified a minimum of three rounds of weekly background sampling. Due to a combination of factors, the dyes could not be introduced at the end of the planned background sampling period; so, background sampling continued on a weekly schedule for a total of 12 weeks. This provided an unexpectedly detailed set of data on the variability of background fluorescence conditions at a waste site.

There was a total of 899 activated carbon samplers and 93 water samples—only water samples could be collected from some wells—analyzed during the background sampling period from a total of 100 sampling stations. One or more fluorescence peaks in or near the ranges of tracer dyes was detected at 20 of the sampling stations. Tracer dyes from the previous work were detected at 11 of the 20 sampling stations. Fluorescein, attributable to runoff from a nearby highway, was detected at one sampling station. Emission peak wavelengths of compounds deemed to not be tracer dyes were found at 8 sampling stations, and all fluorescence peaks were outside of the established acceptable wavelength ranges for positive dye detections and peak shapes were atypical of those for the tracer dyes. Virtually all monitoring wells with fluorescence peaks that were not associated with dye from the previous work had those fluorescence peaks in most or all analyzed samples. In contrast, background fluorescence conditions were much more variable at sampling points in an adjacent river.

Discussion. Based on these data two rounds of background sampling at most groundwater sampling stations appears adequate to characterize background fluorescence. Characterizing fluorescence background in surface streams, and especially those that flow through upstream communities or industrial areas, is more difficult. This limitation can usually be offset by establishing control sampling points on streams upstream of the area being investigated in a tracing program.

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Box 27 - Case History 27: First successful groundwater trace to Big Spring, Missouri, USA

In 1966, the US Forest Service established a study watershed on Hurricane Creek focused on karst issues. Hurricane Creek is a surface water tributary to the Eleven Point River, but only 19 percent of the annual water yield from the 294 km^2 (113 mi^2) basin discharges as surface flow into the river (Aley, 1978). The remainder enters karst groundwater, and much of this flow was presumed to discharge from Big Spring. A groundwater trace was needed to confirm groundwater flow from the Hurricane Creek basin to Big Spring—a tributary to the Current River—and to provide an estimate of groundwater velocity.

The Blowing Spring Estavelle, in the channel of Hurricane Creek, was selected as the dye introduction point. An *estavelle* is a karst feature that functions as a spring in wet weather and as a swallet (a location where a stream "disappears" into the subsurface) under drier conditions. Flow rates of up to 0.57 m^3/sec or 34,200 L/min (20 ft^3/s or 9,000 g/min) have been observed discharging from this estavelle. Approximately 182 km^2 (70 mi^2) of topographic basin is upstream of the estavelle, and except for perennial flow from a small hillside spring 50 m (165 ft) upstream of the estavelle, the channel of Hurricane Creek has no surface flow for approximately 75 percent of the year. Figure Box 27-1 shows the estavelle at a time when the flow rate of sinking water was approximately 57 L/min (15 g/min).

Figure Box 27-1 - Flow of approximately 57 L/min (15 gpm) sinking in the Blowing Spring estavelle.

On June 11, 1968, 4.5 kg (10 lb) of fluorescein dye mixture with a 50-percent dye equivalent was introduced into a flow of approximately 416 L/min (110 g/min) sinking into groundwater at the Blowing Spring estavelle. Dye was first detected in carbon samplers in place in the flow from Big Spring for the period from 7 to 14 days after dye introduction;

the estimated time of first dye arrival was 10 days after dye introduction. The straight-line travel distance was 27.4 km (17.0 mi). The mean flow rate of Big Spring during the tracing period was 11.7 m^3/sec or 702,000 L/min (413 ft^3/s or 185,367 g/min). Figure Box 27-2 shows Big Spring at a flow rate of 14.68 m /sec or 880,800 L/min (518 ft^3/s or 232,494 g/min).

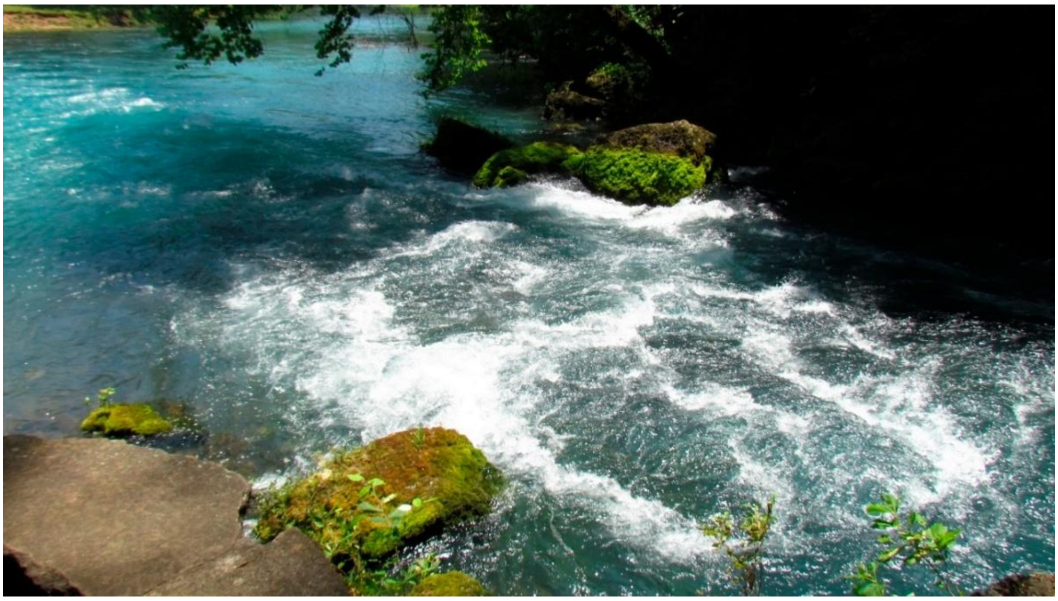

Figure Box 27-2 - Big Spring, Missouri with a Flow Rate of 14.68 m^3/sec or 880,800 L/min (518 ft^3/s or 232,494 g/min).

Discussion. This trace was accomplished with a relatively small amount of dye that addressed the relevant questions even though the dye experienced massive dilution. The trace was made possible by using activated carbon samplers. Fluorescein dye was visible in the carbon sampler elutant within five minutes of covering the carbon with the eluting solution. The trace demonstrated that the large topographic basin upstream of the dye introduction point contributes water to Big Spring under some conditions. Many long-distance traces to large springs in Missouri were conducted with fluorescein and carbon samplers before spectrofluorophotometers capable of synchronous scans were readily available. In this case the positive dye recovery was confirmed with a filter fluorometer.

Groundwater traces can be conducted with fluorescein dye, sampled using activated carbon samplers, and the elutant can be visually examined for the presence of the fluorescein dye. This approach was discussed in Section 3.6. This approach does not work adequately with dyes other than fluorescein or if there are multiple dyes in the carbon sampler. The visual approach can be useful in situations where interim results are needed to guide further sampling. If this approach is used, samplers that have not been eluted should be shipped to a qualified laboratory for analysis to confirm the visual analysis.

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Box 28 - Case History 28: Delineating the recharge area for Mitch Hill Spring, Arkansas, USA

Mitch Hill Spring discharges in the channel of Cane Branch and flows approximately 450 m (1,500 ft) to join the Buffalo River, a portion of which is a unit of the US National Park System. It is one of the largest springs feeding the Buffalo River, and tracing work by the OUL and National Park Service has shown that it has a recharge area of 129 km^2 (49.65 mi^2) (Soto, 2014; Aley, 2018).

A successful trace to Mitch Hill Spring was conducted in 1986 from a small sinkhole adjacent to the proposed landfill. The proposed waste site is underlain by the Boone Formation which is primarily limestone and chert. A general estimate applicable to similar settings in this part of Arkansas is that 65 percent of the total annual water yield moves through the karst groundwater system and the other 35 percent is surface runoff (Figure Box 28-1). Surface stream channels are common, but most have only intermittent water flow. Total water yield averages approximately 33 cm (13 in) per year. Mean annual precipitation is 117 cm (46 in).

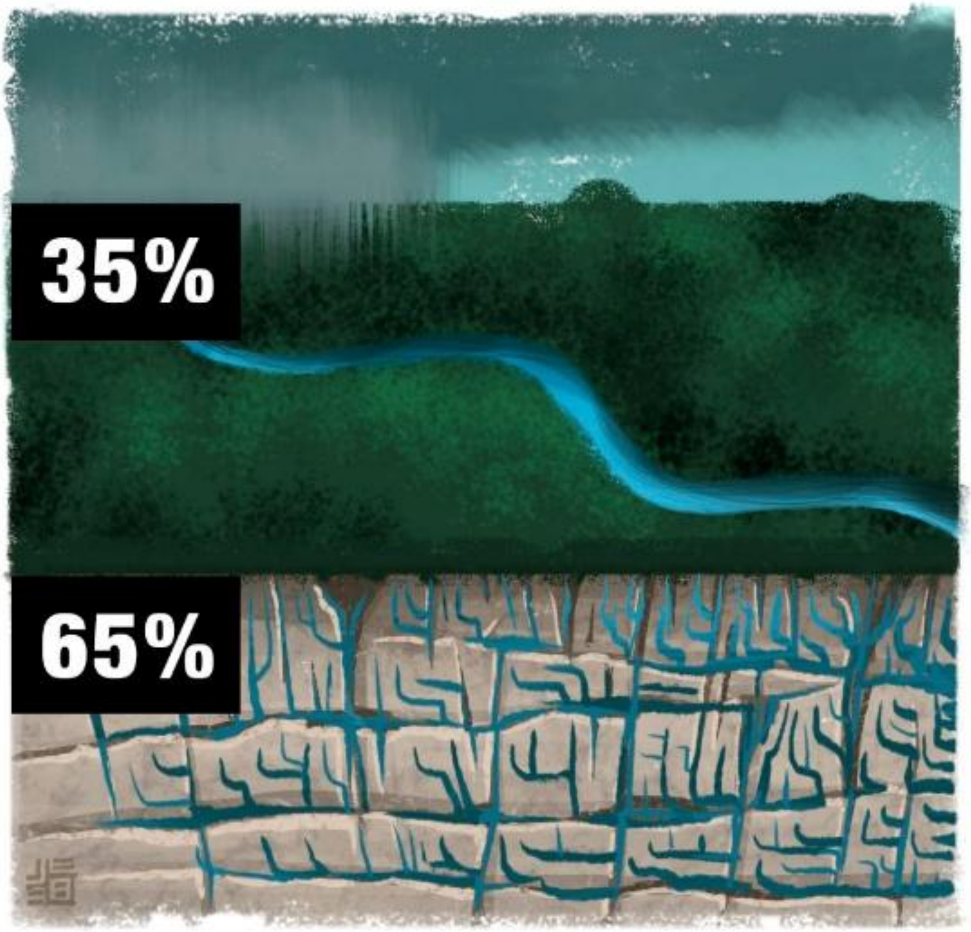

Figure Box 28-1 - Approximately 65 percent of the annual water yield from the Mitch Hill Spring recharge area is through groundwater.

Local concern about the impact of a waste site that would ultimately cover approximately 72.7 hectares (160 acres) of upland karst was the reason the OUL conducted this dye trace. If permitted, the landfill was expected to receive municipal trash and possibly waste from chicken processing. The landowner (the proponent for the landfill) denied access to the proposed landfill site for a dye-tracing investigation. An alternate dye introduction site, a sinkhole approximately 40 m (130 ft) from the edge of the proposed landfill but on a neighboring property, was selected as the dye introduction point. The sinkhole was a minor topographic feature 3.7 m (12 ft) in diameter and 0.6 m (2 ft) deep. 4.1 kg (9 lb) of fluorescein dye mixture containing approximately 50 percent dye was introduced into the sinkhole and flushed with 74,200 L (19,600 gal) of water. Similar volumes of water entered the sinkhole under natural conditions. The water was pumped from a pond, run through fire hoses, and discharged into the sinkhole at a mean rate of 143 L/min (37.75 gpm); all this water entered groundwater through the sinkhole.

Sampling for the tracer dye was conducted at 24 sampling stations and used activated carbon samplers. Dye was detected at eight sampling locations as shown in Table Box 28-1 and Figure Box 28-2. Five of the dye detection locations were domestic water supply wells where the water was used without treatment. Drilling records for the Holder Well (Station 26) documented six cavities in the limestone with a total vertical extent of 17.4 m (57 ft), demonstrating that the area around the proposed landfill was cavernous.

Table Box 28-1 - Detection sites for dye introduced at injection site 86-01.

Dye detection site and station number in Figure Box 27-2	First dye arrival (days after dye introduction)	Distance from dye introduction point	
		Meters	Feet
12. Cannon Spring	3 to 5 days	3,475	8,500
26. Holder Well	Less than 5 days	200	650
19. Jack Keith Spring	25 to 32 days	2,530	8,300
1. Young Well	38 to 41 days	1,600	5,250
29. Nichols Well	38 to 41 days	1,465	4,800
27. Henson Well	38 to 41 days	1,585	5,200
28. Herron Well	38 to 41 days	1,615	5,300
16. Mitch Hill Spring	Approximately 61 days	7,165	23,500

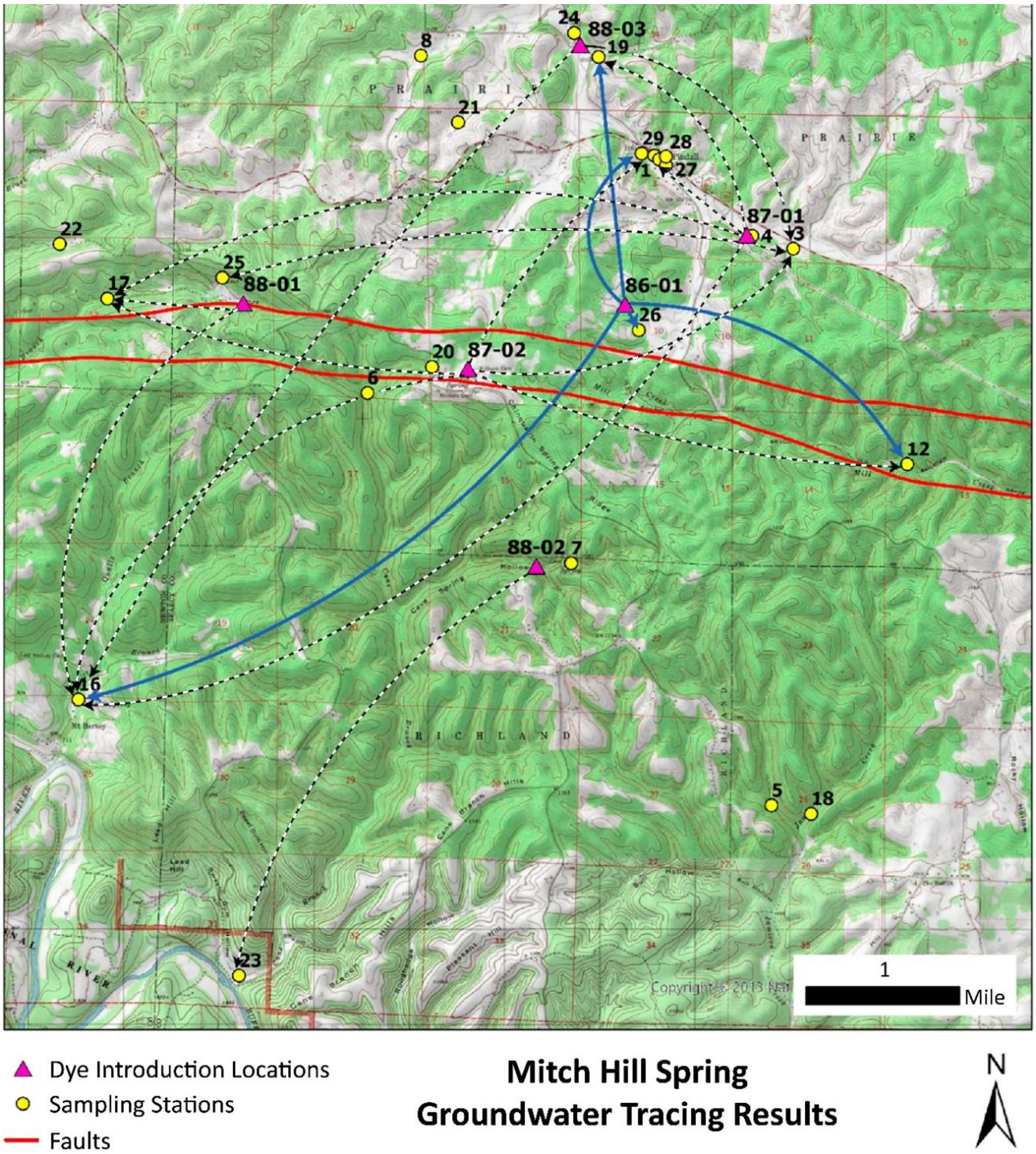

Figure Box 28-2 - Dye trace from a proposed landfill site to Mitch Hill Spring and five subsequent groundwater traces in the region. Mitch Hill Spring is Station 16. Dye from Trace 86-01 was detected at wells 1, 27, 28, and 29; flow lines from 86-01 introduction to positive dye detections are shown in blue lines; not all flow lines are shown for clarity. Black, dashed lines show additional hydrologic connections established from subsequent dye introductions.

The Mill Creek Graben, bounded on the north by the St. Joe Fault and on the south by the Mill Creek Fault, runs roughly east-west and averages about 610 m (2,000 ft) wide. Bedding both north and south of the graben dips away from it and the dye introduction point lies approximately 560 m (1,830 ft) north of the St. Joe Fault. The channel of Mill Creek, a major losing stream in the area, is mostly within the graben and follows it for approximately 6.4 km (4 mi) within the study area.

Not only did this dye-trace discharge from multiple springs, but the springs were in three different topographic basins. Cannon Spring is located on Mill Creek within the graben and is east-southeast of the dye introduction point. Jack Keith Spring is north of the dye introduction point on Clear Creek; this steam is not tributary to the Buffalo River until many miles down-river of Mill Creek. Mitch Hill Spring is southwest of the dye introduction point and is on Cane Branch, a tributary to the Buffalo River.

Discussion. Subsequent work in the area (Aley, 1988; Aley & Aley, 1989) included dye introduction 88-03 into water that had discharged from Jack Keith Spring and was sinking at a point approximately 420 m (1,380 ft) downstream of the spring. That dye introduction used 1.36 kg (3 lb) of fluorescein dye mixture containing approximately 50 percent diluent. That dye was detected in activated carbon samplers at Station 16 Mitch Hill Spring 8.84 km (5.49 mi) from the dye introduction point with first dye arrival within 13 days of dye introduction. Dye from this trace was also detected at Station 3, the SPG public water supply well, 3.23 km (2.01 mi) from the dye introduction point with first arrival occurring between 13 and 24 days after dye introduction.

The SPG well (Station 3) was the only public water supply for the communities of St. Joe, Pindall, and Gilbert, hence the name SPG Well. It was drilled on the floor of the Clear Creek valley at the intersection of two fracture traces and has 152 m (500 ft) of pressure grouted casing. Three dye traces resulted in dye detections at the SPG well and demonstrated a recharge area for the well of approximately 34.8 km^2 (13.4 mi^2). In addition to the trace to the SPG well from downstream of Jack Keith Spring, two other traces to the SPG Well showed travel times of seven days or less for water moving from dye introduction points to dye detections in the well.

This Box 28 illustrates some important considerations in designing and conducting traces to delineate recharge areas for springs as listed here.

1. The anticipated result of the initial trace was that dye would be detected in one or more local wells and probably also in Jack Keith Spring. However, sampling occurred throughout a much larger area in case other groundwater paths existed and would be discovered by a more comprehensive sampling program. It is often difficult to convince project funders of the need for an adequately comprehensive program, but this convincing is often easier if the less-likely possible detection sites are monitored with activated carbon samplers that are collected and replaced only a few times. This is relatively inexpensive sampling and analysis, and carbon samplers in relatively clean water can be left in place for longer periods of time than at waste sites.
2. Groundwater flow paths can change seasonally. The change that occurred in this case was one of the largest the OUL has documented with dye traces. Under wet weather conditions, groundwater flow is generally northward from the Mill Creek Graben to the sinking point on Clear Creek approximately 420 m (1,380 ft)

downstream of Jack Keith Spring. Under low flow conditions dye tracing demonstrated that the flow direction is from the sinking point on Clear Creek southward toward to the SPG Well and across the graben to Mitch Hill Spring. The groundwater divide in this study area can move seasonally by at least 3.41 km (2.12 mi).

3. The use of activated carbon samplers permitted continuous sampling at a substantial number of locations with relatively few sample collection visits. Several of the sampling stations were in remote locations that were difficult and time-consuming to reach.
4. Placing primary sampling reliance on activated carbon samplers also minimized the amount of dye needed for the dye introduction while still achieving the objectives of the trace. Minimizing the amount of dye needed for traces can be especially important if dye may be detected in water supply wells or in springs or caves that provide habitat for aquatic species of conservation concern. Studies have shown that the dyes discussed in this book, when used in quantities needed for groundwater tracing, do not pose environmental risks (Field et al., 1995). However, it is good protocol in designing studies potentially involving human water supplies or sensitive ecosystems to use as little dye as reasonable. That approach is best accomplished by sampling with activated carbon samplers and conducting dye analysis with a spectrofluorophotometer operated under a synchronous-scan protocol.

Important findings from Box 28:

1. The proposed landfill site contributed water to multiple private wells and springs.
2. The proposed landfill site was in the recharge area for a major spring supplying water to the Buffalo National River. This was the primary reason the State of Arkansas rescinded their initial operating permit for the proposed landfill.
3. Surface water can rapidly enter the SPG Well. Recharge area delineation traces in the region (Aley & Aley, 1989) have shown that approximately 9.15 km (5.69 mi) of US Highway 65 is in the recharge area for this public water supply well. Locating wells on fracture traces and lineaments in karst areas often results in higher water yields than from randomly located wells. This benefit can be offset by an enhanced risk of contamination from surface activities, even in wells with substantial amounts of pressure grouted casing.

Back to where text linked to Box 28↥

Box 29 - Case History 29: Determining groundwater travel rates to Silver Springs, Florida, USA

Silver Springs is in central Marion County northeast of Ocala, Florida, USA. Silver Springs includes two large springs and 28 smaller springs and clusters of spring vents located along the upstream 1,220 m (4,000 ft) segment of Silver River. The vents rise from submerged points on the bed of the river. The combined mean annual flow rate of the springs is approximately 23.24 m^3/sec (820 ft^3/sec) (Rosenau et al., 1977). The flow is from the karstic Floridan Aquifer System and the water is extremely clear. Silver Springs has long been a popular tourist attraction where visitors ride glass bottom boats that are slowly propelled along the river and over the spring vents; fish are abundant in the water and alligators can frequently be seen.

The tracing project in the Silver Springs area was focused on delineating groundwater pathways followed by nutrients (URS et al., 2011). To accomplish this objective four different dye introductions were made at four dye introduction points. Each was reflective of a different type of water and nutrient input to groundwater; the dye introduction points were:

1. Rhodamine WT dye was introduced into a stormwater detention basin at the Ocala Public Theater. This basin was capable of rapidly discharging water to the underlying karst aquifer.
2. Fluorescein dye was introduced into a sinkhole at the edge of Orange Lake. This sinkhole routinely conveys large volumes of water from this nutrient-rich lake into groundwater.
3. Eosine dye was introduced into the Tuscawilla Park drainage well. It artificially introduces stormwater runoff from an area with municipal and industrial land uses.
4. Sulforhodamine B dye was introduced into Pontiac Sink Pit. It receives water from a constructed wetland that provides treatment for stormwater runoff.

Tracing conditions at Silver Springs were substantially different from those existing for Box 27 at Big Spring and Box 28 at Mitch Hill Spring—also discussed in Box 3 and Box 14. When designing tracer investigations, it is important to adequately consider the great differences that exist among aquifers even if they have many similarities—in this case they are all large karst aquifers. The porosity in the zones conducting water through the carbonate aquifer at Silver Springs is much greater than in the aquifers supplying Mitch Hill Spring and Big Spring. This difference required the use of more dye and a much longer sampling period at Silver Springs than for the other two locations.

While long underwater cave systems explored and mapped by divers are associated with many springs in Florida, this is not the case for Silver Springs. Instead, flow of the springs is apparently from a three-dimensional network of solutionally enlarged openings in the bedrock that provide the relatively large porosity. Groundwater discharge occurs at multiple individual points called *spring vents*, and they have been divided into three groups based on water quality (URS et al., 2011).

- Group 1 vents have the highest total dissolved solids, dissolved oxygen, calcium, magnesium, bicarbonate, sulfate, nitrate, and phosphorous concentrations.
- Group 2 vents have lower concentrations of most parameters than Group 1 vents and particularly low dissolved oxygen, sulfate, and phosphorous concentrations.
- Group 3 vents have water quality characteristics intermediate between Group 1 and 2.

The postulated explanation for the differences among the three groups is that Group 1 vents represent shallower groundwater, Group 2 vents represent deeper groundwater, and Group 3 vents are a combination of shallow and deep groundwater. Tracing results were consistent with this interpretation.

The groundwater-tracing investigations at Silver Springs independently sampled 25 spring vents or clusters of vents. The vents are in the riverbed and are typically 4.6 to 12 m (15 to 40 feet) below the surface of the Silver River. To provide vent-specific sampling, scuba divers from Karst Environmental Services placed and recovered activated carbon samplers and collected water samples at the mouth of each vent during each of the 46 sampling events. One diver, Pete Butt, suffered an unprovoked alligator attack. He received a broken jaw and was air-lifted to a hospital. He fully recovered, but the 191 kg (420 lb) alligator was subsequently destroyed. Pete reported from the hospital that all samples were collected, and none were lost. Dedicated field work is essential for high quality tracer studies.

Ocala Civic Theater Detention Basin Trace. 9.1 kg (20 lb) of rhodamine WT solution containing approximately 21-percent dye equivalent was introduced into a small sinkhole in a stormwater detention basin at the Ocala Civic Theater and was flushed with approximately 265,500 L (70,140 gal) of potable water on April 23, 2010. This detention basin commonly introduces this volume of water into the karst aquifer after a storm. The quantity of dye was limited to ensure that colored water would not be visually present at any of the spring vents or in the Silver River. The use of this relatively small amount of dye was possible because primary sampling reliance was based on activated carbon samplers rather than water samples. The distance from the dye introduction point to the mid-point of the sampling stations at Silver Springs was 2.4 km (1.5 mi).

Rhodamine WT dye was detected in carbon samplers from all twelve Group 1 vents, from four of the eight Group 2 vents, and from four of the five Group 3 vents. Approximately 95 percent of the dye discharged from Group 1 vents, but this does not mean that 95 percent of water from the Ocala Civic Theater Detention Basin discharges from Group 1 vents. Dye was introduced into the upper groundwater system, and it appears that there was no appreciable mixing of upper and lower groundwater bodies between the dye introduction point and the vents.

The time when rhodamine WT was first detected at individual sampling points varied widely. The first arrival of dye at any of the spring sampling points was in carbon samplers in place for the sampling period from 5–10 days after dye introduction; this occurred at four of the Group 1 vents. The median first arrival time for all Group 1 vents was during the sampling period 16–21 days after dye introduction. First dye arrival times at Group 2 vents ranged from the sampling period 10–16 days after dye introduction to the sampling period 277–295 days after dye introduction. There was a pronounced trend for vents that had earlier first arrival times to also receive larger dye concentrations. Rhodamine WT was detectable in carbon samplers in place at many vents for several months and at some for almost 500 days after dye introduction. Typical plots of mean daily rhodamine WT concentrations from carbon samplers showed a rapid rise to a peak concentration and then a less rapid decline with low dye concentrations persisting at some locations until the end of sampling. Excluding duplicates, rhodamine WT was detected in a total of 284 carbon samplers. Thirty-five water samples were analyzed from vents for sampling periods during some of the largest rhodamine WT concentrations in associated carbon samplers. The detection limit for the rhodamine WT mixture in water samples was 0.015 ppb and no rhodamine WT was detected in any of the analyzed water samples. The trace would have failed if only water samples had been collected and analyzed.

Orange Lake Sink Trace. Thirty pounds (13.6 kg) of fluorescein dye mixture containing approximately 70-percent dye equivalent was introduced into the Heagy-Burry Sink in the edge of Orange Lake on April 23, 2010. The rate of inflow into the sink was approximately 0.68 m^3 (24 ft^3) per second and remained at this rate for several months. Table Box 29-1 shows the high production wells where fluorescein was detected and indicates the time after dye introduction when dye was first detected.

Fluorescein dye was detected in four carbon samplers and one water sample from IFAS Well D; in five carbon samples and two water samples from IFAS Well A; in 10 carbon samplers and 9 water samples from Reddick Elementary School; and in five carbon samples but no water samples from Marion Correction Institute. The Heagy-Burry Sink is 17 miles from Silver Springs. Fluorescein was not detected in any samples at Silver Springs.

Table Box 29-1 - Fluorescein from the Orange Lake Trace detected in water supply wells. The cardinal directions from the dye introduction point are indicated.

Well	Direction	Straight-line distance	Sampling period for first dye arrival (Days)	Mean groundwater velocity per day
IFAS Well D	SE	3.4 km 2.1 mi	6–13 days	260 to 580 m/day 850 to 1,900 ft/day
IFAS Well A	SE	3.5 km 2.2 mi	20–27 days	130 to 175 m/day 430 to 580 ft/day
Reddick Elementary	S	6.8 km 4.2 mi	6–13 days	520 to 1,100 m/day 1,700 to 3,600 ft/day
Marion Correction Inst.	SSE	13.7 km 8.5 mi	181–194 days	70 to 75 m/day 230 to 250 ft/day

Tuscawilla Park Drainage Well Trace. Thirty pounds (13.6 kg) of eosine dye mixture containing a dye equivalent of approximately 75 percent was introduced into the Tuscawilla Park Drainage Well on April 23, 2010. The drainage well receives water from an adjacent stormwater detention pond which, in turn, receives stormwater runoff from a large area with municipal and industrial land uses. Eosine dye was detected in activated carbon samplers in place at Silver Springs Station 32, the South Boathouse Vent, which is 8.2 km (5.1 miles) from the dye introduction point. The first dye detected was in a carbon sampler in place from 295–312 days after dye introduction. Based on this detection, the average groundwater velocity for first dye arrival at the South Boathouse Vent was 26 to 28 m/day (86 to 91 ft/day). The peak dye concentration at the South Boathouse Vent occurred during the period 312–329 days after dye introduction. Not counting duplicate samplers, dye was detected in carbon samplers from Boathouse Vent during five sampling periods. No eosine dye was detected in associated grab samples of water.

Pontiac Pit Sink Trace. Some information on this trace was included in Box 9 where the degradation of sulforhodamine B dye by deaminoalkylation within the groundwater system was discussed. Fifty pounds (22.7 kg) of sulforhodamine B dye mixture was introduced into Pontiac Pit Sink on October 5, 2010. The dye mixture contained approximately 35-percent dye equivalent. A large-constructed wetland that provides treatment for stormwater runoff from an area of about 12.8 hectares (32 acres) is located adjacent to this sink and routinely introduces larger volumes of water into this sinkhole than the volume introduced following the dye introduction (approximately 651,000 L (172,000 gals) of potable water from a fire hydrant). The distance from the dye introduction point to Silver Springs is 10.1 km (6.3 mi). Table Box 29-2 summarizes dye detection information for the three wells where the dye was detected.

Table Box 29-2 - Sulforhodamine B detections at three water supply wells. The cardinal directions from the dye introduction point are indicated.

Well	Direction	Straight-line distance	Sampling period for first dye arrival (days)	Mean groundwater velocity per day
Cedar Hills Well 1	ESE	3.9 km 2.4 mi	Day 50–57	70 to 80 m/day 220 to 250 ft/day
Blue Skies Well 1	NNE	5.6 km 3.5 mi	Day 93–113	50 to 60 m/day 165 to 200 ft/day
Fort King Forest Well	ENE	7.1 km 4.4 mi	Day 147–164	45 to 50 m/day 140 to 160 ft/day

Sulforhodamine B dye was detected in 10 carbon samplers from Cedar Hills Well 1, in 5 carbon samplers from Blue Skies Well 1, and in nine samplers from Fort King Forest Well. No dye was detected in any of the water samples from these wells.

Discussion. In 2006 the Florida Department of Environmental Protection and the St. Johns River Water Management District modeled 2-, 10-, and 100-year capture zones within the Silver Springs basin based on a particle track simulation (MODPATH). One purpose of groundwater tracing at Silver Springs was to compare model results with groundwater-tracing results (URS et al., 2011).

The rhodamine WT trace from the Ocala-Public-Theater stormwater pond to most of the spring vents at Silver Springs was within the two-year capture zone. The delineated area for the two-year capture zone was roughly circular with a radius of approximately 6.3 km (3.9 mi). All dye detections from the rhodamine WT trace were at vents at Silver Springs, and, for most of the traces, the time required for first dye arrival at individual spring vents was within 21 days of dye introduction. The average distance from the dye introduction point to the spring vents was 2.4 km (1.5 mi).

The fluorescein dye introduction point in Orange Lake is located NNW of Silver Springs. The dye introduction point lies approximately 4.3 km (2.7 mi) outside of the outer boundary for the Silver Springs 100-year capture zone. Fluorescein dye was detected at the Marion Correction Institution well which is at the outer boundary for the calculated 10-year capture zone for Silver Springs. First dye arrival at this well was 181–194 days after dye introduction. The Marion-Correction-Institution well is approximately midway between the dye introduction point and Silver Springs. If traced water moves halfway to Silver Springs in about half a year, it is reasonable to estimate that travel time for groundwater to move from Orange Lake to Silver Springs should be on the order of one year for first dye arrival rather than over 100 years as the modeled capture zones imply. While first arrival times for tracer dyes and capture zones are not directly comparable, the differences in groundwater travel rates indicate that the modeled capture zone boundaries in this area do not appear to be a useful groundwater management tool.

The eosine dye introduction made into the Tuscawilla Park Drainage Well was outside of the outer boundary for the two-year Silver Springs capture zone, and by interpolation was at about a predicted three-year time-of-travel point. Eosine dye was first detected at Silver Springs 295–312 days after dye introduction. The tracing results suggest that delineated size of the two-year capture zone is, at least in the vicinity of this well, too small.

The sulforhodamine B introduction made into Pontiac Pit Sink resulted in dye detections at three wells between the dye introduction point and Silver Springs. The dye introduction point is approximately midway between the two-year and 100-year capture zone boundaries. Based on first dye arrivals at Blue Skies and Fort King Wells, the sulforhodamine B dye moved approximately halfway to Silver Springs in approximately half a year. The tracing results suggest that the delineated size of the two-year capture zone is, at least in the vicinity of this well, too small.

The tracer results show that all model-delineated capture zones are too small. All dye introductions were made into nutrient-rich water. The implication is that some major nutrient input locations yield flow and nutrients to Silver Springs far more rapidly than suggested by the capture-zone modeling. Some of the increases noted in nutrient concentrations at Silver Springs are explained by contributions from areas formerly thought to not be relatively rapid contributors of water and nutrients to the springs.

This Box 29 illustrates some important considerations in designing and conducting tracer tests.

1. While Box 27 through Box 29 were all in karst, functioning of the aquifers were substantially different. One major difference is that the groundwater systems at Big Spring and Mitch Hill Spring are far more conduit-dominated than the one supplying Silver Springs. More dye was needed for tracing of similar distances at Silver Springs than at the other two springs. Regardless of the type of aquifer, it is important to carefully evaluate differences between aquifer conditions and not simply presume that since there are similarities between sites that nearly identical tracing approaches can be used at both. Regardless of the type of aquifer, failed tracer studies are often related to inadequately appreciated site conditions.
2. There was substantial stakeholder pressure to minimize dye quantities to ensure there would not be colored water discharging from Silver Springs or public water supply wells. Placing primary sampling reliance on activated carbon samplers allowed the tracing work to be conducted with much less dye than would have been required if primary sampling reliance had been based on water samples. There were almost three hundred carbon samplers from Silver Springs that were positive for one or more of the introduced tracer dyes, but dye was not detectable in any of the corresponding water samples. Most of the water samples analyzed were for sampling periods when the highest measured dye concentrations were present in carbon samplers.
3. Large volumes of water introduced following dye introductions helps ensure successful traces. The volumes of water introduced in conjunction with tracing in the Silver Springs recharge area were unusually large, but they were still in the range of the volumes that would be introduced by many stormwater flows. In general, the more water used the better the dye detection results, as long as the volume of water does not unnaturally alter the flow field, or at waste sites, potentially transport detained wastes.
4. Introducing dye followed by flush water maximizes the amount of dye introduced into the flow system for transport to the sampling points. It is usually the easiest way to conduct traces and it minimizes the amount of equipment that must be cleaned of residual dye. Introducing some water to moisten surfaces prior to introducing dye is also good protocol.
5. Routine sampling for tracer dyes was conducted at 18 public water supply wells and dye was detected in seven of them. Sampling required special installations to permit continuous sampling of water with activated carbon samplers before the water was chlorinated. Chlorination oxidizes and destroys dyes. This sampling approach ensured that two of the four dye introductions were detected at multiple sampling points.
6. High quality field work is key to good quality results. Sampling public water supply wells and diving with scuba equipment to sample spring vents was both essential and

difficult (and in the case of the alligator attack, dangerous). For this project it was done with great care and skill.

7. For the purpose of showing the methodology has produced dye detections, it is desirable to have at least one site where dye is detected for each dye introduction. The introduction of rhodamine WT at a point 2.4 km (1.5 mi) from the springs was relatively certain to result in multiple dye detections even though the mean flow rate of Silver Springs was 23.2 m^3/sec (820 ft^3/sec). Ensuring that there would be at least some positive traces was one of several reasons this dye introduction site was selected.

Back to where text linked to Box 29⮥

Box 30 - Case History 30: Tracing to evaluate a proposed water supply reservoir near Joplin, Missouri, USA

A privately owned public utility provides municipal water to Joplin, Missouri, USA. The current water source is Shoal Creek and a few wells. Population projections indicate that additional water supplies will be needed in the future and the water company proposes to meet this projected need by constructing a reservoir on Baynham Branch, a karst watershed. At design pool elevation the reservoir would cover approximately 480 hectares (1,200 acres). The depth of water at the dam for the planned pool elevation, would be approximately 33 m (100 ft). The watershed area for the impoundment is 40.8 km^2 (15.7 mi^2). The water company plans for the reservoir to be capable of delivering 113.6 million liters (30 million gallons) of water per day during periods of low to moderate flow rates on Shoal Creek. Annual water yield for this basin, if there were no groundwater flow into or out of the basin, would be approximately 32.5 million liters per day (mld) (8.6 million gallons per day [mgd]). Evaporation from a full lake would reduce the available water supply to approximately 17.8 mld (4.7 mgd). The water company plans to pump water from nearby Shoal Creek into the reservoir during high flow periods on that stream. Water would be discharged to Shoal Creek and allowed to flow for approximately 16 km (10 mi) to the company's treatment plant on Shoal Creek.

The water company submitted a pre-application plan to regulatory agencies. They were apparently unaware that there was usually no flowing water in the channel of Baynham Branch at the planned dam site for several months each year (as shown in Figure Box 30-1).

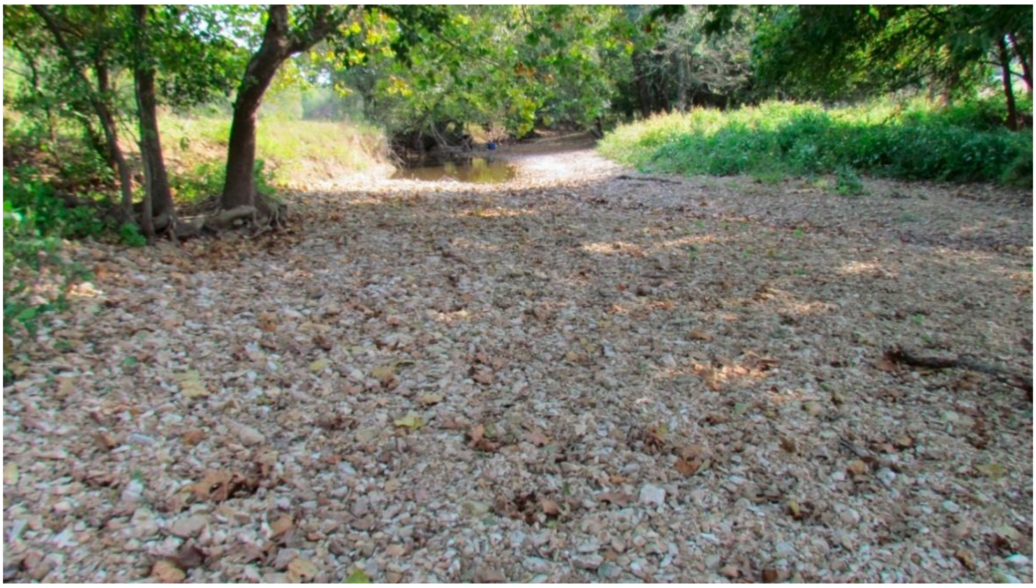

Figure Box 30-1 - Channel of Baynham Branch near centerline of proposed dam on October 8, 2020. View looking upstream.

The pre-application stated that there was 1.17 km (0.73 mi) of losing streams in the area that would be impounded. This did not include the losing stream segment at and upstream of the proposed dam site. The OUL determined that the minimum length of losing stream segments in the area proposed for impoundment is 5.85 km (3.64 mi) and includes three major segments. Figure Box 30-2 shows major losing stream segments within the proposed impoundment area.

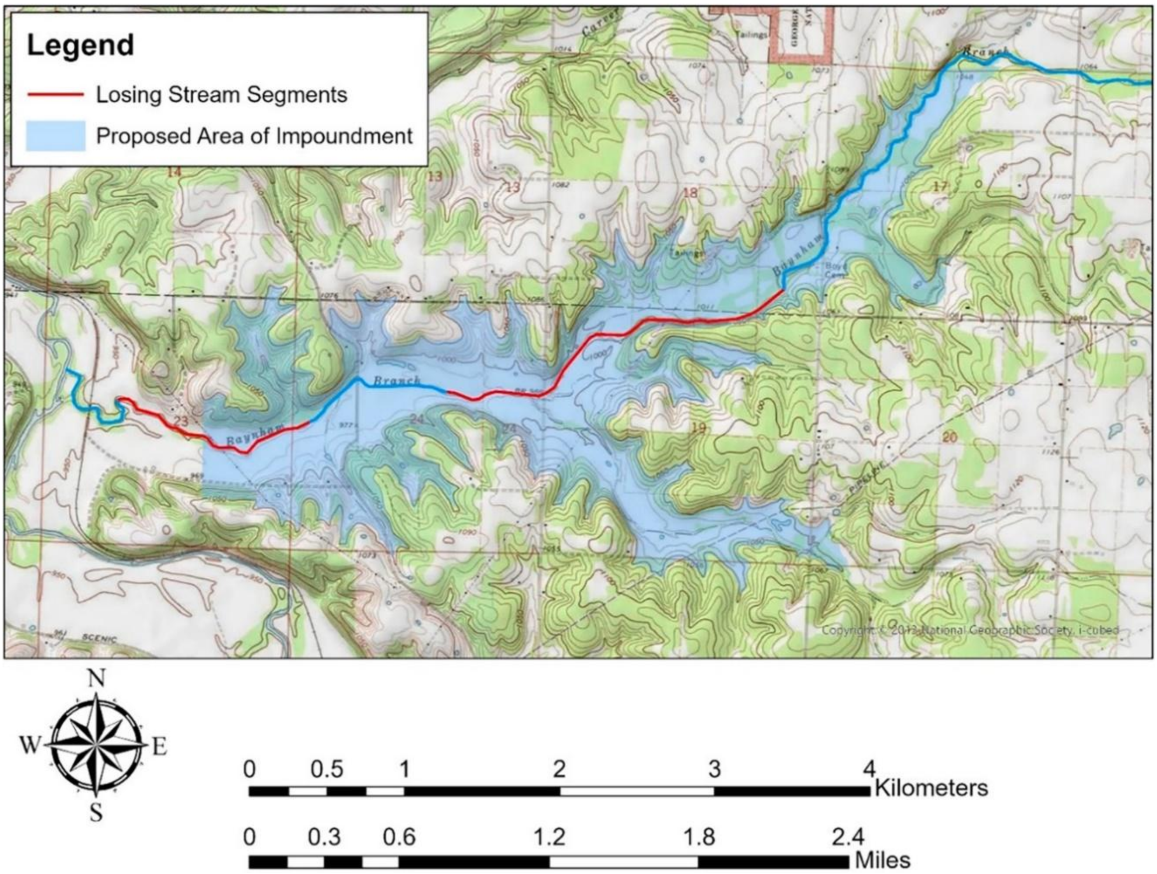

Figure Box 30-2 - Major losing stream segments within the proposed impoundment area.

The OUL was retained to assess the hydrological feasibility of the reservoir project. The OUL introduced a different tracer dye into flowing water upstream of each of the three major losing stream segments. Some of the dye from two of the three dye introductions was detected at downstream sampling points in Baynham Branch that were within the proposed impoundment area. Dye from all three dye introductions was detected in a complex of springs (Harris Family Springs) outside of the proposed impoundment area. Dye-tracing results and flow rate measurements demonstrated that losing stream segments on Baynham Branch supplied almost all flow for the Harris Family Springs. Figure Box 30-3 shows discharge from the lake fed by the largest of these springs.

Figure Box 30-3 - Discharge from the largest of the Harris Family Springs. The measured flow rate at the time of this photo was 43.7 mld (11.57 mgd).

The pre-proposal stated that, as the losing stream segments would all be inundated by the impoundment the losing stream segments were not anticipated to have any impact on reservoir storage. This is incorrect if water sinking in losing stream segments discharges from springs outside of the impounded area. Dye tracing showed this is the case. The losing stream segment at and upstream of the proposed dam is approximately 625 m (2,050 ft) long with its mid-point 1.46 km (0.91 mi) from the Harris Family Springs (Figure Box 30-4).

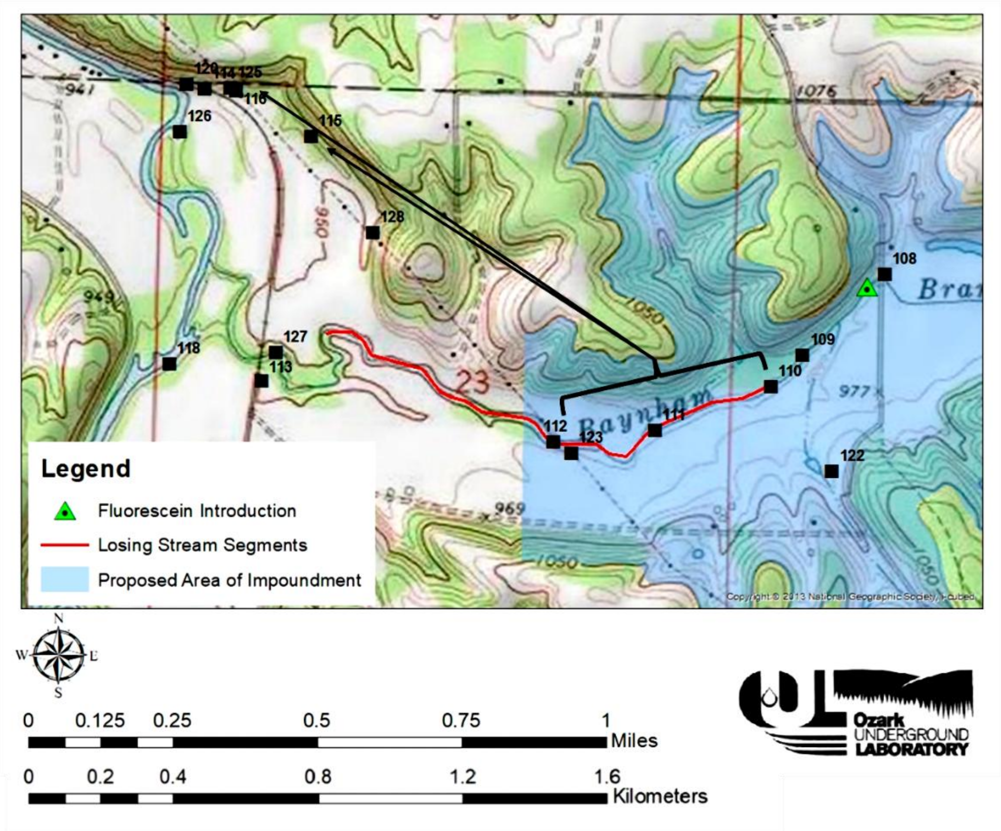

Figure Box 30-4 - Diagram of dye trace from a losing segment of Baynham Branch to Harris Family Springs. Flow rate measurements indicated multiple points where surface flow is entering karst groundwater. The straight black arrows show straight-line flow pathways of dye entering the karst groundwater through losing stream segments (Stations 110, 111, 123, 112) and discharging at Harris Family Springs.

Figure Box 30-5a schematically shows the dye trace from the losing stream segment in the proposed reservoir area to the Harris Family Springs. Figure Box 30-5b is a diagrammatic illustration of groundwater flow from the proposed impoundment if it were holding water.

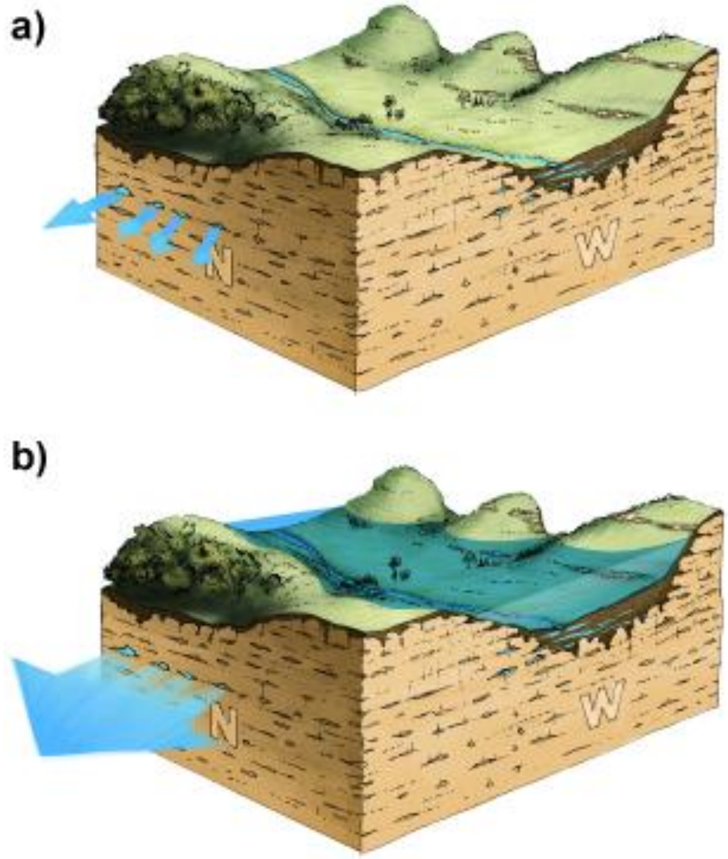

Figure Box 30-5 - Groundwater flow from the proposed Impoundment to Harris Family Springs. The proposed dam is on the west face of the block diagram. The top illustration shows natural leakage without a dam. The bottom illustration shows leakage with a dam. The dam design would not block major groundwater flow paths to the springs and would dramatically increase hydraulic head on groundwater between the impoundment and the springs.

First arrival of dye at the springs from the losing stream segment upstream of the dam occurred within seven days of dye introduction; no samples were collected earlier. Based on ten months of flow rate measurements of the largest of the springs the peak instantaneous flow rate of this spring was 171.8 mld (45.4 mgd) and occurred during a stormflow period when the depth of water in the channel of Baynham Branch was approximately 2.1 m (7 ft) higher than under low flow conditions. If the reservoir were constructed and were at design pool elevation the head in this losing stream segment would be increased by 25.9 to 28.4 m (85 to 93 ft) above current low flow conditions.

Discussion. The proponent for the impoundment has made borings, primarily along the centerline of the dam, and has indicated that they did not identify significant leakage problems. That is not surprising, as the borings are not in the area where groundwater flow occurs between the stream and the springs. It appears that the project proponent intends to proceed with the project. While inter-basin groundwater flow is common in karst, the concept is unfortunately foreign to those who expect 1) surface and groundwater flow paths to be similar, and 2) good damsites exist if two hills are close together. Kuniansky and others (2022) in *Introduction to Karst Aquifers*↗ provides an excellent discussion of common characteristics of karst aquifers and their hydrologic complexity. This publication is available from The Groundwater Project for download at no cost.

Back to where text linked to Box 30↥

Box 31 - Case History 31: Aquifer vulnerability mapping for planned waste rock dumps, Antamina Mine, Peru

Data in this case history are from Evans and others (2005). This is an open pit copper-zinc mine on the east side of the Cordillera Blanca about 270 km (167 mi) north of Lima at an elevation of approximately 4,300 m (14,100 ft). The terrain is rugged, and surface drainages are in the headwater of the Amazon River. The average dip of the bedrock is 70°. Approximately 10 percent of the Peruvian Andes are underlain by karstic limestone, and polymetallic ore deposits occurring as porphyry, skarn, and replacement type deposits are commonly associated with karstic limestone units. The ore deposits at Antamina also include lead, bismuth, and molybdenum.

Because of the association between ore deposits and limestone, waste rock dumps in the Andes are often underlain by limestone. The Antamina Mine anticipates creating 1.37 billion metric tons of waste rock; a metric ton equals 1,000 kg (2,205 lb). The East Dump was in use at the time of the study, and the study focused on two new sites for waste rock dumps. The Tucush dump was planned for the Tucush Valley and is expected to have a surface area of 270 hectares (675 acres) and a height above the valley floor at completion of 300 m (985 ft). The Vallecito dump was planned for the Vallecito Valley and is expected to have a surface area of 50 hectares (125 acres) and a height above the valley floor at completion of 200 m (656 ft). Low grade ore will be temporarily stockpiled in a third area and will be processed at the end of the mining period. Mean annual precipitation in the area varies as a function of elevation, but ranges between 1200 and 1500 mm (47 and 59 in). Although most of the planned dump areas are underlain by low permeability soils, approximately 16 percent of the originally proposed Vallecito site and 22 percent of the originally proposed Tucush site are underlain by bare karstic limestone.

Three dye introductions were made into natural vertical shafts up to 100 m (328 ft) deep, dissolved into the limestone. Because of difficult access only 40 L (10.6 gal) of water was introduced after the dye at two of the three dye introduction points. Subsequent precipitation and runoff mobilized the dyes and transported them. In effect these two dye introductions were modified dry sets. At the third dye introduction approximately 34,000 L (9,000 gal) of flush water was used after dye introduction. All three of the dye introductions resulted in dye detections in springs and spring-fed streams at points up to 4 km (2.5 mi) from the dye introduction points. Dye quantities introduced were 4 kg (9 lb) fluorescein mixture, 1.4 kg (3 lb) of a second fluorescein mixture, and 4 kg (9 lb) rhodamine WT mixture. The dye equivalent in the fluorescein mixture was approximately 70 percent and approximately 20 percent in the rhodamine WT mixture.

Discussion. The dye traces provided flow direction information for the karst drainage system. In the case of a dye introduction on the east side of Vallecito Valley the groundwater flow shown by the trace was 4 km (2.5 mi) north to Callapo Creek rather than south into the Antamina Valley which was only 400 m (1,300 ft) from the dye introduction point. All three traces demonstrated rapid groundwater movement and were used to design a better monitoring program than would otherwise have been possible.

There have been other dye traces conducted at the Antamina mine and other mines in Peru. Most of these have placed primary sampling reliance on activated carbon samplers with secondary reliance on grab samples of water. Dye-tracing investigations using activated carbon samplers are attractive because they are cost-effective, especially in situations where travel to sampling points is difficult and time-consuming.

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Box 32 - Case History 32: Groundwater travel rates in fractured rock units with polymetallic ores, Peru

Eosine, fluorescein, and rhodamine WT were introduced into each of three monitoring wells as a high concentration pulse. After dye introduction a volume of water slightly more than the volume in the well bore was introduced to flush the dye into the aquifer. Dye and water introductions were made by pouring the dye and water into the top of the well bore as shown in Figure Box 32-1. 5.45 kg (12 lb) of rhodamine WT mixture with a 20-percent dye equivalent was introduced into MW-10. 2.72 kg (6 lb) of fluorescein mixture with a 70-percent dye equivalent was introduced into MW-09. 4.09 kg (9 lb) eosine dye mixture with a dye equivalent of 96 percent was introduced into MW-05. Sampling was conducted in two horizontal bores, a network of monitoring wells, and in 11 mine adits that were discharging to the adjacent river.

Figure Box 32-1 - Dye introduction into a well. Tyveks are worn to reduce the chance that workers could contaminate a vehicle or other material with dye. Small amounts of dye can penetrate Tyveks.

The three dye introductions resulted in 10 traced flow paths. Table Box 32-1 summarizes groundwater travel distances and travel rates along these flow paths. Both activated carbon samplers and grab samples of water were analyzed. There were 10 sampling stations where dye was detected in carbon and/or water samples. Dye was detected in carbon samplers from nine of the 10 detection locations and in water samples from seven of the 10 dye detection locations. Where dye was detected in both carbon and water samples the dye was detected an average of four days earlier in carbon samplers than in water samples. At one site, dye was detected earlier in a water sample than in the corresponding carbon sampler.

Table 32-1 - Groundwater travel distances and travel rates from wells at study site.

Parameter	**Maximum**		**Minimum**		**Mean**	
	m	ft	m	ft	m	ft
Distance	625	2050	40	131	263	863
Mean travel rate (distance/day) for first dye arrival	156.3	512.7	1.1	3.6	46.9	153.8
Mean travel rate (distance/day) maximum dye concentration	156.3	512.7	1.1	3.6	30.8	101.0

Discussion. The trace was useful in demonstrating rapid flow through the fracture system. Sampling with both activated carbon samplers and grab samples of water was beneficial to the study. While there was no apparent problem with carbon samplers in wells, care must be used to ensure that the sampler is in the water and not stuck on the side of the well bore above the water level. This is most commonly a possible problem in small diameter and deep wells. It is seldom a significant problem with adequately trained and skilled field personnel.

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Box 33 - Case History 33: Tracing in abandoned zinc–lead mines under Joplin, Missouri, USA

Joplin is at the northeastern end of the Tri-State zinc–lead mining district that extends southwestward into Kansas and Oklahoma, US. The mining beneath Joplin was primarily for zinc. Mining in Joplin began with a lead mine in about 1870, was extensive until about the end of the First World War (1918), and finally ended after the Second World War (1945) (Feder et al., 1969). The host rock was limestone with abundant chert. Essentially all mining was below the original potentiometric surface and heavy groundwater extraction was required to access the minerals. Now that mining has ended, the potentiometric surface varies seasonally and is minimally impacted by groundwater pumping. Natural solutional openings, fractures, and man-made cavities now provide hydrologic connections between mines. The presence of lead and other mineral constituents makes the water unusable for drinking water purposes without substantial treatment. Feder and others (1969) indicate that the pH of mine water in the area ranges from 5.6 to 8.0 with a median value of 7.3. Based on OUL experience, tracing at the lower values requires the use of about 25 percent more dye than would otherwise be needed.

The mineral deposits were mostly flat-lying and mines were connected to the surface by vertical shafts that provided access, ventilation, and hoisting of ore. Prior to about the year 2000, some of the old mine shafts were not sealed. Not surprisingly, some of the old shafts were used as waste disposal sites, and reportedly, as final resting sites for murder victims. One of these shafts, at a former manufacturing plant, received significant amounts of industrial wastes that included the solvent TCE. TCE was widely used during the period that many mine shafts were open, so this solvent undoubtedly entered the flooded mines from multiple points in addition to the one at the former manufacturing plant. To help assess responsibility for TCE cleanup efforts, the OUL conducted several groundwater-tracing studies to determine groundwater flow paths for contaminants migrating from the former manufacturing site.

In 1998 and 1999 dye was introduced at the site and sampling was conducted weekly for 10.5 months beginning in late August and ending in early July. There were 45 routine sampling stations plus four points that were occasionally monitored. Sampling was conducted over a wide area, and sampling points included accessible mine shafts, springs, surface streams, and ponds fed at least in part by groundwater. Most of the sampling points were in urban areas. Primary sampling reliance was based on activated carbon samplers with secondary sampling reliance on grab samples of water. All analysis was by a spectrofluorophotometer operated under a synchronous-scan protocol. The tracing associated with the former waste disposal site identified groundwater flow paths and stream segments where mine water discharged to surface water, but dye was not detected at many locations where TCE had been detected.

Discussion. In some cases, it was not possible to identify the exact point where mine water tagged with dye discharged to surface streams, but such discharges could be identified as occurring at a point or points between two sampling stations. The tracing program was effective in indicating that the manufacturing site was not hydrologically connected with many of the points in the area where TCE had been detected in groundwater. While groundwater flow paths through the abandoned mines might change with hydrologic events or groundwater extraction, the long duration of the study helped ensure that the results were representative of the range of typical conditions.

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Box 34 - Case History 34: Muddy Creek trace, West Virginia, USA

A trace designed to compare the effectiveness of three dyes in water typical of local mine drainage was conducted on Muddy Creek, a tributary to Cheat River in Preston County, West Virginia, USA. The stream receives acid mine water discharges from several abandoned underground coal mines and pH in the stream segment studied varied at the time of the study from 4.2 to 4.6. Rocks in the stream channel were coated with *Yellowboy*, an iron hydroxide deposition common in acid mine drainage from coal mines in West Virginia. Some workers have suggested that iron hydroxide deposits in mine water might be effective in adsorping tracer dyes.

The estimated flow rate of the stream during the study was approximately 0.85 m^3/sec (30 ft^3/sec) at the upstream sampling point and 1.28 m^3/sec (45 ft^3/sec) at the downstream point. Fifty-one grams (0.11 lb) of fluorescein mixture, 188 g (0.41 lb) of eosine mixture, and 454 g (1.00 lb) of sulforhodamine B mixture were introduced at essentially the same time into the surface flow of Muddy Creek. The dye equivalent in the fluorescein mixture was 70 percent, 96 percent in the eosine mixture, and 35 percent in the sulforhodamine B mixture. Activated carbon samplers were placed in the stream at points ranging from 825 m (2,700 ft) to 4 km (13,100 ft) downstream of the dye introduction point. The carbon samplers were all placed prior to dye introduction and were collected and replaced 30 hours later. The second round of samplers, placed at 30-hours post dye introduction, failed to collect any detectable tracer dyes.

The stream distance from the farthest upstream sampling point to the farthest downstream station was 3,175 m (10,400 ft). The concentration of tracer dyes at the downstream sampling station, as a percent of that at the upstream station, was 55 percent for fluorescein, 52 percent for eosine, and 29 percent for sulforhodamine B. The flow rate at the upstream station was approximately 67 percent of that at the downstream location. Much of the loss of fluorescein and eosine is likely attributable to dye degradation or adsorption onto the iron hydroxide deposits in the stream station. In contrast, there was appreciable loss and/or degradation of sulforhodamine B.

Discussion. Based on the weights of the three dyes introduced and conditions present in the water of Muddy Creek, if we were to derive equal concentrations of dye from carbon samplers, we would use dye in approximately the following ratios: 1 kg fluorescein=3.3 kg eosine=13.8 kg sulforhodamine B. This trace illustrated that fluorescein was the best dye for use in this mine water at pH values in the range of 4.2 to 4.6 when sampling utilized activated carbon samplers. Subsequent sampling in the area used this information in designing underground tracer studies. Bench tests are often appropriate when planning tracing work in mine water, and other simple investigations (such as the sampling and analysis on Muddy Creek) can sometimes be very helpful in designing and interpreting groundwater-tracing investigations.

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Box 35 - Case History 35: Groundwater tracing at a former iron mine, Virginia, USA

Dye tracing was used at a large, abandoned, underground iron mine in Virginia, USA. The mine had multiple shafts, several former working levels, and extended to depths of several hundreds of feet below the bed of a nearby river. The purpose of the tracing program was to determine current flow paths through the mine as a part of the site remediation efforts. The pH of mine water varied but in some places was as low as 2.8. Remediation work included increasing the pH of mine water, and this was resulting in large volumes of sludge and treated water. The sludge was being introduced back into the mine through one of the existing shafts. Treated water was discharged to a large, constructed wetland designed to provide further treatment for the water. Excessive iron deposits were creating problems in the wetland, but that was beyond the scope of the groundwater-tracing program.

A key objective of the tracing program was to determine groundwater flow paths from the sludge introduction point. Sampling points included other shafts, points where water was extracted for treatment as part of the remediation program, and a submerged drainage tunnel that discharged to the nearby river. The discharge water entering the river exceeded state standards for pH, iron, and several other heavy metals.

Bench tests using mine water indicated that fluorescein and rhodamine WT could persist in the mine water for periods long enough to permit tracing, but the dyes were lost or destroyed when they encountered the treatment sludge. It was not known if the treatment sludge introduced into the mine was raising the pH of water that would be encountered by the dye in its underground movement. Based on the bench tests, and previous experience with the tracer dyes, the OUL concluded that fluorescein was likely to be the best dye for tracing in this environment.

Thirty pounds (13.6 kg) of fluorescein dye mixture with a dye equivalent of approximately 70 percent was mixed with water on the surface and poured into the shaft that received treatment sludge. Treated water was used to help flush the dye past the area where it was suspected that most of the sludge had accumulated. Sampling for the dye used both activated carbon samplers and grab samples of water.

The pH of water samples was increased to approximately 9.5 prior to dye analysis. This resulted in abundant sludge and turbidity in water samples. Samples were clarified by centrifuging, yet this did not remove all the turbidity. Carbon samplers worked adequately in detecting fluorescein dye but also contained extensive iron-rich deposits after being in place for a few days. This required very thorough washing of the carbon samplers prior to adding the eluting solution. It is likely that some dye was lost to adsorption onto the iron-rich deposits or that those deposits blocked some dye adsorption onto the carbon. However, the tracing program only needed to determine if hydrologic connections existed, and the trace provided this information. The trace demonstrated groundwater movement from the sludge introduction shaft to another shaft and to the discharge at the river. It also provided travel times.

Discussion. Iron deposits and abundant iron bacteria are commonly associated with acid-mine drainage. Since this was a former iron mine, iron deposits were especially abundant. Bench testing of mine water and treatment sludge with two tracer dyes was valuable in showing that dyes could be used for tracing groundwater movement at the site. The bench testing answered an initial question of whether dyes could be used in the environment present at the site. The bench testing also indicated that the treatment sludge could trap or destroy tracer dyes, and this resulted in a decision to flush the dye with treated water. Finally, the bench testing indicated that appreciable amounts of dye would likely be lost as the dyed water moved through the mine. As a result, the quantity of dye used was an order of magnitude larger than would have been used if there had not been treatment sludge present in the mine and if the pH of the water had been less acidic. Fluorescein dye was detected in both carbon samplers and water samples.

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9 Exercise Solutions

Solution Exercise 1

Three actions to minimize the possibility of visual detection by the public without compromising the trace are as follows. 1) Base primary sampling reliance on activated carbon samplers and laboratory analysis of these samplers. 2) Leave carbon samplers in place for at least one day; one week is commonly a desirable period. The longer carbon samplers are in place the more dye they will adsorp. However, carbon samplers left in place in heavily contaminated water for periods longer than one or possibly two weeks are likely to have substantially reduced ability to adsorp tracer dyes. 3) Use a dye such as eosine that is not highly visible.

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Solution Exercise 2

The concentration difference between the visual detection limit for the public for the five tracer dyes and the instrumental detection limit using a laboratory spectrofluorophotometer is about five orders of magnitude.

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Solution Exercise 3

Dyes used in tracing are mixtures. It is important to know and report the percentage of diluent in the dye mixture used for a trace because the diluent percent tells the user how much dye is present in the mixture being used. Proper technical reporting of the weight of the dye mixture used and its diluent (non-dye) percent is essential to properly plan and implement a trace, from choosing a dye quantity to preventing false negative laboratory results.

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Solution Exercise 4

The use of primary sampling reliance based on carbon samplers rather than water samples can yield equal or better tracing results with a similar budget because it reduces the number of samples that must be analyzed and the number of sampling visits to monitoring points. Carbon samplers provide continuous and cumulative sampling, so their use prevents missing short-duration dye pulses at sampling points and allows longer periods between sample collection than is possible with sampling based on grab samples of water. Also, when using carbon samplers at contaminated sites, longer sampling intervals can be used for wells with low concentrations of contaminants than for wells that are more heavily contaminated.

Return to Exercise 4↥

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Solution Exercise 5

The five travel important times to estimate using tracer test results are commonly.

1) The travel time for the first dye arrival at sampling points.
2) Time of peak dye concentration at sampling points.
3) Time when approximately 50 percent of the detected dye mass, of a recorded breakthrough curve, has reached important sampling points. Mass balances show that most of the dye introduced does not reach sampling points within the study periods so percentages are based on the amount of detected dye not introduced dye. At springs these data should be combined with flow rates if they are available.
4) Time at which dye is no longer detectable at sampling points.
5) Time when there are no new dye detections and dye concentrations at most or all sites where dye has been detected are decreasing.

Recently, one additional travel time has become important in artificial groundwater recharge work in California. In this work, one of the key metrics in obtaining credits required in the permitting process of groundwater recharge projects that use a tracing test is the time at which 10 percent of the peak concentration arrives at a sampling location.

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Solution Exercise 6

Backhoe trenches provide significantly more surface area for dye introduction relative to a borehole or well. This increased surface area provides a higher likelihood of intersecting heterogeneities, which in turn provide better likelihood of intersecting connected zones of higher permeability that lead to preferential flow pathways through the flow system that is being tested. Additionally, depending on the objectives of the study, introductions via a backhoe trench may provide a more accurate test of the flow system than would otherwise be possible via a single or multiple well introduction.

Return to Exercise 6↥

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Solution Exercise 7

Several factors should be considered during the selection process of dye type(s) and quantity, including the following.

1. Study objectives.
2. Geological environment.
3. Background sampling.
4. Geochemistry of study-site water.
5. Flow distances.
6. Primary sampling and analytical methods to be used.

Return to Exercise 7↥

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Solution Exercise 8

1) Explain that only a small fraction of the introduced dye will actually reach points where colored water is of concern and estimate what that percent is likely to be. 2) Explain that the dyes used are not harmful in the concentrations used in professionally directed traces. At many sites the dyes are harmless while contaminants of concern in the water being traced have important health and environmental concerns. 3) Use a dye such as eosine that is not highly visible.

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Solution Exercise 9

Losing stream segments and sinkholes are preferred to borings and unused wells due to the well-connected nature of losing stream segments and sinkholes to preferential groundwater flow pathways. In addition, the surface catchment of losing stream segments and sinkholes can be defined using topographic maps. If the dye introduction from a losing stream segment or a sinkhole provides a positive trace to the feature of interest, the entire catchment area for the dye introduction location is within the delineated recharge area. For this reason, well placed dye introductions in losing stream segments and sinkholes usually provide significantly more information than is otherwise obtained using other dye introduction locations.

Lastly, recharge area delineation studies are commonly used to inform vulnerability assessments. Much of the risk included in the vulnerability assessment is determined by the relative travel times for groundwater to reach the feature of interest—that is, *connectedness*. Therefore, dye introduction into features that are poorly connected with the groundwater system may not only prevent an accurate understanding of the recharge area, but will limit the accuracy of risk-based classifications generated for delineated recharge areas.

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Solution Exercise 10

Yes. The two most important factors that indicate the size of a recharge area are the flow rate of the spring(s) draining the recharge area and the way that recharge enters the subsurface.

Supplemental information: In the Ozarks with a mean annual precipitation of 44 in/yr (112 cm/yr), in a system in which most of the recharge enters the groundwater system via losing streams, based on the experience of the OUL the recharge area is likely to be 1 to 2 square miles per ft^3/s of annual mean flow at the receiving spring(s) (equivalent to approximately 9.2 to 18.3 hectares of land for each 1 L/s of annual mean flow). If the recharge primarily enters the groundwater system via sinkholes, the recharge area will be larger per unit flow rate relative to a losing stream dominated system.

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Solution Exercise 11

The seven factors the US Supreme Court has identified as pertinent to obtaining an NPDES permit for discharge to groundwater are: 1) transit time; 2) distance traveled; 3) nature of the material through which the pollutant travels; 4) extent to which the pollutant is diluted or chemically changed as it travels; 5) amount of pollutant entering the navigable water relative to the amount of the pollutant that leaves the point source; 6) manner by or area in which the pollutant enters the navigable water; and 7) degree to which the pollution (at that point) has maintained its specific identity. The court stated that time and distance would be the most important factors in most cases, but not necessarily in every case.

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Solution Exercise 12

1. Comprehensive background sampling.

2. Incorporation of a sampling network and sampling methodologies that identify all sampling points potentially reached by the introduced tracer dyes.

3. Identification of dye introduction locations. If boreholes/wells are to be used as dye introduction locations, it is recommended that injection tests using clean water be completed prior to dye introduction to ensure the proposed dye introduction location(s) are hydraulically well connected to the formation of interest. Tracer studies will not provide accurate data if the dye does not enter the formation of interest in a representative fashion.

4. How the dye will be introduced. Unless the study objectives require the dye to be introduced at a constant concentration and flow rate—such as is required for the determination of volume and mobile porosity—dye is best introduced as a high concentration slug followed by flush water.

5. Not underestimating adsorption and dispersion of the dye in the subsurface when determining dye introduction masses.

6. When dye introductions are associated with remedial agents, it is generally best to introduce the dye as a slug followed by the remediation agent(s). Some remediation agents will degrade the dye limiting both the effectiveness of the dye trace and the remedial agent(s).

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10 Notations

F = fluorescence intensity at temperature T in °C (photons per unit area and time)

F_o = fluorescence intensity at T_o (assumed to be 0 °C) (photons per unit area and time)

h = thickness of the injected interval often assumed to be equal to the screened interval of the injection well (L)

K_d = distribution coefficient for the solute within the aquifer matrix (L^3M^{-1})

n = constant for a given dye (°C^{-1})

p_b = dry bulk density of soil (ML^{-3})

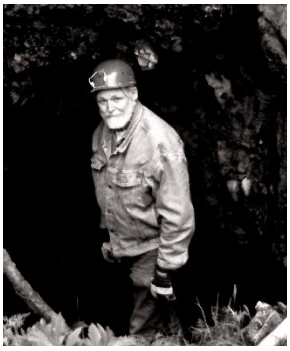
r = radial distance to the dose-response wells (L)

θ = total porosity (dimensionless)

θ_m = mobile porosity (dimensionless)

Vol_{Inj50} = volume injected by the time the observed concentration reaches 50 percent of the maximum concertation at dose-response wells (L^3)

11 About the Authors



Tom Aley is the founder and president of Ozark Underground Laboratory. He has 60 years of experience in groundwater hydrology specializing in karst hydrogeology and groundwater tracing using fluorescent tracer dyes and other tracing agents. He has been involved with approximately 4,000 groundwater traces conducted on every continent except Antarctica. From 1966 to 1973, Tom directed the Hurricane Creek Barometer Watershed study for the US Forest Service. Hurricane Creek was the national-type example study area for water management in karst areas administered by the Forest Service. A substantial part of his Forest Service work focused on developing methods and strategies for cost-effective groundwater tracing to large volume springs and rivers. From 1973 to present, Tom has conducted hundreds of contract studies and consulting assignments through the Ozark Underground Laboratory. He is a professional hydrogeologist, certified by the American Institute of Hydrology; is licensed at a Registered Geologist in Missouri; and is licensed as a professional geologist in Arkansas, Kentucky, and Alabama. Tom has extensive field experience that is reflected in the numerous case histories found in this book.

Trevor Osorno is the director and senior hydrogeologist at Ozark Underground Laboratory. He has 10 years of experience in the groundwater field, specializing in subsurface characterization and contaminant mass flux studies using fluorescent tracer dyes and direct groundwater velocity tools. Mr. Osorno has designed and/or conducted over 400 groundwater traces across a wide range of hydrologic and geologic settings throughout North America, South America, Europe, Australia, and the Pacific Islands. He also co-developed the Point Velocity Probe (PVP) suite of tools, has facilitated the commercial development of the tooling by Solinst Canada, and continues to provide commercial services for the tools across North America and Europe. Mr. Osorno is licensed as a registered geologist in Missouri.

Dr. J. F. Devlin is a professor in the geology department at the University of Kansas, US, and previously served on the faculty of the University of Waterloo. He has 35 years of experience in the groundwater field, has supervised 35 student theses, and published 75 peer-reviewed articles on groundwater research. He has worked collaboratively with academic and government researchers in the US, Canada, France, Denmark, and New Zealand. He has also worked jointly with industrial partners in North America and Europe. His research interests range from organic contaminant geochemistry to aquifer characterization, bioremediation, subsurface mixing in groundwater remediation schemes, and the direct measurement of groundwater velocity. He currently serves on the board of the Tumbling Creek Cave Foundation and has served as an associate editor for *Groundwater Monitoring and Remediation* and the *Hydrogeology Journal* and as editor-in-chief for *Groundwater Monitoring and Remediation*. He the recipient of the W. B. Pearson Medal from the University of Waterloo, an NSF Career Award, the Otto Monsted guest professorship at DTU, Leo M. and Robert M. Orth Water Resources Award, and a Kemper Award for teaching excellence.

Alexa Goers is a senior geologist at Ozark Underground Laboratory where she oversees laboratory services alongside Cathy Aley. She has over six years of experience working with contaminated sites at the Kansas Department of Health and Environment and OUL. Prior to moving into environmental geology, Alexa specialized in carbonate geology and ichnology with an emphasis on the influence of bioturbation on petrophysical characteristics for petroleum geology applications.

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