The Ozark Underground Laboratory's

GROUNDWATER TRACING HANDBOOK

A handbook prepared for the use of clients and colleagues of the Ozark Underground Laboratory
2019

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Cover: Groundwater discharging from a small hillside spring at the Ozark Underground Laboratory. The springhouse contains a hand carved rock basin once used for household drinking water and to chill perishable foods. Groundwater tracing is a valuable tool for understanding and protecting groundwater systems, and for rehabilitating those which have been degraded. Groundwater tracing at the Laboratory is playing a critical role in land restoration work designed to improve groundwater quality for the Tumbling Creek Cavesnail, a federally listed endangered species known only from the cave system beneath this hillside spring.

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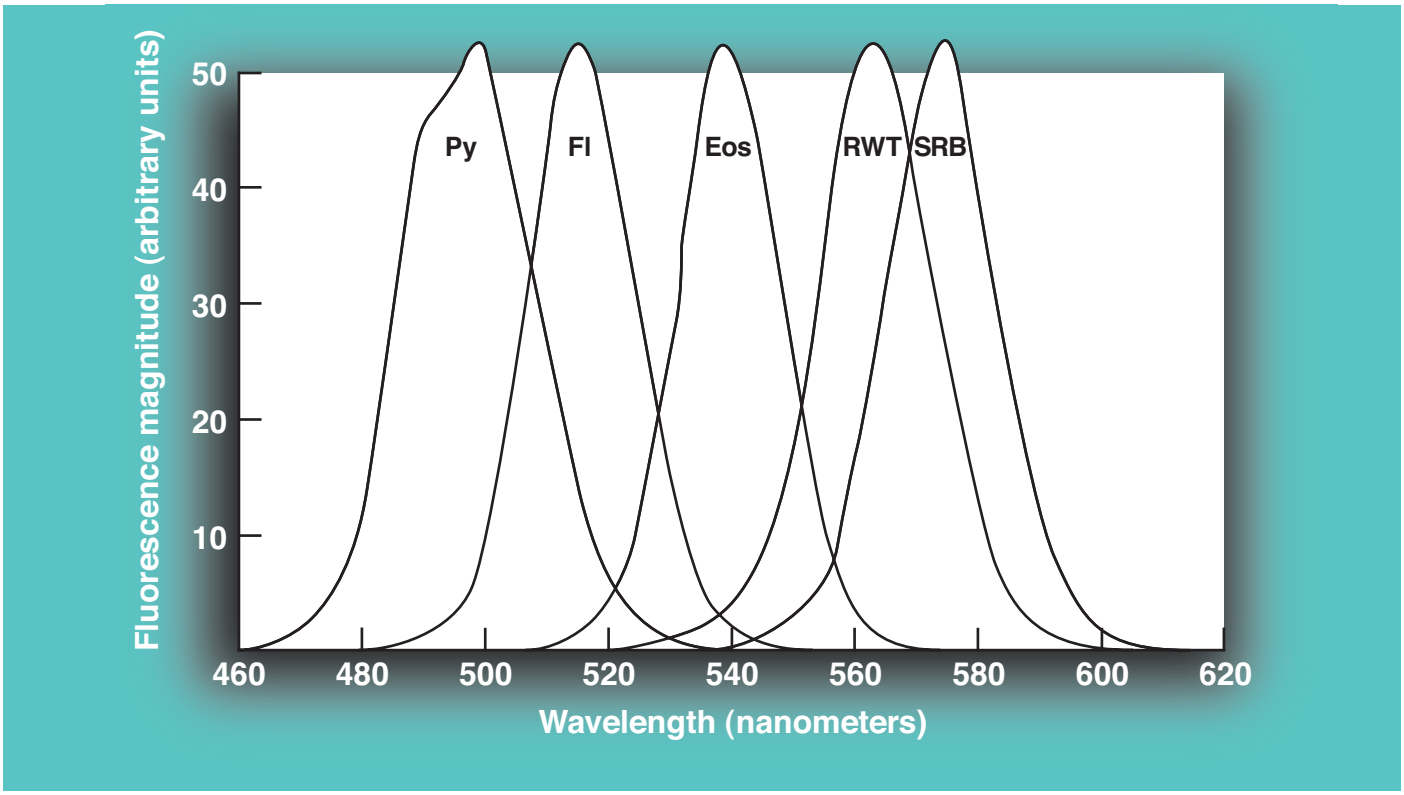
Ozark Underground Laboratory, Inc. *Groundwater tracing with fluorescent dyes*



Frontispiece

Emission fluorescence graphs for five groundwater tracing dyes in the activated carbon sampler eluent used by the Ozark Underground Laboratory. The dyes are pyranine (Py), fluorescein (Fl), eosine (Eos), rhodamine WT (RWT), and sulforhodamine B (SRB). All analysis was done using a 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. The "as sold" dye concentrations in the samples were:

- **Fluorescein** = 8.84 parts per billion (ppb).
- **Eosine** = 28.1 ppb.
- **Pyranine** = 192 ppb.
- **Sulforhodamine B** = 194 ppb.
- **Rhodamine WT** = 226 ppb.

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INTRODUCTION

The Ozark Underground Laboratory

The Ozark Underground Laboratory, Inc. (OUL), is a private consulting and contract studies firm that provides groundwater tracing and other hydrogeological services worldwide. The OUL has been in continuous full-time operation since 1973 under the direction of Tom Aley, who serves as Principal Hydrogeologist for the firm. The OUL typically has a full-time staff of nine people. We are not affiliated with any academic institution, and we have no academic responsibilities which could interfere with full client service. The OUL has designed and either conducted, or assisted with, over 4,000 groundwater traces on every continent except Antarctica. Our clients include many environmental and engineering consulting firms; other corporate and private entities; and federal, state, and local agencies.

Purpose of this Handbook

This handbook is a practical reference on groundwater tracing for our clients and colleagues. It:

- Includes information useful for those involved in problem solving work where fluorescent tracer dyes might be used or where the dyes have been used.
- Answers common questions about groundwater tracing and tracer dyes, and helps users design and manage effective and credible groundwater tracing programs.
- Identifies common problems in the selection of dyes and dye quantities, and in sampling strategies and dye analysis approaches.

Our experience can help make your tracing work successful. Please call us at 417-785-4289; E-mail at Contact@ozarkundergroundlab.com; or Fax at 417-785-4290.

Tracer Dyes: An Under-Utilized Groundwater Tool

Three basic questions commonly encountered in groundwater hydrology are:

- Where does the water go?
- How long does it take to get there?
- What happens along the way?

The use of tracer dyes can answer, or help answer, these three questions. However, there are few practical groundwater hydrology tools which are less commonly and less effectively used than fluorescent tracer dyes. This under-utilization of a cost-effective tool is in part rooted in five incorrect perceptions about tracer dyes and groundwater tracing investigations.

One common misconception is that dyes may be harmful or that they will cause some sort of public relations problem. There is extensive technical literature (such as Field et al., 1995) demonstrating that the dyes present no health or environmental problems at concentrations five orders of magnitude or more above the method detection limits of modern analytical protocols. Dye tracing does not require large quantities of dyes; the dyes discussed in this handbook are safe groundwater tracing agents.

A second common misconception is that dye tracing works only 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 almost any aquifer. 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. The dyes discussed in this handbook have been successfully used as groundwater tracers:

- From sinking streams through the groundwater system for up to 40 miles to Big Spring, Missouri; flow rates of the spring were typically 400 to 650 cubic feet per second (cfs).
- In high-yield limestone aquifers including the Edwards Aquifer and the Floridan Aquifer.

- Through thousands of feet of landslide debris in Alaska.
- For tens of miles through lava flows in Idaho.
- For hundreds of feet through fractured granite aquifers in New Hampshire and Minnesota and for thousands of feet through fractured andesite and rhyolite in New Mexico.
- Through glacial outwash, various alluvial deposits, and deep residuum to water supply and monitoring wells.
- From highway, rail, and pipe line spill sites to streams, springs, and wells.
- From perimeter points around Solid Waste Management Units (SWMUs) at RCRA and CERCLA sites to monitoring wells and other monitored points.
- From on-site sewage systems to bulkhead drains adjacent to marine shellfish beds, Washington. Based upon 1,600 dye introductions, about 23% of the sewage systems were functioning inadequately and yielded dye to sampling stations.
- Through various deposits to verify or refine time of travel calculations for groundwater remediation.
- From leaking sewers to water supply and monitoring wells, springs, streams, and building sumps.
- From leaking impoundments to springs and wells.
- From perennial stream segments to private and public water supply wells.
- For delineating wellhead protection zones.
- For assessing groundwater scenarios where the "worst case" is flow along preferential flow routes.

A third common misconception is that tracer dyes will not work in acidic waters such as those commonly encountered in metal or coal mines. While not all of the dyes are suitable for such conditions, we have conducted many successful traces into and/or through both active and inactive mines. Designing effective mine-related traces and other traces involving acidic waters will be discussed later.

The fourth common misconception is that most dyes are rapidly destroyed by sunlight, and that this precludes their use for tracing water movement into, or out of, surface streams. We commonly design effective groundwater traces where dyes may spend time in surface waters. The topic is discussed later.

The fifth common misconception is that groundwater tracing is impractical because most tracing requires substantial experience, the purchase of many hard to find materials, and analytical work necessitating instruments not normally found in most water testing laboratories. The OUL has solved this problem for our clients. We will work with you in the design of traces and in selecting the dyes to use. We will provide all specialized materials and supplies, and certify that all sampling and analysis materials are free of any extraneous fluorescent materials. Ship us the samples packed in "Blue Ice" by overnight courier and we will do the analysis. We will interpret the results and will provide you with either a Certificate of Analysis or a report on the dye tracing study. Groundwater tracing with the services of the OUL is practical and cost effective.

Tracer dyes have been successfully used in many different groundwater situations.

The Reality of Tracer Dyes

1. *They are safe.*
2. *They work effectively in many hydrogeologic settings.*
3. *They can be used in acidic waters.*
4. *Dye destruction by sunlight does not preclude their use in surface water.*
5. *The services of the Ozark Underground Laboratory make the work simple, credible, and cost effective.*

Five Critical Factors for Successful Groundwater Tracing

There are five factors critical to successful groundwater tracing:

1. Selection of appropriate dyes and adequate quantities of dyes and water. The dyes and their performance are dramatically different from one another. Never assume that a pound of one dye equals a pound of another.
2. Selection of appropriate types of samples. In most cases primary sampling reliance should be on activated carbon samplers rather than on water samples. Activated carbon samplers routinely maximize the detection of tracer dyes and minimize the number of samples, sampling efforts, and project costs.
3. Procedures which insure that no dye is lost or destroyed in samples prior to analysis.
4. Sample analysis instruments and methods which will quantify dye concentrations, distinguish among dyes, and adequately deal with fluctuations in background and interference fluorescence.
5. Study designs that adequately address and credibly answer essential questions. Among other issues, good study designs require selection of appropriate dye introduction points.

SELECTION OF APPROPRIATE DYES AND DYE QUANTITIES

Introduction

Successful groundwater tracing requires the use of appropriate dyes and adequate quantities of both dye and water. The following sections will:

1. Identify the five most generally useful dyes.
2. Discuss dye nomenclature for accurate identification of each of the dyes.
3. Identify and discuss five dye performance factors crucial to the selection of an appropriate dye and dye quantity. These factors are:
   - Relative fluorescence intensities.
   - Resistance to adsorption and other losses.
   - Fluorescence interference.
   - Performance in surface waters.
   - Limitations in acidic waters.
4. Discuss quantities of dye and water needed for dye tracing studies.

The Most Useful Dyes for Problem Solving

While there are many fluorescent dyes which have been used in groundwater tracing, we have limited this handbook to the five dyes most useful for general problem solving. All five of the dyes are anionic compounds and are thus less subject to adsorption onto clays and similar materials than are cationic dyes. The five dyes and the abbreviations used in this handbook are:

• **Eosine** (Eos) • **Fluorescein** (Fl) • **Pyranine** (Py) • **Rhodamine WT** (RWT) • **Sulforhodamine B** (SRB)

Analysis by the OUL for all of the dyes except pyranine uses the same protocol. The necessity of employing a different analysis protocol, plus other factors which can limit the utility of pyranine, make this dye generally less useful than the other four dyes.

Tracing Nomenclature and Dye Concentrations in "As Sold" Mixtures

Dye manufacturers and retailers use a myriad of names for the dyes. This causes confusion among dye users and report readers. Additionally, dyes purchased for groundwater tracing are always mixtures which contain both dye and an associated diluent. Diluents enable the manufacturer to standardize the dye mixture so that there are minimal differences among batches. Additionally, diluents are often designed to make it easier to dissolve the dye mixture in water, or to produce a product which meets a particular market need (and groundwater tracing is only a tiny fraction of the dye market). The percent of dye in "as sold" dye mixtures often varies dramatically among manufacturers and retailers, and retailers are sometimes incorrect about the percent of dye in their products. The material used as a diluent in the powder form dyes also varies. The most common diluent in powder mixtures is sodium sulfate; it is water in liquid mixtures..

Good technical reporting of a tracer dye used in a project should indicate its common name, its color index name and number, and should indicate the approximate percent of dye in the mixture. If the OUL provides dye for your trace, the appropriate information for each of the dyes we routinely use is shown in Table 1. The approximate range of dye concentrations we have encountered in the marketplace is also indicated to illustrate the importance of knowing the characteristics of the dye mixture used. Please check with us to insure that the percent of dye in the "as sold" mixture we are currently using has not changed. Finally, Table 1 indicates the more common alternate names for the five dyes. Figure 1 shows the chemical structure for each of the five dyes discussed in this handbook.

The OUL reports dye concentrations based upon the "as sold" weight of the dye mixture. We of course identify the approximate percent of dye in that mixture. One sometimes encounters reports where the author has calculated dye concentrations in samples based upon the assumed dye fraction in the dye mixture. When this approach is used, the reported dye concentrations are always smaller than if the concentration reflected the dye mixture. Good technical reporting clearly indicates whether the reported dye concentration reflects the "as sold" dye concentration or whether it is based upon the assumed dye concentration in the mixture sold.

One other bit of nomenclature warrants discussion. An ***eluent*** is a liquid used to remove dye from activated carbon. ***Elutant*** is the solution of the eluent and dye. ***Elution*** is the process by which an eluent becomes an elutant. ***Elutriation*** refers to a different process and has nothing to do with dye tracing.

Table 1 Color index identifications and approximate percent dye in "as sold" dye mixtures.

Dye	Color Index Name	Color Index Number	Approximate Percent Dye in "As Sold" Mixtures	
			OUL Mixtures	Market Range
Eosine	Acid Red 87	45380	75%	2 to 75%
Fluorescein	Acid Yellow 73	45350	75%	2 to 80%
Rhodamine WT	Acid Red 388	Not Assigned	20%	3 to 20%
Sulforhodamine B	Acid Red 52	45100	75%	3 to 75%
Pyranine	D&C Green 8	59040	77%	Unknown

CAS Numbers and common alternate names for the five dyes are shown below. Several of the dyes are sometimes sold under Drug and Cosmetic (D&C) names because they are used in such products. Dyes purchased under D&C names are typically more expensive than the generic dyes adequate for groundwater tracing work.

Eosine. CAS Number 17372-87-1. Also known as Eosin, Eosine OJ, and D&C Red 22.

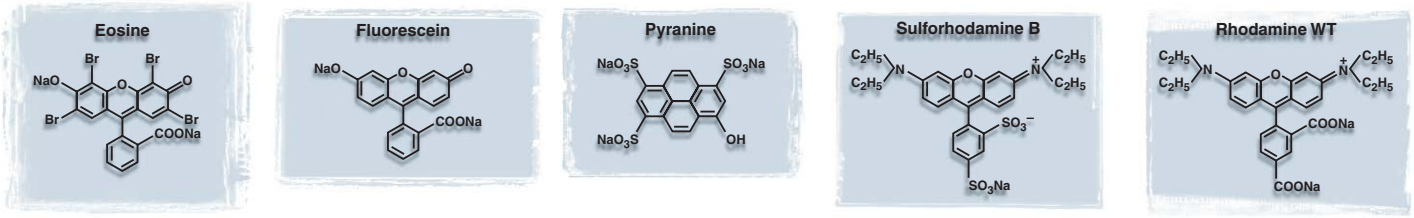
Fluorescein. CAS Number 518-47-8. Also known as Uranine, Uranine C, Sodium Fluorescein, Fluorescein LT, and Fluorescent Yellow/Green. This dye is sometimes sold simply as "green fluorescent dye". Also known as D&C Yellow 8

Rhodamine WT. CAS Number 37299-86-8. Sometimes sold as Fluorescent Red; the name "Fluorescent Red" is sometimes applied to Rhodamine B (Basic Violet 10) which has carcinogenic properties and is not a suitable dye for groundwater tracing. Rhodamine WT is sometimes sold simply as "red fluorescent dye".

Sulforhodamine B. CAS Number 3520-42-1. Also known as Sulfo Rhodamine B, Pontacyl Brilliant Pink B, Lissamine Red 4B, Kiton Rhodamine B, Acid Rhodamine B, Amido Rhodamine B, and Fluoro Brilliant Pink. This dye is sometimes sold simply as "red fluorescent dye".

Pyranine. CAS Number 6358-69-6. Also known as Solvent Green 7 (SG 7) and D&C Green 8.

Figure 1 Chemical structure for the five fluorescent dyes most commonly used in groundwater tracing.



Performance Factors: Relative Fluorescence Intensities

Part of the reason that the five fluorescent dyes are used is that they have high detectability in water samples or in elutant samples from activated carbon samplers. Water samples to be analyzed for fluorescein, eosine, or pyranine should be pH adjusted to a value in excess of 9.5 prior to analysis. The dyes are highly detectable both visually and by analytical instruments. The magnitude of fluorescence intensity varies dramatically among the five dyes; it also varies with the matrix as is indicated by the data in Tables 2 and 3. The intensity of fluorescence can be increased for most dyes through the addition of alcohol and ammonia hydroxide or potassium hydroxide. The presence of other materials in the sample matrix may sometimes alter the fluorescence intensity of the dyes.

Fluorescence intensities vary greatly among the dyes; a pound of one dye does not equal a pound of another.

The frontispiece of this handbook shows emission fluorescence peaks for all five of the dyes in an eluent solution. The samples were spiked to yield peaks of similar heights. Note that the dye concentrations varied dramatically among the five dyes. It required about 25 times more rhodamine WT dye mixture than fluorescein mixture to yield emission fluorescence peaks of approximately equal heights.

The fluorescence magnitude of rhodamine WT and sulforhodamine B in the standard OUL eluent is about twice as great as the respective fluorescence magnitude of these dyes in OUL reagent water (pH about 7.7). In the case of eosine, the fluorescence magnitude in the standard OUL eluent is more than three times as great as the fluorescence magnitude of this dye in OUL water. In distinct contrast, there is little difference between the fluorescence magnitude of fluorescein in water and fluorescein in the standard OUL eluent. The fluorescence intensity of pyranine decreases below a pH of about 9.5. As a result we have not shown any value for this dye in water in Table 2. Water samples to be analyzed for pyranine are routinely pH adjusted to values greater than 9.5 prior to analysis. In addition, the bandwidth separation normally used for pyranine analysis is 35 nm rather than the 17 nm normally used for the other four dyes. The values shown in Tables 2 and 3 are for cases where pyranine is analyzed using a 17 nm bandwidth separation. We have done this to help users anticipate fluorescence interference if pyranine and one or more of the other four dyes are present in a sample analyzed using the OUL protocol with a 17 nm bandwidth separation. Analysis approaches will be discussed in more detail later.

Table 2 Relative magnitude of fluorescence intensity of five tracer dyes compared with rhodamine WT. All values are ratios based upon the "as sold" weight of the dye mixture routinely used by the OUL; rhodamine WT is arbitrarily assigned a value of 1.00 in each solution.

Parameter	RWT	SRB	Py*	Eos	Fl
Emission Peak Area in OUL eluent	1.00	1.14	3.25	7.36	27.08
Emission Peak Area in OUL water***	1.00	1.17	**	4.50	48.15

* Pyranine is typically analyzed by the OUL with a different protocol than that used for the other four dyes. The value shown in this table is for cases where the pyranine is analyzed with the same protocol as the other four dyes.

** Varies with the pH of the water at pH less than 9.5.

*** pH about 7.7.

Table 3 Fluorescence magnitudes of four tracer dyes in the standard OUL eluent compared with fluorescence magnitudes in OUL reagent water. Positive values indicate that the fluorescence magnitude is greater in the OUL eluent than in OUL reagent water. No values are shown for pyranine since water samples are not directly analyzed, but are instead first pH adjusted.

Parameter	RWT	SRB	Eos	Fl
Emission Peak Height	+107%	+157%	+199%	-13%
Emission Peak Area	+98%	+150%	+188%	-10%

Table 4 summarizes detection limits for the five tracer dyes in instrumental analysis of water and elutant samples. This table also estimates detection limits for visual observations. All values are based upon "as sold" weights of the dyes (the dye mixtures used are those identified in Table 1).

Our method detection limits are larger than those reported by some other organizations. There are several reasons. First, our detection limits are based upon conditions typically encountered in "real-world" samples rather than being based upon spiked samples in clean laboratory eluent or water. The values shown are those which can routinely be achieved with "real-world" samples where the dye may have been affected by environmental factors and where other fluorescent compounds may increase the magnitude of background noise in the analytical graph. Secondly, we use narrow excitation and emission slit settings on our analytical instruments. This enhances our ability to separate dyes from one another and from other fluorescent compounds, but it also increases the detection limit value. Finally, our values are based upon the "as sold" dye mixture rather than the dye equivalent in that mixture.

There are three visual detection values shown in Table 4, all of which are for water essentially free of turbidity and color.

Table 4 Detection limits of five tracer dyes under different conditions. Concentrations in ppb of "as sold" dye mixture. Values in water assume pH >9.5 for pyranine, eosine, and fluorescein. Shimadzu RF 5301.

Parameter	RWT	SRB	Py	Eos	Fl
Dye in water; instrument analysis MDL*	0.015	0.008	0.010	0.015	0.002
Dye in elutant; instrument analysis MDL*	0.170	0.080	0.015	0.050	0.025
Dye in water; field conditions, experienced person	125	50	175	135	7
Dye in water; field conditions, general public	2,500	1,000	3,500	13,500	140
Dye in water; Dark room, experienced person	50	5	3	10	2

*MDL = Method Detection Limit; see text.

Note: The values shown for instrumental analysis of pyranine are synchronous scans for water with excitation slit = 5 nm and emission slit = 3 nm. For elutants the excitation slit = 3 nm and the emission slit = 1.5 nm. All bandwidth separations = 35 nm.

The first of these is identified as ***field conditions, experienced person***. These results are based upon OUL experience. The second set of values is identified as ***dark room, experienced person***. These results are consensus results from OUL staff with good color perception. A sample was placed in a glass bottle 2.5 inches in diameter and a microscope light was beamed through the solution. Observations were made by viewing the bottle at 90 degrees to the light beam. The final value is identified as ***field conditions, general public***. Except for eosine, this value is set at 20 times the concentration limit of an experienced person under field conditions. Most people who observe eosine dye in natural waters attribute the combination of green and pink color to algae or some other natural material. As a result, we have set the value for eosine at 100 times the concentration limit of an experienced person. Even at much higher concentrations eosine does not attract the degree of public attention associated with any of the other four tracer dyes.

All five of the dyes have their maximum fluorescence in the visible wavelength range. For most individuals, this is between 380 and 760 nm. There is a persistent myth that an appropriate visual detection method is to look for fluorescein dye (or one of the other four dyes) in a dark room with an ultra-violet light source. This is not true. It is far better, and much more diagnostic, to beam a light such as a small "mag" light into a bottle containing water or elutant with dye. The light should be aimed at 90 degrees to the viewing angle. Dye is most visible in the light beam.

Performance Factors: Resistance to Adsorption and Other Losses

Variables which control dye adsorption include pH, temperature, water quality, degree of water agitation, sediment concentration, sediment type, dye concentration, and dye type. Dye concentration is one of the most important variables. Smart and Laidlaw (1977) found a marked decrease in the percentage of dye lost to adsorbing materials with increasing initial dye concentration (see Table 5). 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.

The OUL has found that if a trace in a karst aquifer is replicated with the use of twice as much of the same dye, the resulting dye recovery concentrations at sampling stations are substantially more than double the concentration in the initial trace. Conversely, if the trace is replicated with the use of half as much dye, the resulting dye recovery concentrations at sampling stations are substantially less than half. Additionally, loss of rhodamine WT (and probably other dyes as well) is greater in karst aquifers at low flow rates than at high flows. The reason for this is more and longer contact between the dyed water and substrates under low flow rates than under high flows.

The data in Table 5 indicate that organic materials adsorb much more dye than do inorganic sediments. This has been attributed to the extremely large surface areas of organic material and to the large number of broken chemical bonds present on these surfaces. Similar findings have been widely 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 seems to have the greatest resistance. Rhodamine WT and, to an even greater degree sulforhodamine B, have lesser resistance to adsorption onto inorganic materials; this is particularly true when there is appreciable contact between such inorganic materials and water containing dyes. Dyed water moving along preferential flow systems loses much less dye to adsorption than in the case where flow systems are more diffuse. As an illustration, much less dye is lost to adsorption in deep clay-rich residuums with well developed and integrated macropore drainage than in similar textured materials with less well developed and integrated macropores.

Fluorescein, eosine, and pyranine have good resistance to adsorption onto inorganic material.

One OUL trace introduced both fluorescein and rhodamine WT at the same time and location in a karst aquifer. Fluorescein was recovered from 18 domestic wells; rhodamine WT was recovered from only 2 of these wells, and there were no wells where rhodamine WT was detected and fluorescein was not. The differences are attributed to greater adsorptive losses of rhodamine WT onto inorganic surfaces within the aquifer.

Table 5 Comparison of tracer dye adsorption onto mineral and organic materials. Values are percent of dye remaining in solution from a 100 ppb initial solution. Adapted from Smart and Laidlaw (1977).

Material	Sediment concentration gm/l	Fl	Py	RWT	SRB
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	—

Note: Eosine dye was not evaluated by these authors.

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

Fluorescein loss: Surface Soil >> Cave Stream Pebbles > Cave Sediment.

Rhodamine WT loss: Surface Soil >> Cave Sediment > Cave Stream Pebbles.

Rates of dye losses in surface soils were greater than with any other substrate. We attribute this to a combination of greater adsorption and more biological decomposition. The loss of dye to cave stream pebbles is attributed to biological decomposition of the dyes or deactivation of their fluorescence. Stream pebbles which had been sterilized by heating did not remove dyes from the test solutions. Cave sediments adsorb dyes, and more readily adsorb rhodamine WT than fluorescein. The rate of dye loss to cave stream pebbles was greater for rhodamine WT than for 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 travel distance was 4,000 feet. The percent of fluorescein dye recovered in both activated carbon samplers and in water samples was greater than the percent of rhodamine WT dye recovered.

Several workers have developed "breakthrough curves" for dyes passed through soil or sediment columns. A conservative tracer, such as chloride or bromide which moves with the water, has been used for comparison purposes in some of these studies. Pyranine, fluorescein, and eosine pass through these columns like, or almost like, truly conservative tracers. Rhodamine WT shows a dual movement curve where approximately half of the dye moves through the column much like a conservative tracer. The other half of the rhodamine WT is appreciably detained by adsorption and subsequent desorption. This performance is apparently due to rhodamine WT being comprised of two isomers, each of which has different resistance

to adsorption. The two isomers may also have slightly different fluorescence characteristics; this would explain apparent shifts in emission fluorescence wavelengths of rhodamine WT as encountered in some tracing investigations. In these cases the rhodamine WT emission fluorescence peaks from activated carbon samplers tend to be at longer wavelengths early in the dye recovery period and at shorter wavelengths later in the study.

Based upon laboratory tests, Smart and Laidlaw (1977) ranked the resistance of dyes to adsorption as shown below. These authors did not evaluate eosine, but based upon the technical literature its performance should be similar, but not quite as good as, fluorescein.

Resistance to Adsorption onto Inorganic Material: Py > Fl > RWT > SRB. Smart and Laidlaw, 1977.

Resistance to Adsorption onto Organic Material: Py > SRB > Fl = RWT. Smart and Laidlaw, 1977.

Behrens (1986) ranked the resistance of a number of tracer dyes to adsorption. His data were largely based upon field work in Europe where much of the sampling uses water samples. His rankings were as follows:

Resistance to Adsorption: Py = Fl > Eos > RWT > SRB. Behrens (1986).

Based upon OUL groundwater experience where much of our tracing places primary reliance on activated carbon samplers with secondary reliance upon water samples, the rankings of Behrens (1986) agree with our findings. However, our data on pyranine resistance to adsorption are limited.

Ability of Activated Carbon to Adsorb Dye in Field Situations and then Release it to the Elution Protocol: Fl > Eos > RWT > SRB > Py. OUL data.

Percentage losses of tracer dyes in groundwater systems increase as:

1. Dye concentrations decrease.
2. Travel distances increase; travel through soil and residuum are more effective in removing dye than travel through most aquifers.
3. Travel times increase.
4. Organic matter increases.
5. Bacterial activity increases.
6. Water follows dispersed, rather than concentrated, flow routes.

Performance Factors: Fluorescence Interference

Background sampling prior to dye introductions sometimes detects tracer dyes or compounds with similar fluorescence characteristics. Common man-made sources of these dyes or compounds are indicated in Table 6. Compounds with fluorescence characteristics similar to one of the five dyes are indicated as though they contained the dye.

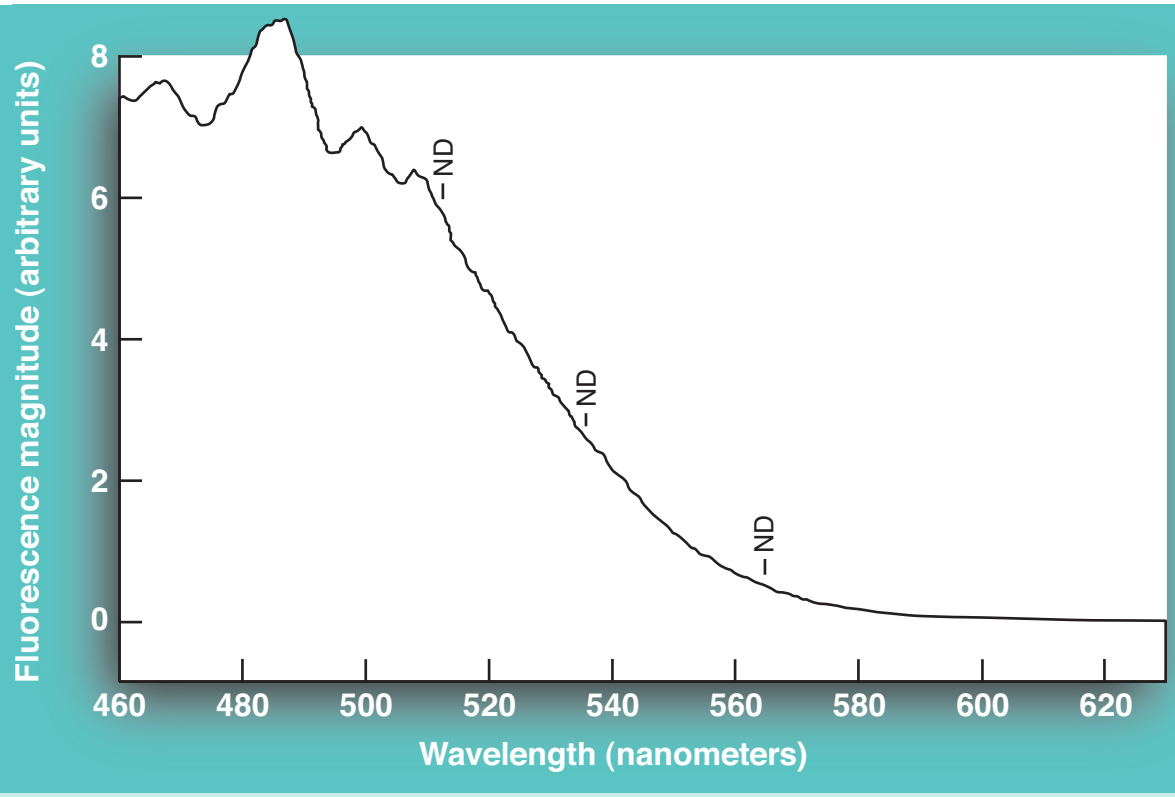
Adequate quantitative background sampling prior to dye introduction is essential.

Table 6 Common fluorescence interference sources. Some of the interference is due to dyes and some is due to other compounds.

Source	Eos	Fl	Py	RWT	SRB
Stormwater runoff from major roads and large parking areas		✔		✔	
Automotive coolants (anti-freeze)	✔	✔		✔	
Residential and municipal sewage and discharge from sewage treatment plants		✔	✔		
Municipal landfill leachate		✔	✔		
Hydraulic fluids from heavy industry plants				✔	✔
Wood treatment plants, especially those which used pentachlorophenol	✔	✔			
Waters in contact with high sulfur coal	✔	✔			
Cooling tower blow-down		✔			
"Leak tracer" dyes used by plumbers and sanitarians		✔		✔	
Agricultural chemicals				✔	
Plastic manufacturing plants and metal casting plants				✔	✔
Colored paper and colored felt-tip pens	✔	✔		✔	

Fluorescence interference also results from natural compounds which include, among many others, some of the humic and fulvic compounds. This resulting background interference is variable both in space and time. Using the OUL analytical protocol the magnitude of background fluorescence decreases as the emission fluorescence wavelength increases (see Figure 2). This has led some users of filter fluorometers to conclude that dyes with fluorescence peaks at longer emission wavelengths are superior to those with peaks at shorter emission wavelengths. However, the OUL conducts analysis work with a spectrofluorophotometer with a synchronous scan protocol which eliminates this difference in performance.

Figure 2 OUL analysis graph of activated carbon elutant sample containing no dyes.



Station 1: Water Supply Spring
OUL number: H3979
Date placed: 9-1-1998
Time placed: 1350

Charcoal Analyzed: 9-9-1998
Date recovered: 9-2-1998
Time recovered: 1020

Peaks within normal range of tracer dyes:

Dye	Peak nm	Left X	Right X	Height	Area	H/A	Conc.
Fl	512.5	510.7	515.0	0	0	0	ND
Eos	536.0	533.0	539.6	0	0	0	ND
RWT	565.0	561.7	568.9	0	0	0	ND

Fluorescence interference from natural compounds sometimes results in fluorescence peaks in or near the acceptable wavelength range of some of the tracer dyes, especially fluorescein and pyranine. Under synchronous scan protocols, the shapes of the fluorescence peaks associated with these natural materials are typically broader, more irregular, and less symmetrical than those associated with the tracer dyes.

Fluorescence interference from natural compounds can be reduced by:

- Washing activated carbon samplers prior to analysis.
- Selecting eluent solutions and protocols which minimize elution of the natural compounds yet are effective in eluting the tracer dyes.
- Keeping collected samplers refrigerated until analysis to minimize biological growths on the carbon.
- Reducing the amount of time that samplers are in place. This not only reduces the amount of natural compounds adsorbed on the activated carbon but also limits the biological growth that occurs on the samplers. Carbon is a macronutrient for plant growth; do not let your samplers become good biological substrates. The shortest duration for leaving a sampler in place is about an hour. Water samples should be used for shorter duration sampling.
- Placing samplers where they are more protected from direct sunlight since light enhances plant growth.

In almost all cases the magnitude of the background fluorescence is small and can easily be overcome by quantifying and statistically characterizing its magnitude prior to dye introduction and then using sufficient dye to produce fluorescence peaks which are at least an order of magnitude larger than the largest background fluorescence peak. The OUL routinely applies the following approach. In rural settings we routinely collect one round of background samples prior to dye introduction. In more heavily populated areas where little or no fluorescence background is anticipated we routinely collect two rounds of background samples. In urban or industrial areas we typically collect three rounds of background samples. Only in rare instances have we found it desirable or necessary to collect more than three rounds of background samples

Performance Factors: Losses in Surface Water.

The focus of this handbook is on groundwater tracing, so a consideration of dye performance in surface water might seem out of place. However, there are many cases where dyes can be used to trace water from a stream into nearby wells or springs. In such cases the stream segment into which dye must be placed is sometimes thousands of feet long. In other cases tracer dyes in the groundwater system may discharge to streams, rivers, or lakes, and the groundwater tracing study must identify the receiving water bodies and the specific or general locations of the dye discharge points.

Processes which remove, degrade, or destroy dyes from surface water include water and dye uptake by plants and dye degradation or destruction by photo-degradation (sunlight). Dye losses in surface water increase with greater water clarity, increases in water temperature and associated biological activity, and increases in the extent of contact between dyed water and the stream or lake substrate. Studies by the OUL have shown that all five of the dyes discussed in this handbook can be detected in the tissue of plants that are transpiring water which contains the tracer dyes. Riparian vegetation and aquatic plants can extract appreciable amounts of dye during dye tracing programs. The dye losses could be particularly extensive under spring and summer conditions where small springs are located in wetland areas, or where a shallow water table is accessible to the roots of phreatophytes.

Several of the tracer dyes can be effectively used to trace water into, or out of, surface water bodies. Dye destruction by sunlight is often less significant than other loss processes.

The rate of dye degradation in sunlight, as measured by fluorescence magnitude, varies dramatically among the five tracer dyes. This is illustrated by the data in Table 7. Shallow plastic trays containing 0.5 inches of dyed water were placed in direct sunlight and were sampled periodically over a five-hour period between 11:00 AM and 4:00 PM on a mostly sunny but hazy day in mid July; the latitude was 36 degrees 33 minutes North.

One should not conclude from the data in Table 7 that sulforhodamine B and rhodamine WT are the only dyes suitable for tracing work that involves surface waters. "Real world" water bodies are typically deeper than the 0.5 inch trays, so sunlight penetration and photo-degradation of the dyes is less. Many streams are deep and shaded thus minimizing photo-degradation. Photochemical decay rates are typically about an order of magnitude greater during sunny conditions than under cloudy conditions. Depending upon the season, groundwater discharge to lakes may stratify beneath the effective depth of sunlight penetration. Finally, dyes discharging to surface waters are not subject to photo-degradation at night. The net result of all these factors is that fluorescein can often be detected for longer distances down a surface stream than either eosine or rhodamine WT, and that eosine and rhodamine WT usually persist better in surface streams than either sulforhodamine B or pyranine. Based upon OUL data, eosine and rhodamine WT concentrations in the less than 1 ppb range in water persisted equally well along a 9,000 foot stream channel segment in Pennsylvania. Based upon 28 sampling periods and correcting for increases in flow rate volumes, dye concentrations at the downstream end of the stream segment averaged 18% of those at the upstream end of the segment.

Table 7 Dye degradation in sunlight as measured by fluorescence magnitude. Water depth 0.5 inches.

Dye	Initial Concentration (ppb)	Percent of Initial Dye Remaining		
		1 hour	3 hours	5 hours
Eosine	1,000	2 %	< 1 %	—
	100	1 %	< 1 %	—
Fluorescein	1,000	19 %	< 1 %*	—
	100	7 %	< 1 %*	—
Pyranine	1,000	68 %	3 %	—
	100	55 %	1 %*	—
Rhodamine WT	1,000	100 %	100 %	83 %
	100	97 %	49 %*	32 %*
Sulforhodamine B	1,000	100 %	97 %	95 %
	100	81 %	70 %	60 %

* Atypical peaks for the dye in question. In the case of rhodamine WT, the 100 ppb dye concentrations exposed to sunlight for three and five hours showed fluorescence shoulders in the general acceptable wavelength range for eosine.

Note: All concentrations are based upon "as sold" weights of dye mixtures. The dye mixtures for eosine, fluorescein, and sulforhodamine B contained approximately 75% dye, the pyranine mixture contained 77% dye, and the rhodamine WT mixture was approximately 20% dye.

Performance Factors: Limitations in Acidic and Mine Waters

The fluorescence magnitude of tracer dyes is dependent upon the pH of the solution in which the dye is found and the nature of that solution. Maximum fluorescence for all five of the dyes occurs at pH values of about 9.5 or higher, although this may be due in part to the bases which are added to the solution to adjust the pH for laboratory measurement. Table 8 provides generalizations about the pH values which substantially decrease the fluorescence magnitude of particular dyes (data derived from Smart and Laidlaw [1977], Behrens [1988], and OUL experience).

Table 8 Influence of pH on fluorescence magnitude.

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	3.5	2.0

If one is tracing in waters with pH values outside the usual range of most natural waters (which is from about 6.5 to 8.0) it is advisable to conduct some laboratory investigations using the water in question. This is illustrated by the following investigation conducted of the five dyes discussed in this handbook in water from a metal mine in California. The pH of the waters from this mine vary from about 2.9 to 4.3; the tested water had a pH of 3.7. The mine waters are moderately mineralized with various elements and compounds which might interfere with one or more of the five dyes.

Solutions of 10 ppb and 100 ppb of each of the five dyes were made in mine water and the fluorescence magnitude was measured two hours after the solutions were mixed and a second time 15 days after the mixing. Dye concentrations reflected the "as sold" dye mixtures. Samples were pH adjusted with potassium hydroxide immediately prior to analysis to a pH of greater than 8. Results are shown in Table 9.

Tracer dyes can be used in acidic waters, but selection of the dyes to be used is critical.

Pyranine was not detectable in any of the samples. The results from the mine waters after two hours suggested that either sulforhodamine B or rhodamine WT might be a suitable dye for use. Earlier discussions indicated that the addition of potassium hydroxide to water samples increases the fluorescence intensity of some of the dyes. This explains apparent concentrations greater than the actual mixed concentration for some of the dyes at some concentrations. However, after 15 days, neither rhodamine WT nor sulforhodamine B dyes were detectable in the mine water. Since anticipated travel times through this mine were on the order of a few weeks, the dye selected was fluorescein, with eosine a second choice.

Iron hydroxide deposits (known in the coal fields as "yellow boy") are capable of adsorbing appreciable amounts of some of the tracer dyes, especially rhodamine WT. As a result, sulforhodamine B commonly performs better in coal mine situations than does rhodamine WT, although the OUL has successfully used rhodamine WT in some coal mine tracing. In some coal mine settings (particularly those with high sulfur coals) there is sometimes appreciable natural fluorescence in or near the acceptable emission wavelength range of fluorescein dye. In such cases one may wish to select a different dye, or else adequately quantify fluorescence background prior to dye introduction and use somewhat more fluorescein than might otherwise be indicated.

Based upon the OUL experience in tracing acidic mine waters (in both metal mines and coal mines) the general suitability of tracer dyes is as follows:

Suitability for Acid Mine Water Tracing: Fl > Eos > SRB > RWT. Py is unsuitable for this use.

In some cases sulforhodamine B is much more suitable for this use than eosine. OUL data.

Table 9 Apparent dye concentrations detected in acid mine waters as compared with standards in OUL reagent water. Water from a northern California metals mine; initial pH 3.7, adjusted to >8 with potassium hydroxide prior to analysis. All values ppb.

Conditions	Eos	Fl	Py	RWT	SRB
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 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 15 days after mixing. pH adjusted to >8 immediately before analysis.	2.3	8.2	ND	ND	ND

ND = None Detected

Selecting Dye Quantities

There are a number of equations which have been used for estimating the quantity of dye needed for a groundwater trace. One review of the equations found that the estimated dye quantities from the equations varied by eleven orders of magnitude. The reasons for this enormous variation include differences among the dyes, dye strengths, detection methods, analytical approaches, and the types of groundwater settings encountered by the particular author. There is no credible standard equation for estimating dye quantities needed for groundwater tracing work.

Many groundwater traces have failed because of inadequate dye quantities for the hydrogeologic setting or for the sampling or analysis approach used. Many of these failures have resulted from assumptions that much or all of the introduced dye would be recovered at an anticipated sampling station. Other traces have failed because the limitations of minimal quantities of dye have been compounded by inadequate quantification of background fluorescence and/or because the minimum dye concentration indicating a positive dye recovery was arbitrarily set at too large a concentration.

Many traces have failed because of inadequate dye quantities. Failures have often been due to an assumption that all introduced dye would discharge from the groundwater system during the sampling period.

Dye recovery amounts as a percent of the amount introduced are typically greater in karst than in most other hydrogeologic settings, yet even in karst the values are typically small. The following are reported percentages for karst settings where dyed water discharged from springs and mass balance calculations were possible:

a. Aley (2017) median 4.9% for mean straight-line travel distances of 0.9 miles; n = 15.

b. Hauwert et al. (2004) median 4.2% for straight-line distances 2.0 to 18.9 miles; n = 20.

c. Aley (1997) traces in seasonally saturated epikarst 1 to 10%.

d. Aley (1997) traces in perennially saturated epikarst 0.1 to 1%.

A few other generalizations can be provided relative to quantities of dye needed for successful groundwater tracing using the approaches outlined in this handbook. Please recognize that alternate approaches (such as the use of filter fluorometers for dye analysis, grab samples of water rather than activated carbon samplers, and the use of most other dyes) are likely to appreciably increase the dye quantity requirements for credible tracer dye detection.

In terms of the "as sold" weight and assuming other conditions are equal, the quantity of dye needed for a successful trace where primary sampling reliance is placed on activated carbon samplers is as follows:

Dye Quantity: Fl < Eos < Py < RWT < SRB.

If multiple traces are being conducted, the three best dyes for concurrent use are generally fluorescein, eosine, and rhodamine WT. Fluorescein should generally be used for the trace which is viewed as likely to be the most difficult, and rhodamine WT should generally be used for the easiest trace. Interference problems between fluorescein and eosine are greater than interference between fluorescein or eosine and rhodamine WT. If it is anticipated that both fluorescein and eosine will be recovered at some of the important sampling stations, adjust the dye quantities used so that the magnitude of the fluorescence peaks will be similar for these two dyes. When using these three dyes, to the extent reasonable, introduce fluorescein and eosine at sites where they are unlikely to both be recovered at the same sampling stations. Alternately, stagger the times of dye introductions so that the eosine dye pulse is detected first followed by the fluorescein pulse. Fluorescein is more strongly fluorescent than is eosine and will tend to over-ride eosine peaks in activated carbon samplers.

Rhodamine WT and sulforhodamine B have emission fluorescence peaks relatively close to one another. As a result, these dyes should generally not be used concurrently if both might be detected at the same sampling stations. These dyes can be used consecutively if only small concentrations of one of the dyes persists at sampling stations and if a sufficient amount of the other dye is introduced for a new trace.

Tracer dyes can be used for many purposes and those purposes and associated distances being traced influence the amount of tracer dyes needed. Most groundwater traces for distances of 500 feet or more require only a few pounds of the "as sold" weights of tracer dyes. We seldom use less than one pound of any of the dyes for any particular dye introduction. In karst areas, most dye introductions use one to five pounds of the selected dye except where the dye is introduced into wells, borings, or backhoe pits. When dye introductions are made into these man-made locations, dye quantities are most commonly in the range of five to ten pounds for fluorescein or eosine and ten to twenty pounds of rhodamine WT. We seldom use pyranine or sulforhodamine B for these types of dye introductions.

Groundwater tracing in non-karst areas typically requires two or three times more dye than in karst areas. The extent to which various dyes tend to be adsorbed onto substrate surfaces often limits the number of different dyes which can be appropriately used. Tracing into or through underground mines can be effectively done, but it typically requires careful selection of the type of dye to be used plus the use of at least two or three times more dye than might be selected for a groundwater tracing investigation in a karst area. Bench tests of dye performance in the waters typical of the mining area are advisable prior to selection of dye type and dye quantities for mine tracing work.

It has been our experience that the magnitude of the fluorescence peak associated with a particular dye trace tends to increase with about the square of the amount of dye used. In other words, a trace conducted with two pounds of a particular dye will yield fluorescence peaks about four times greater than if the same trace is replicated with one pound of the particular dye. At a minimum, the use of twice as much of a particular dye will almost always yield fluorescence peaks which are more than double those resulting from the use of half as much dye. This general relationship does not apply if excessively large quantities of dye are used. The relationship is most applicable where peak dye concentrations in activated carbon samplers are one to three orders of magnitude greater than the detection limit for the dye in question.

Tracer dyes are commonly used to aid in the design and/or evaluation of in situ remediation at waste sites. In some cases the tracer dyes are introduced as high concentration slugs of dye immediately prior to the injection of the remediation agent. This approach negates concern that the remediation agent will destroy some or much of the dye. This approach also gives good time of travel data for dye arrival at particular sampling points and serves to identify monitoring points that could potentially be reached by the remediation agent. An alternate approach is to introduce dye at a standard concentration mixed in with the remediation agent. Sutherson et al. (2014) provide recommendations for designing and optimizing the results from traces using this approach.

Experience is the most effective method for determining appropriate dye quantities. We are happy to help clients and colleagues select appropriate dyes and estimate dye quantities for groundwater traces; give us a phone call.

Experience is the most effective method of determining appropriate dye quantities. We are happy to help.

INTRODUCING TRACER DYES

Sites for Dye Introduction

There are many different ways in which tracer dyes can be introduced into groundwater systems. The dye introduction sites used for a particular tracing project are first determined by the questions to be addressed by the study, and secondly by feasible approaches for the site in question.

It is fundamental logic that one cannot provide an appropriate answer unless one understands the question. Good groundwater tracing tests are designed to answer one or more specific questions. Unfortunately, many groundwater tracing studies have not been designed to answer the most relevant questions, and many studies have been designed and conducted in such a manner that they had little or no chance of credibly answering the questions that they were supposed to address. A common flaw of such studies is failure to use a relevant site for dye introduction.

In karst areas, sinkholes, cave streams, or surface streams which sink into the subsurface are obvious points for dye introduction into the groundwater system. These locations are typically easy and inexpensive to use; such locations are well suited to studies such as delineating recharge areas for springs. However, these sites may be inappropriate for characterizing water movement from a waste site several hundred feet away.

Backhoe trenches and EDIPs are often desirable for dye introduction at waste sites. Monitoring wells are often poor sites.

Many surface streams recharge valley aquifers or springs. It is simple to introduce a tracer dye into a surface stream and then sample appropriate wells, springs, or other features for the dye. In the case of perennial streams the percent of surface flow which enters the groundwater system may be quite small; this can necessitate the use of more dye than if the dye were introduced directly into the groundwater system. Eosine is often an ideal dye for this type of dye trace since this dye is not as noticeable in surface water as the other dyes discussed in this handbook. Dyes can be introduced shortly before dark to minimize photo-decomposition. If it is desirable to dye an appreciable reach of stream it is often beneficial to introduce part of the dye at several points along the stream rather than placing all of it in one location. In some cases wells, borings, or backhoe trenches adjacent to those portions of the stream which recharge groundwater provide ideal dye introduction points. If these features are used, one can sample the adjacent stream to demonstrate that most or all of the dyed water does or does not return to the surface stream.

Many stream channels and road ditches have flow only during and shortly after major storm events. These features can be used for dye introduction if the dye is flushed into the groundwater system with introduced water from a fire hydrant, tanker truck, or nearby pond. If this approach is used, there is the possibility that subsequent storm events will flush some dye further downstream than the segment of the channel dyed during the initial water introduction. This is generally not a major problem, especially if the study design includes several sampling stations on the drainageway downstream of the dye introduction area.

Backhoe trenches can often be constructed adjacent to waste sites and used as dye introduction points. The rate at which water will leak from such trenches should be determined prior to any dye introduction, and the trench should not be used for dye introduction unless the leakage rate is deemed adequate. The OUL has had successful traces with leakage rates as small as 0.04 gallons per minute per square foot of trench bottom, but greater rates are desirable. Dye and flush water can then be added to the trench after adequate leakage rates have been verified. Once most or all of the dyed water has leaked from the trench it can be backfilled with the excavated material. This has the benefit of preventing people or animals from making contact with any residual dyed water and thus possibly contaminating the study.

Many hazardous waste sites underlain by soluble rock units have detected contaminants of concern in the underlying bedrock aquifer. Groundwater tracing from these waste sites is often needed. We frequently advise the use of Epikarstic Dye Introduction Points (EDIPs) for introducing dyes at points which bracket the waste unit or a portion of that unit. EDIPs are basically vertical borings that extend to a few feet below the top of bedrock. The bottom ten feet or so of the EDIP is backfilled with pea gravel and a casing surrounded by bentonite is placed on top of the gravel. Dye and water are then introduced into the EDIP.

EDIPs have several advantages for use in this type of situation. First, you are introducing dyes at exactly the points where you typically need the information. Secondly, in most cases there is no dispute that contaminants of concern have moved downward through the overburden material to reach the bedrock. By introducing the dye at the top of the bedrock one accelerates the study; it is not necessary to trace the dye downward through the overburden. This saves time and permits tracing with less dye. The use of the EDIP strategy is also viable for many non-soluble rock settings.

EDIPs should be tested prior to any dye introduction to insure that they will accept reasonable amounts of water. We have had a number of successful groundwater traces from EDIPs where the rate of water acceptance was as low as one or two gallons per minute, but rates of five gallons per minute or more are desirable. We typically test the EDIP with at least 500 gallons of water.

In most cases monitoring wells are poor dye introduction points. Many of them have their screened openings at lower elevations than the elevations most desirable for introducing tracer dyes. Additionally, when wells (or for that matter EDIPs) are used for dye introduction there will be some dye residual in the well or boring for a long period of time. The use of a monitoring well for dye introduction could reduce the utility of the well for monitoring purposes. Finally, EDIPs are usually much less expensive than monitoring wells and the EDIPs can be abandoned and sealed with regulatory agency concurrence after the tracing work has been completed.

All of the tracer dyes will foam to some extent when added to water and agitated. This characteristic can be important when introducing tracer dyes into EDIPs or wells since there is the potential for such foam to rise in the casing and discharge to the surface. Based upon tests by the OUL, fluorescein and pyranine dye mixtures yield negligible amounts of foam at concentrations of 100 parts per million (ppm) or less; they do produce foam at higher concentrations. In contrast, rhodamine WT dye mixtures of 100 ppm ("as sold" weight) can create a volume of foam equal to about 25% of the volume of the dye and water mixture when vigorously agitated; this foam will persist for a period of a few hours. The introduction of two pounds of 20% rhodamine WT dye mixture into a small stream at the top of a forty foot high waterfall resulted in foam about ten feet deep covering the pool in the narrow canyon at the base of the falls. Such colorful results are not always acceptable.

The tendency of the five dyes to create noticeable foam at concentrations of 100 ppm is as follows:

Water = Fl = Py < Eos << SRB <<RWT.

The tendency to produce foam does not preclude the use of any of the five tracer dyes in EDIPs or wells, especially if the water level in the well is 15 or more feet below the top of the casing. On a number of occasions we have poured ten pounds or more of "as sold" rhodamine WT dye mixture into the tops of EDIPs or wells without having foam rise to near the top of the casing. If necessary, one can minimize the foaming problem by inserting a tube (such as a segment of 5/8 inch garden hose) into the well or EDIP and introducing the dye through the tube. This technique can also be used to introduce dyes directly into the screened interval of a well or into a known fracture zone or cavity.

Selecting Water Quantities

Tracer dyes are introduced to tag water and then trace it through the groundwater system. In general, the more water used the more effectively it can be traced through the groundwater system. In some cases the volume of water used is limited by the purposes of the study or by logistical considerations.

In general, the more water introduced the more effectively it can be traced.

Many successful dye introductions have used on the order of 1,000 to 4,000 gallons of water, although this must not be viewed as a restrictive "target range". At hazardous waste sites multiple dye introductions are sometimes needed and the study designs sometimes specify that water is to be added slowly to the dye introduction points. In such cases portable storage tanks can often be rented, filled with water by a tanker truck prior to the start of the dye introductions, and then slowly emptied through hoses as needed. While chlorinated drinking water will destroy a small amount of dye, the amount of dye lost during a dye introduction is inconsequential and does not preclude the use of such water.

Use of "Dry Sets"

Many of the dye introductions in karst areas are made during periods when there is overland flow. However, it is often difficult to personally encounter flow at the most ideal locations. This problem can often be offset by using "dry sets". A dry set involves the placement of dye in such a fashion that it will be flushed into a surface drainageway or sinkhole by the first stormflow event. Additionally, dry sets placed in highway culverts or road ditches are particularly useful in assessing the impacts of stormwater runoff.

Dry sets are most easily conducted with fluorescein, eosine, and sulforhodamine B since these dyes are provided by the OUL as powders. We prefer to have the dry sets protected from direct rainfall, yet be where they will be taken into solution by the first storm flow. One approach is to use a short piece of four inch diameter plastic pipe (usually one or two feet long, but the length is dependent upon the amount of dye to be used). The powder form of the dye mixture is poured into the pipe segment, and the segment is anchored into the stream channel where water will run through it when a storm flow occurs. The dye should fill no more than the bottom half of the pipe cross section so as to not obstruct water flow. The powdered dyes will absorb moisture and usually become solid within a couple of days. When flow occurs through the pipe it will typically take up to an hour for all of the dye to be taken into solution; the flow rate is obviously an important variable.

If rhodamine WT is to be used for a dry set it should not be poured onto the ground where much of it would be lost, nor should it be allowed to sit in a container where it could freeze and then leak. One approach is to use a plastic bottle such as a vinegar jug which has a handle; milk jugs are not desirable since the plastic is thin and may become brittle. The bottle is partially filled with rhodamine WT and is placed upright in a plunge pool or an excavated hole in the selected stream channel. The bottle is not capped, but an over-sized lid is used to minimize evaporation and protect it from direct precipitation. A cord is run from the handle of the bottle to a large rock or steel anchor point on the bottom of the plunge pool. When flow occurs the pool starts to fill with water, the bottle containing the dye floats and then is turned upside down by the cord, and the dye is introduced into the stream flow.

"Dry sets" can be used where flow is intermittent.

Dry sets can be hidden under leaves or brush to minimize the chance that someone will discover and tamper with them. Activated carbon samplers can be placed at downstream locations to determine how far downstream the introduced dye was detectable in the surface flow. In most cases the downstream migration distance is short, often 200 feet or less, because most of the dye is introduced with the leading edge of the stormflow.

Mixing Dyes

In most cases it is better to introduce the tracer dye as a slug rather than mixing the dye and water in a tank prior to dye introduction. Slug introduction minimizes cleanup and prevents discharge of residual dye at some off-site location. Strong concentrations of dye will adsorb onto dry earth materials. For this reason we typically introduce about 10 to 15% of the water we will use prior to introducing the dye; we then introduce the remaining water to flush the dye into and/or through the groundwater system.

Four of the five dyes discussed in this handbook are provided in a powder form; only rhodamine WT is provided as a liquid. In many cases the easiest way to introduce the powder dyes is to first mix them with water. The OUL typically ships dyes to the study site as powders in 15 or 20 liter carboys which are packed in coolers to prevent shipping damage. These coolers are marked so that they will never be used for holding or shipping samples. Up to eight pounds of dye can be put into a single carboy. At the site the cap is removed from the carboy, a four-inch diameter disposable plastic funnel is inserted into the carboy opening, and water is poured through the funnel and into the carboy until the carboy is about 80% full. The funnel prevents dye powder from fluffing out of the carboy when water is being added. The carboy is then capped and periodically shaken to help mix the dye. It will require at least an hour to obtain a good mixture of dye which is essentially free of lumps of powder; allowing the mixture to stand overnight is ideal. The tendency of dyes to foam when agitated was discussed earlier; allowing the dye mixture in a carboy to stand quietly overnight will dissipate any foam and enhance the ease of the dye introduction.

Introducing water and fluorescein dye into a sinking stream point, Edwards Aquifer, Texas. Note the dye and water mixture in the 20-liter carboy.

SAMPLING FOR TRACER DYES

Sampling for the five tracer dyes can be done either with activated carbon samplers or with water samples. In most cases primary sampling reliance should be placed on activated carbon samplers with secondary reliance upon water samples.

Activated Carbon Samplers

Activated carbon samplers are fundamental to most cost-effective groundwater tracing studies. The samplers continuously adsorb and accumulate all five of the dyes discussed in this handbook. The samplers are inexpensive, easy to use, and adsorb tracer dyes even in the presence of most common water contaminants (including petroleum products and solvents commonly encountered in contaminated groundwater).

The OUL manufactures activated carbon samplers containing 4.25 grams of Calgon Carbon 207C. This is a coconut shell charcoal with a 6 to 12 mesh size range. The particle size distribution in this product is 5% maximum on the top screen (6 mesh, 3.350 mm) and 5% maximum through the bottom screen (12 mesh, 1.700 mm). This charcoal has a surface area of 1150 square meters per gram. The fiberglass screening used by the OUL for activated carbon samplers has openings approximately 1.3 mm to 1.5 mm wide; based upon OUL tests, this screening will contain approximately 99% by weight of the activated carbon as supplied by the manufacturer. Insignificant amounts of activated carbon are lost from the samplers unless they are torn in the field or the activated carbon is abraded by being placed in very high velocity waters. These problems are uncommon if field personnel use reasonable care.

Activated carbon samplers are fundamental to most cost-effective groundwater tracing studies.

One manufacturer indicates that activated carbon has a shelf life of five years when stored in sealed containers under cool conditions. The OUL once studied unused activated carbon stored for eleven years under such conditions and found its performance to be equal to newly purchased activated carbon. However, reasonably fresh activated carbon and other tracing chemicals should always be used in professional work.

Activated carbon contains three groups of pores in which adsorption can occur. These are macropores, transitional pores, and micropores. The major portion of the surface area is in micropores and transitional pores; activated carbons produced from coconut shells contain a preponderance of micropores. Based upon OUL tests, activated carbon made from coconut shells appears to be the best carbon for detecting tracer dyes in the low concentrations likely to be encountered under field conditions. Activated carbon made from coal is less effective for dye tracing use under typical conditions. Activated carbon used in water treatment plants will adsorb tracer dyes but is not nearly as effectively as the coconut shell activated carbon that we specify.

The samplers can be placed in springs, surface streams, the flow from pumping wells, and in monitoring wells or any other appropriate points. The effectiveness of the samplers is a combination of their ability to adsorb the particular dyes and to then release the dyes when eluted in the laboratory. Based on OUL data (which are limited for pyranine), the effectiveness of the activated carbon samplers and the OUL elution protocol are ranked as follows:

Effectiveness of Activated Carbon Samplers: Fl > RWT > Eos > SRB > Py. (OUL).

Sampler Placement

The activated carbon samplers adsorb the dyes rather than filtering them out of the passing water. As a result, a sampler placed in swift flowing water will not adsorb appreciably more dye than a sampler placed in slower moving water. The exception to this is when the water being sampled is moving so slowly that water adjacent to the activated carbon is depleted of dye due to adsorption. These conditions commonly occur in monitoring wells which are not being pumped. The OUL samplers are constructed so that the water being sampled has good access to all of the carbon; tightly packed carbon in samplers reduces the total amount of dye adsorbed per unit weight of carbon by minimizing the exchange of water around carbon in the interior of the samplers.

Samplers in streams and springs should be placed where there is a reasonable current. Typical vertical velocity-curves for streams indicate that a sampler placed on the bottom of a stream is likely to encounter velocities about half the mean velocity in the vertical profile at that point in the stream. Avoid settings where the sampler may be unduly battered by swift water, or where debris is likely to accumulate on the sampler. Stream velocities between 0.1 and 1.0 foot per second are the most desirable. Velocities greater than one foot per second may cause abrasion and subsequent loss of some of the activated carbon in the sampler. If the stream or spring has a soft bottom, place the samplers where they are at least a few inches above the soft materials. Figure 3 shows a "gumdrop" sampler made famous by the late Dr. Jim Quinlan. Gumdrop samplers are ideal for channels floored with soft materials. At streams and springs always place at least two independently anchored samplers in the event that one is lost during the sampling period.

In many areas surface streams can readily be sampled at public road crossings. As discussed earlier in conjunction with dye losses in surface water, there is no standard interval between sampling stations on surface streams. However, spacing sampling stations more than about a mile apart should be avoided as much as possible. Fluorescent dyes from vehicle coolants may enter streams at or near road crossings; one can test for this by placing sampling stations both upstream and downstream of the point at which the road drainage enters the stream.

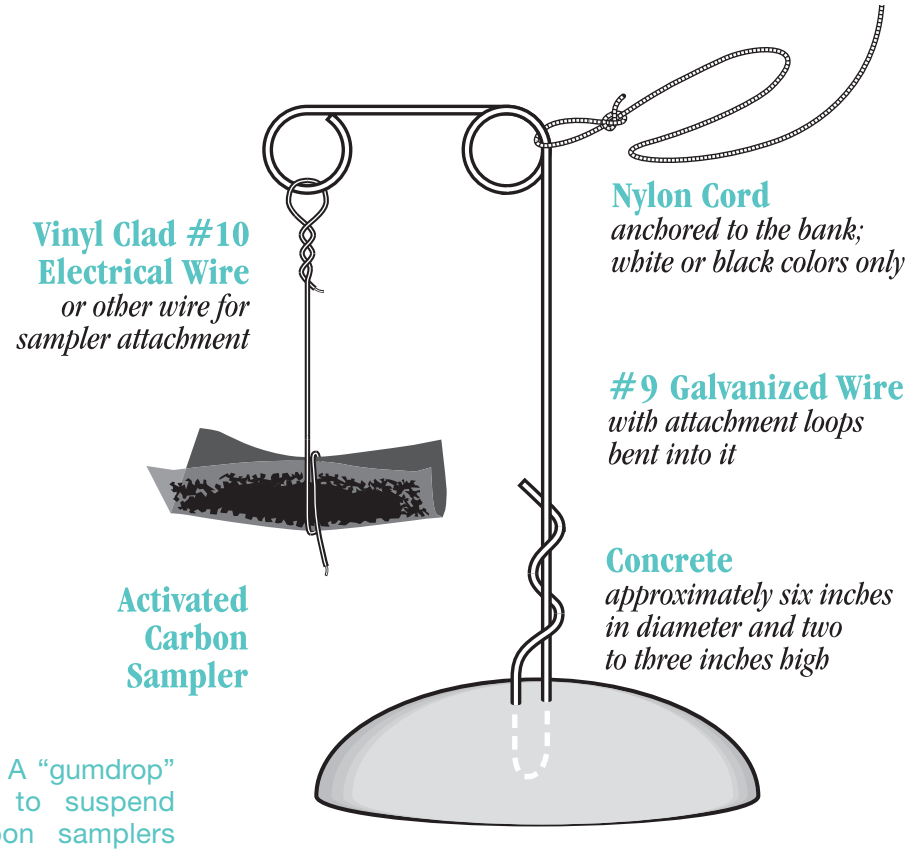


Figure 3 A "gumdrop" sampler used to suspend activated carbon samplers above the stream bed.

Pumping wells can be monitored with activated carbon samplers by placing a sampler in a trickle of water derived from the well. A flow rate of one gallon per minute is routinely adequate if the water is continuously in contact with the activated carbon sampler. Sampler holders made of plastic pipe fittings are ideal and can be attached to a hose bib. At residential wells one can sample from outside hydrants and not need access to the home. One must ensure that the samplers are always completely in the water being sampled. This can be accomplished by connecting a hose to the downstream end of the sampler holder and insuring that a portion of the hose is higher than the sampler.

Some arid urban areas regulate lawn watering dates and times. There are programmable devices which one can use to set the times and durations of water flow from a hydrant. These devices are useful when an individual is unwilling to have his well run water continuously for sampling because of low yield or other considerations.

Sampling of residential wells is sometimes conducted by placing activated carbon samplers in toilet reservoirs. The amount of water passing through a toilet tank is limited. The toilet reservoir sampling strategy is not generally recommended since it is likely to miss relatively small concentrations of dye. Furthermore, people sometimes place toilet cleanser compounds in the toilet reservoirs; some of these contain dyes which may interfere with the sampling.

Monitoring wells can be sampled by attaching an activated carbon sampler to the top of a disposable bailer with a clear or white plastic cable tie. The sampler is then lowered to the center of the screened interval or to the middle of the saturated zone of the well. The well does not need to be pumped or purged. Activated carbon samplers to be used in monitoring wells should be treated as follows prior to use to prevent adding small amounts of activated carbon powder to the well. Add 25 samplers to a bucket containing one gallon of distilled or de-ionized (DI) water, slosh them around, and let stand for 10 minutes. Discard the dirty water, add a second gallon of clean distilled or DI water, slosh, and let stand for 10 minutes. Repeat the procedure with a third gallon of water. Store the samplers wet and in a plastic bag under refrigeration; use them within a week.

How Quantitative Are the Samplers?

Aley (2017) compared the concentrations of dyes in carbon sampler elutants that had been in place for durations of 6 to 8 days with mean dye concentrations in water samples from the same sampling locations collected at the beginning and end of the 6 to 8 day periods. The dye concentration in the elutant from the carbon samplers, divided by the mean water concentration, equaled a dye "Accumulation Factor". The study included only fluorescein, eosine, and rhodamine WT dyes; it included data for 281 sampling periods in springs and 383 sampling periods in monitoring wells. The data were from numerous studies at multiple sites. The weighted mean Accumulation Factor for carbon samplers in springs was 445; the median was 311. The weighted mean Accumulation Factor for carbon samplers in monitoring wells was 166 and the median was 14. Short-duration pulses of dye at sampling stations (especially in wells) could be responsible for some very high Accumulation Factor values and the observation that mean Accumulation Factors were higher than median values.

The longer an activated carbon sampler is in place the slower the rate at which it will adsorb tracer dyes. One sampling approach sometimes used in OUL-directed traces is to conduct more intensive sampling in the first week after dye introduction. If Day 0 is the date of dye introduction, samplers are collected for the periods from 0 to 1 day, 1 to 2 days, 2 to 4 days, and from 4 to 7 days after dye introduction. Comparison samplers are placed at the same stations for the entire seven day period. We routinely find that the sum of the dye concentrations from the short duration samplers is greater than the dye concentration in the sampler in place for the entire week. There are several possible explanations for this finding; all of these possibilities may be involved to some extent. One possibility is that, with time, the dye in the charcoal sampler migrates to activation sites where it is more tightly bonded and less effectively removed by elution. The second possibility is that, with time, the amount of adsorptive area decreases and additional dye is adsorbed more slowly; this is an important consideration when sampling waters contaminated with compounds which may be adsorbed onto the activated carbon. Another possibility is that, through time, desorption or biological decomposition of the dyes occurs.

In many traces we have analyzed 5% duplicate activated carbon samplers and calculated Relative Percent Difference (RPD) values. The RPD value equals the difference between the two concentrations divided by the mean of the two concentrations. The duplicate activated carbon analysis essentially never encounters dye in one sampler and not in the other

unless the dye detected in the positive sampler is very close to the detection limit. Typical RPD values for activated carbon samplers are:

Eosine = 29 to 36%

Fluorescein = 26 to 34%

Rhodamine WT = 37 to 49%

Sulforhodamine B = 35 to 46%

Pyranine = 34 to 47%

How Often Should Samplers Be Changed?

Part of the answer to this question is dependent upon the questions being addressed by the study. Frequent changing of samplers is needed if time of travel is an important consideration. In some cases the sampling frequency can be more intensive early in the trace and less intensive after the dyes have been detected. If one wishes to compare dye concentrations among the sampling periods ideally they should all be of similar length. An acceptable alternate approach is to normalize the concentration results by dividing them by the number of days the sampler was in place and then making the comparisons. When there is intensive sampling during the early part of the trace we commonly have some samplers at all stations which are in place for the typical sampling period and other samplers at those stations which are in place for the shorter sampling periods.

In most cases collection of activated carbon samplers and placement of new samplers approximately once per week is logistically feasible and is adequate to insure that the samplers are effective in adsorbing and retaining tracer dyes throughout the entire sampling period. Longer sampling intervals may be appropriate for tracing in remote areas where the water is of good quality. Intervals of less than once per week may be prudent at some heavily contaminated sampling stations, especially if rhodamine WT or eosine dyes are being used. It has been our experience that these dyes are more sensitive to decreased adsorption rates with increased exposure time of the samplers than is the case for fluorescein. We have no data on the response of sulforhodamine B and pyranine. In one study we found that activated carbon samplers in a stream heavily contaminated with discharge from a chicken processing plant lost much of the rhodamine WT they had adsorbed (but little if any of the fluorescein) if they were left in place in the stream for more than a few hours.

Sampling interval is an important study design parameter. Weekly intervals are often appropriate with activated carbon samplers.

The sampling interval should not be so long that most of the detectable dye might discharge during a single sampling period. Confidence in results is enhanced by having multiple positive dye recoveries from every positive dye recovery site. Furthermore, samplers will sometimes be lost even if (as we recommend) two or more are placed at each spring or stream sampling station. In general, the longer the sampling interval the more serious is the loss of a set of samplers.

It is a poor protocol to place a substantial number of samplers at a sampling station and then recover one or more of them each time the station is visited during the study. The longer the sampler is in place the less effective it is in adsorbing and retaining tracer dyes. The proper protocol is to collect used samplers and place new samplers each time the station is visited.

When Do Samplers Miss Dyes?

When the samplers are in pumping wells, springs, streams, or similar moving water settings the answer is essentially never. It is water samples, rather than carbon samplers, that are most likely to miss detecting small concentrations of tracer dyes. Even in monitoring wells carbon samplers are less likely to miss detecting dye than is the case for water samples. This

assumes that samplers in appreciably contaminated waters are changed, and new samplers placed, at least once per week.

Aley (2017) compared the frequency with which tracer dyes were detected in carbon samplers with the frequency of detections in grab samples of water. Water samples were analyzed for both the beginning and the end of the carbon sampler placement periods and dye from on-going groundwater traces was detected in one or both of the water samples and/or in the associated carbon sampler. Based on 1002 sampling periods at springs, dye was detectable in 98.9% of the carbon samplers but in only 44.3% of the associated water samples. Based on 939 sampling period at monitoring wells, dye was detectable in 95.7% of the carbon samplers but in only 80.9% of the water samples. Results for eosine, fluorescein, rhodamine WT, and sulforhodamine B were similar. The difference in results between springs and monitoring wells is largely a reflection of the extent of water movement within the monitoring wells and how minimal water movement decreases the ability of the carbon to adsorb tracer dyes. If the water column in monitoring wells were agitated dye concentrations on carbon samplers would undoubtedly increase.

When feasible, it is a good idea to analyze both carbon and water samples from wells that experience very low water level recovery after pumping. This helps ensure that small concentrations of dye are not missed.

Maintaining the Integrity of Samplers

Other than following good sampling and custody protocols, the following steps will help maintain the integrity of activated carbon samplers:

1. Label the outside of sample bags; do not put any labels inside. Do not use any colored pen or marker such as a "Sharpie" (which has some of the tracer dyes in the ink). Black Sharpies are good for labelling; we have not tested other brands of black marker pens.
2. Keep the collected samplers under refrigeration until analysis if the tracing work is associated with a sensitive issue or regulatory concerns. The activated carbon provides a substrate for biological growth; this is minimized if the samplers are refrigerated. Biological growths have the potential to degrade, diminish, or destroy tracer dyes. Unrefrigerated samplers are accepted at the OUL and they routinely yield adequate and credible data.
3. Use cold packs such as "Blue Ice" to keep samples cool during field work and sample shipping. Do not use real ice since it will melt and could cross contaminate samples. We recommend shipping samplers by second day air in the USA and Canada. Provide adequate packing so that the "Blue Ice" packages will not slide around and potentially crush activated carbon samplers or vials of water. Do not use "Green Ice"; it contains tracer dyes. Do not use colored Styrofoam peanuts for packing.
4. Do not wash samplers in chlorinated tap water. Studies by the OUL of paired activated carbon samplers has shown appreciable dye losses (and in a few cases total dye loss) when activated carbon is treated with chlorinated water.
5. The issue of sampler custody may arise, especially in court testimony. Samplers are often left in place and out of the custody of the investigator for several days at a time; this can open the possibility that someone might have tampered with the sampler. If water samples are collected each time a sampling station is visited, the investigator has custody of them and can use data from their analysis to conclude that the results from the activated carbon samplers are or are not consistent with the results from the water samples.

There are five important steps in maintaining the integrity of activated carbon samplers.

Water Samples

We recommend that investigators using our services consider collecting 50 ml. vials of water each time they visit a sampling station. The OUL provides appropriate disposable vials which have been randomly tested (1% tested) to demonstrate that they are free of any fluorescent compounds which could interfere with tracing work. The samples should be kept under refrigeration and in total darkness, and are shipped to the OUL with the activated carbon samplers. There is no need to collect the samples in amber bottles so long as the samples are always kept in total darkness. The water samples provide data on dye concentrations in the water being tested at a known point in time. In contrast, the activated carbon samplers are the best way of determining whether or not any dye has reached the sampling station at any time during the sampling period.

The OUL does not charge for archiving water samples collected concurrently with activated carbon samplers. In many cases the only water samples analyzed are those from stations where one or more of the tracer dyes are detected in activated carbon samplers. In some cases it is desirable to analyze both activated carbon samplers and water samples for monitoring wells which may receive only very small concentrations of dye.

Frequent collection of water samples can be done with programmable automatic pumped water samplers (such as those manufactured by ISCO). Dye concentrations from these instruments do not generally degrade appreciably between instrument visits even though the water may be held in the instrument unrefrigerated for several days at a time. One can periodically collect and refrigerate grab samples of water from the sampling station for analysis and comparison with the water collected by the automatic sampler. Another approach is to leave part of the last sample collected in the sampler until the next time the sampler is visited. The difference in dye concentration in this sample between the first and second visit provides a measure of the amount of dye deterioration. Dye deterioration in such instruments is enhanced by the total exposure time, by summer temperatures, by organic matter in the water, and by water containing large numbers of bacteria.

One potential stability problem with water samples is that the tracer dyes (and especially rhodamine WT) can be metabolized by *Pseudomonas* sp. and especially by *Pseudomonas fluorescens*. These bacteria are almost ubiquitous in hospital and laboratory distilled water systems, and they undoubtedly exist in some natural waters as well. While there are no established holding times for dye samples (either in water or in charcoal samplers), refrigeration of samples and analysis as soon as possible is clearly advisable.

In some tracing studies we have analyzed replicate samples of water for tracer dyes and have calculated RPD values (explained earlier in the section on the quantitative nature of activated carbon samplers). Many of the samples were from monitoring wells and had very small dye concentrations. Small dye concentrations tend to yield higher RPD values than do larger concentrations. Typical RPD values for water samples are:

Eosine = 3.0 to 4.5%

Fluorescein = 1.7 to 2.7%

Rhodamine WT = 4.5 to 6.0%

Sulforhodamine B = 4.2 to 5.5%

Pyranine = 4.1 to 5.4%

DYE ANALYSIS

All activated carbon and water samples shipped to the OUL are analyzed on one of our spectrofluorophotometers operated under a synchronous scan protocol. The protocol is detailed in a procedures and criteria document which is available from the OUL; this document is periodically updated and revised as necessary. The following is a general discussion of methods, but does not include the detail found in the Procedures and Criteria document.

Sampler Washing

Activated carbon samplers are washed at the OUL in strong jets of dye-free unchlorinated water prior to being eluted. The purpose of the washing is to remove sediment and organic matter from the samplers. These materials interfere with fluorescence analysis by decreasing light penetration into the resulting elutant and by increasing background fluorescence. Background fluorescence typically decreases with increases in the emission wavelength. Decreases in light penetration into unwashed samples containing sediments tend to be independent of the emission wavelength. As a result, unwashed samples generally cause a higher percentage of interference with dyes in the longer emission wavelengths (characteristic of rhodamine WT and sulforhodamine B) than in the shorter wavelengths (characteristic of pyranine, fluorescein, and eosine).

Increased amounts of plant materials and humic and fulvic compounds in unwashed activated carbon samplers tend to impact pyranine, fluorescein, and eosine analysis more than rhodamine WT and sulforhodamine B analysis. Elution times in excess of one hour (especially in unwashed samples) also tend to increase the background fluorescence due to plant materials and humic and fulvic compounds.

Our experience and studies convince us that only unchlorinated waters should be used to wash activated carbon samplers. The OUL uses unchlorinated water from a dolomite aquifer for the sampler washing. Sodium hypochlorite solutions are routinely used to destroy tracer dyes in laboratory cleanup work. Furthermore, chlorination of public drinking water supplies routinely removes most or all tracer dyes (such as those derived from wellhead delineation studies) present in such waters.

The OUL conducted a study to assess the potential impact of chlorine residual in wash water on dye concentrations in activated carbon samplers. Paired charcoal samplers known to contain various concentrations of tracer dyes were tested. One packet was washed in unchlorinated well water from a karst aquifer **(Untreated Sample)**. The paired packet was washed in the unchlorinated well water then emptied into a beaker and allowed to stand for five minutes in 15 ml of a water solution containing 4 part per million (ppm) sodium hypochlorite **(Treated Sample)**. The chlorine residual in drinking water typically contains 0.4 ppm chlorine or less. After treatment, the charcoal samplers were eluted and analyzed following the normal OUL protocol. The treated and untreated samples were then compared; the results are shown in Table 10.

Large volumes of reagent water are needed for washing samplers. This water should be free of chlorine.

Table 10 Tracer dye losses from activated carbon samplers treated with a 4 ppm sodium hypochlorite solution.

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 dye concentration (ppb)	67.8	59.8	55.0
Minimum dye concentration tested (ppb)	2.67	0.29	2.57

Note: Dye loss percentages = Untreated Sample Concentration minus Treated Sample Concentration divided by Untreated Sample Concentration

The OUL study indicates that sodium hypochlorite solutions destroy some (and sometimes all) of three tracer dyes adsorbed on activated carbon samplers. The chlorine residual at a particular tap drawing water from a public water supply can vary substantially from time to time. In view of these findings and conditions, the use of chlorinated tap water for washing activated carbon samplers is at best an undesirable analytical methodology. It should be noted that there are papers in the literature which indicate that the destructive effects of chlorine on tracer dyes may be less than we found in our limited study, yet these other studies did not test the impacts on dyes adsorbed on activated carbon samplers.

To the best of our knowledge, the OUL is the only tracer dye analysis laboratory which uses only water which has never been chlorinated. At least one other laboratory dechlorinates wash water prior to use. Some laboratories using chlorinated tap water dry their samplers in ovens after washing and prior to elution. It seems possible that this protocol might increase the destruction of tracer dyes. OUL clients should not pre-wash samplers with municipal water prior to shipment for analysis since such waters could destroy adsorbed dyes. Furthermore, some municipal waters could contain dyes or fluorescently similar compounds.

Sampler Elution

The solution which the OUL uses to elute all five of the tracer dyes from activated carbon is a mixture of 5% aqua ammonia and 95% isopropyl alcohol solution and sufficient potassium hydroxide flakes to saturate the solution. The isopropyl alcohol is 70% alcohol and 30% water. The aqua ammonia solution is 29% ammonia. 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.

There are many different solutions used for eluting dyes from activated carbon. Most of these are some mixture of alcohol, water, and a strong base. The most commonly used alcohols are isopropyl, ethyl, and 1-propanol. The most commonly used bases are ammonia hydroxide and potassium hydroxide. Evaluations of various eluents have been the topics of masters thesis projects and other experiments. Some laboratories (including the OUL) elute samples while they are still wet from being washed. Others dry the samplers in ovens prior to adding the eluent.

The "ideal" eluent is a function of the dyes being used, the types of hydrological and biological conditions under which the tracing is typically done, and a host of practical considerations. The OUL eluent was selected for our work because it:

- Will elute all five dyes.
- Minimizes fluorescence peaks from natural and man-made compounds in or near the emission fluorescence range of the five dyes.

- Has proven effective over a broad range of hydrogeologic and biologic conditions.
- Provides effective elution in a reasonable period of time (one hour).
- Does not create fumes noxious to personnel or damaging to laboratory instruments.

Water Samples

The fluorescence intensity of several of the commonly used fluorescent tracer dyes is pH dependent. Table 11, adapted from Aley (2019), illustrates the impact of low pH values on the fluorescence intensity of the five commonly used dyes. The pH of water samples analyzed for fluorescein, eosine, and pyranine dyes are adjusted to a pH of greater than 9.5 prior to analysis in order to obtain maximum fluorescence intensities. The pH adjustment is achieved by placing water samples in uncapped 50 ml vials in a high ammonia atmosphere for at least two hours in order to increase the pH of the sample. This does not change the volume of the sample. Reagent water standards are placed in the same atmosphere as the samples. If dye concentrations in a sample are off-scale and require dilution for quantification of the dye concentration, the diluting water used is OUL reagent water that has been pH adjusted in a high ammonia atmosphere.

Table 11 Reduction of fluorescence intensity of dyes in water as a function of pH.

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	3.5	2.0

Table 5 (presented earlier) characterized the percent of various dyes lost to various mineral and organic materials when in aqueous suspensions. In view of these data, one must recognize that some of the dye in a turbid sample may become adsorbed onto the materials responsible for the turbidity and thus be undetectable in the water sample.

We routinely use one or more of the following three approaches for the analysis of noticeably turbid samples. First, much of the turbidity may settle out of a water sample if it is allowed to sit undisturbed for a day or two in the refrigerator prior to analysis. Secondly, samples can be centrifuged prior to analysis. In most cases we find that five minutes at 5,000 revolutions per minute is adequate to reduce most excessive turbidity. The third approach is to dilute the sample prior to analysis. This latter approach is also useful for samples which have high color due to constituents other than one of the tracer dyes.

Analytical Instruments

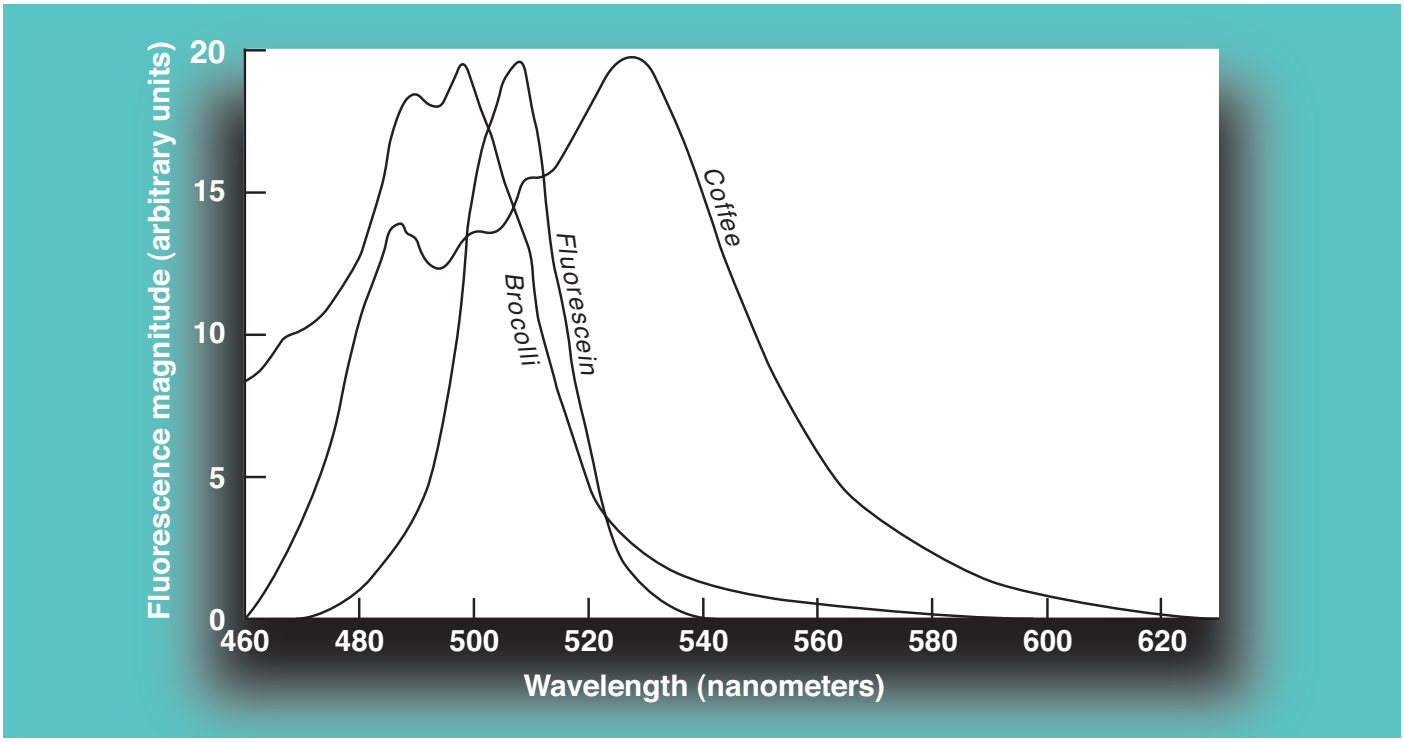
Filter fluorometers and spectrofluorophotometers are the two types of laboratory instruments most commonly used for detecting the five fluorescent dyes. These are not comparable instruments. Some recording field instruments exist that electronically replicate the abilities of filter fluorometers to detect and quantify specific tracer dyes. Since they pick only a single point on the fluorescence peak they have similar limitations to those discussed below for filter fluorometers.

Filter fluorometers and spectrofluorophotometers are not comparable instruments.

The filter fluorometer uses optical filters to limit the wavelengths of light which excite fluorescence in the sample and which are emitted from the sample. Filter fluorometers yield a single number as a fluorescence intensity value. The filter fluorometer is useful if there is little or no change in the background fluorescence during the entire sampling period, and if there are no pulses of fluorescent materials other than dyes. In our experience, many sampling stations show one to two orders of magnitude fluctuations in background fluorescence over periods as short as a few hours. Changes in sample turbidity can also dramatically alter fluorescence intensity. Finally, the filter fluorometer cannot separate tracer dyes from many other fluorescent compounds.

Figure 4 shows fluorescence peaks associated with fluorescein in elutant, brewed coffee, and water in which broccoli has been cooked; these graphs were developed with a spectrofluorophotometer operated under a synchronous scan protocol. A filter fluorometer could not identify which (if any) of these three peaks is due to fluorescein dye. In this case the filter fluorometer can only demonstrate that the fluorescence intensity has changed in or near the wavelength ranges in which the fluorescein excitation and emission are maximized. Coffee and broccoli water are only two of the many materials which can yield fluorescence peaks which could be mistaken for one of the five tracer dyes if analysis work is done with a filter fluorometer.

Figure 4 Fluorescence peaks associated with fluorescein dye, brewed coffee, and water in which broccoli has been cooked.



Filter fluorometers are appropriate instruments for some studies. They have the advantage of being rugged and some are well designed for field use. The credibility of results from filter fluorometers can be enhanced by analyzing some samples with both a filter fluorometer and with a spectrofluorophotometer operated with an appropriate protocol.

All dye analysis work done by the OUL uses a spectrofluorophotometer operated under a synchronous scan protocol. Fluorescence excitation and emission wavelength slits are set to specified widths and the bandwidth between the excitation and emission wavelengths are set at a constant value. This bandwidth separation is designed to be at or near the wavelength difference between the maximum excitation and maximum emission wavelengths of the dyes for which one is sampling. The sample is then synchronously scanned over a typical range of about 170 nm, and the intensity of emission fluorescence is then printed out as a graph. The curves in Figure 4 are examples of synchronous scans with a spectrofluorophotometer.

Instrumental Analysis of Samples

In 1986 the OUL began conducting all dye analysis work with a spectrofluorophotometer operated with a synchronous scan protocol. We immediately found the results to be extremely valuable in tracing work which required rigorous data capable of withstanding technical challenges. The OUL owns several spectrofluorophotometers. Instrumental settings for analysis vary slightly among the instruments; values for the RF 5301 (the most extensively used instrument for information in our data base) are shown in Table 12.

Table 12 Standard OUL settings for Shimadzu RF 5301 for different types of samples.

Sample Type	Dyes	Excitation Slit (nm)	Emission Slit (nm)	Bandwidth Separation (nm)
Water	Eos, Fl, RWT, SRB	5	3	17
Water	Py	5	3	35
Carbon elutant	Eos, Fl, RWT, SRB	3	1.5	17
Carbon elutant	Py	3	1.5	35

The wider the excitation and emission wavelength slits the larger the resulting fluorescence intensity and the lower the detection limit. However, the ability to discriminate between different fluorescent compounds decreases as the slit widths are increased. One must never assume that the best analytical protocol is necessarily the one with the lowest reported detection limit. A common approach for establishing detection limits is to spike laboratory samples with known concentrations of the tracer dye, and to conclude that the detection limit is the dye concentration which yields an instrument response three times the signal to noise ratio of the instrument. Total reliance on this approach ignores the effects of other fluorescent materials routinely encountered in actual field samples. The detection limits used by the OUL have been adjusted to approximate the minimum concentration of the dye credibly detectable in actual field samples.

The wavelength of the emission fluorescence peak for a dye in a particular matrix is diagnostic in determining that the peak is in fact due to a particular dye. Peak wavelengths vary somewhat due to the electronics and optics in the instrument, environmental factors which have acted upon the dyes, and other factors. Peak wavelength differences due to dye concentrations are small, but the wavelengths tend to decrease slightly as the dye concentrations decrease. We have used our data base of successful traces to calculate "acceptable wavelength ranges" for the tracer dyes in the water and elutant matrixes. The acceptable wavelength range is the mean value plus and minus two standard deviations.

Never assume that the best analytical protocol is necessarily the one with the lowest reported detection limit.

Table 13 shows acceptable wavelength ranges for the five dyes discussed in this handbook in both water and the standard OUL eluent. The values are specific to the settings in Table 12, to the OUL RF 5301, and to the OUL eluent. Method detection limits for each dye mixture in each matrix are also indicated in the table; note that the values vary with the matrix. All water samples are pH adjusted to greater than 9.5 prior to analysis.

Table 13 Normal OUL emission peak wavelength ranges and method detection limits for Shimadzu RF 5301 Spectrofluorophotometer. All concentrations are based on the dye mixtures routinely used by the OUL.

Fluorescent Dye Mixture	Normal Acceptable Emission Wavelength Range (nm)		Detection Limit (ppb)	
	Elutant	Water	Elutant	Water
Eosine	539.3 to 545.1	532.5 to 537.0	0.050	0.015
Fluorescein	514.1 to 519.2	505.9 to 509.7	0.025	0.002
Rhodamine WT	564.6 to 571.2	571.9 to 577.2	0.170	0.015
Sulforhodamine B	575.2 to 582.0	580.1 to 583.7	0.080	0.008
Pyranine	502.6 to 508.6	497.7 to 503.7	0.015	0.010

* Fluorescein and eosine detection limits in water are based on samples pH adjusted to greater than 9.5.

Emission wavelength peaks tend to become slightly shorter as dye concentrations decrease. For example, based upon the OUL data base, the mean emission fluorescence peak for fluorescein in the standard OUL eluent decreases by less than 0.9 nanometers with a four orders of magnitude change in the dye concentration.

Several computer programs can be used to analyze complex analytical graphs. Using these programs individual peaks consistent with various fluorescent dyes can be derived from a fluorescence intensity graph. These programs are sometimes valuable, but must be used with extreme care to prevent false positives for some of the tracer dyes. The risk of false positives is increased by samples which may contain dyes with emission fluorescence peaks relatively close to one another (such as rhodamine WT and sulforhodamine B), or by samples that may contain dyes which have yielded fluorescence peaks that differ substantially in size. Waters impacted by urban runoff or industrial activities are often subject to pulses of fluorescent compounds which can be credibly separated from tracer dyes only if the number of dyes used concurrently is limited and if the fluorescence characteristics of the dyes selected have been carefully considered. The use of too many dyes (especially in urban or industrial areas) with the presumption that they can be credibly separated and detected through the use of peak separation programs is at best risky.

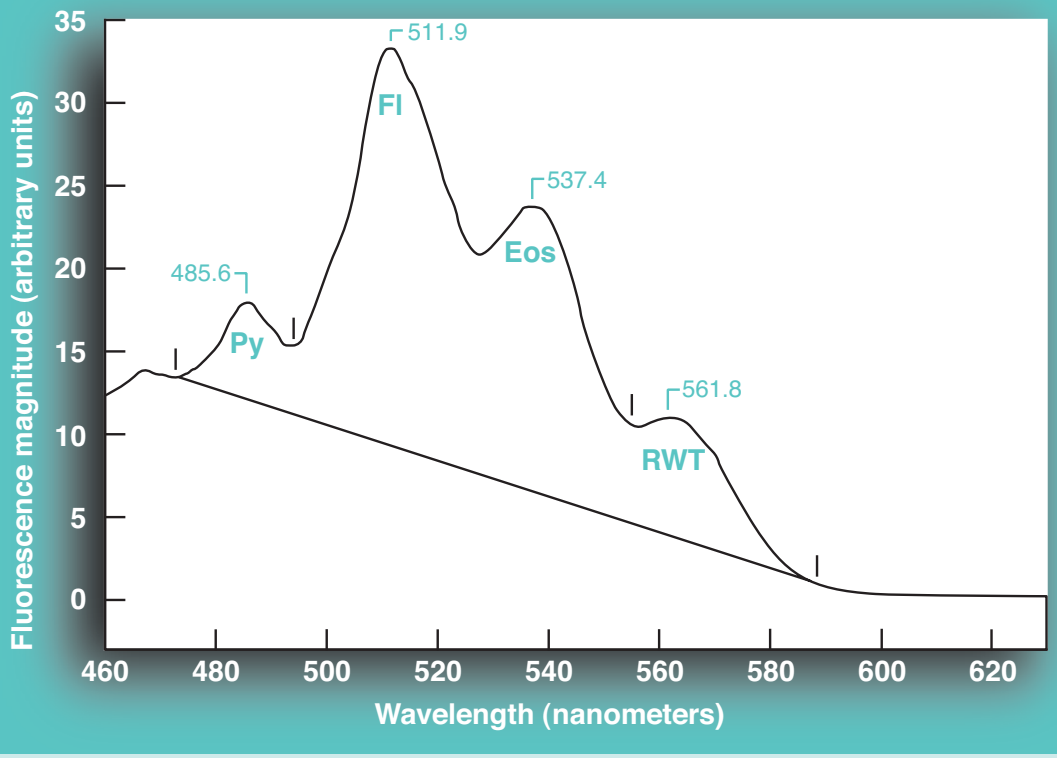
OUL Software and Data Output

The OUL has developed and uses extensive proprietary software which ensures that:

- All instrument settings are correct for all samples.
- Dye concentrations are automatically and accurately calculated from daily standards.
- Data are stored in a manner where they can be quickly retrieved, sorted, and laser printed by project and by sampling station. Figure 5 shows a graph of a sample analyzed on a Shimadzu RF5000U Spectrofluorophotometer with the OUL software. Figure 6 shows a four-per-page print of sample results from a sampling station.

Analytical results from all samples are stored on disk; backed up on additional disks; and subjected to daily, weekly, and monthly saves on magnetic tape. Hard copies of the data are stored in a building separate from the laboratory, and all backup tapes are stored in a fire-proof safe. A copy of the most current weekly backup tape is stored off-site.

Figure 5 Analytical graph of an activated carbon sampler elutant containing fluorescence peaks from pyranine, fluorescein, eosine, and rhodamine WT



Station 8: Tsultan Rising

OUL number: H1996
Date placed: 03-24-1998
Time placed: 1420

Charcoal Analyzed: 04-15-1998
Date recovered: 04-02-1998
Time recovered: 1330

Peaks within normal range of tracer dyes:

Peak nm	Left X	Right X	Height	Area	H/A	Conc.
511.9	494.0	528.2	24.17	539.32	0.04	4.64
537.4	528.2	555.2	17.36	350.95	0.05	11.2
561.8	555.2	588.4	7.12	135.54	0.05	34.1
Peaks close to normal range of tracer dyes:						
485.6	472.8	494.0	5.95	73.97	0.08	3.23

Note: This was a sample from a groundwater tracing study in British Columbia, Canada. The pyranine peak is at 485.6 nm since the bandwidth separation for this sample was 17 nm rather than the 35 nm specified in Table 12.

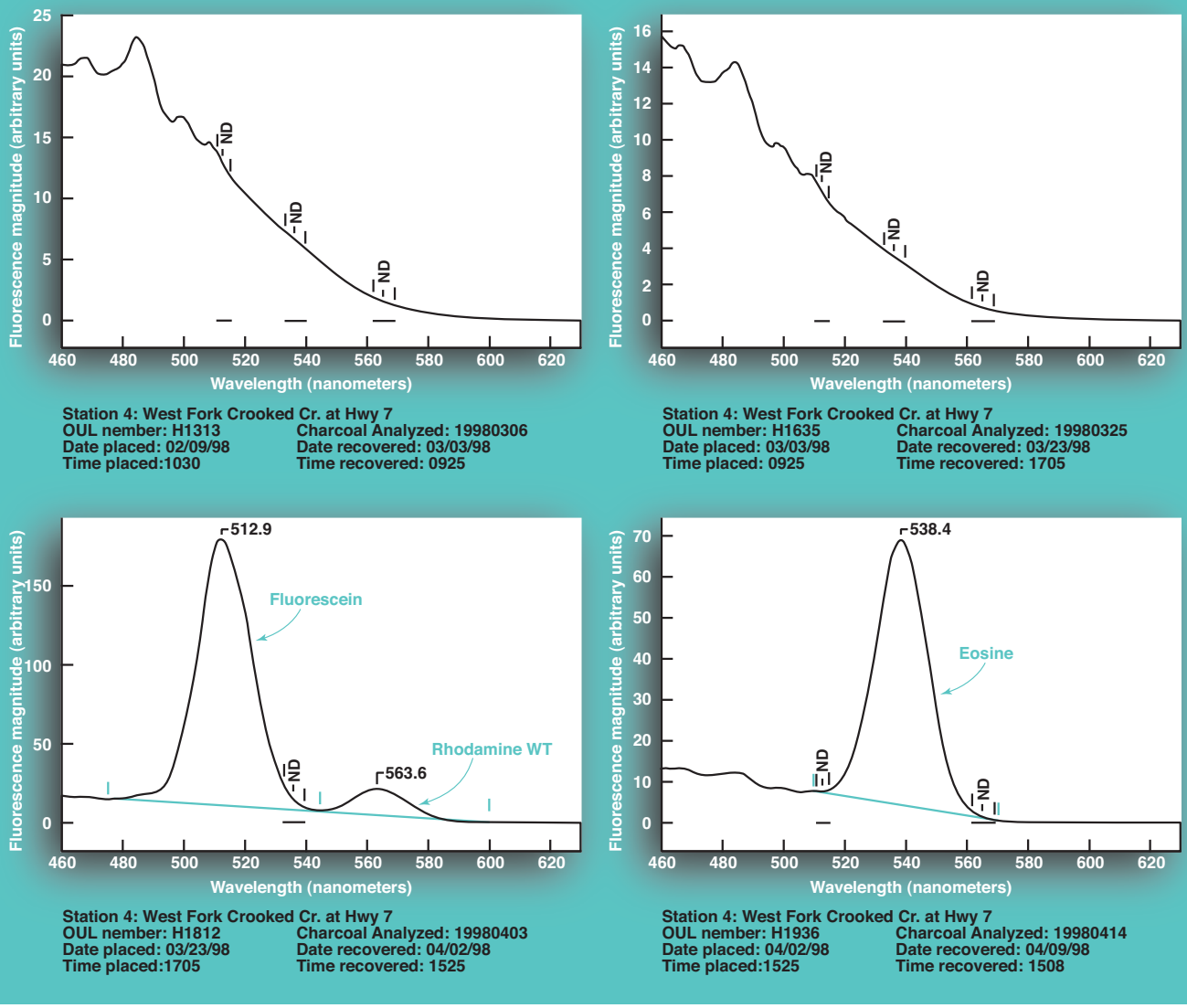
Degradation of Dyes and Resulting Changes in Emission Wavelength Ranges

Rhodamine WT and sulforhodamine B dyes in groundwater systems are both occasionally subject to alteration through a process known as deaminoalkylation (Kass, 1998). The result is that emission fluorescence peaks grow shorter with time even though well shaped fluorescence peaks still persist in samples. This can sometimes occur within a period of a few weeks, yet in most cases is minor in extent and does not occur even when the dye mixture has been in a groundwater system for several years.

The wavelength of eosine dye peaks can also shorten with time within some groundwater systems. This likely occurs under reducing conditions. A bench test was conducted by the OUL where solutions of eosine, fluorescein, rhodamine WT, and sulforhodamine B in water were spiked with zero valent iron (ZVI). ZVI is sometimes used as an in situ treatment agent

at waste sites. In this test 50 ml of 138 μg/L eosine dye solution in water was placed on top of 5 grams of ZVI. Samples of water were periodically tested for dye concentrations and for changes in the peak emission wavelengths. After 48 hours the emission peak wavelength had declined to 0.8 nm shorter than the normal wavelength range for eosine in water. After 168 hours the peak wavelength had declined to 9.2 nm shorter than the normal wavelength range and the dye concentration had decreased to 2% of the initial concentration. Sulforhodamine B dye also experience approximately 98% destruction by the ZVI in 168 hours, but the emission wavelength remained within the normal range. Both fluorescein and rhodamine WT dyes were reasonably stable during the test period.

Figure 6 Four-per-page graphs of activated carbon sampler elutants from a sampling station used in a groundwater tracing study in Arkansas.



Station 4: West Fork Crooked Cr. at Hwy 7
OUL nember: H1313 Charcoal Analyzed: 19980306
Date placed: 02/09/98 Date recovered: 03/03/98
Time placed:1030 Time recovered: 0925

Station 4: West Fork Crooked Cr. at Hwy 7
OUL nember: H1635 Charcoal Analyzed: 19980325
Date placed: 03/03/98 Date recovered: 03/23/98
Time placed: 0925 Time recovered: 1705

Station 4: West Fork Crooked Cr. at Hwy 7
OUL nember: H1812 Charcoal Analyzed: 19980403
Date placed: 03/23/98 Date recovered: 04/02/98
Time placed:1705 Time recovered: 1525

Station 4: West Fork Crooked Cr. at Hwy 7
OUL nember: H1936 Charcoal Analyzed: 19980414
Date placed: 04/02/98 Date recovered: 04/09/98
Time placed:1525 Time recovered: 1508

Note: The top two graphs show no detectable dyes. The graph at bottom left shows fluorescein and rhodamine WT. The graph at bottom right shows eosine only. Please note the different scales on the Y-axis.

DESIGNING EFFECTIVE GROUNDWATER TRACING STUDIES

We have made a list of 12 Rules to Dye By.

"Rules to Dye By"

1. Successful groundwater tracing can be conducted in a wide range of hydrogeologic settings. Its utility is not confined to well developed karst aquifers. In mining situations pay special attention to the pH of the water. Bench tests of dye performance under the conditions to be encountered are strongly recommended.

2. Background sampling and quantitative analysis of the samples is an important component of most professional-grade groundwater traces. Most tracing investigations should have at least two rounds of background sampling prior to any dye introduction. The workplan for the study should allow the project manager to change the type and quantity of dyes based upon the results of background sampling.

3. In most cases fluorescein is the most effective groundwater tracing dye. Eosine and rhodamine WT are commonly very effective and can be used concurrently with fluorescein in many cases. To the extent feasible, if these three dyes are used concurrently, fluorescein should be used for the longest trace or the trace likely to encounter the most adsorptive surfaces. Rhodamine WT should be used for the shortest trace or the one likely to encounter the least adsorptive surfaces. There may be problem interference between fluorescein and eosine if the size of the fluorescence peak of one exceeds that of the other by more than two orders of magnitude. Interference between eosine and rhodamine WT can be a problem if the size of the fluorescence peak of one exceeds that of the other by more than three orders of magnitude. Note that these generalizations are based upon the size of the fluorescence peaks rather than the concentrations of the dyes.

4. Use enough dye, enough water, and dyes which are appropriate to the conditions likely to be encountered. There are no general equations which will give you these values. Except for travel distances of only a few feet one seldom detects most of the introduced dye due to processes that include diffusion into the aquifer matrix, adsorption, and biological destruction. "Enough dye" is a function of the type of dye, the type of dye introduction point, characteristics of the aquifer, the nature of sampling stations, the type of sampling conducted, and the effectiveness of adsorption along the flow routes to be traversed by the dye. "Enough water" may be controlled by the nature of the study or by logistics; try to use as much as is reasonably possible.

5. Use dye introduction points which are appropriate to the questions to be addressed by the investigation. Utilize monitoring wells for dye introduction only when they are clearly appropriate.

6. Sample all the points at which the dye might discharge; if you don't sample you don't know. Do thorough field work prior to dye introduction. Don't just sample monitoring wells if you need to assess off-site migration. Groundwater discharge may occur at obscured points in stream channels; sample the stream at appropriate intervals and always have a control point upstream of any point to which your dye might discharge. Sample all of the monitoring wells; reality may or may not fit the site model, and knowing where dyes were not detected can be valuable information.

7. Sample for an adequate period of time. One approach for dealing with sampling duration is to recognize that tracer dyes are most effective in assessing preferential flow routes. Such flow routes provide relatively rapid water and dye transport; if such routes exist between the dye introduction point and sampling stations then one should be able to estimate that one or more dye recoveries should occur prior to the end of a projected study period. Failure to recover the dye in that period should be viewed as evidence that the hypothetical preferential flow routes either do not exist or else are not integrated into a preferential flow system.

8. For most studies, primary reliance should be on sampling with activated carbon samplers and secondary reliance on grab samples of water. The use of both kinds of samples should be considered and will often enhance the value of the investigation. Samplers must actually be in the water in order to sample it; sloppy sampling by those who are not willing to get wet or dirty in the field is the bane of dye tracing studies. When dealing with springs, never assume that nearby springs (even those only a few feet apart) are receiving waters from identical areas.

9. Tracer studies relying primarily or exclusively on water samples are more likely to not detect dye at all locations to which the dye moves than is the case for sampling based on activated carbon samplers. As a result, tracer studies relying solely on water samples are likely to inadequately characterize the aquifer. This short-coming can be overcome by using several times more dye for traces based on sampling with water samples than is needed for traces based on activated carbon sampling. The same failure to detect problems exists for field fluorometers.

   In a karst aquifer with an appreciable diffuse flow component White et al. (2015) detected eosine in water samples from 15 of the 30 sampling stations where this dye was detected in carbon samplers; in water samples fluorescein was detected from 11 of the 15 sampling stations where this dye was detected in carbon samplers, and with rhodamine WT dye in water samples rhodamine WT was not detected at any of the 11 sampling stations where this dye was detected in carbon samplers. The result was that sampling based exclusively on water samples would have failed to identify more than half of the positive dye detection locations.

10. Collect samplers and place new samplers at intervals frequent enough to ensure that dyes are not missed and that most or all of the dye recovered at a sampling station is not limited to only one sampling period. In most cases weekly intervals are adequate; more frequent sampling can be desirable during the first week or two after dye introduction. Consistent sampling intervals during a study are often desirable.

11. Good analysis for the tracer dyes is essential for professional groundwater tracing. In most cases this means analysis by a spectrofluorophotometer operated under a synchronous scan protocol. The study must establish credible detection thresholds for the various dyes based upon the analytical instrument, field experience, and site-specific background sampling. Detection thresholds should be neither too high nor too low. When water samples are analyzed for fluorescein or eosine the pH should be adjusted to 9.5 of greater to ensure accurate dye concentrations values.

12. Groundwater tracing is a bit like surgery. It is fundamentally simple, yet most patients (clients) would hope that the person doing the work has experience. We are always happy to answer questions about dye tracing and to design a groundwater tracing program for you or help you design one. We have experience from over 4,000 successful groundwater traces; please call on us.

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