



2013

Kentucky Geological Survey Procedures for Groundwater Tracing Using Fluorescent Dyes

James C. Currens

University of Kentucky, currens@uky.edu

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Follow this and additional works at: https://uknowledge.uky.edu/kgs_ic

 Part of the [Hydrology Commons](#)

Repository Citation

Currens, James C., "Kentucky Geological Survey Procedures for Groundwater Tracing Using Fluorescent Dyes" (2013). *Kentucky Geological Survey Information Circular*. 17.

https://uknowledge.uky.edu/kgs_ic/17

This Report is brought to you for free and open access by the Kentucky Geological Survey at UKnowledge. It has been accepted for inclusion in Kentucky Geological Survey Information Circular by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

Kentucky Geological Survey
James C. Cobb, State Geologist and Director
University of Kentucky, Lexington

Kentucky Geological Survey Procedures for Groundwater Tracing Using Fluorescent Dyes

James C. Currens

Dr. Quinlan's First Principle for Groundwater Tracing in Karst:

"Dye-tests should be designed so that there is always a positive result – somewhere."

Alexander and Quinlan (1996)

Our Mission

Our mission is to increase knowledge and understanding of the mineral, energy, and water resources, geologic hazards, and geology of Kentucky for the benefit of the Commonwealth and Nation.

Earth Resources—Our Common Wealth

www.uky.edu/kgs

Technical Level



ISSN 0075-5583

Contents

Introduction	1
A Philosophy to Dye By	1
A Cautionary Note.....	2
Qualitative Tracing Protocol	2
Inform Regulatory Agencies and Other Researchers	2
Expendable Equipment and Supplies.....	2
Construction of Gumdrops.....	3
Construction of Receptors (Bugs).....	3
Quality Control and Quality Assurance.....	4
Initial Field Work	4
Scouting.....	4
Deploy Gumdrops and Receptors.....	5
Logistical Considerations.....	5
Conducting a Trace.....	6
Calculating the Mass of Tracer to Use	6
Handling Tracer in the Field	7
Servicing Receptors	8
Determination of Results	9
Prepare Receptors	9
Analyze Receptors	9
Operation of Fluorometers	9
Criteria for Determination of Tracer Recovery in Passive Activated-Carbon Receptors..	10
Recording and Documenting Results	10
Quantitative Tracing Protocol.....	10
Inform Regulatory Agencies and Other Researchers	11
Discharge Measurement Is Essential.....	11
Selecting Tracer Recovery Equipment.....	11
Selecting a Fluorometer.....	11
Deploying an Autosampler	11
More Programming Considerations	12
Prepare Quantitative Tracer Aliquots.....	13
Conduct the Trace.....	13
Analyze the Concentration and Calculate the Relevant Parameters.....	13
Calculate the Percent Recovery of the Tracer, the Time of Travel, and the Mean Velocity.....	15
Additional Thoughts on Qualitative Groundwater Dye Tracing	16
Summary	18
Selected Bibliography	19
Appendix A: Characteristics of Commonly Used Tracers.....	20
Appendix B: Tracer Mass Calculation Spreadsheet.....	22
Appendix C: Matrix for Estimating Mass of Groundwater Tracer for Connectivity in Karst (10 ppb)	23
Appendix D: Matrix for Estimating Mass of Groundwater Tracer for Connectivity in Karst (100 ppb)	24
Appendix E: Matrix for Estimating Mass of Groundwater Tracer for Time of Travel in Karst (10 ppb)	25
Appendix F: Matrix for Estimating Mass of Groundwater Tracer for Time of Travel in Karst (100 ppb)	26

Contents (Continued)

Appendix G: University of Kentucky, Kentucky Geological Survey, Groundwater Trace Monitoring and Analysis Data Report.....	27
Appendix H: Groundwater Trace Injection Report Form.....	28
Appendix I: Definitions.....	29

Figures

1. Photograph of a gumdrop dye receptor anchor.....	3
2. Example of a simple activated-charcoal bug	4
3. Illustration of packaging dye for transport in the same vehicle as dye receptors.....	5
4. Photograph of Sulforhodamine B being poured into a sinking stream.....	7
5. Textbook example of Sodium Fluorescein injection into a sinking stream	7
6. Photograph of Optical brightener (Tinopal CBS-X) being poured into a sinking stream cave entrance	8
7. Photograph of introduction of a quantitative trace into an inundated swallow hole	14
8. Photograph of automatic water-sampling machines set up to collect two 24-bottle series of samples.....	14
9. Photograph of analog filter fluorometer.....	14
10. Photograph of synchronous scan fluorescence spectrophotometer	15
11. Photograph of submersible fluorometer with 30 m of cable	16

Tables

1. Organic dyes used as tracers by the Kentucky Geological Survey.....	6
2. Serial dilution for Rhodamine WT to produce a 100 µg/L working solution	15

Kentucky Geological Survey Procedures for Groundwater Tracing Using Fluorescent Dyes

James C. Currens

Introduction

Karst terrain often develops from an ancestral landscape of surface-flowing streams, which leaves behind a relict pattern of the surface watershed divides. If caves only developed in ancestral watersheds, then groundwater tracing, for the purpose of groundwater basin mapping, would be unnecessary. But lithologic, structural, and hydrologic factors conspire to ensure that some caves extend headward faster than their neighbors and encroach upon adjacent groundwater basins to pirate drainage under the original surface divides. In many areas, groundwater basin boundaries have been significantly reorganized, to the point that there is little relationship to the ancestral surface watershed boundaries.

Groundwater dye tracing is a powerful hydrogeologic tool for resolving these ambiguities when used in well-planned experiments. Like any powerful tool, it also has its pitfalls, however, and when misapplied can cause considerable problems. These protocols standardize the methods used by the Kentucky Geological Survey for groundwater tracing with fluorescent organic dyes. Although the methods prescribed in this document are rigorous, they are nevertheless necessary to assure the accuracy of the groundwater tracing data gathered by KGS.

A Philosophy to Dye By

Historically, groundwater tracing has been conducted with a variety of substances varying in sophistication from wheat chaff to radioactive isotopes. The most commonly used tracers are fluorescent dyes (Alexander and Quinlan, 1996), and this report is concerned only with them. A full description of many types of tracers may be found in Davis and others (1985). There are few articles in

scientific journals describing qualitative groundwater techniques, the repeatability of the results, or the analytical and quality-control techniques. Many case histories and methodologies are available in speleological journals and agency reports, however. These protocols are the nuts and bolts of conducting successful and defensible qualitative traces. The guidelines that follow promote an intuitive understanding of how to apply the techniques and a sense of what the techniques can and cannot do.

Organic fluorescent dyes were developed for commercial applications (only Rhodamine WT was designed for water tracing) and were subsequently applied to groundwater problems by various researchers beginning in the early 1900's. The ideal groundwater tracer is soluble in cold water, is nontoxic to plants, animals, and people, does not occur in the natural environment, is not present as a pollutant, does not degrade over the duration of the trace, is easily detected at low concentrations, is imperceptible downstream of the resurgence, does not adsorb onto the aquifer substrate, does not have to be continuously monitored, and is inexpensive. Such a tracer does not exist, but several fluorescent dyes come close. Researchers are continually trying other dyes, with varying degrees of success. Appendix A summarizes the characteristics of six dyes commonly used for several types of tracing problems. If you use another tracer, above all else, be certain the tracer is safe (nontoxic) for your intended purpose.

Qualitative groundwater tracing methods are widely accepted by the karst hydrology community. Some researchers have criticized qualitative groundwater tracing (Field, 2003), whereas other practitioners defend it vigorously (Worthington and Smart, 2003). In my experience, groundwater dye tracing is highly efficient and reliable. Qualita-

tive tracing is the appropriate tool for determining point-to-point connectivity. Quantitative tracing is appropriate when time of travel must be accurately determined, when hydrograph separation analysis must be conducted, or when conduit hydraulics must be determined for a flow path that is already known. Quantitative tracing requires considerable logistics, is labor intensive, and has its own vulnerabilities. The process requires both collecting samples and measuring flow past the discharge point. Submersible fluorometers or automatic water samplers are typically deployed at the spring closest to the injection site, along with a stage recorder and equipment to measure discharge. Although the resulting breakthrough curve from analysis of tracer concentration in a series of water samples conclusively shows that the tracer detected is the tracer injected, it is impractical for most reconnaissance studies covering large areas.

Qualitative tracing is the right tool when:

- Connectivity between a swallow hole, or other inflow point, and a spring, or other discharge point, must be determined.
- The watershed for a spring must be mapped.
- A suspected source of contamination must be linked to a spring.
- Suspected leakage from an impoundment must be confirmed.
- An approximate groundwater flow velocity is sufficient.

Quantitative tracing is the right tool when:

- The spring or other potential recovery point is already known.
- The velocity of groundwater flow in conduits or other hydraulic parameters must be precisely measured.
- Various hydraulic coefficients and parameters must be determined.

Negative results at a monitored spring can always be questioned in the absence of corroborating data. There are many possible reasons for a negative result; for example, the dye may be discharged to an unknown spring. In addition to being less defensible, a missed trace is undesirable because the same tracers cannot be used in the same groundwater basin again for a long time or until a major precipitation event occurs. Keep in mind that to bracket a basin boundary with traces, positive re-

sults at surrounding springs are also needed. Although knowing that a trace from a swallow hole was not detected at a specific spring is valuable information, a positive result at another spring will strongly support your conclusion.

A Cautionary Note

The following guidelines are intended for use in Kentucky by the staff of the Kentucky Geological Survey. They have been modified from other sources for the hydrogeology and the logistics of working in Kentucky and are not intended to be comprehensive or universally applicable. Use these guidelines in your project area outside of Kentucky only after thorough review and testing has shown they are appropriate for your area.

Qualitative Tracing Protocol

Before tracer is injected, significant preparation is required. A tracing project will more likely be successful and defensible if you adhere to some basic rules.

Inform Regulatory Agencies and Other Researchers

During the project planning stage, inform local environmental and emergency officials in writing about the planned work. During field work, each tracer injection must also be reported. When tracing is restarted following a significant hiatus, a reminder is highly recommended. Current regulations require submitting your planned tracer injection to the Kentucky Division of Water via an online form at dep.gateway.ky.gov/dyetrace/login.aspx [accessed 01/18/2012].

Expendable Equipment and Supplies

- (1) Use laboratory-grade activated carbon (Fisher Scientific catalog number 05 685A, 6- to 14-mesh) for charcoal receptors.
- (2) Fabric receptors should be either untreated cotton swab or bleached cotton broadcloth test fabric (Testfabrics Inc. catalog number 419).
- (3) Use only laboratory-grade alcohol, basic compounds, and other chemicals, excluding the tracers.
- (4) Use Smart Solution, which is a mixture by volume of 5:2:3 of 1-propanol, concentrated

NH_4OH , and distilled water as the elutant, for all tracers.

- (5) If the initial result for a trace that used Fluorescein or Eosine is a weak positive, then a 5 percent solution of KOH in 70 percent isopropyl (by mass) may be used on residual charcoal to elute more dye. The use of the reserved remainder of the charcoal for this purpose should be carefully considered.
- (6) Purchase groundwater tracers from established chemical suppliers. Specify the Color Index and common name. Also ask the supplier to report the fraction of the product that is active ingredient percentage by mass.
- (7) All other materials are common hardware and general-purpose items.

Construction of Gumdrops

- (1) Gumdrop anchors are preferred, and must be used when the receptor could be buried by sediment. Gumdrops must be made of a dense anchoring material, such as a brick or concrete cast for the purpose. Use the design of Quinlan (1986) (Fig. 1). An eight- to 10-gage, galvanized steel wire should extend vertically from the weight, and have loops bent in it for attaching receptors.
- (2) Keep a supply of gumdrops in the vehicle when scouting or deploying receptors. Keep them away from tracer materials.

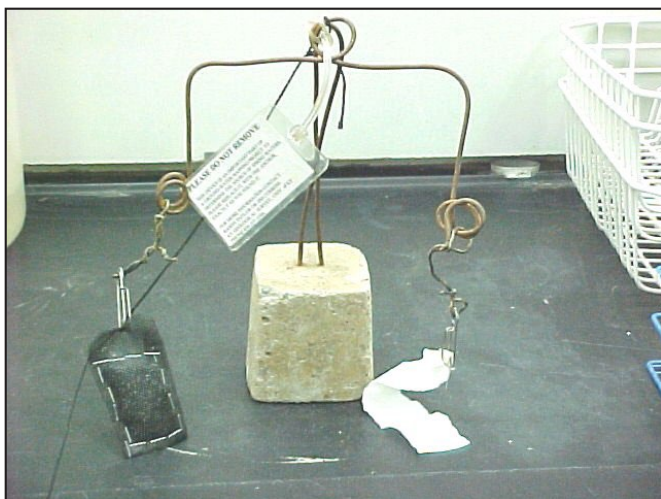


Figure 1. A gumdrop dye receptor anchor complete with a lanyard, an identification tag printed with a “replace as you found it” message, and bugs, both cotton and charcoal. Two steel wires are used to provide attachment points for multiple bugs.

- (3) Field-expedient anchors may be fashioned from material found onsite when hiding the anchor is necessary. They may also be used as temporary anchors when a spring is discovered during reconnaissance and no gumdrop is available.
- (4) Field-expedient anchors must be clean and free of apparent sources of contamination. They should be replaced with a gumdrop as soon as possible if there is a chance they could be buried by sediment.
- (5) Gumdrops must have a lanyard of dull-color nylon cord that will be tied to an immovable object above the estimated greatest flood stage.
- (6) Passive tracer receptors should be attached to the gumdrop with a section of electrical wire or fishing trotline clip, as available.
- (7) Gumdrops that are visible to casual observers must have an identification tag attached that asks that the gumdrop be replaced as it was and provides contact information.

Construction of Receptors (Bugs)

- (1) The envelope for charcoal receptors must be constructed of synthetic window screen. The charcoal compartment should be a flat rectangular packet approximately 3 x 2 in., with a tapered loop extending from one edge for affixing the completed receptor to the gumdrop (Fig. 2).
- (2) The receptor envelope can be assembled by sewing or stapling. Stainless steel or brass staples are preferred.
- (3) The window-screen envelope must be filled with a minimum of 1 teaspoon and a maximum of 3 teaspoons of charcoal (approximate mass of 15 g).
- (4) If broadcloth is used, a “bow tie” is fashioned from a 1.5-in.-wide by 12- to 18-in.-long strip of fabric. A loop is formed in the middle by tying an overhand knot on a bight.
- (5) Place receptors in zippered plastic bags for storage, protection, and transport to and from the field. Each bag may contain two receptors, one of cotton and one of charcoal.
- (6) Put the individual receptor bags in a large zippered plastic bag and the larger bag inside a light-resistant container, such as an ice chest.

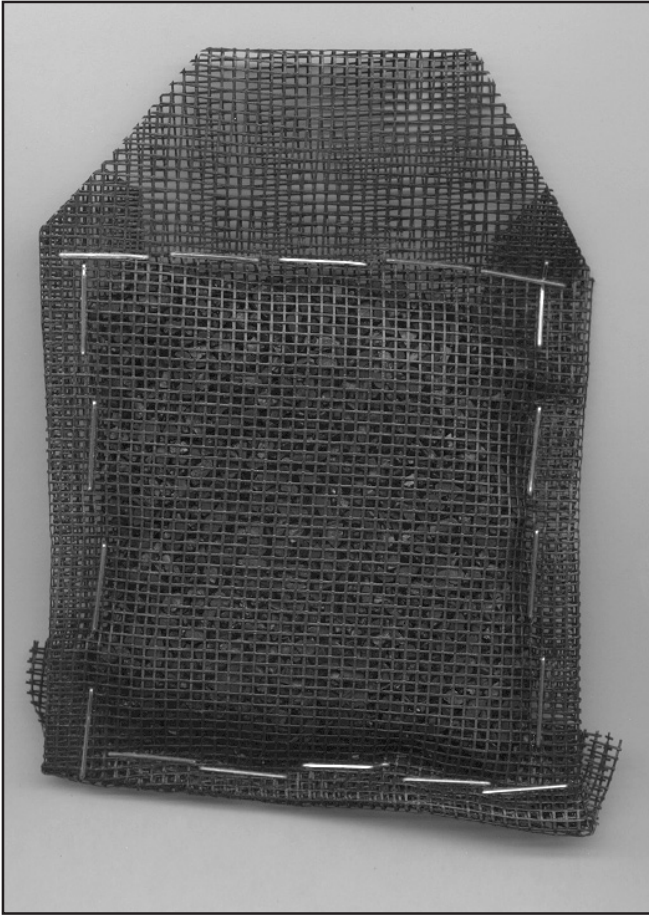


Figure 2. Example of a simple activated-charcoal bug. The taper at the top facilitates attaching the bug to the gumdrop with vinyl-clad multistrand copper wire. Staples should be brass or stainless steel.

- (7) Charcoal receptors may be used in place of cotton fabric receptors, if a scanning spectrophotofluorometer is available and if the tracer has a well-defined narrow range of wavelength for fluorescence emission.

Quality Control and Quality Assurance

- (1) The following types of blank receptors should represent 5 to 10 percent of the receptors deployed over the course of the tracing project. A range is allowed because a trace that is recovered quickly requires fewer receptors, whereas the minimum number of blanks and background receptors is comparatively constant.
 - (a) Trip blanks
 - (b) Background
 - (c) Duplicate
 - (d) Replicates

- (2) Trip blanks are standard receptors (charcoal or cotton) placed without an individual package between the outer bag and the bags with the individually packaged receptors. They are treated and analyzed in the same way as the sample receptors. One trip blank must be used for each field trip in which receptors are recovered.
- (3) Background blanks are standard receptors deployed in the field prior to any tracer injection. They are treated and analyzed in the same way as the sample receptors. One negative background blank should be recovered from each spring prior to initiating a groundwater trace.
- (4) Duplicates are second or third receptors deployed at springs expected to have high rates of human or animal visitation and at all other springs when tampering actually occurs. The duplicate receptor should be well hidden and placed in a different location than the primary receptor.
- (5) Replicates are second or third receptors deployed at springs to provide charcoal for additional analysis. The replicate receptor should be hung with the sample receptor at exactly the same location. As an alternative, a slightly larger envelope may be used for the replicate, provided water circulates freely through the package.
- (6) Duplicates and replicates of traces thought to be positive, and which are not needed to replace damaged or lost receptors, may be submitted to a second laboratory for replicate analysis.
- (7) The presence of fluorescence in the eluent from a charcoal receptor deployed for background determination will exclude the intentional use of the corresponding tracer in that groundwater basin as long as the background presence persists.

Initial Field Work

Scouting

- (1) Conduct a thorough field reconnaissance. The initial search should be by automobile. Springs are easily hidden by vegetation and may not be seen from a vehicle. Search all suspect areas on foot.

- (2) Scout reaches of streams that likely function as local base level in the project area by boat, or when possible, on foot.
- (3) Use topographic and geologic maps and aerial photography to search for springs and swallow holes. Large springs can be located with topographic maps, but these maps are not very useful for finding small springs. Aerial photographs should be used when available, and are the most helpful if taken when vegetation was dormant.
- (4) Record the locations of field sites (springs, swallow holes, sinking streams, and other hydrologically important features) on a form and on either a topographic map or in a Geographic Information System database.
- (5) Ask residents of the study area about the locations of springs; this can save a significant amount of time. Knock on doors, be polite, and fully inform people of what you are doing and why. The source of the water discharging from a karst spring on their property is a great mystery to many people, and they will be more willing to help if you provide them with the results. As a bonus, you will be able to educate them about the methods you are using so they will not be concerned if they see colored water discharging from their springs.

Deploy Gumdrops and Receptors

- (1) All springs in the study area that are likely to receive tracer, and some unlikely resurgences, should be located and bugged.
- (2) Carry receptors and anchors when on reconnaissance trips and set background bugs as new springs are found.
- (3) Deploy the gumdrop in the channel of the spring run so that the dye receptor is totally immersed in the outflow, for maximum exposure to the resurgent tracer.
- (4) Take precautions so that the receptor is not buried by sediment, stranded by low flow, or dislodged by wildlife, livestock, or vandals. If possible, place the receptor in the shade.
- (5) Always place "failsafe" bugs at intervals along base-level streams that are apparent boundaries to unconfined flow.
- (6) Deploy two receptors at springs expected to have high rates of human or animal visitation

and at all other springs if tampering actually occurs.

Logistical Considerations

- (1) Before going to the field, repackage tracers into incremental aliquots in small, sturdy, unbreakable containers. Use half-liter Nalgene bottles for powder and gallon jugs for tracers that are to be mixed in the field. The exterior of all reusable containers must be thoroughly washed after being refilled.
- (2) Use aliquots of tracer packaged in quantities that approximate the mass of tracer desired and that can be composited from the packaged units. Increments of Fluorescein should be 25, 50, 75, 100, and 200 g, for example. This allows for adjustments in tracer mass and minimizes handling of the tracers.
- (3) The tracer packaging should be in four layers (Fig. 3). Put bottles inside a zippered plastic bag, and the bagged bottles inside another zippered plastic bag while wearing disposable gloves. Put the individual packages in a garbage bag and the entire package inside a plastic tub or dedicated ice chest. Several small bottles can be placed in one large zippered plastic bag. Put one pair of disposable gloves inside the garbage bags, and extra pairs in a zippered plastic bag inside the plastic tub.
- (4) *Do not* transport tracer to the field in the bulk shipping container.



Figure 3. Packaging dye for transport in the same vehicle as dye receptors. The hand symbols represent packages of disposable gloves and the green bottle shapes are the dye inside zippered plastic bags. The large bag should be placed in a plastic storage tub. See text for further details.

- (5) Do not open any of the tracer packages while inside the vehicle.
- (6) Tracer and receptors can be transported in the same vehicle without compromising the results, but the practice is discouraged. When transported together to and from the field, both dye and bugs must be carefully packaged, and the vehicle interior must be thoroughly cleaned.
- (7) Vehicles used to transport tracers must be examined with an ultraviolet light before use to verify absence of tracer from door handles, gear shifters, steering wheels, etc. Any surface showing fluorescence must be cleaned with water containing bleach. Keep a record of when the vehicle was cleaned and if any tracer contamination was found with the vehicle mileage log.

Conducting a Trace

Calculating the Mass of Tracer to Use

Choosing the type and correct mass of dye is perhaps the most difficult decision made in groundwater tracing. Table 1 lists the dyes used by the Kentucky Geological Survey. The objective is to produce a concentration at the spring that is invisible to the human eye, yet detectable by instrumentation. A trace that fails because too little tracer was used is nearly as bad as an easily visible trace because of the wasted time, expense, and the recurrence of risk from a second trace. Use enough dye, but try to anticipate the consequences of using too much.

- (1) This protocol uses the equations of Worthington and Smart (2003, p. 287–295). Their algo-

rithms were derived from the correlation of mass injected (as calculated) versus mass recovered for 209 groundwater traces in karst. Mass to inject is based on Sodium Fluorescein.

Qualitative traces (point to point):

$$\text{Eq. 1: } M=19 (\text{LQC})^{0.95}$$

Quantitative traces (time of travel):

$$\text{Eq. 2: } M=0.73 (\text{TQC})^{0.97}$$

Where: M=mass of tracer to use, in grams

L=distance in meters

Q=discharge in m³/sec

T=expected travel time in seconds

C=desired concentration at the spring in g/m³ or ppb.

- (2) An Excel workbook, "Tracer Mass Calculation.xls," has been prepared that uses these equations to produce a matrix for estimating the mass of Fluorescein to use. The goal is to produce a specified peak concentration at the spring in the parts per billion (µg/L or mg/m³) range. Worksheets have been developed for peak concentration at the spring or 10 ppb (0.01 mg/m³) and 100 ppb (0.1 mg/m³). Copies of these spreadsheets are in Appendix B.
- (3) Other worksheets in the workbook are set up to use a second equation for quantitative traces, which estimates an approximate flow velocity from prior qualitative traces. The maximum time of travel is estimated from these data and is used with the discharge to calculate the quantity of tracer to inject.
- (4) If a proposed trace has the possibility of flowing to either a nearby spring or a very distant spring, use the median distance. The intent is to provide enough tracer mass for a positive

Table 1. Organic dyes used as tracers by the Kentucky Geological Survey.

Common Name	Color Index	Color in Solution	Excitation (nm)	Emission (nm)
Fluorescein	Acid Yellow 73	brilliant green	485 ¹	513 ¹
Rhodamine WT	Acid Red 388	vivid orange-pink	558 ¹	565 ¹
Sulforhodamine B	Acid Red 52	vivid orange-pink	548 ¹	578 ¹
Tinopal CBS-X	Fabric Brighter 351	blue-white	360 ²	450 ²
Eosine	Acid Red 87	greenish orange to red	512 ¹	538 ¹
Solophenyl*	Direct Yellow 96	greenish yellow	397	490
Pyranine	D&C Green 8	green	(pH adjusted) 455 ²	501 ¹

¹Aley (1999)

²Ford and Williams (1989)

*Although formally renamed Solophenyl, the older name, Diphenyl Brilliant Flavine 7GFF, is still widely used.

result at the more distant spring while reducing the coloration at the nearer spring, should the trace in fact follow the shorter route.

- (5) When conducting numerous traces in a study area, use alternate dyes for traces that may flow to the same spring (Figs. 4–6). This reduces the chance that residual dye in the system could result in a false positive. In dry weather, this practice shortens the time needed to complete the tests.

Handling Tracer in the Field

- (1) Before injecting *any* tracer, notify the Kentucky Division of Water–Groundwater Branch via fax (502-564-0111), telephone, or e-mail. If by e-mail, send a copy to the project manager at KGS (www.uky.edu/kgs/water). If by telephone, write down who you spoke to, the time, and which tracers were discussed. If someone reports strangely colored water discharging from a spring, Division of Water officials will then call you to determine if it is just a groundwater dye trace before raising an alarm. Agency staff may be aware of other persons conducting dye traces in the watershed, which obviously could result in very confusing results. *If you cause a problem for the water protection agency, they will cause a problem for you!*
- (2) *Do not open* any of the packaging of the tracers while the packages are inside a vehicle.
- (3) Although tracers delivered from the manufacturer as powder may be injected dry, they are much easier to handle if premixed with water.
- (4) Mixing with water onsite can be done by placing the dye in a suitable jug and adding water from the inflow to the sink or a potable source. Any heavy plastic container can be used, but a carboy is preferable for large quantities of tracer. If bleach bottles are used, they must be thoroughly rinsed before use. Do not use milk jugs, because they are too fragile.
- (5) Never inject Solophenyl (Direct Yellow 96) dry. It is difficult to dissolve and will lay in clumps in the stream bed.
- (6) Use boots and gloves that can be easily washed or disposed of.
- (7) Always keep the wind to your back and the tracer container downwind when pouring into the water.
- (8) Potential injection points that can receive natural run-in include sinking streams, swallow holes, dolines, karst windows, caves, and dry (stormwater disposal) wells.
- (9) All of the locations mentioned in (8), and also auger holes and trenches, can be traced with hauled, potable water. Wet any soil or organic material in the injection feature with an absolute minimum of 300 gal of clean, potable water before injection. Flush the tracer with an additional 600 to 2,000 gal.



Figure 4. Sulforhodamine B being poured into a sinking stream in Hardin County. Notice the stance of the researcher above the water, with feet away from the tracer. The wind direction should be toward the camera. Photo by David Lutz.



Figure 5. Textbook example of Sodium Fluorescein injection into a sinking stream. The dark object at the distant terminus of the dye is the swallow hole. Fort Knox Military Reservation. Photo by David Lutz.



Figure 6. Optical brightener (Tinopal CBS-X) being poured into a sinking stream cave entrance, Bourbon County. Photo by J.C. Currens.

- (10) Prior to injecting tracer into trenches or auger holes (epikarstic dye injection points), establish that the excavation will accept inflow. The minimum acceptable rate of infiltration is 1 L/min/m².
 - (11) *Do not inject tracer into a water well without a written memorandum of understanding signed by the well owner stating that tracer concentrations may remain high for an extended period.* Also secure the permission of the Kentucky Division of Water before injecting in any well. The only exception is stormwater disposal dry wells (EPA Class V).
 - (12) Wash your hands thoroughly after handling tracers to avoid cross contamination from door handles and steering wheels, for example.
 - (13) If you have injected tracer, do not touch receptors or the containers they are packaged in until you bathe and change clothes.
- ### Servicing Receptors
- (1) The default schedule for changing receptors is once a week. This is the optimum frequency for logistics, dye adsorption, and the security of the receptors.
 - (2) If a receptor is to be changed more frequently than once every 24 hours, a duplicate receptor should be deployed before the tracer arrives. The duplicate must be left in place until the tracer has passed or changed each week, whichever is sooner.
 - (3) Do *not* leave receptors in the field for longer than 2 weeks. Long deployments make failure of the trace more likely because of degradation, loss, or theft of the receptor.
 - (4) Always replace one set of receptors with another until you are certain the tracer has come through. Continue to exchange receptors until the trace is detected and the concentration has returned to pre-trace levels or the program is discontinued.
 - (5) Many traces take longer than expected and may be lost if you end the experiment prematurely. If the conceptual model does not predict slow travel times, after 6 weeks of negative results the trace may be considered lost.
 - (6) Handle the receptors with clean hands washed immediately before handling the bug, or wear disposable gloves. To protect the receptors from contamination, always place them in a sample bag immediately after removal from the gumdrop. Never lay a receptor on the ground or on top of the gumdrop. They are easily lost when not in a bag.
 - (7) *Carry a clean, empty zippered plastic bag in which to place the old receptor.* Keep a supply of fresh bags if there is any chance of litigation. Otherwise, save the clean bag that the new receptor was taken from and use it for the recovered receptor at the next site. Keep the empty bag closed and do not put anything else in it.
 - (8) Label the exterior of the bag with a waterproof marker. Provide the site name, the date, and the time. Do not insert any other material into the bag with the receptor.

- (9) Use a black permanent marker to label the bags and *do not* use a colored pen.
- (10) Transport receptors inside the individual sample bags placed inside a larger sample bag. Place the large bag inside a lightproof container.
- (11) Do not ice receptors unless there is going to be a 24-hour or longer delay in preparation for analysis. If using water ice to chill receptors, be certain each individual zippered plastic bag is sealed and each large bag is tightly sealed so that meltwater is excluded.
- (12) If traces are to be conducted on the same trip that receptors are changed, *do not* inject any tracer until all receptors have been serviced. The recovered receptors should be completely repackaged and a trip blank included before the tracer is handled. Always have one person handle the tracer and another handle the receptors.

Determination of Results

Prepare Receptors

- (1) If receptors must be stored longer than 24 hours before analysis, keep them on ice or refrigerated. The larger zippered plastic bag must be waterproof if iced. The temperature should be near 5°C.
- (2) To prepare the receptors for analysis, wash the window-screen packet or cotton test fabric strip thoroughly with a high-pressure stream of clean, nonchlorinated water. Do not use chlorinated tap water.
- (3) Do not put the receptors down on a countertop or lay them in a sink. Use latex gloves or wash your hands thoroughly before and after handling receptors. You can place the clean receptors back in the individual zippered plastic bags in which they were delivered.
- (4) Put a tag on the receptors or affix them to a clean sample bag with the identification information clearly recorded. Hang the receptors in a dark room or storage cabinet to air dry. Never allow more than one receptor to be simultaneously involved in relabeling because they may become mixed up.
- (5) Optionally, the receptors can be oven dried to uniform moisture content if long-term storage or semiquantitative comparison of tracer con-

centration is desired. A temperature of 80°C is adequate.

Analyze Receptors

- (1) Open the receptor envelope with a staple puller or cut off a corner with scissors. Pour about half of the charcoal (exactly 5 gm if semiquantitative comparison is desired) into a disposable plastic condiment cup or a glass jar that is designated for the purpose (washed with bleach and thoroughly rinsed).
- (2) Pour enough eluent into the container to cover the charcoal. Use exactly 10 ml if it is important to have a semiquantitative comparison of one receptor to another.
- (3) Allow elution to proceed for no less than 20 minutes and no more than 1 hour. If an approximate breakthrough curve from receptors that have been changed every 30 minutes is needed, the timing of elution must be consistent, and 20 minutes is recommended.
- (4) Gently pour the elutant from the container into a clean, disposable cuvette and analyze.
- (5) All trace receptors collected should be eluted and analyzed. Leaving some of the receptors until a later date is not recommended because there may be more than one positive.
- (6) Confirm all visually positive traces by determining fluorescence of the elutant with a filter fluorometer or scanning spectrophotometer.
- (7) Examine cotton receptors under a handheld ultraviolet light. Use UV-protective eyeglasses.
- (8) It is highly recommended that cotton receptors that are positive by visual inspection be confirmed with a scanning spectrophotometer with a dry-sample adapter. Analyzing an activated carbon receptor that was deployed in place of another deployment of the cotton is acceptable.
- (9) Record all visually positive traces and those detected by fluorometer on the Groundwater Trace Analysis Report.

Operation of Fluorometers

- (1) Operate the Varian Cary Eclipse scanning spectrophotometer according to the manufacturer's hardware and software operating manuals dated May 2000 and February 2000, respectively.

- (2) Operate the Turner Designs SCUFA (Self-Contained Underwater Fluorescence Apparatus) filter fluorometer according to the manufacturer's user's manual dated April 15, 2000.
- (3) Operate the Turner Designs model 10-070A water-resistant, field-portable filter fluorometer according to the manufacturer's Operating and Service Manual dated October 1981.
- (4) Calibrate the instruments used in a project according to the manufacturer's instructions at the beginning of its usage period.
- (5) Battery-powered equipment should have fresh batteries installed before calibration when possible, and if depleted by the calibration process, replace batteries immediately before deployment.
- (6) Print the analytical report for archiving when possible. Keep a digital copy of the report files permanently on a suitable storage medium, and on the hard drive of a dedicated computer.
- (7) Complete the Groundwater Trace Analysis Report, which is the official record of the trace. Retain the printouts from the instruments and the archived remainder of the charcoal and cloth receptors for 3 years or until after the end of the project, when a final report is drafted.
- (4) A positive trace must have the minimum fluorescence equivalent of 1 ppb concentration and at least 10 times the ambient background fluorescence intensity.
- (5) The tracer should be present in more than one receptor for the trace to be considered positive. Exception may be made for receptors that are deployed in poorly accessible locations, such as underground, for longer than the standard 1 week. An exception may also be made for trace distances less than 1,000 m.
- (6) After a trace to a spring has been conducted, wait until a negative receptor has been recovered from that spring before using the same tracer again in the basin.

Criteria for Determination of Tracer Recovery in Passive Activated-Carbon Receptors

- (1) A spectrophotometer emission scan must show a peak at the appropriate wavelength of the injected dye in the sample matrix. The pH of the matrix affects the wavelength within a known and predictable range. Deviation from the ideal wavelength of ± 2 nm is acceptable.
- (2) The spectrophotometer scan must reveal a peak with the same curve shape as standard solutions of the tracer, or a composite of several known tracers.
- (3) The tracer should be absent from all background receptors collected at likely resurgent springs before the injection. If repeated use of a tracer is unavoidable, the receptors must have known and predictable background fluorescence intensity.

Recording and Documenting Results

- (1) Keep the receptors in a secure room, preferably in a locked cabinet, when they are not in the possession of someone who will sign or has signed the chain of custody bug sheet.
- (2) The attached receptor recovery sheet functions as a chain-of-custody form. Spaces are provided for the signatures of the persons relinquishing and receiving the receptors. If the same person is performing more than one task, that person will sign at all of the lines.
- (3) When the receptors are received, the person making the delivery signs the relinquishing custody line and the receiving person signs the appropriate line. Ask the person making the delivery if he or she wants a photocopy as a receipt.
- (4) If the receptors are to be analyzed by another staff person, the person who received the receptors should have the receiving staff person sign the appropriate line. Give a photocopy of the form to the person making the delivery.
- (5) When the analysis of the listed receptors is complete, indicate the results on the Groundwater Trace Analysis Report and sign the line attesting to the findings. Make a photocopy for the GIS database manager, and deliver the original to the person conducting the trace.

Quantitative Tracing Protocol

Most of the precautions discussed under **Qualitative Groundwater Tracing** can also be applied to quantitative work. It is assumed that the

researcher knows from previous qualitative work where the trace is going to go. Quality control and quality assurance are just as important for quantitative tracings as for qualitative, perhaps more so.

Inform Regulatory Agencies and Other Researchers

Just as with qualitative work, inform local environmental and emergency officials in writing about the planned project. Current regulations require submitting your planned tracer injection to the Kentucky Division of Water via an online form at dep.gateway.ky.gov/dyetrace/login.aspx [accessed 06/08/2012].

Discharge Measurement Is Essential

- (1) Discharge measurement is essential to accurately calculate the center of mass used to determine time of travel. Although a semiquantitative trace can be useful, the resulting time of travel determined from first arrival or peak concentration can be significantly different from the center-of-mass time of travel.
- (2) Discharge can be obtained in several ways.
 - (a) Use an existing gaging station for which a rating curve has already been established.
 - (b) Make a series of discharge measurements during the tracer recovery.
 - (c) Install a staff gage and make discharge measurements at a range of stage depths. Use these data to develop a rating curve so that wading is not necessary to manually obtain the discharge during the trace.
 - (d) Record the stage continuously by installing a stage recorder that is correlated with the staff gage.
 - (e) Install a discharge control structure (flume or weir) with appropriate stage recorder. If personnel are not able to monitor the tracer recovery, (d) or (e) is compulsory.
- (3) Correlate the concentration of the recovered tracer with the total discharge between each time period. For example, the discharge is determined every 10 minutes, but tracer concentration is recorded every 30 minutes. The three interim discharge measurements following determination of the dye concentration would

be summed and the total discharge during the 30-minute interval would be multiplied by the prevailing concentration. If making discharge measurements manually, collect a sample at the beginning of each discharge measurement as well as a final sample.

Selecting Tracer Recovery Equipment

- (1) In addition to the discharge, the prevailing tracer concentration must be determined. This can be accomplished by collecting samples or can be observed in the field directly with portable fluorometers.
- (2) Containers for tracers should be glass bottles or culture tubes, regardless of sampling method.
- (3) Keep water samples containing tracer out of direct sunlight, preferably on ice in an ice chest. Close the caps securely when using ice, and lay the bottles on their side.

Selecting a Fluorometer

- (1) A fluorometer capable of determining the concentration of the tracer is essential for this work.
 - (a) Most fluorometers require tracer concentration standards to be mixed, with which the machine is calibrated. See the discussion on mixing standards.
 - (b) Most field-deployable fluorometers are filter fluorometers, and they can be configured for only one dye at a time.
- (2) Decide if you will use a field fluorometer or an autosampler.
- (3) Portable fluorometers are available that use a flow-through cell inserted into the machine. The other type is a submersible fluorometer.
- (4) If multiple dyes are being used simultaneously or sequentially in rapid succession, a scanning fluorometer is essential, which dictates that water samples be collected.

Deploying an Autosampler

- (1) There are two common and several less common makes of autosamplers, and it is possible to build your own.
- (2) When you have obtained an autosampler, it should first be set up in the laboratory and tested. Any worn or broken parts should be replaced, desiccant pack refreshed, and bat-

teries tested for amperage and for the ability to sustain a charge if set to be triggered by a rise in stage.

- (3) Practice programming the machine. The most common source of failure to collect samples is a programming error, followed by jamming of the distributor and holes in the intake hose.
- (4) Thoroughly clean the hoses and bottles in soapy water. Rinse with a dilute solution of household bleach followed by complete rinsing with distilled water.
- (5) When deploying, consider the following precautions:
 - (a) Choose a location for the machine that is above base-flow elevation as high as the machine will pump, typically 20 feet above the intake.
 - (b) Choose a location that is at least partly obscured by underbrush or other cover and that has a tree or other anchor point to which the machine can be chained.
 - (c) Test the machine by collecting a manual sample and advancing the distributor to the last bottle. Be certain that the distributor will clear the mouths of the sample bottles *all the way around*.
 - (d) If using a water-level trigger of any type, make sure the machine turns on when the trigger is touched by water.
 - (e) Be certain the clocks on the autosampler, field fluorometer, stage recorder, and wristwatches are all synchronized. Havoc will ensue if the dye injection was recorded in daylight-saving time while the sampler and stage recorder are on standard time.
 - (f) Calibrate the sample volume; do not depend on the autovolume functions, but use them if the existing calibration is grossly incorrect. If insufficient sample is collected, check the intake hose for cuts and pinholes. If too much sample is collected, you may have entered the wrong hose dimensions or bottle type.
 - (g) Repeat calibration until the desired volume is obtained. When calibrating, keep the autosampler pump at the same elevation as when the machine is deployed.
 - (h) After calibration and testing are complete, turn the machine off and replace the battery with a completely charged one. Use a lead-acid battery if the machine will be deployed for a week or more between inspections. Nickel-cadmium batteries have a tendency to discharge spontaneously, but when fresh have a higher amp/hour rating than lead-acid batteries.
 - (i) After restoring power, press "exit program" and "start sampling." Enter a brief interval to start time. When the start time arrives, if an actuator is being used, some indication should be displayed that the machine is disabled. Use the latch function on the water sensor.
 - (j) Cover the pump controller, fasten any security cables, and run a chain or wire rope through the handles, around the anchor point, and meet the ends at the padlock, along with the security cables.

More Programming Considerations

- (1) Larger volumes of sample require either larger sample bottles (plastic 1 liter for ISCO brand) or multiplexing. Multiplexing is the use of more than one bottle for a sample, or alternately, more than one sample in a single bottle.
- (2) The more bottles multiplexed per sample, the fewer samples that can be collected for a given period. Avoid multiplexing for quantitative tracing.
- (3) The first time a quantitative trace is attempted, estimate the travel time using results from any qualitative tracing results available. Sampling should be timed to begin at about half of the expected travel time. By spacing samples a half hour to one hour apart, the tracer is more likely to be sampled, which provides data to plan more precise experiments later.
- (4) If more than one quantitative trace is planned, subsequent sampling intervals should be shorter. Shorter sampling intervals significantly increase the detail and the accuracy of dye-recovery calculations.
- (5) Construct a chart of travel time/stage or travel time/discharge rating curve for the quanti-

tative traces. Use the chart for programming the start time and the sample intervals on the autosampler. When enough data to construct such a rating curve have been measured, the task is nearly finished, in any case.

- (6) The ideal sampling sequence would begin about three sampling intervals before the tracer arrives, and the sampling interval would match the period on the stage recorder.
- (7) If only one attempt is possible, consider using multiple autosamplers. For example, one sampler could be set to sample hourly, so as to have a high probability of bracketing the tracer breakthrough curve. A second sampler could be programmed to sample at a shorter interval, which is the best estimate of the travel time. Alternatively, two or more samplers could be programmed to sample in sequence at shorter intervals for however long necessary to assure the breakthrough of tracer is covered and that the available equipment will permit. If one of the short-interval samplers fails, the data from a widely spaced interval sampler could provide some backup. Widely spaced data may be all that is needed to define the slope of the breakthrough curve on the trailing side of the peak concentration.

Prepare Quantitative Tracer Aliquots

- (1) Determine which tracers are to be used. Be aware that many tracers have bulking agents (commonly cornstarch) mixed with the active ingredient, and the percent mass of the components is not readily available.
- (2) Determine the percent active ingredient or plan to treat samples as if the mass injected is 100 percent active ingredient. Rhodamine WT is 20 percent by volume, at a specific gravity of 1.19 g/cm³ (varies from 1.1 to 1.2). Use Fluorescein (Uranine) as if it is 100 percent active ingredient. Treat Eosine and Sulforhodamine B the same way. If both the standard and the samples are treated as 100 percent active ingredient, the dye recovery percentage and the arrival time of the center of mass will be the same.
- (3) Calculate the mass to be injected based on the expected discharge during the recovery period. The goal is to minimize the visibility

of the tracer, yet have sufficient concentration so that the falling limb of the breakthrough curve is distinct. See Appendices D and E.

- (4) Always know exactly the mass of the tracer actually injected. If conducting a constant-rate injection, collect a sample of the tracer as it is introduced into the groundwater.

Conduct the Trace

- (1) After the recovery location is identified and all necessary equipment is in place, verify that the clocks are synchronized and the sampler or fluorometer is properly programmed.
- (2) Set the delayed start time of an autosampler to allow enough time for personnel to travel to the injection site plus half of the expected travel time of the tracer. Program the sample collection interval to be in multiples of the stage recording interval.
- (3) Have personnel and equipment on hand to measure discharge, or apply the previously developed rating equation to the stage data being recorded.
- (4) Travel to the injection site and introduce the tracer (Fig. 7). Be careful to keep powdered tracers downwind. Hold the mouth of the container near the water surface. Rinse the bottle with water and pour any residue into the sinking flow as quickly as possible. Keep in mind that the percentage of dye recovered is valuable information in addition to the center of mass.
- (5) Put the empty bottle and used gloves in a bag, and place the bag in the larger dye container. Save the bottle for reuse. Return to the tracer recovery site at the earliest opportunity.
- (6) Upon arrival at the recovery site, verify that the sampler or fluorometer and stage recorder are running (Fig. 8). If time permits, wait until colored water arrives to be certain the sampler has come on in time.

Analyze the Concentration and Calculate the Relevant Parameters

- (1) Standards are required for all fluorometers that are used to measure the concentration of fluorescent tracers (Figs. 9–10). Mixing standards is not difficult, but is time-consuming, and blunders are possible.



Figure 7. Introduction of a quantitative trace into an inundated swallow hole, Hardin County. The water was over 5 m deep and the boat was positioned using landmarks. A garden hose was inserted into the swallow hole, and a funnel was used to pour the Rhodamine WT into the hose. The total head in the swallow hole was less than at the surface, and the dye was quickly drawn down the hose. Photo by David Lutz.

- (2) Determine if the tracer you are using is 100 percent active ingredient or something else. If it is less than pure, calculate the grams per kilogram of actual dye and prepare your dilution series using that mass of active ingredient. Alternatively, prepare the standards as if the tracer is 100 percent active ingredient.
- (3) Keep one factory-packaged container for quantitative traces only. Mix the contents of

the factory container thoroughly each time the initial quantity of tracer is obtained, but if using Rhodamine WT, let the container be still long enough for foam to dissipate.

- (4) The most difficult step is obtaining the pipet of concentrated Rhodamine WT from the original container. Use a to-detai pipet with white graduations. A pipet with multiple graduations is easier to use than a single-volume pipet at this point in the process. Be as accurate as possible. Blow out the residual dye in the pipet, but do not rinse it into the mixing flask.



Figure 8. Automatic water-sampling machines set up to collect two 24-bottle series of samples. Photo by J.C. Currens.



Figure 9. Analog filter fluorometer. The instrument shown can be configured for Fluorescein and Rhodamine WT. Photo by J.C. Currens.



Figure 10. Synchronous scan fluorescence spectrophotometer. The system is entirely digital. Photo by J.C. Currens.

- (5) Use amber glass bottles for all standards. The bottles should be 2 L or larger for the first, second, and third dilution and decreasing in volume to 250 ml for the lesser concentrations, except that the 1 $\mu\text{g}/\text{L}$ concentration will require a 1 L bottle.
- (6) A dilution of 100 $\mu\text{g}/\text{L}$ is the working solution. It may require a 4 L bottle. This aliquot can last a long time and be the source of all subsequent series for that tracer.
- (7) Organic fluorescent dyes decrease in concentration quickly when put into a new bottle. In my experience, subsequent aliquots of the same concentration are more stable when put into a used and unwashed bottle that formerly held the same concentration. I always keep my 10 $\mu\text{g}/\text{L}$ standard in the same 10 $\mu\text{g}/\text{L}$ bottle and the 5 $\mu\text{g}/\text{L}$ standard in the 5 $\mu\text{g}/\text{L}$ bottle, and so on. This simple convention has extended the shelf life of standards and the accuracy of the calibrations made with them. Keep your standards in a dark cabinet.
- (8) The most efficient way to prepare dilution series repeatedly is to set up a cookbook table listing the volume of diluted tracer in so many milliliters of distilled water to create the desired concentration (Table 2). Also see Kilpatrick and Cobb (1985), Kilpatrick and Wilson (1989), and Wilson and others (1986).
- (9) The standards should bracket the expected range of concentrations to be measured. Standards above 100 $\mu\text{g}/\text{L}$ may have to be specially made, as the published dilution series do not have aliquots above 100 $\mu\text{g}/\text{L}$.
- (10) Choice of which series to use is dependent on the glassware and storage available for the residual solution from each step in the series. If a large number of sets of standards is expected to be mixed, then the first or second line could be used. At KGS, the last series in Table 2 will produce more than enough batches of the final concentration.
- (11) Follow the procedures recommended by the fluorometer manufacturer to calibrate the machine.

Calculate the Percent Recovery of the Tracer, the Time of Travel, and the Mean Velocity

- (1) Measure the concentration of dye in the samples, or download the concentration data from your submersible fluorometer (Fig. 11) or other field fluorometer.
- (2) Multiply the concentration by the discharge during the interval between samples to determine mass conveyed during the sampling interval.
- (3) Calculate the percentage of tracer recovered. Sum the mass recovered for each sample time

Table 2. Serial dilution for Rhodamine WT to produce a 100 $\mu\text{g}/\text{L}$ working solution.*

Initial Concentration	First Dilution		Second Dilution		Third Dilution		Final Concentration
	Volume Dye (ml)	Volume Water (ml)	Volume Dye (ml)	Volume Water (ml)	Volume Dye (ml)	Volume Water (ml)	Working Solution
Rhodamine WT 20% by volume. SG 1.19 g/cm ³	50	3,792	20	3,500	20	3,500	100 $\mu\text{g}/\text{L}$
	25	2,585	20	3,000	20	3,000	100 $\mu\text{g}/\text{L}$
	20	2,068	20	3,000	20	3,000	100 $\mu\text{g}/\text{L}$
	20	1,158	10	2,000	10	2,000	100 $\mu\text{g}/\text{L}$

*Modified from Wilson and others (1986).



Figure 11. Submersible fluorometer with 30 m of cable. The device requires a 12-volt auxiliary power supply and is programmed and downloaded using software installed on a laptop computer. Photo by Collie Rulo, Kentucky Geological Survey.

increment. Divide the mass recovered by the mass injected.

- (4) Determine the overall length of the breakthrough curve in units of time. Sum up the time units needed to represent half of the mass of tracer injected. The elapsed time at which half of the total tracer injected has been accounted for is the center of mass.
- (5) Calculate the velocity by dividing the distance from the injection point to the recovery point by the time of travel. Adjustments for sinuosity of the flow route may be needed.
- (6) Calculate the cross-section area of the conduit by dividing the instantaneous discharge by the mean velocity. If the cross section is assumed to be circular, a diameter may be calculated.

Additional Thoughts on Qualitative Groundwater Dye Tracing

- (1) Do not embark on a groundwater-tracing project just because it might be fun or it is the only test that has not been tried. To be cost- and technically effective, formulate a hypothesis to test and contemplate if knowing the answer will justify the effort needed to conduct the trace.
- (2) Conduct a thorough field reconnaissance on foot. Springs are easily concealed by vegetation and may not be visible from a car or boat. Topographic maps are less useful than photographs for finding springs, but are handy

for recording their location once found. Aerial photographs and online satellite imagery should be used when available, but are not very helpful unless the picture was taken when the vegetation was dormant. All likely dye recovery points and some unlikely resurgences should be located and bugged. Always place “failsafe” bugs at intervals along the regional base-level streams. The most common cause of a lost trace is an unmonitored spring.

- (3) The existence and locations of springs can be quickly obtained from area residents, and a few minutes of casual conversation can save significant time. Knock on doors, be polite, and fully inform people what you are doing and why. Most people, even the very skeptical, will eventually be won over. The source of the water discharging from their karst spring is a great mystery to many people, and they will be delighted to help if you provide them with the results. A bonus benefit to talking to residents is the opportunity to educate them about the methods you are using and the appearance and safety of the dye. The well water of one Logan County resident was colored by a trace I conducted, but because I had taken the time several months earlier to let the community know what to expect, he was neither alarmed nor angry.
- (4) Inform local environmental and emergency officials about your work without fail! In Kentucky, you are required to give the Division of Water in Frankfort advance notice. If you are doing more than a couple of traces, obtain a user ID and password for the online reporting form at dep.gateway.ky.gov/dyetrace/login.aspx [accessed 06/08/2012]. If someone reports colored water discharging from a spring, emergency response officials will then call you to determine if it is just a groundwater dye trace before raising an alarm. Agency staff may be aware of other persons conducting dye traces in the watershed, which obviously could result in very confusing results. After a significant hiatus in your program, you should remind the agency people. If you cause a problem for the water protection agency, you will be found and they will cause a problem for you.

- (5) Every dye trace should be designed to have a positive result—somewhere. Although knowing that a swallow hole does not flow to your spring is valuable information, you will always wonder if the dye was diluted or adsorbed, rather than discharged to an unknown spring. Furthermore, a positive result at another spring will strongly support your conclusion that the injection point is indeed outside of your groundwater basin. Keep in mind that to constrain the basin boundary, it is necessary to bracket it with traces flowing in opposite directions. You need positive results to surrounding springs.
- (6) Deploy a set of background bugs before beginning tracing, especially when optical brighteners are being used as tracers, or if there is a chance any fluorescent dye will be a contaminant in the aquifer. I commonly carry bugs and anchors on reconnaissance trips and set background bugs as I find new springs. High background fluorescence can result from a variety of sources such as antifreeze, domestic sewage, and crop seed markers. The ubiquity of several tracers in the groundwater from commercial applications is beginning to interfere with their usefulness.
- (7) Conduct your first tests over short distances, and then work your way headward in the basin. The initial groundwater traces can be ongoing with the continuing reconnaissance, provided the first traces are over short distances and well within the interior of the hypothesized basin. This builds your knowledge of the flow system so that when you begin tracing along the basin divides, you can make an educated guess as to which springs the dye will flow. If carefully planned, this procedure can reduce the number of springs you will need to bug, or enable you to conduct multiple groundwater traces simultaneously. By running many traces simultaneously, you can save much time and labor.
- (8) When conducting repeated traces, try to alternate dyes that may flow to the same spring. This reduces the chance that some residual dye in the system may result in a false positive, and in dry weather, shortens the amount of time needed to complete the tests. Collect a bug that is negative for the dye you wish to use before tracing to the same spring with it again.
- (9) Always replace one set of bugs with another until you are absolutely certain the dye has come through. Many traces take longer than expected and may be lost by prematurely ending the experiment. Of course, after 4 to 6 weeks of negative results, you will have to decide if the trace is lost.
- (10) Be very careful about how the bugs are placed. At the spring, deploy the bug in a shaded area and where it is not likely to be covered by silt. If tampering with the bug is likely at a site, or the bug could be lost to high flow, place a second or third hidden bug. The frequency of bug changes is dependent upon the precision needed for groundwater travel time. Changing bugs frequently, however, may result in insufficient time for dye adsorption onto the bug if the peak dye concentration for the trace is highly diluted at the spring. Do not change bugs more frequently than every 24 hours unless you have access to a scanning fluorometer. Leaving bugs in the field for long periods (more than 2 weeks) may result in their degradation, loss, or theft. For qualitative traces, changing bugs on a weekly schedule is the optimum for logistics, dye adsorption, and the security of the bugs.
- (11) Always place bugs before handling the dye. If the bugs must be transported in the same vehicle as the dye, be certain both containers are redundantly sealed and spillproof.
- (12) Use enough dye, but try to anticipate the consequences of using too much. Choosing the correct volume of dye is perhaps the most difficult decision made in groundwater dye tracing. Traces that are unexpectedly short are the worst culprits, because the amount of dye needed to reach the hypothesized resurgence is commonly more than enough to very vividly color the nearby actual resurgence. See (3) above. Algorithms to calculate the amount needed are found in the appendices, but may still result in amounts that are too small for very long traces or high flow. A trace that is lost because too little dye was used is just as

annoying as one that flowed to an unmonitored spring.

Summary

The goal of the KGS groundwater tracing protocol is to produce reliable results without imposing procedures that are excessively time-consuming. The methods are appropriate for studies not involving litigation or where ambiguous traces can be redone. For point-to-point qualitative traces, the important result is connectivity, whereas precise determination of tracer concentration is valuable but less time-efficient at this point in a study. KGS also recognizes there are compounds in groundwater that fluoresce at wavelengths near those of

some tracers, and therefore the detection of tracer at very low fluorescence intensity may be suspect. For the vast majority of tracing projects, the time lost adopting stringent minimal concentrations, for example, is thought to be greater than the infrequent occasions when a trace must be repeated. The KGS protocol does not preclude, however, applying more stringent standards (for example, a standardized mass of charcoal to elute) when the hypothesis being tested requires it. The protocol presented in this document assures that any fluorescence detected in the receptor at the wavelength of the tracer is from the tracer. Use enough dye, but not too much.

Selected Bibliography

- Alexander, E.C., and Quinlan, J.F., 1996, Practical tracing of groundwater with emphasis on karst terranes, *in* Eckenfelder Inc., Guidelines for wellhead and springhead protection area delineation in carbonate rocks: U.S. Environmental Protection Agency, Groundwater Protection Branch, Region 4, Atlanta, Georgia, 904-B-97-003, appendix B, 38 p.
- Aley, T., and Fletcher, M.W., 1976, The water tracer's cookbook: Missouri Speleology, v. 16, no. 6, 36 p.
- Davis, S.N., Campbell, D.J., Bentley, H.W., and Flynn, T.J., 1985, Groundwater tracers: U.S. Environmental Protection Agency, Office of Research and Development, and National Water Well Association, 200 p.
- Field, M.S., 2003, Tracer-test planning using the efficient hydrologic tracer-test design (EHTD) program: U.S. Environmental Protection Agency, National Center for Environmental Assessment–Office of Research and Development, EPA/600/R-03/034, 175 p.
- Ford, D.C., and Williams, P.W., 1989, Karst geomorphology and hydrology: London, Unwin Hyman Ltd., 601 p.
- Jones, W.K., 1984, Dye tracer tests in karst areas: The National Speleological Society Bulletin, Journal of Caves and Karst Studies, v. 46, no. 2, p. 3–9.
- Kilpatrick, F.A., and Cobb, E.D., 1985, Measurement of discharge using tracers: U.S. Geological Survey, Techniques of Water-Resources Investigations, TWI 03-A16, 52 p.
- Kilpatrick, F.A., and Wilson, J.F., Jr., 1989, Measurement of time of travel in streams by dye tracing: U.S. Geological Survey, Techniques of Water-Resources Investigations, TWI 03-A9, 27 p.
- Mull, D.S., Smoot, J.L., and Liebermann, T.D., 1988, Dye tracing techniques used to determine groundwater flow in a carbonate aquifer system near Elizabethtown, Kentucky: U.S. Geological Survey Water-Resources Investigations Report 87-4174, 95 p.
- Quinlan, J.F., ed., 1986, Qualitative water-tracing with dyes in karst terranes: Practical karst hydrogeology, with emphasis on groundwater monitoring: National Water Well Association, 26 p.
- Quinlan, J.F., 1989, Ground-water monitoring in karst terranes: Recommended protocols and implicit assumptions: U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, 600/X-89/050, 77 p.
- Smart, C., and Worthington, S.R.H., 2004, Water tracing, *in* Gunn, J., ed., Encyclopedia of caves and karst science: New York, Fitzroy Dearborn, Taylor and Francis Group, p. 769–771.
- Smart, P.L., 1984, A review of the toxicity of twelve fluorescent dyes used for water tracing: The National Speleological Society Bulletin, Journal of Caves and Karst Studies, v. 46, no. 2, p. 21–33.
- Spangler, L.E., Byrd, P.E., and Thrailkill, J., 1984, Use of Optical Brightener and Direct Yellow dyes for water tracing in the Inner Bluegrass karst region, Kentucky: The National Speleological Society Bulletin, Journal of Caves and Karst Studies, v. 46, no. 2, p. 10–16.
- Thrailkill, J., Byrd, P.E., Sullivan, S.B., Spangler, L.E., Taylor, C.J., Nelson, G.K., and Pogue, K.R., 1983, Studies in dye-tracing techniques and karst hydrogeology: University of Kentucky Water Resources Research Institute, Research Report 140, 89 p.
- Thrailkill, J., Spangler, L.E., Hopper, W.M., Jr., McCann, M.R., Troester, J.W., and Gouzie, D.R., 1982, Groundwater in the Inner Bluegrass karst region, Kentucky: University of Kentucky Water Resources Research Institute, Research Report 136, 108 p.
- Wilson, J.F., Jr., Cobb, E.D., and Kilpatrick, F.A., 1986, Fluorometric procedures for dye tracing: Techniques of water-resources investigations of the United States Geological Survey: Applications of Hydraulics, book 3, chapter A12, 34 p.
- Worthington, S.R.H., and Smart, C.C., 2003, Empirical determination of tracer mass for sink to spring tests in karst, *in* Beck, B.F., ed., Proceedings of the Ninth Multidisciplinary Conference, Sinkholes and the Engineering and Environmental Impacts of Karst: American Society of Civil Engineers, Geotechnical Special Publication 122, p. 287–295.

Appendix A: Characteristics of Commonly Used Tracers
Revised December 15, 2005

Name	Fluorescein	Rhodamine WT	Sulforhodamine B
C.I.	(Acid Yellow 73)	(Acid Red 388)	(Acid Red 52)
CAS Number	518-47-8	37299-86-8	3520-42-1
Color	rust-brown powder	purple-red solution	purple-red solution
Fluorescence Color	brilliant green	vivid orange-pink	vivid orange-pink
Visibility in Water (naked eye)	high	high	high
Toxicity	low	very low	very low
Carcinogenic & Mutagenic Hazard	none	minimal	unknown
Detector Types	charcoal & autosampler	charcoal & autosampler	charcoal & autosampler
Recommended Elutant	ethyl alcohol & 5% KOH	1-propanol & NH ₄ OH	1-propanol & NH ₄ OH
Minimum Detection Limit	0.1 ppb	0.01 ppb	0.08 ppb
Background Interference	chlorophyll, antifreeze	organics, crop seed	hydraulic fluid, anhydrous ammonia
Solubility	good	> 25% by weight	good
Adsorption	clays	clays	organic detritus
Fluorescence Stability	quenched at pH < 6	lower at higher temperature	quenched in low pH
Photochemical Decay	very high	very low	very low
Excitation Wavelength	485 nm ¹	558 nm ¹	548 nm ¹
Emission Wavelength in Water	508 nm ¹	572 nm ¹	572 nm ¹
KGS Emission Wavelength in Elutant (Varian Eclipse)	500 nm	550 nm	563 nm
Emission Wavelength in Elutant (from literature)	513 nm	565 nm	578 nm ¹
Cost (2005)	\$30.00/lb	\$33.00/lb	\$22.50/lb

¹Aley (1999)

²Ford and Williams (1989)

Appendix A: Characteristics of Commonly Used Tracers
Revised December 15, 2005

Name	Tinopal CBS-X	Eosine	Solophenyl
C.I.	(Fabric Brightener 351)	(Acid Red 37)	(Direct Yellow 96) Name has been changed
CAS Number	38755-42-1	17372-87-1	not found
Color	yellow-white powder	brown-green powder	yellow powder
Fluorescence Color	blue-white	greenish orange to red	greenish yellow
Visibility in Water (naked eye)	very low	high	low
Toxicity	low	very low	low
Carcinogenic & Mutagenic Hazard	none	minimal	unknown
Detector Types	cotton & autosampler	charcoal & autosampler	cotton
Recommended Elutant	UV lamp; 365 nm "long setting"	1-propanol & NH ₄ OH	UV lamp; 365 nm "long setting"
Minimum Detection Limit	0.1 ppb	0.04 ppb	1.0 ppb
Background Interference	detergent	pentachlorophenol	none
Solubility	25 g/L	good	requires prolonged mixing
Adsorption	organic detritus	minor adsorption	none known
Fluorescence Stability	quenched in low pH	quenched in low pH	quenched in low pH
Photochemical Decay	high	very high	low
Excitation Wavelength	360 nm ²	512 nm ¹	397 nm
Emission Wavelength in Water	435 nm ²	533 nm ¹	490 nm
KGS Emission Wavelength in Elutant (Varian Eclipse)	435 nm	523 nm	nm
Emission Wavelength in Elutant (from literature)	450 nm	538 nm ¹	nm
Cost (2005)	\$22.50/lb	\$22.00/lb	\$25.50/lb
¹ Aley (1999)			
² Ford and Williams (1989)			

Appendix B: Tracer Mass Calculation Spreadsheet

Access the Excel spreadsheet at kgs.uky.edu/kgsweb/olops/pub/kgs/IC26_12_Appendix_B.xls.

The effectiveness multiplier and equation to convert mass of tracer to the common concentrate solution of Rhodamine WT are to adjust the mass calculations for less effective or diluted tracers. The mass read from the matrix should be multiplied by the appropriate effectiveness. For example, the liquid equivalent of 119 g of tracer for Rhodamine is 500 ml. The mass of Tinopal CBS-X is 3 times, or 357 g for the same matrix value.

Appendix C: Matrix for Estimating Mass of Groundwater Tracer for Connectivity in Karst (10 ppb)

This estimate is dependent on the accuracy of your prediction of the distance to the resurgence and its discharge.

Desired concentration is 10 ppb or 0.01 g/m³ **0.01**
Equation: M=19 (LQC)^{0.85} (Worthington and Smart, 2003)
 Units: M=grams, L=meters, Q=m³/sec, C=g/m³, T=sec
 1 hour=3,600 sec, 1 day=86,400 sec, m=meters

Spreadsheet: James C. Currens, KGS, 2004
 1 g/m³=1 mg/L
 1 mg/L=1,000 µg/L
 0.1 g/m³=100 µg/L
 0.01 g/m³=10 µg/L

Effectiveness Multiplier	
Fluorescein	X 1
Eosine	X 1
SR-B	X 1
Brightener	X 3
DY 96	X 4

Minimum mass: 25g 50g 75g

		Estimated Discharge at the Expected Resurgence, CFS (Top Row), CMS (Bottom Row)																
	CFS >	1	2	4	8	10	15	20	30	40	50	60	70	80	90	100		
Est. Dist. Feet	Est. Dist. Meters	0.028	0.057	0.113	0.227	0.283	0.425	0.566	0.850	1.133	1.416	1.699	1.982	2.265	2.549	2.832		
500	152	1	2	4	7	9	13	17	24	32	39	47	54	62	69	76		
1,000	305	2	4	7	13	17	24	32	47	62	76	91	105	119	133	147		
1,500	457	3	5	10	20	24	36	47	69	91	112	133	154	175	196	216		
2,000	610	4	7	13	26	32	47	62	91	119	147	175	203	230	257	284		
2,500	762	4	9	17	32	39	58	76	112	147	182	216	251	284	318	352		
3,000	914	5	10	20	38	47	69	91	133	175	216	257	298	338	378	418		
3,500	1,067	6	12	23	44	54	80	105	154	203	251	298	345	392	438	484		
4,000	1,219	7	13	26	50	62	91	119	175	230	284	338	392	445	497	550		
4,500	1,372	8	15	29	56	69	101	133	196	257	318	378	438	497	556	615		
5,000	1,524	9	17	32	62	76	112	147	216	284	352	418	484	550	615	679		
5,500	1,676	9	18	35	67	83	123	161	237	311	385	458	530	602	673	744		
6,000	1,829	10	20	38	73	91	133	175	257	338	418	497	576	653	731	808		
6,500	1,981	11	21	41	79	98	144	189	278	365	451	536	621	705	789	872		
7,000	2,134	12	23	44	85	105	154	203	298	392	484	576	666	756	846	935		
7,500	2,286	13	24	47	91	112	165	216	318	418	517	615	711	808	903	998		
8,000	2,438	13	26	50	96	119	175	230	338	445	550	653	756	859	960	1,062		
8,500	2,591	14	27	53	102	126	185	244	358	471	582	692	801	910	1,017	1,125		
9,000	2,743	15	29	56	108	133	196	257	378	497	615	731	846	960	1,074	1,187		
10,000	3,048	17	32	62	119	147	216	284	418	550	679	808	935	1,062	1,187	1,312		
15,000	4,572	24	47	91	175	216	318	418	615	808	998	1,187	1,375	1,560	1,745	1,929		
20,000	6,096	32	62	119	230	284	418	550	808	1,062	1,312	1,560	1,806	2,051	2,294	2,535		
25,000	7,620	39	76	147	284	352	517	679	998	1,312	1,622	1,929	2,233	2,535	2,835	3,134		
30,000	9,144	47	91	175	338	418	615	808	1,187	1,560	1,929	2,294	2,655	3,015	3,371	3,726		

Appendix D: Matrix for Estimating Mass of Groundwater Tracer for Connectivity in Karst (100 ppb)

This estimate is dependent on the accuracy of your prediction of the distance to the resurgence and its discharge.

Desired concentration is 100 ppb or 0.1 g/m³ 0.1
Equation: M=19 (1QC)^{0.95} (Worthington and Smart, 2003)
 Units: M=grams, L=meters, Q=m³/sec, C=g/m³, T=sec
 1 hour=3,600 sec, 1 day=86,400 sec, m=meters
 Minimum mass: 25g 50g 75g

Spreadsheet: James C. Currans, KGS, 2004
 1 g/m³=1 mg/L
 To convert grams to ml
 of 20% RWt; grams X 4.2
 (Mass/1.19 s.g.)/0.2
 ml=(g/1.19 g/ml)/0.2

Effectiveness Multiplier	
Fluorescein	X 1
Eosine	X 1
SR-B	X 1
Brightener	X 3
DY 96	X 4

Estimated Discharge at the Expected Resurgence, CFS (Top Row), CMS (Bottom Row)																
	CFS >	1	2	4	8	10	15	20	30	40	50	60	70	80	90	100
Est. Dist. Feet		0.028	0.057	0.113	0.227	0.283	0.425	0.566	0.850	1.133	1.416	1.699	1.982	2.265	2.549	2.832
500	152	9	17	32	62	76	112	147	216	284	352	418	484	550	615	679
1,000	305	17	32	62	119	147	216	284	418	550	679	808	935	1,062	1,187	1,312
1,500	457	24	47	91	175	216	318	418	615	808	998	1,187	1,375	1,560	1,745	1,929
2,000	610	32	62	119	230	284	418	550	808	1,062	1,312	1,560	1,806	2,051	2,294	2,535
2,500	762	39	76	147	284	352	517	679	998	1,312	1,622	1,929	2,233	2,535	2,835	3,134
3,000	914	47	91	175	338	418	615	808	1,187	1,560	1,929	2,294	2,655	3,015	3,371	3,726
3,500	1,067	54	105	203	392	484	711	935	1,375	1,806	2,233	2,655	3,074	3,490	3,903	4,314
4,000	1,219	62	119	230	445	550	808	1,062	1,560	2,051	2,535	3,015	3,490	3,962	4,431	4,897
4,500	1,372	69	133	257	497	615	903	1,187	1,745	2,294	2,835	3,371	3,903	4,431	4,956	5,477
5,000	1,524	76	147	284	550	679	998	1,312	1,929	2,535	3,134	3,726	4,314	4,897	5,477	6,054
5,500	1,676	83	161	311	602	744	1,093	1,437	2,112	2,775	3,431	4,079	4,723	5,362	5,996	6,628
6,000	1,829	91	175	338	653	808	1,187	1,560	2,294	3,015	3,726	4,431	5,130	5,824	6,513	7,199
6,500	1,981	98	189	365	705	872	1,281	1,684	2,475	3,253	4,021	4,781	5,535	6,284	7,028	7,768
7,000	2,134	105	203	392	756	935	1,375	1,806	2,655	3,490	4,314	5,130	5,939	6,742	7,540	8,334
7,500	2,286	112	216	418	808	998	1,468	1,929	2,835	3,726	4,606	5,477	6,341	7,199	8,051	8,899
8,000	2,438	119	230	445	859	1,062	1,560	2,051	3,015	3,962	4,897	5,824	6,742	7,654	8,560	9,461
8,500	2,591	126	244	471	910	1,125	1,653	2,172	3,193	4,197	5,188	6,169	7,142	8,108	9,068	10,022
9,000	2,743	133	257	497	960	1,187	1,745	2,294	3,371	4,431	5,477	6,513	7,540	8,560	9,574	10,581
10,000	3,048	147	284	550	1,062	1,312	1,929	2,535	3,726	4,897	6,054	7,199	8,334	9,461	10,581	11,695
15,000	4,572	216	418	808	1,560	1,929	2,835	3,726	5,477	7,199	8,899	10,581	12,250	13,907	15,554	17,191
20,000	6,096	284	550	1,062	2,051	2,535	3,726	4,897	7,199	9,461	11,695	13,907	16,100	18,278	20,442	22,594
25,000	7,620	352	679	1,312	2,535	3,134	4,606	6,054	8,899	11,695	14,457	17,191	19,902	22,594	25,269	27,929
30,000	9,144	418	808	1,560	3,015	3,726	5,477	7,199	10,581	13,907	17,191	20,442	23,666	26,867	30,048	33,211

Appendix E: Matrix for Estimating Mass of Groundwater Tracer for Time of Travel in Karst (10 ppb)

This estimate is dependent on the accuracy of your prediction of the travel time to the resurgence and its discharge.

Desired peak concentration is 10 ppb or 0.01 g/m³ 0.01

Velocity is assumed to be 0.1 m/sec to estimate TOT from distance

Equation: $M=0.73 (TQC)^{0.97}$ (Worthington and Smart, 2003)

Units: M=grams, L=meters, Q=m³/sec, C=g/m³, T=sec

1 hour=3,600 sec, 1 day=86,400 sec, m=meters

Minimum mass: 25g 50g 75g

Spreadsheet: James C. Currens, KGS, 2004

1 g/m³=1 mg/L

1 mg/L=1,000 µg/L

0.1 g/m³=100 µg/L

0.01 g/m³=10 µg/L

Effectiveness Multiplier

Fluorescein X 1

Eosine X 1

SR-B X 1

Brightener X 3

DY 96 X 4

To convert grams to ml of 20% RWt; grams X 4.2 (Mass/1.19 s.g.)/(0.2) ml=(g/1.19 g/ml)/0.2

Estimated Distance		Estimated Discharge at the Expected Resurgence, CFS (Top Row), CMS (Bottom Row)															
Feet	Meters	CFS >	1	2	4	8	10	15	20	30	40	50	60	70	80	90	100
500	152	1,524	0	1	1	2	3	4	6	9	12	14	17	20	23	25	28
1,000	305	3,048	1	1	2	5	6	9	12	17	23	28	34	39	44	50	55
1,500	457	4,572	1	2	4	7	9	13	17	25	34	42	50	58	66	74	82
2,000	610	6,096	1	2	5	9	12	17	23	34	44	55	66	76	87	97	108
2,500	762	7,620	2	3	6	12	14	21	28	42	55	68	82	95	108	121	134
3,000	914	9,144	2	4	7	14	17	25	34	50	66	82	97	113	129	144	160
3,500	1,067	10,668	2	4	8	16	20	30	39	58	76	95	113	131	150	168	186
4,000	1,219	12,192	2	5	9	18	23	34	44	66	87	108	129	150	170	191	211
4,500	1,372	13,716	3	5	10	20	25	38	50	74	97	121	144	168	191	214	237
5,000	1,524	15,240	3	6	12	23	28	42	55	82	108	134	160	186	211	237	263
5,500	1,676	16,764	3	6	13	25	31	46	60	90	118	147	175	204	232	260	288
6,000	1,829	18,288	4	7	14	27	34	50	66	97	129	160	191	222	252	283	313
6,500	1,981	19,812	4	8	15	29	36	54	71	105	139	173	206	240	273	306	339
7,000	2,134	21,336	4	8	16	31	39	58	76	113	150	186	222	258	293	329	364
7,500	2,286	22,860	4	9	17	34	42	62	82	121	160	199	237	275	313	351	389
8,000	2,438	24,384	5	9	18	36	44	66	87	129	170	211	252	293	334	374	414
9,000	2,743	27,432	5	10	20	40	50	74	97	144	191	237	283	329	374	419	464
10,000	3,048	30,480	6	12	23	44	55	82	108	160	211	263	313	364	414	464	514
15,000	4,572	45,721	9	17	34	66	82	121	160	237	313	389	464	539	614	688	762
20,000	6,096	60,961	12	23	44	87	108	160	211	313	414	514	614	713	812	910	1,008
25,000	7,620	76,201	14	28	55	108	134	199	263	389	514	639	762	885	1,008	1,130	1,251

Appendix F: Matrix for Estimating Mass of Groundwater Tracer for Time of Travel in Karst (100 ppb)

This estimate is dependent on the accuracy of your prediction of the travel time to the resurgence and its discharge.

Desired peak concentration is 100 ppb or 0.1 g/m³ 0.1
Velocity is assumed to be 0.1 m/ sec to estimate TOT from distance
Equation: $M=0.73 (TQC)^{0.97}$ (Worthington and Smart, 2003)
 Units: M=grams, L=meters, Q=m³/sec, C=g/m³, T=sec
 1 hour=3,600 sec, 1 day=86,400 sec, m=meters
 Minimum mass: 25g 50g 75g

Spreadsheet: James C. Currans, KGS, 2004
 1 g/m³=1 mg/L
 To convert grams to ml
 of 20% RWt; grams X 4.2
 (Mass/1.19 s.g.)/0.2)
 ml=(g/1.19 g/ml)/0.2

Effectiveness Multiplier	
Fluorescein	X 1
Eosine	X 1
SR-B	X 1
Brightener	X 3
DY 96	X 4

Estimated Distance		Estimated Discharge at the Expected Resurgence, CFS (Top Row), CMS (Bottom Row)															
		CFS >	1	2	4	8	10	15	20	30	40	50	60	70	80	90	100
Feet	Meters	0.028	0.057	0.113	0.227	0.283	0.425	0.566	0.850	1.133	1.416	1.699	1.982	2.265	2.549	2.832	
500	152	1,524	3	6	12	23	42	55	82	108	134	160	186	211	237	263	
1,000	305	3,048	6	12	23	44	82	108	160	211	263	313	364	414	464	514	
1,500	457	4,572	9	17	34	66	121	160	237	313	389	464	539	614	688	762	
2,000	610	6,096	12	23	44	87	160	211	313	414	514	614	713	812	910	1,008	
2,500	762	7,620	14	28	55	108	199	263	389	514	639	762	885	1,008	1,130	1,251	
3,000	914	9,144	17	34	66	129	237	313	464	614	762	910	1,056	1,203	1,348	1,493	
3,500	1,067	10,668	20	39	76	150	275	364	539	713	885	1,056	1,227	1,397	1,566	1,734	
4,000	1,219	12,192	23	44	87	170	313	414	614	812	1,008	1,203	1,397	1,590	1,782	1,974	
4,500	1,372	13,716	25	50	97	191	351	464	688	910	1,130	1,348	1,566	1,782	1,998	2,213	
5,000	1,524	15,240	28	55	108	211	389	514	762	1,008	1,251	1,493	1,734	1,974	2,213	2,451	
6,000	1,829	18,288	34	66	129	252	464	614	910	1,203	1,493	1,782	2,069	2,356	2,641	2,925	
7,000	2,134	21,336	39	76	150	293	539	713	1,056	1,397	1,734	2,069	2,403	2,736	3,067	3,397	
8,000	2,438	24,384	44	87	170	334	614	812	1,203	1,590	1,974	2,356	2,736	3,114	3,491	3,866	
9,000	2,743	27,432	50	97	191	374	688	910	1,348	1,782	2,213	2,641	3,067	3,491	3,913	4,334	
10,000	3,048	30,480	55	108	211	414	762	1,008	1,493	1,974	2,451	2,925	3,397	3,866	4,334	4,801	
15,000	4,572	45,721	82	160	313	614	762	1,130	2,213	2,925	3,632	4,334	5,033	5,729	6,423	7,114	
20,000	6,096	60,961	108	211	414	812	1,008	1,493	2,925	3,866	4,801	5,729	6,654	7,574	8,490	9,404	

Appendix H: Groundwater Trace Injection Report Form

○, √, X, or underline wherever possible

Injection Site Location

Reporters: _____ Report date: _____

Field ID: _____ Trace serial no.: _____

Site name: _____ Type of karst feature: _____

County: _____ Quadrangle: _____ Corridor or project: _____

Geodetic location: N latitude: _____ ° _____ ' _____ " W longitude: _____ ° _____ ' _____ "

UTM location: Zone: _____, easting _____, northing _____

Elevation: _____, Accuracy est. _____ GPS Topo Other _____

Additional forms completed for this location:

Spring Inspection

Cover-Collapse Report

Bug Sheet

Karst Feature Inventory

Tracer Injection

Weather or field conditions: _____

Date of injection: _____ Time (military) of injection: _____

Amount of tracer: _____ Batch or lot no. (optional): _____

Tracer Used

Eosine-Y (Acid Red 87)

Sulforhodamine B (Acid Red 52)

Rhodamine WT (Acid Red 388)

Tinopal CBS-X (Fabric Brightener 351)

Sodium Fluorescein (Acid Yellow 73)

Other tracer (specify)

Solophenyl (Direct Yellow 96)

Tracer Recovery

Name of positive spring(s): _____ AKGWA no.: _____

Detector placement: Date: _____ Time (military): _____

Detector recovery: Date: _____ Time (military): _____

Other positive sites? _____

For a complete list of all monitored sites, see the Monitoring Report dated: _____

Comment or Interpretation

Appendix I: Definitions

Aliquot: A portion of a larger whole, especially a sample taken for chemical analysis.

Base flow: Normal rate of outflow of groundwater from a spring that is not influenced by rapid recharge from a storm or snowmelt. Base flow is a steady or slowly decreasing discharge rate and has uniform physical water-quality characteristics (i.e., low turbidity). Discharge measurements should be made between the complete recession of any peaks from the runoff of a storm up to the next runoff event. Summer base flow in Kentucky is from mid-June through mid-October, whereas winter base flow is from mid-December through mid-March.

Bedrock-collapse sinkhole: Formed by the collapse of a bedrock roof into an underlying cave. Bedrock collapse in karst is rare, but is the origin of some sinkholes, principally **karst windows**.

Cave: A natural opening created by dissolution or erosion of bedrock and large enough for an adult person to enter. The flow of water in a cave may be year-round, seasonal, high-flow (flood) only, or permanently dry. A diameter of 50 cm and a length (depth) of 2 m are approximate minimal dimensions. In Kentucky, caves can exceed 30 m in width and hundreds of kilometers in aggregated length. Orientation of the cave passage in space is not definitive; therefore, an open air pit with minimal overhanging ledges is a cave if the minimum dimensions are met. The orientation relevant to the outcrop is significant in that an opening 2 m wide and 0.6 m deep in a cliff is an overhang or rock shelter, not a cave.

Conduit: A tubular opening created by dissolution of the bedrock, which carries, can carry, or has carried water. Conduits have a minimum diameter of 1 cm up to a maximum diameter of 0.5 m. Flow in a conduit may be year-round, seasonal, high-flow only, or permanently dry. The minimum diameter is the approximated critical diameter of 1 cm, at which fundamental changes in the mechanisms of carbonate dissolution and groundwater flow occur.

Cover-collapse sinkhole: Formed by the collapse of the unconsolidated cover (soil, residuum, loess,

or till) that formed the roof of a soil void or conduit at the soil-bedrock interface, or spanned a grike (fissure) or other karst void in the bedrock.

Cuvette: A test tube or chamber that holds a sample and fits into a receiving chamber of an analytical machine.

Dissolution sinkhole (synonym: **doline**): Sinkhole resulting exclusively from gradual dissolution of the bedrock and removal of the dissolved rock and insoluble residuum via the **sinkhole throat** and **karst aquifer** conduits. Dissolution sinkholes may be totally buried and filled, or the bedrock can be totally exposed. Most dissolution sinkholes have the classic bowl-shaped contour, with a variable thickness of soil or other unconsolidated residual covering the bedrock. Also see **epikarst**.

Eluent: The solution applied to activated carbon charcoal to extract fluorescent tracing dyes adsorbed on the charcoal. Common mixtures are 5 percent by volume potassium hydroxide and 70 percent isopropyl alcohol, the balance being distilled water, and for Smart Solution, 50 percent of 1-propanol to 20 percent ammonia hydroxide (NH₄OH) to 30 percent water, by volume. There are other solutions. The choice depends on the dye being extracted.

Elutant: The liquid with tracer decanted from the charcoal after elution.

Epikarst (synonym: **subcutaneous zone**): The interval below the organic soil and above the mass of largely unweathered soluble bedrock, consisting of highly corroded bedrock, residuum, subsoil, float, and unconsolidated material of other origins. Thickness of the epikarst varies from absent to a reported 30 m. The epikarst is important for the storage and transport of soil water and groundwater in the karst system and is relevant to foundation stability.

Epikarstic dye injection point: A shallow excavation, typically a trench dug with a backhoe, but including auger holes into soil and very shallow excavations into bedrock. Tracer is mixed with water in the trench or a tank, then poured into the hole.

Additional water is added until the movement of the tracer into the epikarst is assured.

Fluorescent dye (synonym: **tracer**): One of several organic dyes that fluoresce under short-wavelength light, particularly when dissolved in water or other solvent. Detection of the dye is confirmed by analysis of elutant or water samples with a fluorometer.

Gumdrop: Anchor fashioned from concrete and heavy-gage wire that suspends a passive dye receptor above the bottom of the channel. Usually a lanyard is tied from the gumdrop to a higher elevation so that the dye receptor can be changed during high water.

Karst aquifer: A body of soluble rock that conducts water principally via a connected network of tributary conduits, formed by the dissolution of the rock, which drain a groundwater basin and discharge to at least one perennial spring. The conduits may be partly or completely water-filled. The karst aquifer may also have primary (intergranular) and secondary porosity (fracture), which is saturated with water when below the potentiometric surface.

Karst terrain (synonym: **karst terrane**): A landscape generally underlain by limestone or dolomite, in which the topography is chiefly formed by dissolving the rock and which may be characterized by sinkholes, sinking streams, closed depressions, subterranean drainage, and caves (Monroe, 1970). The term "terrain" implies that only the surface is considered, whereas "terrane" includes the subsurface (caves or aquifer) as a single system. Karst also forms on gypsum and salt bedrock, although not in Kentucky.

Karst valley: A mid-sized to valley-scale closed depression otherwise meeting the definition of a sinkhole but also enclosing and including more than one smaller sinkholes and/or a sinking stream.

Passive tracer receptor (synonyms: **bugs**, **dye detector**, **dye receptor**, **passive charcoal detector**): Consists of two general types. The activated carbon receptor is constructed of a few grams of coconut shell charcoal enclosed in a mesh bag typically made of nylon screen. The cotton receptor is a section of untreated surgical cotton or bleached

broadcloth. Tracer receptors are fastened to a gumdrop or field-expedient anchor.

Ponor: See **sinkhole throat**.

Qualitative trace: Tracer experiment to establish the point-to-point connectivity, or flow vector, from the input point of the tracer to the resurgence. It identifies only the presence of tracer in the water (Smart and Worthington, 2004). The method of sampling and determination of the tracer is immaterial to the concept, but is generally assumed to be passive tracer receptors or bugs.

Quantitative trace: Combines concentration measurements of tracer in water at the resurgence with flow-rate measurements, permitting the compilation of mass-flux curves. The area under the mass-flux curve represents the total mass of tracer measured at a site (Field, 2002; Smart and Worthington, 2004). The incremental mass flux of tracer, the total mass of tracer recovered, and the center of mass of the tracer breakthrough curve are among the quantitative measures calculated from the concentration and discharge data.

Semiquantitative trace: The concentration of the tracer in water samples collected over time is determined as in a **quantitative trace**. Discharge is not included in the calculation. The resulting time versus concentration curve provides additional data for recognition of the tracer, and in some cases can aid in determining characteristics of the traced route (Smart and Worthington, 2004).

Sinkhole: Any closed depression in soil or bedrock formed by the erosion and transport of earth material from below the land surface, which is circumscribed by a closed topographic contour and drains to the subsurface. Morphologies of sinkholes formed in soluble rock include **dissolution sinkhole** (gently sloping depression wider than deep), **karst window** (sinkhole exposing an underground stream), vertical shaft (depression in bedrock much deeper than wide and roughly circular in plan), and grike (depression in soil and bedrock much deeper than wide and roughly lenticular in plan). Also see **cover-collapse sinkhole** and **bedrock-collapse sinkhole**.

Sinkhole cluster area: A group of two or more sinkholes clustered so that the average spacing

among them is closer than the average spacing of sinkholes in the immediate area as a whole. A sinkhole cluster is likely to have a common groundwater basin.

Sinkhole throat (informal usage): Outlet or outlets for a sinkhole allowing runoff from the **sinkhole watershed** to flow into the ground. Not all sinkhole throats have a discernable opening or an opening large enough to enter, but some have large dimensions and all are a sink point for an intermittent or

perennial stream varying in flow rate from rivulet to rivers.

Sinkhole watershed: An area bounded by a projected line demarcating a change in slope from toward the center of the sinkhole to away from the sinkhole, which represents a local topographic drainage divide. Precipitation falling on the surface sloping toward the sinkhole is likely to run into the sinkhole throat or infiltrate the soil and move through subsoil conduits to the throat.