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INTRODUCTION

The tracing of underground drainage in karst regions is a technique belonging to both the hydrologist and the caver. The disappearance of surface streams into caves and sinks and the presence of large carbonate springs has intrigued man through the centuries. Hay, wheat chaff, sawdust, duck feathers (and occasionally ducks), and carved logs have been used successfully as tracers. A few of the more interesting tracers used in recent years have included computer card chips, dog biscuits, oranges, tagged eels, and a "geobomb" which was set to explode underground at some predetermined time after release. The source of a spring supplying water to a high school in Tennessee was found when revenue agents poured 2,000 gallons of illegal whiskey into a nearby sinkhole (Aley and Fletcher, 1976).

One of the earliest reported tracer experiments used chaff that Philips, the Tetrarch of Trachonitis, threw into Ram Crater Lake (Fijala); subsequently the chaff reappeared in one of the springs at the headwaters of the Jordan River. Mazor (1976) demonstrated by isotopic analysis of the waters that the reported connection was highly unlikely. It is interesting to note that this first reported successful(?) tracer test from almost 2,000 years ago is controversial to this day.

The Aach (a tributary of the Rhine) was traced 12 km to the Danube in 1877 by C. Ten Brink. Dionis des Carrieres used fluorescein to establish the water origin of typhoid fever at Auxerre, France in 1882. The use of fluorescein as a tracer in North America was first described by Dole (1906).

Many of the early tracer tests used large quantities of dye. The Henne Morte (Pyrenees) was traced using 100 kg (220 lbs) of fluorescein—travel time was 28 hours and extensive coloring of the surface stream was reported. Casteret used 54 kg of fluorescein to trace the Trou du Toro. The dye reappeared 3 km away in less than 24 hours and colored the Garonne River for over 48 km downstream. About 160 kg of fluorescein were used in a single test in the Popovo Polje (Yugoslavia) in 1971 (Milanovic, 1981).

Tracer techniques have become more sophisticated in recent years. Tests have been conducted by Aley in Missouri at distances over 60 km using less than 7 kg of fluorescein. New fluorescent dyes have been added to the repertoire of tracing agents, and it is now possible to test several sinking streams simultaneously using different colored dyes. The progress of knowledge of the areal drainage systems in the Mammoth Cave Region is described in Figure 1.

This special issue describes tracer techniques primarily for using fluorescent tracers. Fluorescent dyes are the "traditional" tracers for use in karst areas. The fluorescent tracers are relatively inexpensive, easy to use, and nontoxic. The following papers present discussions of the use and applications of fluorescent tracers in karst studies and reviews of the recent literature. The "Handbook" format of this issue departs somewhat from the normal presentation used for reporting scientific studies in the *NSS Bulletin*.

I would like to thank all of the researchers who have contributed papers and review comments to this issue.

William K. Jones
Guest Editor

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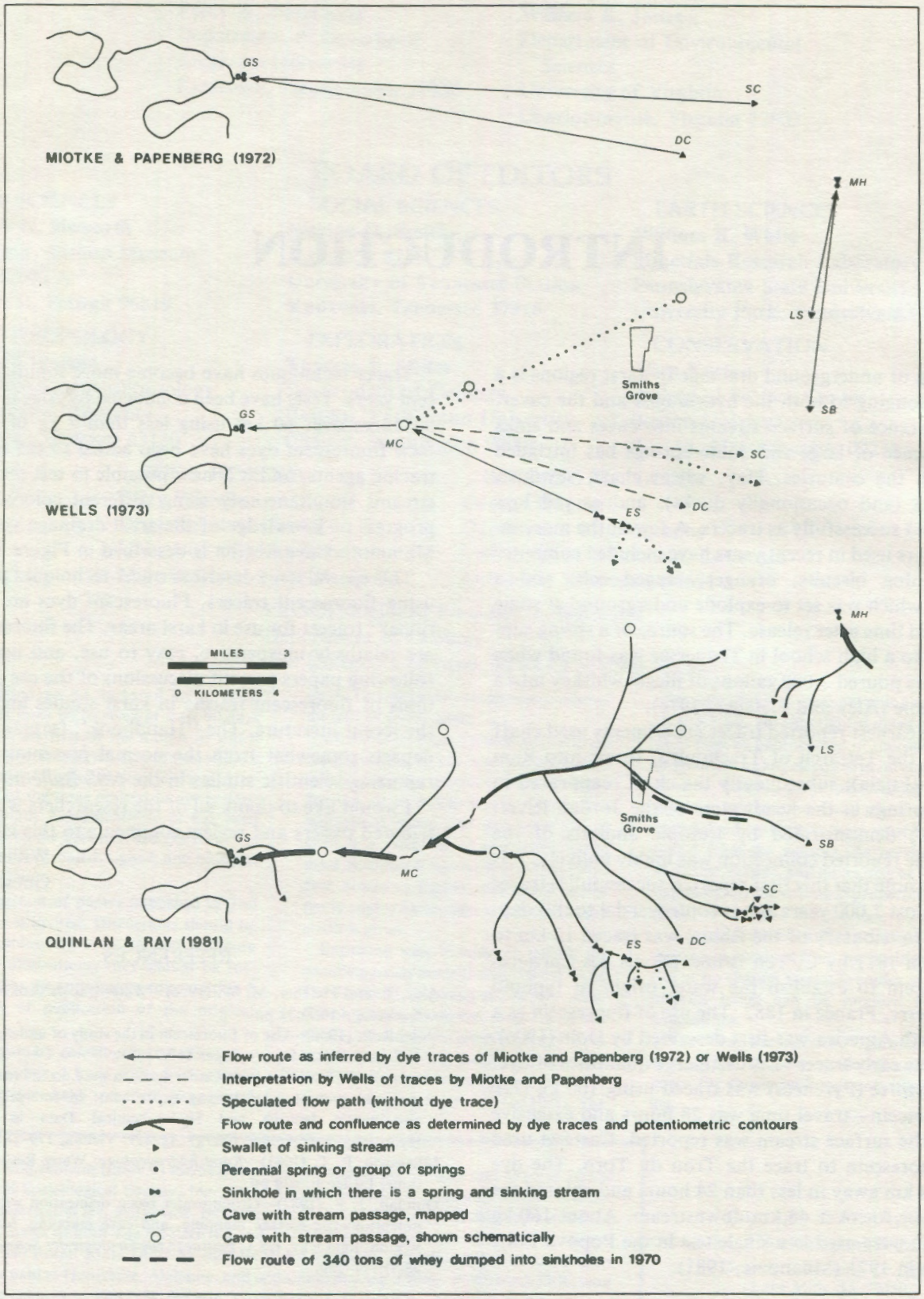

Figure 1. **Evolution of knowledge of flow routes in the Graham Springs groundwater basin. DC—Doty Creek, ES—Elk Spring, GS—Graham Springs, LS—Little Sinking Creek, MC—Mill Cave, MH—Mill Hole, SB—Sinking Branch, SC—Sinking Creek (after Quinlan, 1982).**

Jones, William K.—Dye Tracer Tests in Karst Areas: *NSS Bulletin* 46: 3-9.

DYE TRACER TESTS IN KARST AREAS

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Dye tracer techniques have evolved from simple labeling experiments used to determine the source of resurgences or the outlet for sinking streams to sophisticated tools for studying the hydraulics of karst aquifers. Karst regions are generally first studied using qualitative methods and passive detectors to monitor springs. Quantitative tests, often using several dyes simultaneously, may then be conducted to study the flow characteristics of the aquifers. The tracer dyes used most often in North America for reconnaissance type studies include fluorescein sodium, direct yellow 96, and optical brighteners. These dyes are readily collected on passive detectors and analyzed without using instruments. Rhodamine WT may be used simultaneously with the above dyes and fluorometric analysis in quantitative studies.

INTRODUCTION

Tracer tests fall into several categories with different data requirements depending on the objectives of the study. The simplest type of test is usually to determine if a water connection exists between two points, as perhaps between two cave passages or between a sinking stream and a spring. After a series of point-to-point tests have been run in an area it may be possible to determine the direction of ground water movement (at least the part flowing through discrete conduits) and the karst ground water catchment areas. Contour maps of the potentiometric surface may be combined with tracer test data to further define the recharge areas (Quinlan, 1982). The time required for the tracer to travel from the injection point (input) to the recovery point (output) may be used to calculate average groundwater velocity and, if velocity data is available for several different flow levels (discharge), inferences can be made about the internal flow characteristics of the aquifer. If the recovery concentration of the tracer is measured at close enough time intervals to characterize the shape of the tracer recovery curve versus time, further insight into the flow characteristics of the aquifer will be gained. The simple point-to-point type of tests are generally conducted as a first step in studying an area and are qualitative in nature. The quantitative dye recovery tests are much more time consuming and expensive and should be based on the results of earlier qualitative works.

The first step in any water tracing program should be to collect all of the available geologic and hydrologic data for the study area and define the objectives of the tracer tests. Aley and Fletcher (1976) suggest that most tracer tests attempted in the United States fail due to: 1) insufficient hydrologic field work before the tracer is injected, 2) failure to allocate sufficient time to the tracing effort, and 3) tracing attempts during low flow conditions. These problems are not directly related to the type of tracer or the techniques employed, but to an insufficient understanding of the hydrogeologic setting and boundary conditions of the study area. Any tracer tests conducted in large or complex karst areas are time consuming and expensive. Researchers who have limited background in karst studies should enlist help from properly qualified individuals before attempting any large scale tracer tests.

The purpose of this paper is to provide detailed directions on the techniques currently used in karst areas for water tracing with fluorescent dyes. The paper begins with a description of basic qualitative procedures and advances to cover some quantitative techniques. The references provide a guide to more advanced techniques. The papers by Wilson (1968) and Smart and Laidlaw (1977) are indispensable references for anyone wishing to go beyond the basic qualitative methods. The works by Jones (1976), Aley and Fletcher (1976), and Milanovic (1981) provide detailed basic

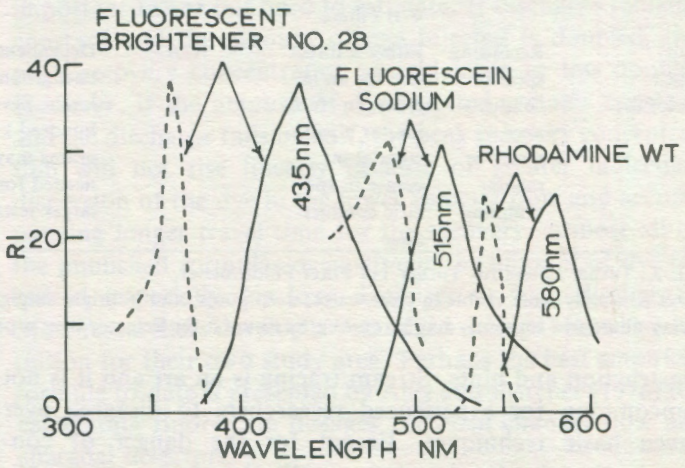

Figure 1. **Excitation (dashed lines) and emission (solid lines) spectra of fluorescent brightener no. 28, fluorescein sodium, and Rhodamine WT. Samples scanned using an American instrument company SPF-125 Spectrofluorometer and 2 mm slit widths for excitation and emission.**

Table 1. Evaluation of Principal Water Tracers Used in North American Karst Studies

Tracer & Color	Passive Detector	Test (elutriant)[1]	Maximum Excitation & Emission nm[2]	Detectable Conc.[3]	Advantages	Disadvantages	Remarks
Fluorescein Sodium $C_{20}H_{10}Na_2O_5$ Yellow-Green Xanthene	Activated cocoanut charcoal 6-14 mesh	Ethyl alcohol and 5% KOH. Visual test or fluorometer & 2A-47B; 2A-12, 65A filters	485 515	0.1 μg/1 Dependent on background levels. "Controls" must be used to determine background.	1) Does not require constant monitoring or any special equipment. 2) Inexpensive.	1) Dye is photochemically unstable. 2) Moderate sorption on clay. 3) pH sensitive.	This is the most popular method used in the USA. Carbon detectors first suggested by Dunn, 1957.
Rhodamine WT $C_{28}H_{31}N_2O_3Cl$ Red-Purple Xanthene	Activated coconut charcoal 6-14 mesh	Ethyl alcohol & 5% KOH or 1-Propanol + NH_4OH. Solution tested using fluorometer and 546-590 filters.	550 580	.01 μg/1. Dependent on background levels and fluctuation.	1) Dye is photochemically stable. 2) Dye may be used in low pH waters.	1) Requires the use of a flurometer. 2) Moderate clay sorption.	Rhodamine has been used extensively in Canada & USA. This is not a suitable method for amateurs without access to a flourometer.
Lycopodium Spores *Lycopodium Calvitum*	Plankton netting nylon-25 micron	Spores & sediment are washed from the nets. Microscopic examination is used to identify spores.	N/A	Dependent on background levels. Several kilograms of spores are usually used.	1) Several simultaneous tests may be conducted using different colored spores 2) No coloring of water occurs.	1) Spores may be prematurely filtered out. 2) Field collection system elaborate. 3) System is generally more expensive.	Spores have not been extensively used in North America.
Optical Brighteners Colorless normal light	Unbleached cotton	Visual examination of detectors under UV light or 7-37; 2A + 47B Filters.	360 435	Dependent on background levels, but generally at least .1 μg/1.	1) Inexpensive. 2) No coloring of water occurs.	1) Background readings may be excessively high. 2) Adsorbed onto organics.	May be used simultaneously with a green & red dye using fluoro-metric separation.
Direct Yellow (DY 96) Low Visibility Stilbene derivative	Unbleached cotton	Visual examination of detectors under UV light or 7-37; 2A + 47B Filters	N/A	1.0 μg/1 on cotton, and with fluorometric analysis.	1) Little natural background. 2) Good stability and low sorption. 3) No coloring of water.	1) Moderate cost. 2) Sensitive to pH.	Has been used extensively in Kentucky.
Salt NaCl Colorless	Recording specific conductance meter or regular sampling	Either a direct test for an increase in chloride, or a substantial increase in specific conductance	N/A	Dependent on background levels. Several hundred kilograms may be needed for larger tests.	1) Generally considered safe for use on public water systems. 2) Useful where fluorescent background conditions exclude other methods	1) Large quantities usually needed. 2) Background specific conductance is often high.	Salt is occasionally used by the US Geological Survey for tests dealing with public water supplies

[1]G. K. Turner Filters for Turner 111 Filter Fluorometer.
[2]Dye is usually most visible in clear water, deep pools, and in bright sunlight. These figures are not exact.
[3]Very dilute dye solutions may be concentrated upon the detector over a period of time.

instruction and hints. Stream tracing is an art and it is not uncommon for experienced researchers to disagree over even basic techniques. Except for the danger of contaminating the dye detectors, qualitative stream tracing using one tracer at a time is fairly certain (if a positive test appears). The quantitative methods using fluorometric separation of different dyes may give somewhat ambiguous results. In short, if a simple qualitative test will answer the question being asked, don't make the test needlessly complicated. Sawdust or computer card chips may be all that's needed to establish the connection between two cave passages. As with working a jigsaw puzzle, the first pieces are the hardest to get, but it becomes more predictable as the pattern begins to emerge.

The fluorescent dyes have an absorbance peak (maxima) at one wavelength and reemit the energy at a longer wavelength (some energy is always lost). Many dyes may be excited at several different wavelengths, but the emission

Table 2. Survey of Groundwater Tracers

NATURAL TRACERS		INJECTED TRACERS					
Stable Isotopes		**Radioactive**		**Activatable**		**Inactive**	
						Ionized Substances	**Drift Material**
Deuterium	^{2}H	Tritium	^{3}H	Bromine	^{35}Br	Salts: Na^+Cl^-	Lycopodium Spores
Oxygen—18	^{18}O	Sodium—24	^{24}Na	Indium	^{49}In	K^+Cl^-	Bacteria
Carbon—13	^{13}C	Chromium—51	^{51}Cr	Manganese	^{25}Mn	Li^+Cl^-	Viruses
Nitrogen—15	^{15}N	Cobalt—58	^{58}Co	Lanthanum	^{57}La	Na^+I^-	Fungi
Strontium—88	^{88}Sr	Cobalt—60	^{60}Co	Dysprosium	^{66}Dy	K^+Br^-	Sawdust
Sulfur—34	^{34}S	Bromine—82	^{82}Br				
Radioactive Isotopes						**Fluorescent Dyes:**	
Tritium—3	^{3}H	Gold—198	^{198}Au			Optical Brighteners	
Carbon—14	^{14}C	Iodine—131	^{131}I			Direct Yellow 96	
Silicon—32	^{32}Si	Phosphorus-32	^{32}p			Fluorescein	
Chlorine—36	^{36}Cl					Lissamine FF	
Argon—37	^{37}Ar					Rhodamine WT	
Argon—39	^{39}Ar						**Physical Characteristics**
Krypton—81	^{81}Kr						Water Temperature
Krypton—85	^{85}Kr						Flood Pulse

wavelength remains constant. Fluorescein always appears green whether it is excited at 365 nm or 485 nm. The optical brighteners (blue dyes) are invisible under "normal" light and generally have peak emission wavelengths around 435 nm. Fluorescein has an emission peak about 515 nm and Rhodamine WT at about 590 nm (Figure 1). Table 1 reviews the characteristics of commonly used tracers in karst studies, and Table 2 categorizes groundwater tracers in general.

Qualitative Techniques Using Fluorescent Dyes

Precautions—Check that no public or private water intakes will be affected by the dyes. Diplomatically notify the state water regulatory agencies of the study. Don't ask permission *per se* to use such and such dye—most agencies will just say "no" to protect themselves. Try to calculate dye concentrations so surface streams will not be colored. Check to make sure no one else is conducting tracer tests in the area.

Planning—Review all the previous tracer tests and hydrogeologic data. Try to locate all possible resurgences. Avoid conducting the initial tests under extremely high or low flow conditions. Measure or estimate discharge at the resurgences and calculate expected travel time for the dye (it may take several tests to get a feel for this). Good experimental control must be planned into the test. The detectors should all be made and handled in the same manner. A few detectors placed upstream of suspected resurgences should provide background values during the testing period.

Dyes—The characteristics of the tracers most frequently used in North American karst studies are summarized in Table 1. Fluorescein sodium (CI45350) is a green dye (Plate 7) which is almost "traditional" for use in karst areas. It is readily adsorbed on activated charcoal and eluted with a basic alcohol solution for visual examination. The dye has a low sorptive tendency, is photochemically unstable, and may lose fluorescence in low pH (< 5) waters.

Direct yellow 96 is a yellow dye which imparts a bright canary yellow to unbleached cotton detectors. The dye is detectable at 10 ppb on cotton examined under ultra-violet (UV) light. No chemical treatment is used. Almost invisible after dilution in water, optical brighteners are fluorescent in the ultraviolet range and impart a blue-white color to unbleached cotton detectors. There may be high natural background levels which interfere with the blue dyes—good "control" detectors must be maintained. These dyes are photochemically unstable and invisible under normal light.

Amount of Dye—The minimum amount of dye which can be detected for the dyes listed above is about .1 ppb (depending on background). The ability of passive detectors to collect dye depends on the length of time they are exposed to the tracer as well as the concentration of the tracer. The average velocity of the water (a function of discharge) is an important factor but hard to estimate. If discharge remains constant and the amount of dye injected is doubled, the peak recovery concentration should more or less double. However, if the amount of dye injected remains constant and the discharge falls by half, the peak recovery concentration will not rise linearly because of greater molecular dispersion of the dye in the lower velocity flow and accompanying longer travel time for the recovery. Almost all of the published formulas overestimate the amount of dye required (especially for long distances or high discharge). Most researchers develop a "standard dosage" based on intuition for their own study area. Perhaps the best empirical formula to-date is presented by Aley and Fletcher (1976) for calculating fluorescein dosages for open channel flow and charcoal detectors

$$Wd = 1.478\,(DQ/V)^{.5}$$

where Wd is the weight of fluorescein dye in Kg, D is the straight line distance in km from sink to resurgence, Q is discharge in m^3 second, and V is estimated velocity of flow in M/hour.

The mean velocity depends on discharge, gradient, and

flow characteristics. Even within a given region, considerable range may be found in groundwater velocities.

If diffuse flow conditions are expected, or if the dye must be injected through a soil cover (such as in the bottom of a doline), the required amount of dye will be considerably greater than needed for open channel flow conditions. Leibundgut and Wernli (1982) present a detailed analysis of the problems of calculating dye injection concentrations.

Injecting the Dye—Generally, the dye should be diluted about 10:1 with water before injection. If possible, the dye should be poured into a rapidly flowing turbulent reach of the stream to be traced. The tracer may be injected in normally dry dolines by using a tank truck to provide at least 2000 l of injection water (Plate 6). About 400 l of water should be dumped before the dye is added. This will help determine that the water drains rapidly from the doline and reduce adsorptive losses from the soil cover. Injecting the dye is often a messy job—place the detectors before injecting the dye.

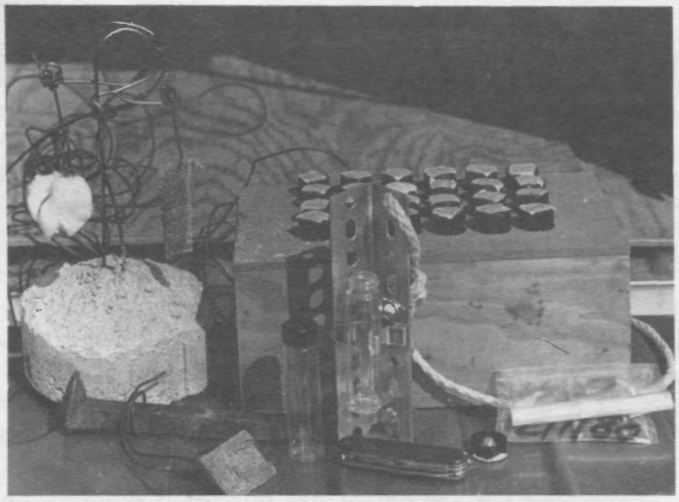

Figure 2. **Photograph showing sampling equipment and passive detectors. From left to right: "Quinlan Gumdrop" detector holder with cotton ball on left arm and charcoal detector on right arm (railroad spike with charcoal detector), 30 ml glass sample vial, vial holder for use in wells or from bridges, plastic bag for transport of individual detectors, wooden sample holder to transport samples with minimal exposure to light.**

Detectors—Passive activated charcoal detectors are used for fluorescein and unbleached cotton for direct yellow 96 and the optical brightness. The fresh activated coconut charcoal (6–14 mesh) is held in plastic or aluminum window screening folded into "envelopes" about 5 cm x 5 cm. The cotton detectors may be unbleached cotton balls or a small sheet of cotton stretched over a frame. The detectors may be tied to rocks and anchored to the bottom of small streams and springs. The "Quinlan Gumdrop" (Figure 2) may be tied to the shore and is a better anchor for use in larger springs or streams with silty beds.

The changing schedule for the detectors depends somewhat on the sediment, pollution load, and amount of algal growth in the springs. In clear water, the charcoal detectors are sensitive for several weeks, but the ability to adsorb dye and maintain low background interference decreases steadily with time. Once the dye has been adsorbed onto the detector it seems to remain almost indefinitely. Detectors have been retrieved from cave passages over one year following a fluorescein tracer study and tested positive. However, charcoal readily adsorbs and concentrates many compounds over time, and cotton is subject to siltation and algae growth. In most areas the detectors should be changed at least weekly.

The detector should be placed in the main current but shielded from high velocity, turbulent water. The detectors should not be in direct sunlight and may have to be "hidden" to prevent tampering. Smart and Smith (1976) found that charcoal detectors did not work well in tropical regions.

Analysis—The cotton detectors are rinsed under a jet of tap water and air-dried in the dark. They are examined visually with a hand-held ultraviolet lamp (usually at both 254 and 366 nm) and the intensity of dye fluorescence recorded (none, weak, medium, strong). The optical brighteners fluoresce a blue-white; direct yellow 96 fluoresces a bright canary yellow. If both dyes are present, the cotton fluoresces a characteristic white (Quinlan, 1977).

Every researcher seems to use a different procedure for testing charcoal detectors. The charcoal is rinsed in water and shaken into a test tube, jar, or petrie dish (some workers dry the charcoal first). The charcoal is covered with a basic alcohol solution and allowed to sit for from one-half hour to several days. It is then examined under sunlight or a high intensity white light (slide projector beam). The appearance of a yellow-green "glow" at the top of the charcoal signifies a positive test (Plate 3). The principal interference appears to be wastes from livestock which appear as a greenish-yellow color—the eye can tell the difference, but this "interference" can give very high readings on a filter fluorometer using the fluorescien filter combination. Visual examination of the charcoal for fluorescein is probably more reliable than instrumental readings of the elutent (at least using the filter fluorometer). The samples should not be shaken if they are to be examined visually, and should be stored in the dark.

A series of laboratory tests were conducted to evaluate the efficiency of different elutriants for fluorescein determination. Fresh activated coconut charcoal (Fisher catalog no. 5685A) was exposed for .5 hours to a fluorescein solution (10 ppb). The charcoal was rinsed in distilled water and separated into test tubes. The samples were treated with different combinations of alcohol and base elutriants and evaluated visually and with a fluorometer. This test suggested that the greatest intensity of fluorescence was pro-

duced by a solution of approximately 25% distilled water, 25% NH_4OH and 50% l-propanol (Smart and Brown, 1973). The "traditional" mixture of 95% ethanol and 5%KOH (Dunn, 1957) and isopropyl alcohol and NH_4OH (Aley and Fletcher, 1976) both performed satisfactorily for the visual test but ranked well below the l-propanol solution on fluorometric analysis.

In strongly positive tests, the maximum color intensity develops within about one-half hour and then slowly decreases, probably due to readsorption of the dye by the charcoal. However, some weakly positive tests take from several hours to a day to develop a clearly visible color. The detectors should be kept at least 24 hours before a final evaluation is made. Examination under UV light is not usually advantageous—the excitation peak for fluorescein is 485 nm.

There appears to be a complete lack of correlation of detector intensity either with discharge, distance, peak concentration, or length of or time of exposure to the dye (Spangler et al., 1983). Smart (1976) described a method to quantify the peak concentration of optical brightener on cotton detectors using fluorometric procedures, however, the generally high background fluctuations in this wavelength (415 nm) makes quantification of the optical brighteners difficult at best.

The intensity of the dye eluted from charcoal is also a function of the "freshness" of the charcoal, the elutriant mixture, the ratio of elutant to charcoal, and the time of exposure to the elutriant. To achieve maximum elutant fluorescence, Smart and Brown (1973) recommended: 1) dye concentrations should be as high as possible for as long as possible; 2) detectors should be changed often (1 or 2 days); 3) detectors should be dried on removal if analysis is not immediate and; 4) stream flow through the detector should be maximized.

Negative Tests—Negative tests do not necessarily prove that a connection does not exist. Tracer tests may fail for a number of reasons (Jones, 1976): 1) the correct resurgence(s) not monitored; 2) inadequate quantity of dye; 3) sorption losses to clays or organic material; 4) diffuse ground water flow conditions causing very slow movement of the tracer; 5) sufficient dye travel time not allowed; 6) tracer "masked" by high background conditions; 7) insulation (coating) of the detectors by sediments; 8) biochemical decay of the tracers; 9) dilution of the dye from flooding; 10) inadequate time for clearing of the tracers between tests in the same areas.

QUANTITATIVE PROCEDURES

One of the outstanding advantages of fluorescent tracers is the ease with which they may be quantitatively analyzed. The lower limit of detection of any tracer depends on the natural "background" level inherent in the analytical procedure, or more specifically on the fluctuation of this background. In most temperate zone karst areas (and populated areas), the blue wavelengths have the greatest noise-to-signal ratio, the green wavelengths, the intermediate, and red wavelengths the lowest. The red dyes should therefore be detectable at lower concentrations and be the easiest to quantify.

Fluorescent tracers are usually analyzed using a fluorometer. Spectrafluorometers are generally laboratory instruments which can scan (manually or automatically) through the spectrum of interest. Filter fluorometers (Figure 3) are smaller and many are suitable for field use. They are

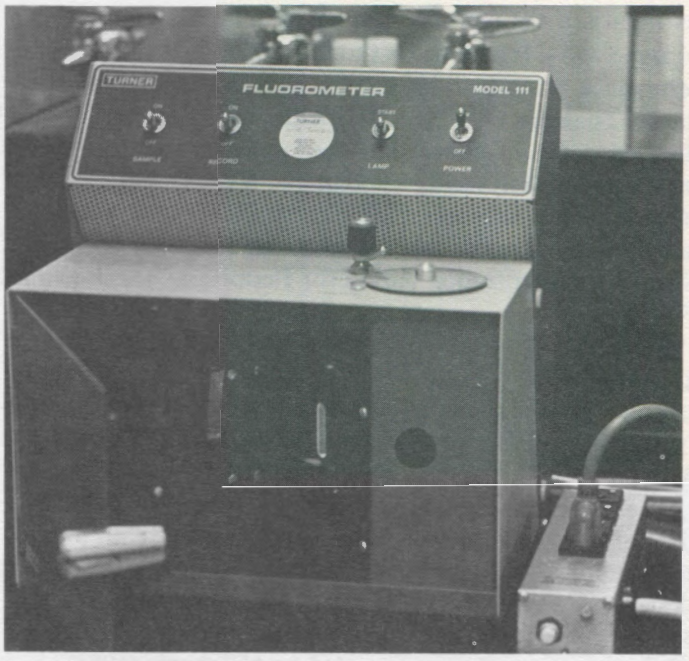

Figure 3. **A filter fluorometer shown with sample door open. Light from the lamp passes through a primary filter to the sample culvet (in door) and is then reflected through the secondary filter to the photomultiplier which sends a signal to the readout devise. Voltage surge protectors may be added at the outlet box (right).**

set at a given wavelength by selecting appropriate filter combinations. For practical purposes, the minimum detectable concentrations of dyes will be a function of the background levels, so both types of instruments have essentially the same sensitivity. The spectrafluorometer's advantage is the ability to scan water samples—a real advantage when some types of interference are present in the sample. The filter unit is more portable, less expensive, and well suited to analyzing large batches of samples. Filter units are the most used instruments in dye-tracing work.

The work by Wilson (1968) is a good basic manual of fluorometric procedures. Also, the instruction manuals provided with the instruments are a good source of information. Several papers describing quantitative procedures are presented in Gospodaric and Habic (1976). Behrens (1982) and Kass (1982) describe techniques for separating and

quantifying samples containing more than one dye. The following comments apply primarily to filter fluorometers.

Filters—The primary filters must be matched to the emission peaks of the lamps (The uneven light output severely limits the ability to do a true double scan with spectrafluorometers.) There is some spectral overlap between the blue, green, and red dyes (Figure 1). Some of this overlap can be eliminated by using "narrow pass" filters to narrow the range of wavelengths transmitted to the photomultiplier. It may be possible to shift the excitation or emission spectrums somewhat away from the maximums for the dye (the sensitivity of the instrument is rarely a limiting factor).

Standards—It may be best to use water collected from the resurgence prior to injecting the dye to prepare the standards. Spangler et. al., 1983 found that the lower pH of distilled water standards quenched some of the fluorescent intensity of fluorescein. Rhodamine WT standards appear to be stable for several months, but standards for fluorscein and the blue dyes should be prepared fresh for each test. Sample standardization curves for fluorescein are shown in Figure 4.

Sampling—The samples should be stored in the dark in glass vials. Figure 2 shows storage boxes for transporting the samples. Automatic samples can save a lot of labor. Several types are available commercially. Crawford (1979) gives a description of a "homemade" sampler.

The sampling interval should be adjusted to the travel time of the tracer. Fifteen minute sampling intervals may be needed for travel times under 24 hours. Tests which are drawn out over a period of months may use 2 or 3 day sampling intervals, although two samples per day is the minumum interval recommended by Milanovic (1981).

Suppression Interference—The samples may sometimes be directly treated to suppress background or selectively enhance fluorescence. The fluorescent intensity of Rhodamine WT is very temperature dependent, so an elevation in sample temperature should have a much greater effect on the fluorescent intensity of Rhodamine WT than on background fluorescence (Calvin Alexander, written communication). Fluorescein is very dependent on the pH of the sample, so (Behrens, 1982) has suggested that acidifying the sample should quench the presence of fluorescein.

Fluorometric analysis of the elutant from charcoal detectors poses several problems, but it's almost a required procedure for using Rhodamine dye. The charcoal concentrates many substances causing background fluoroscence, and some of the elutant solutions may have high background fluorescence. In examining the elutant from charcoal for fluorescein, the unaided eye appears to be more adept at discerning fluorescene dye from livestock waste products than does a filter fluorometer. If the detector is to be tested for both fluoroscein and Rhodamine, Jones (1976) recommended a visual examination for fluorescein and a fluorometric examination for Rhodamine.

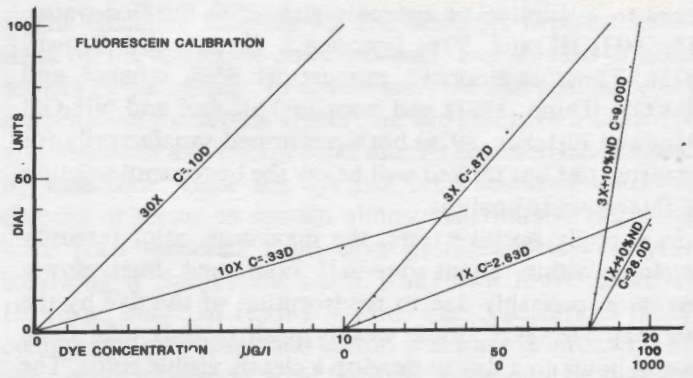

***Figure 4.* Typical set of calibration curves for fluorescein sodium. Each batch of dye, and each instrument requires a unique standardization to convert the dial readings (or relative fluorescent intensity) to concentration.**

CONCLUSIONS

Dye tracing techniques have become increasingly sophisticated in recent years. The skill, however, is still as much an art as a science and technology cannot be substituted for a thorough knowledge of the study area. Tracer tests must be carefully planned and be appropriate to the local hydrologic boundary conditions. The simple coloring of the water can be a great nuisance in the case of a water supply, and amateur speleogists are well advised not to risk tests in areas where public water supply springs are located.

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USE OF OPTICAL BRIGHTENER AND DIRECT YELLOW DYES FOR WATER TRACING IN THE INNER BLUEGRASS KARST REGION, KENTUCKY

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Optical brightener and direct yellow dyes were utilized for water tracing studies in the Inner Bluegrass Karst Region, Kentucky. Cotton fabric detectors were employed for the adsorption of both of these dyes and were utilized for quantitative studies using a spectrofluorometer. Direct relationships between intensity, concentration, and exposure time were obtained from the analysis of optical brightener dye adsorbed onto cotton fabric under laboratory conditions. Relationships between these factors for direct yellow dye, however, yielded poor correlations. In addition, relationships between visual intensity, dye quantity, spring discharge, and length of trace showed weak correlations for optical brightener and no correlations for direct yellow dyes. Comparisons between visual fluorescence and instrumental intensity of detectors indicate that optical brightener dye may be detected analytically below visual methods, while direct yellow appears detectable visually as well as instrumentally to the lowest concentrations.

INTRODUCTION:

Optical brightener (Fluorescent Brightener 28) and direct yellow (Direct Yellow 96) dyes were utilized in water tracing experiments in a study of northern Fayette and southern Scott counties, in the Inner Bluegrass Karst Region, Kentucky, from September 1979 to May 1981 (Spangler, 1982). From January 1980 to May 1981, these dyes were also investigated in the laboratory with the aid of a spectrofluorometer (Byrd, 1981). Both of these dyes have been used extensively for qualitative water tracing experiments in the Mammoth Cave Region by Quinlan (Quinlan, 1976; Quinlan and Rowe, 1977). They were subsequently adopted for use in the Inner Bluegrass Karst Region by Thrailkill (Thrailkill et al., 1982). Prior to Byrd (1981), water tracing studies within the Inner Bluegrass had been largely qualitative or semi-quantitative at most, involving the use of passive dye detectors which fluoresce under ultraviolet illumination when exposed to certain water tracing agents such as the above-mentioned dyes. This paper is devoted to a discussion of the methods and results of both qualitative dye tracing and quantitative laboratory investigations of optical brightener and direct yellow dyes.

FIELD METHODS

Optical brighteners were first utilized in Europe (Crabtree, 1970; Glover, 1972) before being employed in this country. Direct yellow dye was first used for tracing in the Mammoth Cave Region by Quinlan (1976). Both of these dyes have similar characteristics which enable them to be utilized as successful water tracing agents. Some advantages of these dyes are that: they are colorless in dilution, capable of adsorption onto cotton, detectable in very low concentrations over long distances, non-toxic to plants or animals, and have low adsorption onto clays. In addition, direct yellow dye can be used in areas where significant optical brightener contamination (derived from detergents) of the groundwater is present. Optical brightener is manufactured as a liquid dye, while direct yellow dye occurs in the form of a yellow powder which must be thoroughly mixed with water before introduction to the groundwater system.

As mentioned above, a distinct advantage of these dyes is their ability to be adsorbed onto cotton. Quinlan (Quinlan and Rowe, 1977) utilized surgical cotton detectors, and similar detectors were used in the Inner Bluegrass Karst Region prior to 1978. In order to allow instrumental, as well as visual, evaluation, a cotton fabric detector was designed and used for all subsequent traces. The detectors are constructed from bleached mercerized combed 3.11 cotton broadcloth (Testfabrics, Inc., 419-A) which has not been treated with optical brighteners. The fabric is cut into cross-shaped pieces consisting of a 5 x 5 cm square with four arms of the same size attached. The side squares are folded toward the center and fastened with brass staples to form sleeves. Two squared C-shaped frame halves constructed of No. 9 aluminum wire are inserted into the sleeves, and the resulting detector and frame assembly is supported in the water by overlapping loops at the end of each frame half.

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Stages in the construction of the detector are shown in Figure 1.

The fabric detector is as subject to algal attack as the surgical cotton detector and decomposes substantially after one week at some sites. In all other respects, however, the fabric detector is judged to be more satisfactory. Mud adheres to it less, it is more easily rinsed, and, if properly constructed, it shows little deterioration (except for algal attack) with time. Positive fabric detectors exhibit a more even fluorescence, and contamination from handling is more easily evaluated. In addition, instrumental evaluation (see below) is relatively simple.

The cotton fabric detectors were supported by "gumdrops" constructed of concrete and steel wire, designed to keep upright against the flow of current and keep the detectors floating free in the water for maximum exposure to dye adsorption. These are similar to those used by Quinlan, except that his design (Quinlan, 1976) was slightly modified by embedding two or more wires in the concrete base for additional strength and to provide additional arms to support detectors. A length of cord was attached to the "gumdrop" supporting the detector, and tied to an object (usually a tree) near the output.

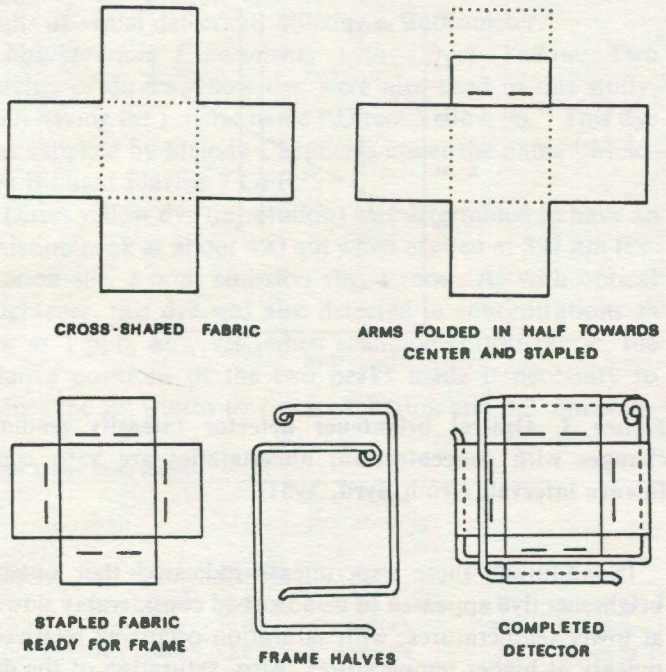

Figure 1. **Procedure for the construction of passive dye detectors from cotton fabric (from Byrd, 1981).**

When a dye trace was undertaken, detectors were placed in all suspected outputs and dye was introduced to an input, usually a sinking stream. The amount of dye used was based upon flow conditions (output discharge) and distance from input to output. Detectors were changed on a weekly basis. After washing and drying in the laboratory, a successful dye trace could be determined by exposing the cotton detector to long and short wave light sources. A positive optical brightener trace fluoresces bluish-white while a direct yellow trace fluoresces yellowish-green. The strength of the fluorescence was rated as negative, weak positive (A), moderately positive (B), or strongly positive (C); and appeared dependent on the amount of dye introduced and the detector exposure time.

LABORATORY METHODS

An equilibration system designed to simulate field conditions was set up in the laboratory in order to investigate various parameters of dye concentration, intensity, and exposure time (Byrd, 1981). Results from these investigations were then correlated with results obtained from field investigations, so that more quantitative information could be obtained about the behavior of these dyes under natural conditions.

The equilibration system consisted of three tanks containing a total of 231 liters of water, which were interconnected with tubing to allow continuous flow from one tank to another. Water temperature was continually monitored. Fabric detectors were suspended in one of the tanks and a stirrer was used to help circulate water around the detectors. The system was buffered to about 8.1 to approximate the pH of the groundwater in the Inner Bluegrass Karst. A standard dye solution was prepared and added to the tank system prior to the addition of the detectors. Several early experimental difficulties were encountered in the laboratory equilibration system. Chlorine from tap water appeared to quench fluorescence, so distilled water was used in the tanks. Continued erratic intensity values suggested the possibility of pH variations and buffering of the distilled water helped produce more stable results. In addition, variations in apparent dye concentrations due to unknown conditions in the equilibrium system, contributed to erratic results.

An Aminco SPF-125S scanning spectrophotofluorometer was utilized for the detection of optical brightener and direct yellow dye from the cotton fabric. Both exitation and emission wavelengths between the range of 200 to 800 nm can be determined by the use of monochromators. Once either the exitation or emission wavelength is determined (by manual methods), a scan can be performed to establish the other wavelength. The intensity of the dye fluorescence can then be determined. In addition, the bandpass of the excited and emitted light can be controlled by adjustment of the slit width, which controls the amount of light passing through the sample and, consequently, the width of the intensity curve. The resultant intensity scan was then recorded on a Beckman 10-inch potentiometric recorder connected to the spectrofluorometer.

A sample holder composed of two aluminum plates was constructed for holding the cotton fabric detector and de-

signed to fit into the sample compartment within the spectrofluorometer. The four sleeves trimmed from the central 5 x 5 cm square of a detector were folded over a 2.5 x 3.5 cm piece of cardboard and inserted in the sample holder. Up to four positions on the detector could be examined by reversing and inverting the mounted detector. Byrd (1981) gives more information concerning the dye equilibration system and spectrofluorometric analysis.

RESULTS AND DISCUSSION

Equilibration Experiments with Optical Brightener: Two batches of optical brightener dye were used in this study, both having the generic name "Fluorescent Brightener 28" and a color index number of 40622. One batch of the dye was supplied by American Cyanamid under the name "Calcofluor White ST," while the other batch was supplied by Mobay Chemical under the name "Phorwite BA."

Optical brightener (in solution) was determined to have an emission peak at about 420 nm (1 mm slit width) when excited at 335 nm (2 mm slit width), and be detectable in concentrations as low as 1 ppb (Byrd, 1981). On cotton fabric, however, the emission peak occurred at 430 nm when excited at 390 nm. The cotton detectors in this study were evaluated at the former peaks. Cotton fabric not exposed to optical brightener was observed to have an intensity (instrumental intensity) of about 40.0. Five experiments involving various concentrations of optical brightener in distilled water suggested that intensity increased slowly with short exposure time, gradually increasing until saturation (generally before five days), after which, the intensity leveled off or decreased (Figure 2). This relationship also seems to be valid with respect to concentration, where intensity varied the least during low and high concentrations, and increased the most where concentration also substantially increased between 15 and 17 ppb (Figure 3).

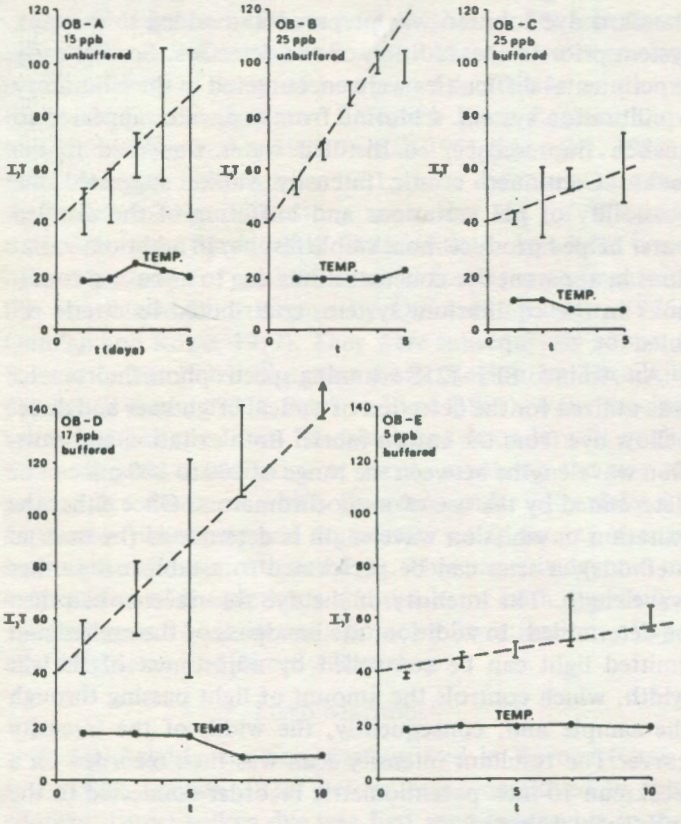

Figure 2. **Optical brightener detector intensity changes with exposure time and temperature; uncertainties are 95% confidence intervals (from Byrd, 1981).**

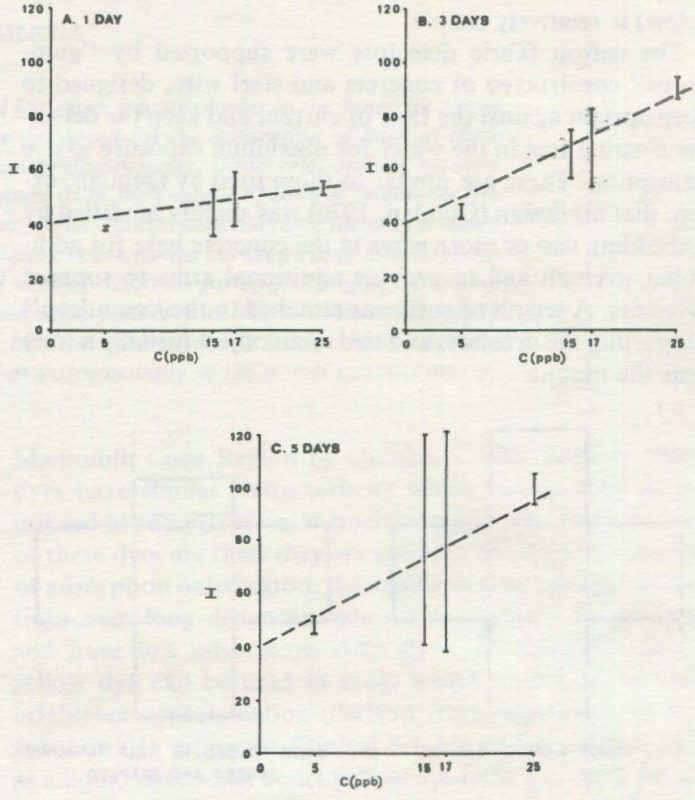

Figure 3. **Optical brightener detector intensity reading changes with concentration; uncertainties are 95% confidence intervals (from Byrd, 1981).**

In addition, these experiments indicated that optical brightener dye appeared to be adsorbed considerably slower at lower temperatures, with saturation occurring relatively quickly at higher temperatures. Also, saturation of the detectors was longer at lower concentrations. The increase in intensity with exposure time (t) in days was determined to be approximately 10 t at 5 ppb, 18 t at 15 to 17 ppb, and 25 t at 25 ppb. The increase in intensity with concentration (c) in ppb was also observed to be about 0.5C after one day and 2C after three and five days.

Table 1 shows a comparison between visual intensity rated as weak (A), moderate (B), and strong positive (C), and instrumental intensities determined by scanning the dyed

Table 1. Comparison of Visual and Instrumental Intensities of Fabric Detectors.

Optical Brightener		Direct Yellow	
Instrumental	Visual	Instrumental	Visual
38.0	NEG.	0.37	A
41.2	NEG.	0.39	A
44.0	A	0.44	A
44.2	A	0.46	A
47.5	A	0.47	A
58.4	A	0.49	A
64.0	A	0.52	A
67.6	B		
71.1	B		
79.4	B		
81.4	B		
96.7	C		
110.4	C		
124.4	C		
136.0	C		

fabric. The increase in instrumental intensity can be correlated directly with the results of visual intensity, with higher intensity readings indicating greater fluorescence. Note that visual intensity of fluorescence was not possible at an instrumental intensity of 38.0, implying that no die was adsorbed. The lack of visual intensity at 41.2 instrumental also suggests that optical brightener dye is detectable below the limits of visual detection, utilizing a fluorometer.

Equilibration Experiments with Direct Yellow: Two batches of direct yellow dye were also used in this study, both having the generic name "Direct Yellow 96." This dye was supplied by Melody Chemicals under the name "Melodye Brilliant Flavine 7 GFF."

Direct yellow dye (in solution) was determined to have an emission peak at about 490 nm when excited at 397 nm (excitation slit, 2 mm; emission slit, 1 mm). As with optical brightener, this dye was also detected in concentrations as low as 1 ppb; however, when scanning cotton fabric, the relative positions of the two peaks made it necessary to reduce the slit widths to 1 mm excitation and 0.2 mm emission. In addition, a fabric peak was consistently noted at about 475 nm but did not appear to interfere with detection of the dye. Cotton fabric not exposed to direct yellow dye yielded an intensity value of 0.44. Three experiments were conducted to investigate intensity versus concentration and exposure time (Figure 4); however, no correlations or trends between these various aspects were noted, presumably due to variabilities or uncertainties in the procedure or dye. Intensity values did not appear to increase with time, suggesting that most of the dye adsorption took place very early. Note that detectors immersed in 25 ppb concentrations for less than three days showed a decrease in instrumental intensity. In addition, the constant temperature throughout the experiment did not appear to have influenced dye adsorption.

A comparison between visual and instrumental intensities for selected direct yellow traces is also presented in Table 1. Detectors exhibiting instrumental intensity values less than 0.44 (undyed fabric) showed a positive visual intensity. Also, the slowly increasing instrumental values never yielded more than a weak positive visual intensity. These data seem to suggest that the detection of direct yellow dye in low concentrations is as effective on cotton fabric as it is with the spectrofluorometer, though the latter method is more quantitative.

The experiments involving the detection of direct yellow dye by fluorometric scanning seems to verify the above findings. A scan of the detector used in the 15.0 km trace to Royal Spring (Table 2) yielded very weak fluorescence under visual examination. When scanned at the above wavelengths established for direct yellow, however, a small peak was observed, verifying the presence of the dye. It is doubtful if the dye would have been detected by visual or spectrofluorometric means had the amount of dye used (and therefore, peak concentration) been much less.

Field Results: Fifteen dye traces employing optical brightener and direct yellow dyes were used to delineate

Table 2. Dye Trace Results.

Trace	Type of Dye	Quantity	Input	Q (1/s)	Output	Recovery Q (1/s)	Maximum Travel Time (DYS)	Minimum Velocity (m/s)	Distance (Km)	Fall (m)	Result
1	Direct Yellow	2.0 Kg	Deep Sps Swallet	2	Russell Sp	400	7.02	.012	7.15	11	B
2	Direct Yellow	2.0 Kg	Joyland	20	Russell Sp	240	7.12	.008	5.22	12	B
3	Direct Yellow	2.0 Kg	Scotts Sw	5	Russell Sp	350	6.89	.008	4.67	12	B
4	Optical Brightener	3.5 L	Towns Sw	5	Russell Sp	400	1.92	.012	1.95	9	B
5	Optical Brightener	3.5 L	Snowden Sw	30	Russell Sp	420	1.96	.010	1.75	9	B
6	Direct Yellow	2.0 Kg	Todd Sw	30	Russell Sp	710	6.96	.008	5.08	15	A
7	Optical Brightener	3.5 L	Ivy Sw	1	Russell Sp	120	6.96	.004	2.18	10	B
8	Direct Yellow	4.0 Kg	Cane Run Sw	140	Royal Sp	990	4.89	.028	12.00	19	A
9	Direct Yellow	6.0 Kg	Cane Run Sw	45	Royal Sp	1600	5.88	.030	15.00	31	A
10	Direct Yellow	4.0 Kg	Bruner Sw	15	Royal Sp	1100	5.07	.024	10.57	19	A
11	Optical Brightener	7.0 L	Hughes Sw	5	Silver Sps	100	4.06	.007	2.45	16	C
12	Optical Brightener	7.0 L	Blackburn	7	Silver Sps	130	4.21	.008	3.00	16	C
13	Optical Brightener	3.5 L	Seabold Sw	2	Silver Sps	150	6.92	.005	3.04	19	C
14	Optical Brightener	3.5 L	Quarry Sw	1	Silver Sps	170	7.02	.004	2.53	15	B
15	Optical Brightener	3.5 L	Hughes Sp	1	Silver Sps	130	6.97	.005	3.23	22	B

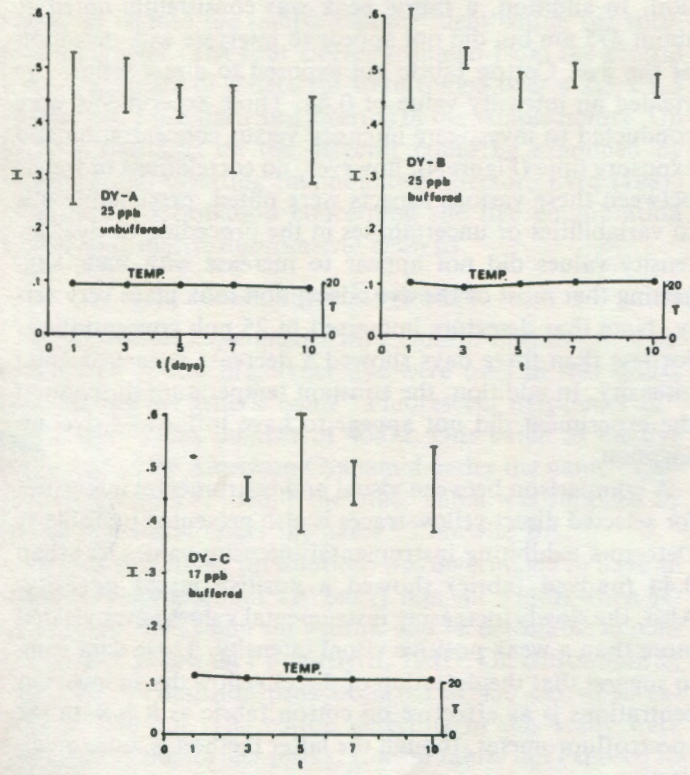

Figure 4. **Direct yellow detector intensity changes with time and temperature (Excitation 397 nm, 1 mm slit; Emission 475 nm, 0.2 mm slit); 95% confidence intervals (from Byrd, 1981).**

three of the larger groundwater basins in the area under discussion. Dye trace data for the Russell Cave Spring, Royal Spring, and Silver Springs groundwater basins are presented in Table 2. Within the Russell Cave Spring groundwater basin, seven dye traces were conducted (four direct yellow; three optical brightener) ranging in length from 1.75 km to 7.15 km. This spring has an optical brightener background due to contamination from septic tanks; however, the shorter traces were performed with this dye with successful results. The remaining longer traces employed direct yellow dye. Quantities of 2 kg of dye were observed to yield sufficient visible fluorescence. The weak trace result from Todd Swallet is interpreted to be due to the higher spring output and resultant lower peak concentration of dye. The maximum travel time represents the amount of time the detector was left in the spring, with the minimum velocity necessary for the dye to travel the input-output distance during this time span.

Three direct yellow dye traces were performed in the Royal Spring groundwater basin, including the longest trace in the study area of over 15.0 km. This spring also exhibits a considerable optical brightener background and required the use of direct yellow dye. The larger quantities of dye necessary for visible results in tracing in this basin reflect the longer distances and greater discharges involved. The weak fluorescence of the cotton detectors suggests that 4 kg is the minimum amount of dye required for visible detection under these conditions. Also note that in all cases, travel time was less than one week; consequently, velocities are considerably greater to travel the required distance.

Five other dye traces using optical brightener dye were performed in the Silver Springs groundwater basin. As no background contamination is present within this basin, this dye yielded excellent results. Dye traces in this basin averaged about 2.85 km. Initially, 7.0 liters of dye were used in tracing to this spring, yielding very positive results. Subsequent dye quantities were reduced by 50% to 3.5 liters for later traces, still yielding positive results. Undoubtedly, substantially less dye could be used and still detected visually.

As previously indicated, all dye traces were detected within the period of one week, indicating relatively fast flow-through conditions. Generally, flow times are based on distance from input, discharge of spring, and nature of the conduit. Many of these traces had, undoubtedly, flow-through times of only several days, especially during higher discharge periods. Investigations by Sullivan (1983) using fluorescein and rhodamine WT dyes (with the aid of an automatic water sampler) within the Russell Cave Spring and Royal Spring groundwater basins, subsequently indicated that travel times in these basins were considerably less than one week, suggesting that most of the dye pulse had passed by the fabric detectors before they were collected.

Relationships among visual detector intensity, dye quantity, discharge at the detection point, and distance (length of trace) for all traces conducted in the Inner Bluegrass Karst Region to date (Thrailkill et al., 1982, Appendix 1) are shown in Figure 5 for optical brightener and Figure 6 for direct yellow. In each diagram, the upper three histograms show the relationships between detector intensity and the ratio of dye quantity used, to discharge at the detection point, on a logarithmic scale. Because of the dilutive effect of large discharge, it would be expected that the most strongly dyed detectors (C) would be associated with largest values of the ratio, the weakest detectors (A) with lower values of the ratio, and detectors evaluated as moderate (B) would result from intermediate values. Although there is some suggestion of this in the optical brightener distribution, the order of the medians (M) shows no relationship (Figure 5). There is even less correspondence for the direct yellow detector intensities (Figure 6).

The lower three histograms in each diagram show variations in detector intensity with the ratio of dye quantity to length of the trace. Here again there may be a weak relationship for optical brightener (Figure 5), but little or none for direct yellow (Figure 6).

The reasons for the lack of correlation of the detector intensity either with discharge or distance are not obvious. There are other factors which would be expected to in-

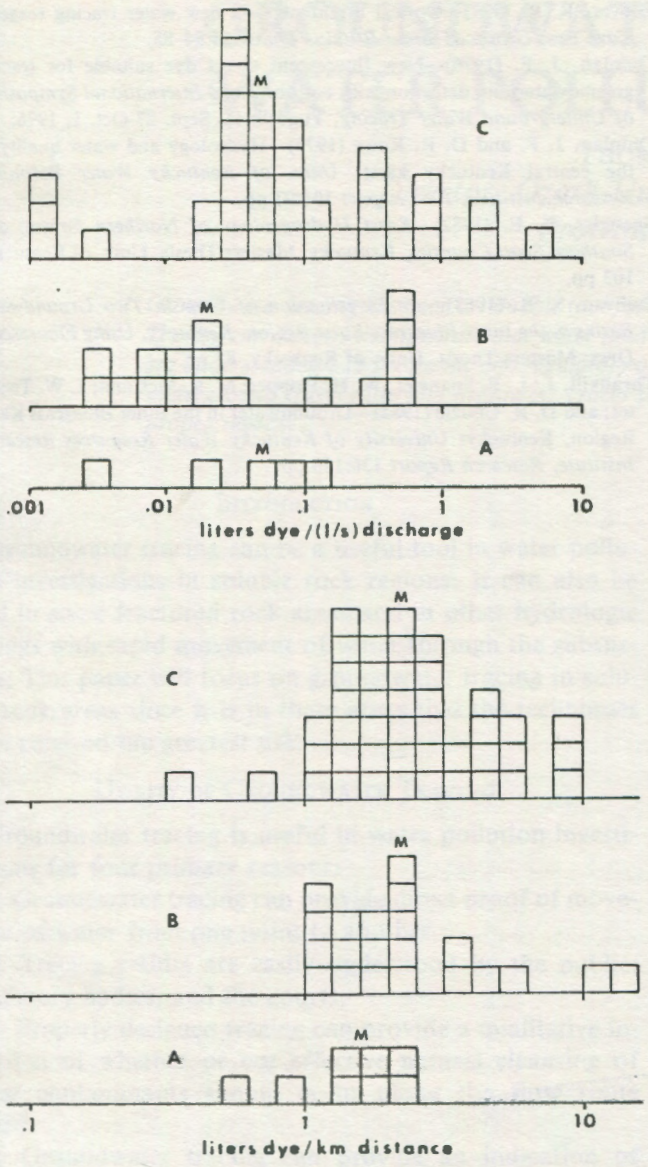

Figure 5. **Relationships between visual intensity, dye quantity, spring discharge, and length of trace, for optical brightener dye.**

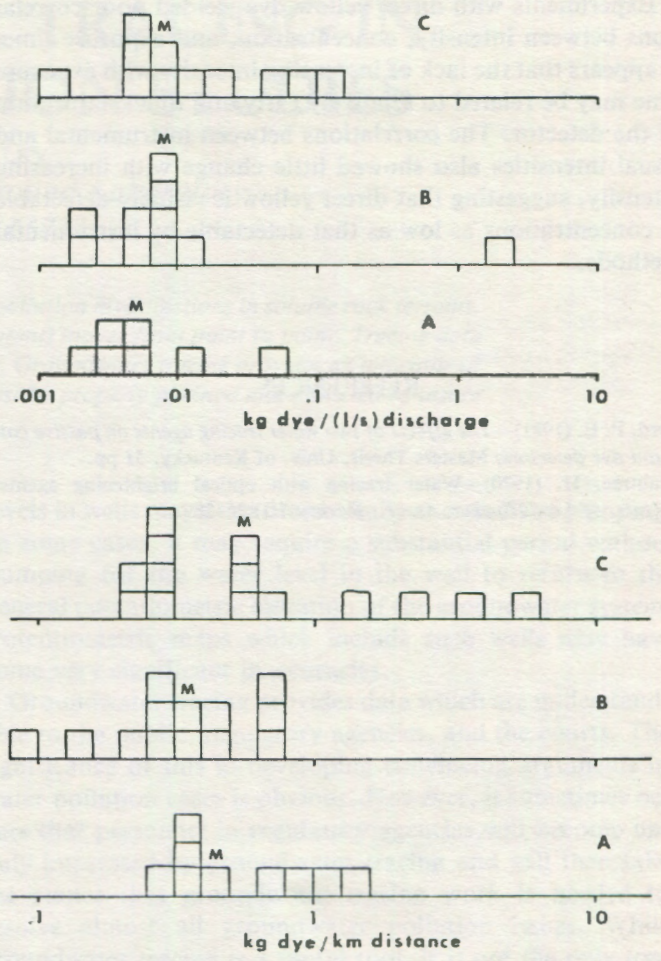

Figure 6. **Relationships between visual intensity, dye quantity, spring discharge, and length of trace, for direct yellow dye.**

fluence detector response, including detector immersion time, temperature, water chemistry, and adsorption and dispersion of dye during the trace. Most of these, however, tend to be relatively constant for the various traces. Furthermore, although the visual evaluation scale is admittedly imprecise, its correlation with instrumental intensities for optical brightener and direct yellow is good (Table 1), so it is unlikely that detectors were grossly misevaluated.

CONCLUSIONS

The use of optical brightener and direct yellow dyes for water tracing purposes yielded excellent results when detected by adsorption onto cotton fabric detectors. Quantities of 2 to 4 kg of direct yellow dye and 3.5 to 7.0 liters of optical brightener dye were observed to be quite sufficient for the discharge and distance parameters present in this area. The detection of these dyes within time spans less than one week indicates fast flow through time and suggests that most of the dye passes the detector during this period; however, relationships between visual intensity, dye quantity, spring discharge, and length of trace showed weak correlations for optical brightener and no correlations for direct yellow dyes.

Analysis of optical brightener dye with the aid of a spectrofluorometer showed a direct relationship between intensity, concentration, and exposure time. Intensity generally increased as concentration increased, and also increased with longer exposure time until saturation, after which intensity decreased as a result of less dye adsorption. Also the increase in instrumental intensity correlated directly with an increase in visual intensity, indicating that optical brightener dye could be detected below the limits of visual detection.

Experiments with direct yellow dye yielded poor correlations between intensity, concentration, and exposure time. It appears that the lack of increasing intensity with exposure time may be related to relatively early and quick saturation of the detector. The correlations between instrumental and visual intensities also showed little change with increasing intensity, suggesting that direct yellow is visually detectable in concentrations as low as that detectable by instrumental methods.

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Aley, Thomas—Groundwater Tracing in Water Pollution Studies: *NSS Bulletin*: 46 17-20.

GROUNDWATER TRACING IN WATER POLLUTION STUDIES

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Groundwater tracing has been a valuable tool for water pollution investigations in soluble rock terrains. Tracing can directly demonstrate that water (and a pollutant) moves from point to point. Tracing data are understandable to the public and regulatory agencies. Groundwater tracing provides an indicator of underground travel rates and distances. Tracer tests must be properly planned and executed to insure proper results.

INTRODUCTION

Groundwater tracing can be a useful tool in water pollution investigations in soluble rock regions. It can also be used in some fractured rock areas and in other hydrologic settings with rapid movement of water through the subsurface. This paper will focus on groundwater tracing in soluble rock areas since it is in these areas that the techniques have received the greatest use.

UTILITY OF GROUNDWATER TRACING

Groundwater tracing is useful in water pollution investigations for four primary reasons:

1. Groundwater tracing can provide direct proof of movement of water from one point to another.
2. Tracing results are easily understood by the public, regulatory bodies, and the courts.
3. Properly designed tracing can provide a qualitative indication of whether or not effective natural cleansing of water contaminants should occur along the flow route traced.
4. Groundwater tracing can provide an indication of underground travel rates; such rates are often grossly underestimated by people who are not adequately familiar with the hydrology of soluble rock terrains.

Groundwater tracing can be extremely valuable in directly demonstrating that water from one point moves to another. This is particularly valuable in soluble and/or fractured rock landscapes where subsurface flow directions may or may not tend to parallel surface flow directions. There is unfortunately a prevalent myth that groundwater basin divides are either directly or almost directly beneath surface basin divides. This is an adequate general characterization for soluble rock lands.

Water pollution work must often be conducted in areas where there are very few wells where depths to water can be measured. In such areas, groundwater tracing is frequently the only practical method for delineating those areas which contribute water to the particular groundwater basins of concern. Furthermore, in some soluble rock areas, water levels in wells may drop dramatically as a result of pumping. In some cases, it may require a substantial period without pumping for the water level in the well to return to the general potentiometric elevation of the groundwater system. Potentiometric maps which include such wells may have some very significant inaccuracies.

Groundwater tracing provides data which are understandable to the public, regulatory agencies, and the courts. The significance of this in developing convincing arguments in water pollution cases is obvious. However, it sometimes occurs that personnel in regulatory agencies will become unduly impressed by groundwater tracing and will then take the stance that groundwater tracing work is needed to resolve almost all groundwater pollution issues. While groundwater tracing is a useful tool, it is not the only tool available to groundwater hydrologists working on pollution issues, and this must be recognized.

Properly designed groundwater tracing tests can provide a qualitative indication of whether or not effective natural cleansing occurs as waters travel through the groundwater system from one point to another. Some years ago I conducted several groundwater traces from dumps in sinkholes to springs (Aley, 1969; 1972; 1972a). One of the arguments raised about the tracing work was that although the dye went from a sinkhole dump to a spring, "anything really bad" in the water could probably get "filtered out" as the water moved through the groundwater system. This argument led to groundwater tracing with stained *Lycopodium* spores; the method is explained in Aley and Fletcher (1976). The *Lycopodium* spores were selected as a tracing agent because their mean diameter is 33 microns, and they are thus 10 to 15 times larger than most pathogenic bacteria (Aley et al., 1972). If the spores can traverse a particular groundwater travel route without being removed by filtration, then one can presume that smaller materials (such as bacteria and viruses) can also be transported along the same route without being removed by filtration. This does not mean that the smaller materials may not be removed from the water by adsorption or some other process.

There are several dyes used in groundwater tracing work. Some of these (such as optical brighteners, direct yellow 96, and fluorescent) have greater sorption tendencies than other dyes (such as rhodamine WT) (Smart, 1972; Jones, 1976). If the relative effectiveness of adsorption is an important issue in a particular case, one may be well advised to use a dye which is subject to appreciable adsorption. It has been my experience that it is difficult to recover optical brighteners and fluorescein from septic field systems which do not intersect discrete recharge zones (Aley, 1974). Discrete recharge zones are localized areas which can transmit appreciable volumes of water into the groundwater system (Aley, 1977; 1978). Discrete recharge zones typically do not provide effective natural adsorption, and this accounts for their ability to transport dyes with some of the higher sorption tendencies.

At present we do not have sufficient information to enable us to use some particular type and amount of dye to quantitatively measure the adsorptive effectiveness of a septic field or other waste site. There are enough variables that such an approach will probably never be possible. However, through experience with various tracing agents, we can develop qualitative insights into the relative effectiveness of adsorption in particular traces, and this obviously can be beneficial in resolving practical problems.

Finally, groundwater tracing is often useful in water pollution investigations because it provides an indication of underground travel rates and distances. It is unfortunate, but many people who are involved in questions of subsurface migration of pollutants are either unaware of, or choose to ignore, the rapid travel rates which often characterize subsurface water movement in soluble rock areas. As an example, Pye et al. (1983) in a report which purported to characterize groundwater contamination in the United States stated (page 4): "Once in the aquifer a contaminant will generally move with the groundwater and at a similar speed, which varies between a fraction of an inch to a few feet per day."

Groundwater travel rates in soluble rock regions are often very rapid, as anyone familiar with the karst hydrology literature knows. Numerous groundwater traces that I have conducted in soluble rock areas in Missouri, Arkansas, Indiana, and Wyoming have often demonstrated straight-line travel rates in excess of 1 km/day (0.62 miles/day). Rapid travel rates can also characterize long distance groundwater transport under gentle gradient conditions in soluble rock areas. As an illustration, dye and *Lycopodium* spores injected in a losing stream segment of the Eleven Point River in Missouri discharged from Big Spring, a tributary to the Current River. The straight-line travel distance for this groundwater trace was 63.6 km; the mean gradient was 1.93 m/km; and the mean travel rate for the first arrival of the tracing agents was 4.9 km/day.

Groundwater travel rates encountered in soluble rock lands can be a thousand times more rapid than Pye et al. (1983) indicate. Soluble rocks underlie perhaps 15% of the United States, and it is foolish to ignore conditions which often typify a major portion of this nation. Slow groundwater travel rates characterize some hydrogeologic settings and some groundwater components. However, in the case of water pollution investigations in soluble rock regions, most of the problems are associated with rapid groundwater movement through flow systems which provide ineffective natural cleansing.

Limitations of Groundwater Tracing

When groundwater tracing is used in conjunction with water pollution investigations, the tracing work is often not begun until after a problem has developed or a dispute has occurred. The person conducting the tracing work in this case is typically faced with a collection of problems not normally encountered during basic hydrologic studies. The nature of these problems should be understood and fully appreciated before any attempts are made to conduct groundwater tracing.

The first and perhaps the most significant problem is that both successful and unsuccessful groundwater traces will be viewed as "proof" by some of the people involved in the problem or dispute. If dye is injected in a drill hole at a waste site and is not recovered from any sampling wells or springs, one must anticipate that one side of the issue will use this unsuccessful trace as "proof" that the site causes no problems. It may be elementary logic that you cannot prove a negative; the fact remains that unsuccessful groundwater traces will not be overlooked or disregarded in reaching decisions about a particular pollution problem.

The second problem is that an investigator often does not have an ideal site for injecting a groundwater tracing agent. Often this is due to physical limitations of the site. At other times one cannot gain permission to inject the tracer at the site where the work most needs to be done. In other cases (but less commonly) there may be problems in getting permission to sample waters where the tracer might be recovered. Furthermore, one often faces not only site limitations but also hydrologic limitations. A particular pollution problem may be associated with wet weather conditions, but the investigator is forced to do the groundwater work during dry weather conditions.

The third problem is that groundwater tracing often requires sampling at a number of sites for a period of days or weeks. As a result, the investigator cannot stay with his sampling equipment. There is always the possibility that people involved in the dispute will tamper (either intentionally or unintentionally) with the tracing effort.

The fourth problem is that the tracing effort must be done right the first time. Often, one cannot try the trace again because something went wrong in the first attempt.

PREVENTING PROBLEMS IN GROUNDWATER TRACING INVESTIGATIONS

There are five recommendations for preventing problems with groundwater tracing investigations conducted as a part of water pollution studies:

1. Do not conduct a groundwater trace in a water pollution investigation unless it is needed to answer a relevant question.
2. Do not conduct a groundwater trace unless it is designed in such a manner that it will produce results.
3. Any tracing attempts should be preceded by thorough field work to locate all possible sites where the tracing agent might be recovered.
4. Avoid injecting tracing agents into man-made pits or wells that may not be integrated with the groundwater transport system.
5. Design and conduct the tracing work in such a way as to minimize the chance of incorrect conclusions resulting from tampering.

Do not conduct a groundwater trace in a water pollution investigation unless it is needed to answer a relevant question. In groundwater tracing one injects an exotic agent into the water and then samples for it at possible recovery sites. In water pollution cases there are often exotic agents present in the water which serve quite adequately as tracers; these should be used to the fullest extent possible. This is particularly true in cases where good injection sites may not be present.

As an example, I was once involved in a case where a sewage lagoon system serving a hospital was suspected of leaking into a series of springs which had suddenly appeared a few hundred feet away. A state agency, utilizing personnel without expertise in groundwater hydrology, injected dyes in the lagoon system on several occasions. The hospital used a water softener, and as a result the lagoon had an atypically large chloride concentration; so did the springs. There were other water quality similarities between the lagoon and the springs; there was no reason for the injection of dyes. Due to poor design of the tracing attempt and inadequate sampling, the agency did not recover their dye from the samples springs, and concluded that the lagoon did not leak. Subsequent independent sampling of the springs found detectable concentrations of one of the two dyes injected in the lagoon. The result was a muddled problem which may well require litigation to resolve. Not only was the tracing attempt unnecessary, but it was improperly conducted and was conducted in an area where tracing difficulties were predictable.

It is often difficult to trace water from lakes and lagoons into groundwater systems. In many cases it is better to make an assessment of whether or not a lake or lagoon is leaking by analyzing water budgets rather than from groundwater tracing attempts. The quantity of water or the timing of flow pulses can be an effective groundwater tracing tool which is sometimes superior to any tracing agent.

Do not conduct a groundwater trace unless it is designed in such a manner that you will get results. Use enough tracing agent, and use a tracing agent which is appropriate for the problem at hand. Make certain that you have an adequate supply of water to transport the tracing agent. Make certain that all possible recovery sites are sampled and that the sampling duration is adequate.

Finally, make certain that the injection site will answer the questions that are relevant. As an example, if the issue is whether some part of a proposed landfill operation is within the recharge area for a spring used as a public drinking water supply, the injection site for the groundwater tracing agent must be near the landfill but between the landfill and the spring. A site on the far side of the proposed landfill from the spring may be useful in answering other questions, but it will not tell us if part of the proposed landfill area contributes water to the spring. I have seen this exact situation occur, and the consulting geologist for the landfill operator selected his injection site on the wrong side of the landfill. Since the relevant question was not answered, litigation has resulted.

There may be occasional situations where the failure to recover a tracer from any of the sampling sites could be a valid objective of the groundwater tracing effort. As an example, we have injected dyes in municipal sewers and sampled for them at springs as a reconnaissance test for sewer line exfiltration problems. Such tracing efforts must be conducted very carefully, and to the extent possible other evidence should confirm any negative results from tracing efforts.

Any tracing attempts should be preceded by thorough field work to locate all possible sites where the groundwater tracing agent might be recovered. Unsuspected springs are sometimes found discharging from the bed of perennial streams, and field work and/or sampling procedures must be adequate.

Due to the nature of flow systems in soluble rock lands (Aley, 1977; 1978), wells are often poor sampling sites in groundwater traces. If groundwater tracers are injected in a natural drainage feature (such as a sinkhole, losing stream, or other discrete recharge zone) they may be recovered at a spring some distance away but not at any of the wells lying between the injection and recovery sites. This is because the flow route between the natural drainage feature and the spring follows localized conduits. Most wells will not intersect the conduits which are transporting the tracer-tagged water flow, and for this reason tracers will not be recovered in such wells. Although this does not mean that wells should not be sampled, great care needs to be used in interpreting negative tracing results from wells in soluble rock lands. If wells must be sampled, it is best to pump them heavily during the tracing attempt. In soluble rock lands it has been my experience that non-pumping wells are almost worthless in groundwater tracing programs.

Avoid injecting tracing agents into man-made pits or wells that may not be integrated with the groundwater transport system. It is generally unlikely that a small man-made pit or well will be significantly integrated with the groundwater transport system in a soluble rock area. If such sites are used for groundwater tracer injections, the tracing agents may be detained for long periods of time in the storage component of the groundwater system rather than in the transport component needed for successful groundwater tracing.

If man-made pits or wells must be used in groundwater tracing work, it is best to inject tracers in several of them rather than rely on a single injection site. Furthermore, particularly when man-made pits or wells are used, all injections should be accompanied with substantial volumes of water (preferably at least 4,000 to 8,000 liters). Although I have successfully conducted groundwater traces using such procedures in the past, I have generally found that such work requires larger quantities of tracing agents than are required when more desirable injection sites are available.

Design and conduct the tracing work in such a way as to minimize the chance of incorrect conclusions resulting from tampering. In water pollution cases, tracing investigations should be conducted in such a way as first to minimize the chance of tampering and secondly to detect tampering if it occurs. Each groundwater tracing situation is different, and the strategy which must be employed to protect against tampering will be different. Extra sampling stations upstream and downstream of important sampling sites can be useful if they are known only to the investigator. I do not make it a practice to explain in detail to anyone potentially involved in a particular issue exactly how I plan to conduct the tracing work. In some cases I quietly re-sample to provide confirming evidence.

CONCLUSIONS

Groundwater tracing can be an important tool in water pollution studies. The tracing can provide types of information which are not obtainable or not readily obtainable using other approaches.

Prudent groundwater tracing requires a good grasp of the limitations of tracing work in water pollution studies. Groundwater tracing requires thorough and careful work to insure proper results.

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A REVIEW OF THE TOXICITY OF TWELVE FLUORESCENT DYES USED FOR WATER TRACING

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Toxicological information is reviewed for twelve fluorescent dyes used in water tracing, Fluorescent Brightener 28, Tinopal CBS-X, Amino G Acid, Diphenyl Brilliant Flavine 7GFF, Pyranine, Lissamine Yellow FF, Fluorescein, Eosine, Rhodamine WT, Rhodamine B, Sulphorhodamine B and Sulphorhodamine G. Mammalian tests indicate a low level of both acute and chronic toxicity. However, only three tracers could be demonstrated not to provide a carcinogenic or mutagenic hazard. These were Tinopal CBS-X, Fluorescein and Rhodamine WT. Rhodamine B is a known carcinogen and should not be used. In aquatic ecosystems, larval stages of shellfish and algae were the most sensitive. Persistent dye concentrations in tracer studies should not cause problems provided they are below 100 µg/l.

INTRODUCTION

Fluorescent dyes have been widely employed in the tracing of water because of their high detectability. There is, however, increasing concern on the effects of chemicals introduced into natural waters. This prompted a previous review of the toxicity of fluorescent dye tracers (Smart, 1982). This paper updates the earlier work and also incorporates material not previously included due to limitations on space. Information is provided on the 12 fluorescent dyes listed in Table 1. The chemical structures of these dyes are given in Figure 1. Dyes for which the exact structural formula is not known, such as CI Fluorescent Brightener 15, are not included. In order to update this data base, I would appreciate receiving details of any toxicological information which has not been included, or which has become available since publication.

Before examining the data, it is important to remember that differences in test protocols and methods, in test species, route of administration and dose, all make comparison of toxicity data from different studies difficult. Furthermore, the standard of toxicity tests has improved with time, and some of the earlier studies may follow protocols no longer acceptable in modern toxicology. A more difficult problem is that the toxicity of dyes may well vary with manufacturer, or even the batch of dye tested, due to the presence of impurities. In the case of biological stains, for instance, Marshall and Lewis (1974) have shown that many substances other than the stated dye can be present. This has been confirmed for several of the dyes discussed in this paper by Lutty (1978) and Nestman et al. (1979), who quote purities as low as 75%. The presence of impurities explains why some workers reported Rhodamine B to be mutagenic in the *Salmonella typhum*—mammalian microsome test, while the pure dye was demonstrated to be non-mutagenic (Nestman et al., 1979). There are also differences in the concentration of commercial dyestuffs due to incorporation of additives, which makes comparison of different products difficult. No attempt had been made in this review to deal

Table 1. Colour index (3rd ed.) designations, dye type and bibliographical code used for the fluorescent dye tracers.

Name	CI Number	Dye Type	Code
Calcophor White ST CI Fluorescent Brightener	28	Stilbene derivative	FB28
Tinopal CBS-X CI Fluorescent Brightener	351	Sulphostyryl derivative	FB351
Amino G Acid	—	Dye intermediate	AGA
Diphenyl Brilliant Flavine 7GFF	DY 96	Stilbene derivative	DY96
Pyranine	CI 59040	Pyrene	P
Lissamine Yellow FF	CI 56205	Aminoketone	LYFF
Fluorescein Sodium	CI 45350	Xanthene	FL
Eosine Sodium	CI 45380	Xanthene	E
Rhodamine WT*	—	Xanthene	RWT
Rhodamine B	CI 45170	Xanthene	RB
Sulphorhodamine B	CI 45100	Xanthene	SRB
Sulphorhodamine G	CI 45220	Xanthene	SRG

*Acid Red 388

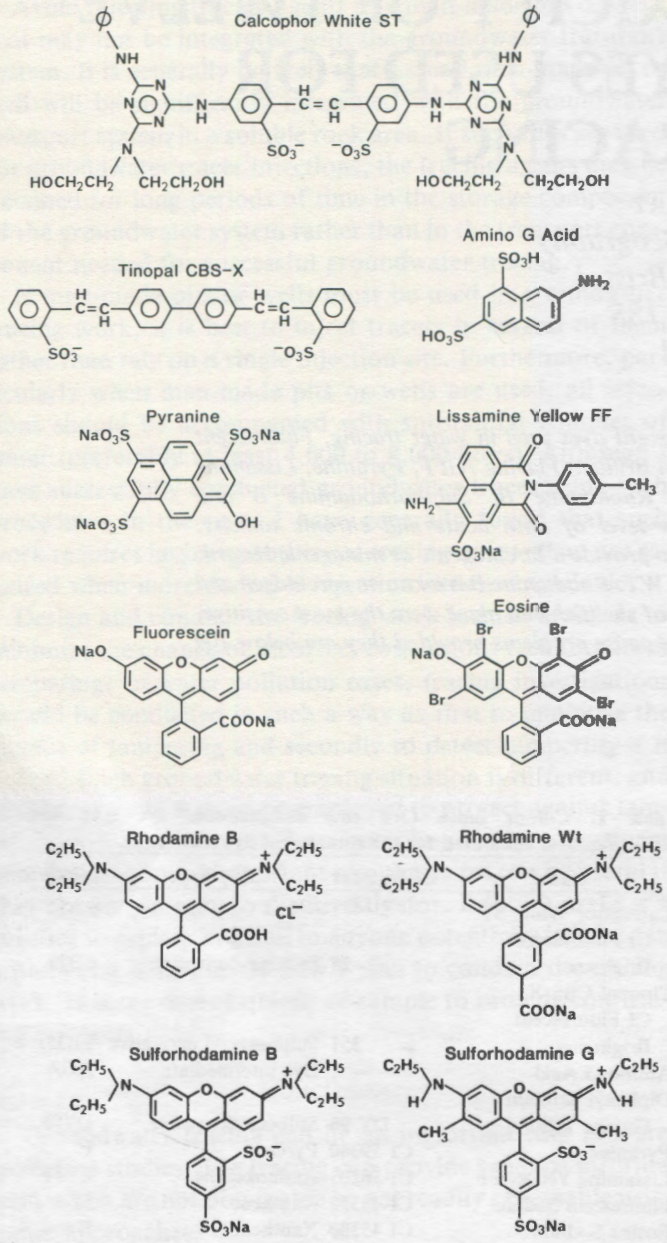

Figure 1.

with these problems, but referral back to the original source will often clarify these points. There are clearly advantages in utilising a product for which a hazard data sheet detailing toxicity information is available from the manufacturer.

The paper is divided into three sections, corresponding to the three main toxicological areas of concern in using tracer dyes. First, the toxicity of dyes in mammal systems is discussed in order to assess the acute and chronic effects of dye ingestion in man, and the possible hazards associated with handling the dyes. The second section reviews the data on carcinogenicity and mutagenicity, hazards which may be caused by long-term low-level exposure. Finally, the possible toxicity of tracers introduced into aquatic ecosystems is discussed. The toxicity criteria and measures included in the

Table 2. Toxicity of fluorescent tracer dyes in mammals.

Test	Animal	FB28	FB351	AGA	DY96	P	LYFF	FL	E	RWT	RB	SRB	SRG	Source
Acute Oral LD50 g/kg	Rat	14.5	5.58	—	> 15.0	> 15.0	8.56	6.72	> 1.0	> 25.0	> 0.5	> 10.0	> 10.0	16, 19, 23, 27, 28 43, 53, 106, 118.
	Mouse	> 10.0	> 5.0	—	—	—	—	4.74	—	—	0.89	—	—	15, 53, 94.
Acute Intravenous LD50 mg/kg	Mouse	—	—	—	—	1050	110	300	550	430	a	b	b	48, 66, 71.
No Effect Acute Intraperitoneal mg/kg	Mouse	375	—	—	—	> 50	> 50	> 91	> 50	> 167	5	> 75	> 75	24, 25, 57, 67.
	Rat	400	—	—	—	—	—	500	250	—	< 95	—	—	57, 94.
No Effect Chronic Oral.	Rat	> 1.0	> 0.5	—	—	—	—	—	> 2.0	—	0.1	—	—	68.
Dose (%)		106	12.9						18		18			
Time (Weeks)	Dog	—	> 1.0	—	—	—	—	—	—	—	—	—	—	
	Hampster	—	—	—	—	—	—	< 0.75	—	—	—	—	—	
								8.6						
No Effect on Reproduction or Teratogenicity	Chronic	1%	0.1%	—	—	—	—	—	—	—	e	—	—	68, 93.
	Rat	18m	3 gen											
Dose/Time	Acute													
	Rat	—	—	—	—	—	—	0.5g/kg	—	—	—	—	—	74, 96.
Irritation c	Eyes	m	e	—	o	s	s	—	—	se	—	s	s	16, 19, 27,
	Skin	o	s	—	o	o	o	—	—	m	—	o	o	39, 43, 106.
Photosensitivity d		o	v	—	—	—	—	+	+	v	—	—		31, 32, 33, 43, 56 77, 78, 102, 107.

Notes: a. LD50 Less than RWT. b. LD50 Greater than RB. c. o = none, s = slight, m = moderate, se = severe, e = extreme.
d. + = toxicity increased with light exposure. 0 = no effect. v = toxicity decreased with light exposure.
e. 'Highest no effect level in prior two-year study.' Exact dose not quoted.

summary tables have been selected to permit wide comparisons of all the dyes. Where several sources of information are available, these have been checked for conformity and the most toxic result recorded. The tables provide a summary only, and reference should also be made to the original sources from which the information has been abstracted. These sources are coded according to the numbers given in the bibliography.

Toxicity in Mammals

Of the acute lethal data reported (Table 2), the LD50 (Lethal Dose—50) value for oral administration is the best general indication of dye toxicity. Although the maximum experimental values for Eosine and Rhodamine B are lower than desirable, the LD50 for all twelve days is very high. None of the dyes would be regarded as toxic using this criterion, the corresponding value for common salt being 8 to 10 g/kg. The intravenous and intraperitoneal administration routes provide a test of the most severe situation, where there is no barrier to movement of dyes from the gut into the body. There is again no indication of substantial toxicity, and Lutty (1978) concluded that Pyranine, Lissamine Yellow FF, Eosine and Rhodamine WT, could be safely used for angiography in the human eye. Fluorescein is already widely used for this purpose.

Given the higher intraperitoneal toxicity of Rhodamine B compared to the other tracer dyes (Table 2), the more severe effects observed for this dye in both acute and chronic oral studies suggest that it is readily adsorbed in the gut. This is confirmed by metabolic studies (Table 3). The two optical brighteners, Fluorescein and Eosine are adsorbed to only a limited extent, but information is not available for the other tracer dyes. Eosine is discharged from the body via the bile duct, a pattern associated with its bromine substituents (Iga et al. 1971). All other dyes are cleared via the kidneys into the urine. Pathological examination of rats fed high dietary levels of Rhodamine B, showed enlargement of both the liver (where metabolism occurs) and the kidneys (Webb et al. 1961), but the metabolites were markedly less toxic. In man, Fluorescein is metabolised predominantly to the monoglucoride (Sheng-Chin Chen et al. 1980), with the basic fluoran structure remaining intact, as is also the case in the metabolism of Rhodamine B and Eosine.

No teratogenic or other effects on reproduction were observed in multi-generation tests on Calcophor White ST, Tinopal CBS-X, Fluorescein and Rhodamine B. Rhodamine WT and Tinopal CBS-X are severe irritants to the eye and moderately irritating to the skin. Both Fluorescein and Eosine are more toxic when contact is combined with exposure to light. In the case of Eosine, this may be due to release of halogen atoms during photo-decomposition (Tonogai et al., 1978), but Takashi and Kobayashi (1977) have demonstrated the importance of the singlet oxygen formed on light excitation of both these dyes. Rhodamine B does not show evidence of enhanced phototoxicity. This has also been demonstrated by an extensive series of tests for the two fluorescent brighteners.

All personnel handling dyes should wear protective gloves and clothing. Excessive inhalation of dust should be avoided, or a face mask employed during repacking of bulk supplies. All skin areas inadvertently contaminated by dye should be washed immediately with soap and water. Any splashes in the eyes should be flushed with copious quantities of water.

Based on the experimental results reviewed above, there is no evidence of either a short or long term toxic hazard to dye users or those drinking water containing tracer dyes. Even those employing tracers routinely in their work would not be likely to ingest sufficient dye to cause concern. For Rhodamine B (the most toxic of the tracers), the long term oral feeding studies yielded 'safe' continuous ingestion levels of 0.75 mg per day (U.S. Department of Health Education and Welfare, 1966), equivalent to 370 ug/1 for normal consumption of drinking water. Comparable levels of the other fluorescent tracer dyes would therefore be acceptable.

Mutagenicity/Carcinogenicity

Early experiments by Umeda (1956) demonstrated development of Sarcoma (cancer) in long-term experiments with rats fed 0.2% of Rhodamine B, Eosine and Fluorescein. However, these studies were found lacking with respect to numbers of animals surviving, provision of adequate control, and experimental duration, when reviewed by IARC (1977 and 1978).

Table 3. Uptake and excretion of the tracer dyes in mammals.

	FB28	FB351	P	LYFF	FL	E	RWT	RB	SRB	SRG	Source
Gut adsorption[a]	O	O	—	—	O	O	—	+	—	—	16, 26, 79, 113
Excretion[b]	—	—	U	U	U	B	U	U	U	U	48, 49, 57, 66 67, 98, 104, 105, 110, 112, 113, 114.
Metabolism[c]	—	O	O	M	M	O	O	M	—	M	11, 43, 66, 67, 112, 113

Notes: a. O = Less than 10% uptake + = Greater than 10% uptake
b. U = In urine via kidneys B = In faeces via bile
c. O = Not metabolised M = Metabolised

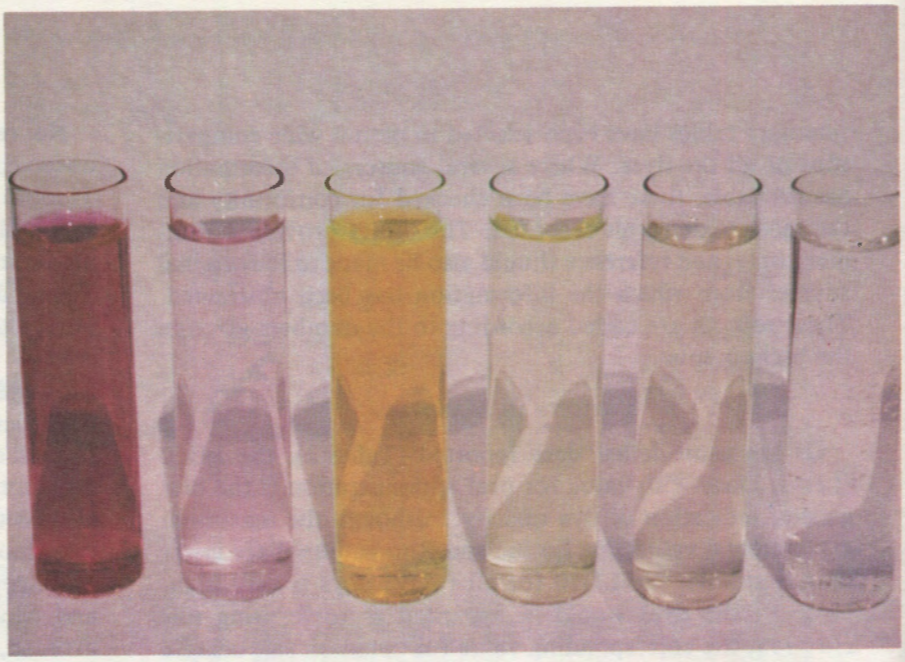

Plate 1. **Rhodamine WT, Fox Cave. Photo by W. K. Jones.**

Plate 2. **Rhodamine WT (red) and Fluorescein (yellow-green) at concentrations of 2 x 10^5 ug/l and 2 x 10^3 ug/l, Optical brightener #28 at 2 x 10^5 ug/l and distilled water (right).**

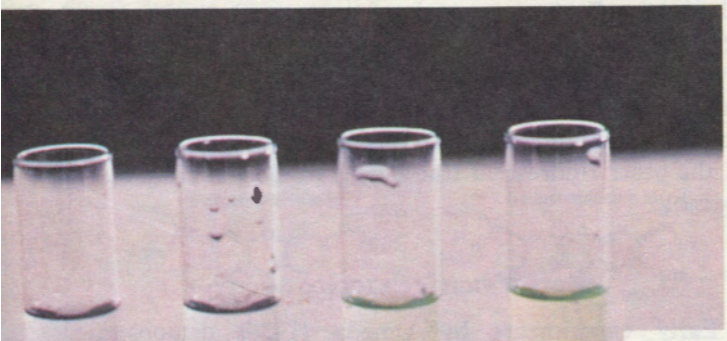

Plate 3. **Positive visual charcoal tests for Fluorescein dye (right) and negative control left.**

Plate 4. **Injecting direct yellow 96 in Central Kentucky Bluegrass Region. Photo by L. E. Spangler.**

Plate 5. **Direct Yellow 96. Photo by L. E. Spangler.**

Plate 6. **Rhodamine WT injection into sinkhole using water from tank truck. Photo by W. K. Jones.**

Plate 7. **Fluorescein sodium is a red powder until it goes into solution. Photo by W. K. Jones.**

Plate 8. **Fluorescein dye. Photo by W. K. Jones.**

Table 4. Carcinogenicity data for the tracer dyes

Test	FB28	FB351	AGA	DY96	P	LYFF	FL	E	RWT	RB	SRB	SRG	Source
Dominant Lethal Mutagenicity Test Rat and Mouse—No effect g/kg	1.0	1.5	—	—	—	—	—	—	—	—	—	—	4, 16, 53, 64, 80.
Carcinogenicity—rat	0/3	0/3	—	—	—	—	0/1	0/3	—	1/3	—	—	7, 13, 16, 33, 42, 46,
mouse	—	—	—	—	—	—	?/1	?/1	—	3/4	—	—	47, 68, 110, 115.

Key: O/n = noncarcinogenic: ?/n = possibly carcinogenic: x/n = carcinogenic in x tests out of n reported

Subsequent experiments (Anonn, 1981) have confirmed that Rhodamine B is carcinogenic. Results of more recent feeding studies, with mouse and rat (US National Toxicology Program) are not yet available for Fluorescein, while no further testing of Eosine has been undertaken.

Fluorescent whiteners have been extensively tested in mammals because of their widespread use and large production. Both Calcophor White ST and Tinopal CBS-X have been demonstrated to be non-carcinogenic in long-term feeding studies in rat, and in the Dominant Lethal Mutagenicity test in rat and mouse (Table 4). Information on carcinogenicity is not available for the remaining dyes, and an evaluation of their safety must be based on mutagenicity screening tests, which are rapid and less costly (Hoffman, 1982). These frequently employ micro-organisms or *in vitro* cultures of mammalian cells, although several *in vivo* tests are also available. There are strong correlations between the mutagenicity of chemicals in short-term tests and their carcinogenicity in mammals (Bartsch et al., 1980).

The three mutagenicity test results reported for Tinopal CBS-X are negative (Table 5), as would be expected from the carcinogenicity data discussed above. Amino G Acid and Lissamine Yellow FF have only been subject to a preliminary screening using the Ames Test (Kilbey, pers. comm., 1981). Amino G Acid was non-mutagenic, confirming the statement of Combes and Haveland-Smith (1982) that sulphonation of amino-napthalenes generally renders them non-mutagenic. Lissamine Yellow FF yielded uncertain results.

Fluorescein has been extensively tested in a variety of micro-organism tests for mutation and DNA alteration (Table 5). The results of the DNA-cell binding (DCB) test suggested that this dye was a possible mutagen on metabolic activation (Kubinski et al., 1981), but the authors were unable to confirm this result using gel-electrophoresis. Yoshikawa et al. (1978) reported no inactivation of transforming DNA *in vitro* using *Bacillus subtlis*. Other systems were also negative for mutagenic activity, with the exception of the results using photoactivation reported by Nishioka (1976) for unspecified mixtures of xanthene dyes. Photoactivation was not demonstrated in the Rec-Assay or inactivation of transforming DNA in *Bacillus subtlis* (Yoshikawa et al., 1978). Fluorescein has not been tested in mammalian systems for its effects on chromosomes, but the balance of the test results indicates it does not constitute a mutagenic hazard.

Eosine has also been widely tested and, unlike Fluorescein, demonstrates consistent photoactivation of mutagenicity in three different systems and four tests (Table 5). It is, however, uncertain to what extent these *in vivo* results can be applied to *in vitro* systems. No clastogenic effects were observed in tests using Chinese hampster ovary cells (Au and Hsu, 1979), nor were mutations induced *in vivo* in *Allium apa* or *Vicia faba* (Landa et al., 1965). Dye concentrations in the latter tests were, however, rather low, although a mutagenic effect was obtained for Rhodamine B.

Rhodamine WT has been the subject of extensive study by Douglas et al. (1983). Nestmann and Kowbel (1979) reported that the dye was mutagenic in the *Salmonella typhum*/mammalian microsome Ames test. However, in a battery of *in vitro* and *in vivo* tests in mammalian systems, Douglas et al. (1983) were only able to demonstrate a weak *in vitro* mutagenicity on using very high dye concentrations. No evidence of *in vivo* genetic activity was observed in terms of sperm abnormalities or bone marrow micro-nuclei in mice. They conclude that 'Rhodamine WT appears not to represent a major genotoxic hazard.'

Rhodamine B has been widely tested for mutagenicity as it is a known carcinogen. Out of twelve systems investigated, mutagenic activity was demonstrated in only five, including *in vivo* in *Drosophila melanogaster* (Clark, 1953)—although these experiments have been criticized by Lee et al. (1983). This demonstrates the limitations of mutagenicity tests and emphasizes the need for a wide suite of test results before deciding on the status of test materials. Furthermore, in the Ames test different results were reported for dye from a number of sources, with only two out of seven tests demonstrating mutagenic activity. Nestman et al. (1979) and Douglas et al. (1980) demonstrated that the mutagen was an impurity in the technical grade dye employed. This is an important finding because the concentration of impurities in commercial dyes may well vary with both manufacturer and dye batch. The advantages of utilising dyes from a manufacturer who has obtained relevant toxicity test information are thus clear.

Table 5. Mutagenicity data for the tracer dyes

Test	FB128	FB351	AGA	DY96	P	LYFF	FL	E	RWT	RB	SRB	SRG	Source
Rec-assay—*Bacillus subtilis*	—	—	—	—	—	—	0/3	1/2[b]	—	1/1	0/1	—	52, 103, 109, 119.
—*Escherichia coli*	—	—	—	—	—	—	0/1	0/1	—	0/1	—	—	51.
Dne test—*Escherichia coli*	—	—	—	—	—	—	0	?	—	?	—	—	65.
Petite mutations and gene conversions—Yeasts	—	0/1	—	—	—	—	0/1	1/2[b]	—	0/1	—	—	54, 56, 82, 84, 102.
Ames test—*Salmonella typhum*	—	0/1	0/1	—	—	?/1	1/4[b]	1/4[b]	A1/1	2/6[a]	?1/2	—	26, 38, 55, 56, 75, 83, 85 86, 87, 88, 92, 97, 101.
Chromosome aberrations —Chinese Hampster cells	—	0/1	—	—	—	—	—	0/1	0/1	3/3	1/1	—	8, 25, 26, 52, 62.
—Human bone cells	—	—	—	—	—	—	—	—	—	0/1	0/1	—	52, 97.
Sister Chromatid exchange —human or hampster cells	—	—	—	—	—	—	—	—	0/1	+/2	0/1	—	25, 52, 92, 97.
DCB Test for DNA alteration	—	—	—	—	—	—	A?/1	—	—	0/1	—		5, 92.
Inactivation of transforming DNA	—	—	—	—	—	—	0/1	1/1[b]	—	—	—	—	119.
DNA damage Chinese hampster cells	—	—	—	—	—	—	—	—	?/1	1/1	—	—	25, 85.
Chromosome alterations in vivo in bone marrow—rat	—	—	—	—	—	—	0/1	—	—	0/1	0/1	—	25, 52, 92.
—mouse	—	—	—	—	—	—	—	—	0/1	0/1	—	—	
Mutations in vivo—silkworm	—	—	—	—	—	—	0/1	—	—	0/1	0/1	—	52.

Key: O/n = nonmutagenic: ?/n = possibly mutagenic: x/n = mutagenic in x tests out of n reported:
A = activation required

Notes: a. Mutagenic impurities present in some commercial products (14, 47)
b. Photoactivation required to produce mutagenicity (S,Y,AC,7)

Sulphorhodamine B has been tested in six systems. It shows no *in vivo* chromosome alteration in rat or *in vitro* in human bone-marrow cells, findings similar to those for Rhodamine B. However, unlike Rhodamine WT, it does cause chromosome aberrations *in vitro* in Chinese hampster cells. Sako et al. (1980) have also demonstrated a cytotoxic effect of Sulphorhodamine B in fetal rate hepatocytes, although this was significantly smaller than for halogen-bearing xanthene dyes. There is therefore some evidence from *in vitro* systems that Sulphorhodamine B is a mutagen, but this has not been demonstrated *in vivo*.

Table 6. Status of the tracer dyes with respect to carcinogenicity and mutagenicity.

Dye	Status	
FB28	Non-carcinogenic	No data
FB351	Non-carcinogenic	Non-mutagenic
AGA	No data	Probably non-mutagenic
LYFF	No data	Possibly mutagenic
FL	Non-Carcinogenic	Non-mutagenic
E	Uncertain	Possibly mutagenic
RWT	No data	Possibly non-mutagenic
RB	Carcinogenic	Possibly mutagenic
SRB	No data	Possibly mutagenic

No information on the mutagenicity of Direct Yellow 96, Pyranine or Sulphorhodamine G has been obtained.

The discussion above illustrates the difficulty of assessing the status of chemicals when a complex and differential genotoxic response is shown, as appears to be the general case with rhodamine dyes. This difficulty is enhanced when the products tested are of technical grade and may contain impurities. Table 6 summarises the findings of this review. Three dyes can be identified as causing minimal carcinogenic and mutagenic hazard; Tinopal CBS-X, Fluorescein, and Rhodamine WT. Conversely, Rhodamine B is known to be carcinogenic and possibly mutagenic and should not be used.

Aquatic Organisms

There is a considerable body of data on the toxicity of the tracer dyes to fish. Most comparative data is available for the 48 and 96 hour LC50 (Lethal Concentration—50) in rainbow trout (*Salmo gairdneri*) (Table 7). In these tests, Rhodamine B and Tinopal CBS-X were the most toxic tracers, a finding supported by studies with bluegill (*Lepomis macrochirus*) and chanel catfish (*Ictalurus punctatus*. However, the LC50 values of these two tracers are still relatively high and they would not generally be considered toxic. Furthermore, dye concentrations of over 100 mg/1 would only be achieved during the injection phase of a tracer test, and would be very unlikely to persist for several days. Data for Calcophor White ST and Pyranine is limited to a single species but, again, indicates low acute toxicity.

The only long-term fish exposure experiments are those of Benoit-Guyod et al. (1979) using guppy (*Lebistes reticulatus*) and are particularly useful as they relate to the most widely used tracers. After thirty days, there was some evidence of continuing mortality with additional exposure, but the TLM values at this time are the most useful guide to long-term toxicity in fish. Rhodamine B, Eosine and the two

Sulphorhodamine dyes are significantly more toxic than Rhodamine WT or Fluorescein. However, the TLM values, even for the more toxic tracers, are still three orders of magnitude greater than the visible dye concentration and five orders of magnitude in excess of those commonly expected in long-term tracer experiments. Indeed, as pointed out by Abram and Rhodes (1978), aesthetic considerations relating to the visible colouration of natural waters are more likely to limit tracer concentrations than dye toxicity. These considerations, however, would not apply to the blue fluorescent tracers which are colourless in solution.

Data are also presented in Table 7 for three aquatic invertebrates. *Asellus aquaticus* (the water hog louse) is generally considered a robust organism, while *Daphnia magna* (water flea) is more sensitive, and has been widely employed in bioassay work. The 72 hour LC50 values for *Daphnia* are generally lower than those for fish (as is also the case for *Artemia salina*). However, the egg and larval stages of organisms are even more sensitive as demonstrated by the limited data for a number of shellfish (Table 7). Concentrations of 1 to 10 mg/1 of Rhodamine WT, Rhodamine B and Fluorescein (depending on the test organism) do not affect development or cause mortality in shellfish eggs and larvae after forty-eight hours' exposure. Whilst further data are needed for the other tracer dyes in these sensitive systems, it can be concluded that dye concentrations as high as 1 mg/1 can be tolerated for two days without damage to aquatic organisms.

Several studies have been conducted to examine the degree of uptake and elimination of Rhodamine B by shellfish. Waugh and Key (1967) and Geckler and Wandstrat (1964) found that no staining occurred at concentrations of 0.02 mg/1 in European flat oysters (*Ostrea edulis L*) and quahog clams (*Mercenaria mercenaria*) respectively. Even at much higher initial concentrations, the dye was eliminated within twenty-four hours on transfer to clean water, although Galassi and Canzonier (1976) demonstrated retention for up to three days in the blue mussel (*Mytilus edulis galloprovin cialis*). Comparable data is not available for the other tracers, although Feron and Hitz (1975) and Ganz et al. (1975) found that Tinopal CBS-X was not significantly accumulated and was rapidly expelled from bluegill (*Lepomus macrochirus*), even after prolonged exposure at 0.1 mg/1. Smart and Smith (in prep.) observed that the strong staining of trout (*Salmo gairdneri*) exposed to 500 mg/1 of Rhodamine B declined on transfer to clean water.

The bioaccumulation tendency of dyestuff can be indicated from the partition coefficient in n-octonal/water (Anliker et al., 1981). This coefficient has been determined for several tracers by Benoit-Guyod (1979) (Table 8). As expected, the high water solubility and anionic character considered desirable in a tracer, give low partition coefficients. Also shown in Table 8 is a rating for protein binding, which is derived from *in vitro* experiments (Lutty, 1978; Lutty,

Table 7. Toxicity of tracer dyes in aquatic organisms.

		FB128	FB351	AGA	DY96	P	LYFF	FL	E	RWT	RB	SRB	SRG	Source
Fish														
TL50 mg/1—	30 days. *Lebistes reticulatus*	—	—	—	—	—	—	752	138	1360	24	116-58	88	12.
LC50 mg/1—	96 hr. *Salmo gairdneri*	—	130	>1000	—	—	>1000	1372	>100	>320	155	—	—	9, 53, 70,
	48 hr. *Salmo gairdneri*	—	—	>1000	>1000	—	>1000	3420	>100	>320	506	450	—	100. 19, 70,
	96 hr. *Lepomis macrochirus*	>1000	241	—	—	—	—	3433	—	—	379	—	—	100, 107. 16,
	96 hr. *Ictalurus punctatus*	—	126	—	—	—	—	2267	—	—	526	—	—	70. 53, 70.
	48 hr. *Oryzias latipes*	—	—	>3000	—	—	—	3000	1800[a]	—	—	>3000	—	107, 108.
Maximum Non-lethal Concentration mg/1	48 hr. *Leuciscus idus*	—	—	—	—	—	—	—	—	—	—	—	>500	11, 43.
	96 hr. *Idus melanshes*	—	—	—	—	>500	>500	—	—	—	—	—	—	11, 43.
Aquatic Invertebrates														
LC50 mg/1	96 hr. *Asellus aquaticus*	—	—	>3000	—	—	>1000	—	—	>2000	550	—	—	100, 117.
	24 hr. *Artemia salina*	—	—	—	—	—	—	100-300	—	—	180	—	—	89.
	72 hr. *Daphia magna*	—	—	386[c]	—	—	>1000[b]	165	90	170	29	139	88	12, 100.
Shell Fish														
No effect	48 hr. *Crassostrea virginica*	—	—	—	—	—	—	—	—	—	1.0	—	—	90.
Development of eggs mg/1	48 hr. *Crassostrea gigas*	—	—	—	—	—	—	—	—	10.0	—	—	—	91.
	48 hr. *Hemicentrotus pulcherrimus*	—	—	—	—	—	—	10.0	—	—	10.0	—	—	89.
	48 hr. *Mytilus edulis*	—	—	—	—	—	—	1.0	—	—	3.2	—	—	89.
LC50 mg/1	96 hr. *Corbicula manilensis*	—	—	—	—	—	—	—	—	—	>500	—	—	18
	120 hr. *Australorbis glabratus*	—	—	—	—	—	—	10< >1.0	—	—	10< >1.0	—	—	76.

Notes: a. 1200 when bromine released during photodecomposition. b. 36 hr. c. 48 hr.

1979; Tonogai et al., 1979b; Tatsuji et al., 1971; Gangolli et al., 1972). These results relate well to the partition coefficients, although Eosine appears to be bound to a greater extent than Fluorescein, despite its somewhat smaller partition coefficient. They also indicate that Lissamine Yellow FF has some potential for bioaccumulation. However, even in the case of Rhodamine B, the most lipophilic of the dyes tested, the bioaccumulation factor would be below 100 and further testing *in vivo* would not be considered necessary.

Table 8. Partition coefficients (n-octonal/water) and protein binding of the tracer dyes.

	Partition Coefficient	Protein binding[a]
P	—	0
LYFF	—	+
FL	4.1×10^{-1}	±
E	4.7×10^{-2}	±
RWT	4.7×10^{-2}	±
RB	$1.9 \times 10^{+2}$	+
SRB	6.2×10^{-3}	0
SRG	9.5×10^{-3}	0

Notes: a + = Protein bound. ± = Weakly bound. 0 = Not protein bound.

Table 9. Effect of tracer dyes on algae (dye concentration 10 mg/1, exposure period 7 days).

	FB28	FL	E	RB	Source
Selanastrum capricornutum	++	—	—	+0	63
Chlorella sp.	—	++	++	++	72
Scenodesums sp.	—	0	0	+	72
Chlorococcum sp.	—	0	+0	+	72
Nostoc sp.	—	0	0	+0	72
Anabaena sp.	—	0	++	+0	72
Oscillatona sp.	—	0	0	+0	72

Key: ++ = No growth retardation
+ = Some growth retardation
+0 = Severe growth retardation
0 = No growth

In practice, the empirical studies of the elimination of Rhodamine B from shellfish and trout discussed above indicate that there is little possibility for bioaccumulation of this dye once environmental concentrations fall on completion of a tracer test.

Only two studies have reported the effects of tracer dyes on algal growth (Table 9). Little and Chillingworth (1974) showed that whilst Calcophor White ST caused no growth inhibition in *Selanastrum capricornutum* over a fourteen-day exposure period, Rhodamine B caused severe growth retardation at 10 mg/1. There was no effect for Rhodamine B at 1 mg/1. In an earlier study, Mason-Williams (1969) showed a differential response to dye exposure for a number of species found in Welsh streams. Surprisingly, Rhodamine B was less toxic overall to algae than Eosine, which was marginally less toxic than Fluorescein, the reverse of the order in higher organisms. Algal growth appears to be affected at similar concentrations by tracer dyes as the sensitive larval stages of shellfish. This reinforces the conclusion drawn above that enduring tracer concentrations as high as 1 mg/1 would not be detrimental to aquatic ecosystems.

The toxicity of dyes to bacteria has been investigated primarily to determine the possible suppression of bacterial decomposition in sewage works. At present, only limited data is available, but following the adoption of a test protocol by ETAD (Brown et al., 1981), more comparable data should become available. No data is available for Lissamine Yellow FF or Rhodamine WT. However, as only the basic dye Rhodamine B appears to affect aerobic decomposition (Table 10), a finding supported by the wider survey of Hunter (1974) for other acid and basic dyestuffs, no detrimental effects would be expected for these dyes. The limited data for *Salmonella typhum* employed in the Ames test protocol suggest that there are no large differences in the toxicity of the various tracers to bacteria. It is not possible to convert the plate concentrations to environmental levels, but overall the data do suggest that bacteria are less sensitive to the tracer dyes than either algae or shellfish eggs and larvae.

In conclusion, there is no evidence of significant bioaccumulation for any of the tracer dyes in fish. The most sensitive aquatic organisms to the dyes are the developmental stages of shellfish, and algae. These, therefore, determine the maximum prolonged dye concentration which can be recommended. This limit is set at 1 mg/1, well above the persistent dye concentrations commonly used in tracer tests, and at least one order of magnitude above the visible threshold. There is no evidence that short-term exposure to concentrations in excess of 1 mg/1, such as could occur transiently at injection sites, are harmful, but prior dilution should be employed if rapid dispersion and dilution of the tracer dye is not expected.

Discussion

Before discussing the toxicity information described above, attention must be drawn to the possibility that chemical transformation of the tracer dyes may occur after release, producing compounds which are intrinsically more toxic.

Information on the toxicity of photo-decomposition products of tracer dyes has been reviewed above, with Eosine appearing to exhibit both photo-activation of mutagenic activity and phototoxicity due to release of bromine atoms. Smart and Smith (in prep.) showed in acute tests using *Asellus aquaticus* that for Lissamine Yellow FF the photodegradation products were similar in toxicity to the parent dye; but for Amino G Acid, they were significantly less toxic. In other tests, photo-decomposition products may well have been present and are therefore incorporated into toxicity figures in a non-systematic manner. The eventual photo-decomposition products of Fluorescein (and, by analogy with metabolism, of rhodamine dyes) are pthalic acid and rescorcinol (Ishibashi 1965). The latter was found to be non-mutagenic when test data were reviewed by Hed-

Table 10. Effect of tracer dyes on bacterial decomposition (dye concentration 100 mg/1, exposure period 3 hours), and in *Salmonella typhum* (Ames test).

	FB28	FB351	DY96	P	FL	E	RWT	RB	SRB	SRG	Source
Activated sludge[a]	+[b]	+	+	+	+	+	+	0	+	+[c]	11, 14, 16, 19,
(Aerobic)	+	0	—	—	—	—	—	—	—	—	29, 43, 45, 72
Salmonella typhum[d]	—	>2	—	—	10	>1	21	>1	—	—	56, 83, 86, 87

Notes: a. Upper line = effect on bacterial decomposition (+ no effect, 0 significant effect)
Lower line = Biodegradation of dye (+ biodegraded, 0 not biodegraded)
b. Aerobic and anaerobic decomposition.
c. Anaerobic decomposition only.
d. Concentration of dye retarding growth (mg / plate).

dle et al. (1983). Therefore, photo-decomposition product toxicity appears only to be a problem for Eosine.

A particular hazard has been identified by Abidi (1982) due to the production of diethylnitrosamine (DENA) from the reaction of nitrites with the diethylamino moieties present in all rhodamine dyes. DENA is a potent animal carcinogen (Magee and Barnes, 1967). Abidi (1982) demonstrated the production of DENA in both natural and distilled water systems with nitrite levels in excess of 11 μg/1 and Rhodamine B with WT concentrations greater than 1 μg/1. It is, however, difficult to reconcile the high reaction yields (up to 96%) quoted in the laboratory experiments, with the known long-term persistence of rhodamine dyes in tracer tests. The DENA formed in the laboratory experiments was found to photo-decompose relatively slowly under ultraviolet irradiation. In the presence of ascorbic acid nitrosation was inhibited. Thus the observed persistence of anionic rhodamine dyes may be related to competing reactions which retard nitrosation of the dye, reducing the DENA hazard correspondingly. Determination of DENA levels during field trials with rhodamine dye tracers is urgently needed.

The third problem associated with the chemical transformation of tracer dyes is related to the production of chlorophenol compounds on chlorination of water for domestic supply. No experimental work has been conducted on this problem, although taste experiments with Rhodamine B performed by Wilson (1968) suggest that this reaction does occur. This confirms the statement of Murphy et al. (1975) that ring structures with electron-activating substituents (primarily OH and amino groups) are likely to be chlorinated. Further work is needed on this problem.

The acute and chronic toxicity of all the tracer dyes in mammal systems is sufficiently low that no danger should result in their use, providing normal precautions are observed during dye handling. However, only three tracers can be demonstrated to cause minimal carcinogenic and mutagenic hazard, Tinopal CBS-X, Flourescein and Rhodamine WT. Conversely, Rhodamine B is known to be carcinogenic and should not be used. The status of the remaining tracers is uncertain and they should be used only after careful consideration of the probable exposure risk, both to tracing personnel and to the wider population. In aquatic ecosystems, bioaccumulation is not a problem with any of the dyestuffs. There is no acute hazard associated with short exposures to very high dye concentrations, such as can occur on injection. For longer exposures, the development stages of shellfish and algae appear to be more sensitive than fish. Even in these systems, a concentration of 1 mg/1 for 48 hours can be endured. It should not be a problem to keep persistent dye concentrations well below 100 μg/1, particularly as this is well above the visible threshold where aesthetic considerations may become dominant.

Further information is needed both on the mutagenicity of tracer dyes, and on the effects of long-term exposure in sensitive larval stages of aquatic organisms. There is also a need to assess the toxicological significance of compounds formed in the environment by reactions with dyestuffs.

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NOTES ADDED IN PROOF

1) Theiss et al. (1981) have reported a weak tumorigenic response in mice for Amino G Acid. In view of this finding, one of the other blue fluorescent tracer dyes should be used in preference to Amino G Acid.
2) A draft report detailing the toxicology and carcinogenesis trials on Fluorescein has now been prepared (National Toxicity Program Technical Report Series 265, available from National Toxicity Program, P.O. Box 12233, Research Triangle, Park, NC 27709, USA). The study found equivocal evidence of carcinogenicity in male rats, but no evidence in female rats or in male or female mice. Fluorescein was also found to be non-mutagenic in the *Salmonella typhum* mammalian microsome assay, confirming the earlier results of Nestmann et al. (1980).
3) Crompton and Knowles Corporation have obtained expert opinion on the probable cancer risks arising from formation of diethylnitrosamine from Rhodamine WT. They conclude that incremental lifetime risks due to diethylnitrosamine exposure from Rhodamine WT would be far less than 10^{-6} (considered by the EPA as acceptable).

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TRACING RECHARGE WATER TO POROUS COLLUVIUM IN ARCHAEOLOGICAL SITES*

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Fluorescein dye tracing can be used to demonstrate or negate a hydrogeological connection between a coal strata and a porous rockshelter colluvium. These data have important implications for the determination of source areas for achaeological charcoal contamination by coal.

INTRODUCTION

Chemical and physical weathering of coal releases both particulate coal and volatiles which are dissolved or suspended in and transported by groundwater. Therefore, coal has been suggested as a source of contamination in radiocarbon samples from archaeological sites (Tankersley, 1983; Tankersley *et. al.*, 1984), in particular one of the apparently oldest rockshelter sites in the Eastern United States (Haynes, 1980, p. 585). Stuckenrath (1977, p. 183) has emphasized that the sample collector should initially assess the possibilities and probabilities of radiocarbon sample contamination. One method to determine coal contamination is analysis of the isotopic composition of the archaeological radiocarbon sample, the coal source area, and the groundwater (Coleman, 1976). Such isotopic studies are costly, however, and more importantly consume a significant quantity of archaeological carbon, which may exist only in very small amounts. This paper describes a field method to determine whether or not groundwater which flows over or through coal deposits can be traced to archaeological deposits.

STUDY PROBLEM

In the Eastern United States, rockshelters (or caveshelters) provide the archaeologist with dry conditions which enhance preservation of organic materials. Rockshelters have always been important sites of human activity throughout prehistory because they provide semi-enclosed habitation areas that are protected from the weather. Additionally, they are archaeologically important because they often contain relatively undisturbed stratigraphy resulting from weathering and colluvial deposition of the overhanging rocks and deposition of human origin. Rockshelter development commonly occurs at the base of cliff exposures along retreating Pennsylvanian sandstone escarpments and stratigraphic outliers. These geographic areas coincide with bituminous coal strata and often have a common source of groundwater. Volatile organic matter released from the coal by the natural chemical and physical weathering processes are frequently dissolved in and transported by groundwater which recharge in porous rockshelter colluvium. For example, ground water passing through a mined coal exposure or natural outcrop can enter the porous colluvium of a rockshelter through the following hydrogeological processes:

1) percolation of groundwater through bedrock capillaries and fractures which recharge within the rockshelter, either on the ceiling or walls or at the interface of the rockshelter wall and colluvium.

2) discharge of groundwater above the rockshelter, enters the colluvium at the dripline, and percolates through these deposits.

3) a coal stratum in the rockshelter's wall acts as an aquifer in formation of springs above or within the colluvium.

Reynolds (1966) has shown that fluorescent dyes can be used to trace percolation water. This study used fluorescein dye to determine if two of the hydrogeological processes can be traced. The third process can be recognized by identifying the petrological composition of the rockshelter—dye tracing is not necessary. Injection and detection methods are described for tracing these other two processes.

INJECTION

Fluorescein's limitations of photostability, pH-controlled structural change, and adsorptive losses necessitate several simple precautions to be taken before dye injection. However, these limitations do not inhibit fluorescein's suitability for tracing percolation water in sandstones.

Smart and Laidlaw (1977, p. 15) have noted that fluorescein has high visibility in low solution concentrations but poor stability under sunlight. Therefore, it is necessary to

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keep the dyed surface water to a minimum. This can be accomplished by injecting the dye at the stream's locus of complete disappearance. Upland escarpment streams are often intermittent making spring the preferable time of the year for successful dye tracing. The locus of stream disappearance in the spring will generally be closer to the rockshelter, thus reducing the sorptive loss of fluorescein. The locus of stream disappearance is usually associated with stream ponding creating an ideal injection site.

Iron sulfide (pyrite) inclusions in bituminous coal form sulfuric acid in surface runoff. Fluorescein structurally changes to a leucocompound in highly acidic streams of coal mining areas. However, stream acidity decreases with its distance from the coal source area and fluorescein's structural change can be reversed in the test by an alkaline solution.

Simple precautions against adsorptive dye loss onto sediment and bedrock surfaces should be taken, although sandstones are poor inorganic adsorbents and fluorescein is relatively resistant to humus adsorption (Smart and Laidlow 1977, p. 27). It is recommended that a minimum of 1000 ml of fluorescein powder (600 mesh or finer) be used for a first order stream. The fluorescein should be wrapped in a gauze or coarse fabric, tied, weighted with a lead fishing weight, and placed in the injection site. The fabric helps prevent immediate organic adsorption (keeps the fluorescein clean), provides a "time release" mechanism, and the great volume of fluorescein guards against complete adsorptive dye loss during percolation.

DETECTION

The rate of percolation volume per time ‡ space varies from locus to locus making activated charcoal adsorption traps necessary. Approximately 50g of activated charcoal (a double handful) contained in a finely-meshed nylon fabric (nylon stocking) is perfectly suited for the recovery of dyed recharge water in porous rockshelter colluvium. The nylon fabric is resistant to short-term decay and prevents an initial physical mixing of the charcoal with the sand, silt, and clay of the colluvium. Suitable places for recovery traps in colluvial deposits may be damp spots along the dripline, near the rockshelter wall, and in areas near springs. At all locales the charcoal adsorption traps should be placed in the colluvium to a depth which permits the surface area of the charcoal to be completely exposed to percolation water. If several potential contamination locales exist within the rockshelter, all locales should be tested in order to assess and reconstruct the hydrogeological processes which affect archaeological carbon.

Adsorbed fluorescein dye can be retrieved from the charcoal with a standard alkaline solution of 10% potassium hydroxide and ethanol. An acceptable percolation time will be dependent upon the distance between the injection and detection sites and the relative abundance of capillaries and fractures in the bedrock. After the trap has been removed from the colluvium the charcoal should be placed on a piece of filter paper at the bottom portion of a petri dish (9 cm in diameter). The alkaline solution can then be poured into the petri dish dampening the filter paper and releasing the adsorbed fluorescein dye from the charcoal, if it is present. If the concentration of fluorescein is too low to be detected in visible light, then a portable ultraviolet lamp can be used to excite the dye. Fluorometric analysis is not necessary since the nature of this experiment is to demonstrate the presence or absence of fluorescein in the recharge water establishing a groundwater connection between a coal outcrop and rockshelter colluvium.

SUMMARY

Coal volatiles in groundwater represent a potential radiocarbon contaminant of archaeological charcoal in rockshelters in the Eastern United States. Archaeologists have a responsibility to recognize the hydrogeological processes that can transport radiocarbon contaminants to a charcoal sample. Fluorescein dye tracing provides an effective means of demonstrating whether or not there is contact between groundwater in coal source areas and rockshelter deposits.

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A COMPARISON BETWEEN FLUORESCEIN DYE AND AMORPHOUS SILICA FOR GROUNDWATER TRACING

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Two hydrologically different localities were chosen to compare the tracing capability of fluorescein dye to amorphous silica, a potentially new, groundwater tracer. Amorphous silica is commercially produced and is non-toxic, colorless, stable, and not easily filtered or absorbed. Although the amorphous silica was not detected in either test case, it is felt that in the more common, hydrologic karst systems with low water storage and single conduit transport, that the silica would be detectable over background. The silica should also prove useful in tracing between two wells in a low to medium storage, karstic aquifer.

INTRODUCTION

Often in groundwater tracing studies, it is absolutely essential that the water supply is not colored by the tracing material. Two hydrologically different study sites in Texas were chosen to test the theory that amorphous silica, a colorless, non-toxic substance, could be used as such as tracer. In order to better quantify the success or failure of the material, fluorescein green dye was simultaneously injected at each site. The purposes of this paper are to review the properties of amorphous silica and to compare the effect of the two different ground materials on the tracers.

PROPERTIES OF AMORPHOUS SILICA: TRACER POTENTIAL

Amorphous silica (fumed silica) is produced by several companies in the United States and is used in a wide range of applications in industry. The silica utilized in this experiment was manufactured by Cabot Corporation in Tuscola, Illinois. The Cab-O-Sil brand of amorphous silica is non-toxic and has been approved by the United States Food and Drug Administration as a food additive at levels up to 2 percent by weight. Example foods using amorphous silica include: chocolate powders, dessert mixes, dried dairy products, flour, instant coffee, instant soup mixes, sugar and many others.

Toxicological studies conducted with Cab-O-Sil indicate that for acute oral toxicity, Oral LD 50 is greater than 5 g/kg (Cabot Corporation, undated). Ingestion of water carrying 1 percent silica as a tracer would require the ingestion of greater than one-half of the body weight of an individual to exceed the 5 g/kg test concentration. Therefore, a 68 kg person would have to drink 34 kg of water with 10,000 mg/1 SiO_2 per day to exceed the test concentration. This equals approximately 34 liters of water per day.

The silica is formed by the hydrolysis of chlorosilanes in a flame of hydrogen and oxygen. The chlorosilane utilized is silicon tetrachloride vapor and the reaction is:

$$SiCl_4 + 2H_2 + 02 \xrightarrow{1800^\circ C} SiO_2 \downarrow + 4HC1 \uparrow$$

(Cabot Corporation, undated). This produces spheres of silica which, by controlling process parameters, range from 7 to 21 millimicrons in diameter with surface areas ranging from 130 to 400 square meters per gram. During cooling, the spheres may collide and form aggregate chains, which vary in size but generally remain in the submicron range.

The small size of the silica spheres (14 millimicrons for this experiment) and the sub-micron size of aggregate chains can be compared to the 30 micron diameter of *Lycopodium* spores. Amorphous silica, when properly dispersed in water, should move with flowing water very well. This is because the very small size of the particles easily allows transport, and because the surface of the silica is hydrophilic and hydrogen bonds to water molecules as well as other hydrogen bonding liquids (Cabot Corporation, undated).

It is believed that filtration of amorphous silica does not occur to any significant degree in limestone aquifers where groundwater may flow through conduits. Filtration of the amorphous silica may occur in very fine-grained porous media such as alluvium or sandstone. Sorbtion of amor-

phous silica is also not believed to be a problem since the manufacturer considers the material to be "chemically inert" (Cabot Corporation, undated).

The stability of silica over the duration of a trace is certainly not a problem. Silica is one of the most abundant compounds known and is considered completely stable. Amorphous silica is approximately 99.8 percent pure SiO_2 and does not undergo chemical alteration under natural conditions.

Amorphous silica is relatively expensive when compared to some other tracers. For this experiment, a chemical supplier charged $3.60 per pound. However, much more silica must be utilized for a trace than fluorescent dye because the detection limit of fluorescent dye is much lower than the detection limit of silica, detection limits being controlled by background concentrations.

Detection of amorphous silica is, at this time, not particularly easy. The standard method for analysis of silica in water is a colorimetric procedure for dissolved silica, with a working range of 2 to 25 mg/1 silica (American Public Health Association, 1975). Because the amorphous silica would exist in significantly higher concentrations and is predominantly in a colloidal suspension, not dissolved, the standard method is not acceptable. For this experiment, detection was accomplished using Inductively Coupled Plasma Emission Spectrometry (ICPES) which analyzes for silicon, and approximately 40 other metals, for both suspended and dissolved species.

It is imperative that the background concentration of a tracer species be low. *Lycopodium* spores and some fluorescent dyes have proved successful primarily because there is essentially no natural background concentration of these materials in groundwater. Amorphous silica can be easily masked by a relatively high background of dissolved silica. This background concentration, controlled by the solubility of natural amorphous silica, not quartz (Garrels and Christ, 1965) varies from one aquifer to another, but appears to be relatively stable in a given aquifer or area. For aquifers, SiO_2 is commonly less than 30 mg/1 (Marquardt and Elder, 1979). In the Edwards Aquifer, dissolved silica concentrations range from 10 to 15 mg/1.

In summary, amorphous silica appears to be favorable as a tracer in regard to toxicity, tracer movement, chemical stability, and filtration/sorbtion. The need of a tracer with the properties of amorphous silica may outweigh its higher expense and time associated with having to regularly collect samples. Although background concentrations may at first appear to be a problem, the small variation in silica content in most karst waters allows for even slight changes due to tracer injection, to be detected. The following two case histories test the use of fluorescein and amorphous silica in a high-storage karst system and a low-storage alluvial system.

CASE 1—CAMP WOOD, TEXAS

GEOLOGY AND HYDROLOGY

Old Faithful Spring, the water supply for the town of Camp Wood, Texas, was first briefly described by Long (1958), and discussed in a little more detail by Brune (1975). Brune (1975) states that Old Faithful Spring (Camp Wood Spring) issues through alluvium, but its source is from cavernous strata of the Glen Rose Limestone.

The Camp Wood Creek basin is 74 square kilometers and is underlain primarily by Glen Rose Limestone (Figure 1). Roaring Springs, Cave Springs, and the South Prong Springs are the three largest springs producing the base flow of Camp Wood Creek. The water sinks for short distances in the chert-rich river gravels and reappears on the surface until the final sink point is reached. The stream is normally dry between this point and Old Faithful Spring which is about 7 kilometers away. Only during major storm events does Camp Wood Creek flow all the way to the Nueces River.

Figure 1. **Drainage basin of Camp Wood Creek and Old Faithful Spring.**

METHODS AND RESULTS

In order to establish a connection between the final sinking point of Camp Wood Creek and Old Faithful Spring, fluorescein and the silica were injected into the flowing water. A slurry of silica was mixed by the creek using two electric outboard motors in a 55-gallon drum. A 10% (100,000 mg/1) slurry of silica was placed into the stream and allowed to infiltrate into the streambed. Some of the silica appeared to drop out of suspension soon after injection.

Twenty minutes later, 0.82 kg of concentrated powdered fluorescein was mixed with water and released to the stream which was flowing at 0.068 cms. Quantification of the dye trace was performed using an ISCO automatic water sampler and a Turner Designs fluorometer. The first samples were taken at Old Faithful Springs one and one-half hours after tracer injection. Discharge of the spring was 0.062 cms at that time. The dye began to arrive at the spring about 23 hours after injection (Figure 2). The original dye concentration injected into Camp Wood Creek was 8,500 mg/1. The maximum dye concentration measured at Old Faithful Springs was 0.094 mg/1, so the dye experienced approximately a 5 order magnitude dispersion. Assuming that the silica would be dispersed at the same rate and that the initial silica concentration was 100,000 mg/1 (10%), a silica concentration due to the trace materials of approximately 1 mg/1 was expected. The average background SiO_2 concentration of 13.271 mg/1 would tend to mask a 1 mg/1 maximum increase unless the background SiO_2 concentrations were extremely constant (standard deviation approaching zero). The standard deviation of the silica analyses was 0.365, which would make a 1 mg/1 increase significant (2.74 standard deviations) if observed. During the period of dye arrival, however, the highest silica content (above test average) was 0.350 mg/1. This occurred 9 hours after the dye peak, and could not be construed as a significant increase (Pearce, 1984).

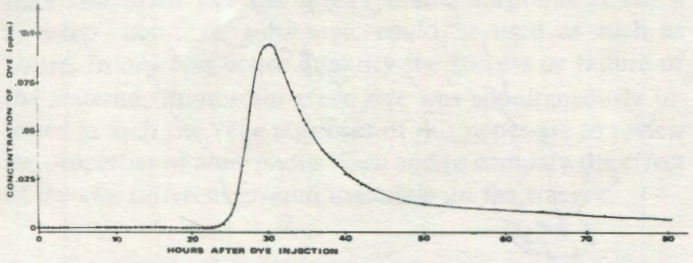

Figure 2. **Fluorescein dye concentration versus time curve at Old Faithful Spring.**

The concentration of dye decreased rapidly and was down to 0.019 mg/1 just 20 hours after peak. Forty percent of the original dye emerged from the spring within the first 100 hours after injection. Probably no more than another 10% emerged after this since the curve was approaching zero concentration. The rest of the dye must have been largely adsorbed by clay minerals and particle surfaces which would be unexpected for flow through an open conduit. Just slightly before the dye reached the spring, a nearby resident complained of her well water turning green. Subsequent discussions with the driller showed that the well had been drilled *only* through river alluvium. This fact, plus the data from the dye-dilution curve, dispel the previously held concept that the groundwater flow was through limestone conduits.

It is our opinion that this trace was a borderline case of silica effectiveness. If the dispersion was slightly less or the initial silica concentration increased, the trace would probably have been successful. It is important to note again that the silica appeared to drop out of suspension along the streambed at the injection site thus lowering the initial concentration. In an open karst system of low storage such as found in West Virginia or Kentucky, the silica concentration would probably remain high enough to be detected at a spring resurgence.

CASE 2—EDWARDS AQUIFER, SAN MARCOS, TEXAS

The Edwards Limestone Aquifer in the Balcones Fault Zone has been well studied and defined as having many cavernous systems that transport water from recharge points to the spring outlets. Although detailed hydrogeologic reports have been published about large areas of the region (Puente, 1969; Reeves, 1976; Maclay and Rappmund, 1979; Klemt et al., 1979; Maclay, 1981) little attention has been drawn to charcterizing the flow conditions between nearby points within the aquifer.

GEOLOGY AND HYDROLOGY

The area around San Marcos is underlain by Cretaceous Aged rocks that have been intensely faulted, causing the Edwards Aquifer to be broken into numerous blocks. The San Marcos Springs issue from the Edwards Limestone Group along the San Marcos Springs Fault (Figure 3) which has created the Balcones Escarpment. On the southeast side of the fault escarpment, quaternary alluvium mantles the flatland areas. Northwest of the escarpment, the hills are underlain by the Del Rio and Buda Formations and the Eagle Ford Group.

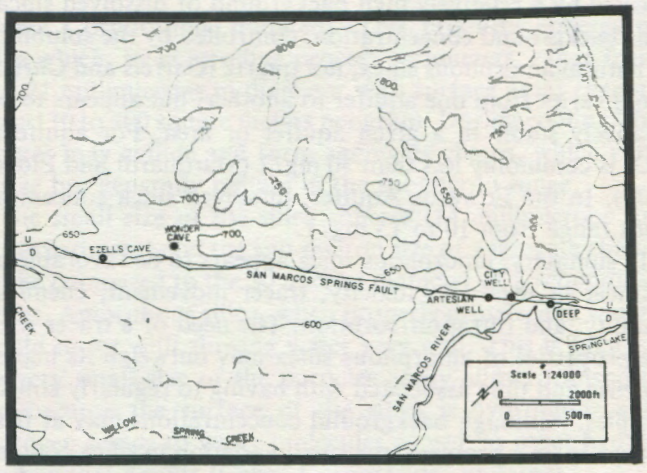

Figure 3. **Location of the San Marcos Springs Fault and points involved with the dye trace.**

The San Marcos Springs is the second largest spring in Texas with an average flow of 4.6 cms (161 cfs) during the

period 1965–74 (Guyton et al., 1979). At the time of dye and silica injection, the spring was flowing at 3.0 cms (105 cfs). Estimated transmissivities for the aquifer along the San Marcos Springs Fault are generally over 1 million gpd/ft. This demonstrates the highly-cavernous nature of the aquifer and the great amount of water in storage around the test site. The San Marcos Springs are actually composed of over a dozen individual spring orifices which are beneath ten to forty feet of water due to an impoundment made by a commercial glass-bottom boat tour operation. Prior to this study, only one trace had been performed to the springs (Ogden, 1984), so flow paths, water-velocities, and the degree of interconnection of spring orifices was essentially unknown.

METHODS AND RESULTS

Ezell's Cave, located 2.5 km southwest of the springs and on the San Marcos Springs Fault (Figure 3), was chosen as the test site because it has a large, deep lake at the bottom that was assumed to be in hydrologic connection with the springs. Approximately 13.6 kg of silica were mixed with approximately 132 liters of water, yielding an initial concentration of 10% silica (100,000 mg/1). Mixing was again by electric outboard motor in a 55 gallon drum. The slurry was pumped down into the cave pool. At the same time (April 1, 1983), 0.91 kg of fluorescein was added to the pool. The following day sampling was initiated at the university's artesian well (Figure 3) using the automatic sampler. Samples were also taken from one of the city's wells by the city employees (Figure 3). Both sites are located along the San Marcos Springs Fault and were sampled every three hours. Charcoal traps were also placed at the two wells and seven of the spring orifices.

For samples collected at the artesian well, SiO_2 values range from 12.806 mg/1 to 14.265 mg/1 with a mean value of 13.439 mg/1 and a standard deviation of 0.368. Samples collected at the city's well ranged from 13.252 mg/1 to 14.650 mg/1 with a mean value of 13.605 mg/1 and a standard deviation of 0.230. The small variation in silica content was evident that no significant trend of increased silica content occurred. Only small variations exist, which are normal for a natural system (Pearce, 1984).

Each sample collected was also treated for fluorescence using the fluorometer. None of the samples displayed any measurable fluorescence (greater than 1 ppb). The great amount of water in storage had caused the dye to be highly dispersed.

Nine days after injection of the dye (April 10th), the charcoal traps had adsorbed and concentrated enough dye to confirm a hydrologic connection. The next day (April 11), the charcoal trap at the city's well showed the dye's presence. The charcoal traps were retrieved by divers from the seven spring orifices on the following day (April 12th) and surprisingly *only one* orifice (Deep Spring, Figure 4)

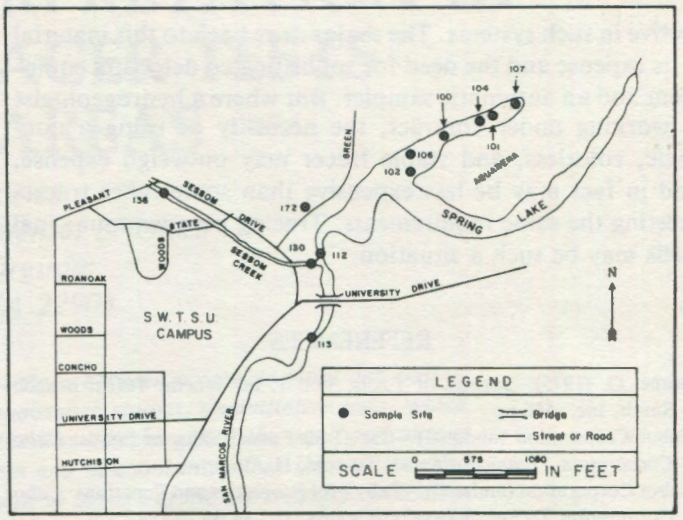

Figure 4. **Location of the San Marcos Springs orifices tested during the dye trace: (100) Divergent Spring, (101) Cabomba Spring, (102) Deep Spring, (104) Johnny W. Spring, (106) Catfish Spring, and (107) Hotel Spring.**

was positive. The dye continued to emerge from Deep Spring for approximately 40 more days as the May 24th samples were negative. Catfish Spring (Figure 8) showed a slight presence of the dye on May 18th and the dye ceased emerging just one week later. Water from the other five spring orifices never tested positive.

Prior to the dye trace, water samples were taken weekly by divers during the previous year. Close inspection of the water chemistry data demonstrated that the Deep and Catfish Spring orifices of the San Marcos Springs are similar chemically, but quite different from the other spring orifices in temperature level and dissolved oxygen content. This information suggests that there are two flow regimes quite isolated from one another. A postulated fault was shown on Guyton's (1979) map that would separate the two sets of springs. This fault could have significant enough displacement to cause isolation of the two flow systems. The conclusion drawn from this dye trace is that the water emerging from the San Marcos Springs is not from a single aquifer zone.

CONCLUSIONS

The two case studies demonstrate that amorphous silica is not a viable tracer in karst systems with high dispersion or for alluvial aquifers with high filtration and adsorption. Most speleologists perform groundwater tracing in open karst aquifers of low storage in which the water is moving under vadose conditions. In these karst aquifers, small surface streams commonly sink into cave openings and travel through long, air-filled passages to emerge as springs with little increase in water volume. Amorphous silica may be ef-

fective in such systems. The major drawback to this material is its expense and the need for sophisticated detection equipment and an automatic sampler. But where a hydrogeologist is working under contract, the necessity of using a nontoxic, colorless, and stable tracer may outweigh expense, and in fact may be less expensive than some other tracers meeting the same requirements. Tracing between municipal wells may be such a situation.

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ANALYSIS AND INTERPRETATION OF DATA FROM TRACER TESTS IN KARST AREAS

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Qualitative tracer tests are used to determine flow connections between accessible input and output points and to delineate karst drainage basins for reconnaissance studies. Quantitative tests, which measure the amount of tracer recovered over time and the flow (discharge) of the input and output sources, provide additional insight into the flow processes and mechanisms within the aquifer. The processes acting on a conservative tracer determine the shape or pattern of the dye recovery curve. The dye recovery pattern is determined by 1) the conduit network pattern, 2) the flow characteristics of the aquifer, and 3) the adsorption and dispersion characteristics of the tracer.

INTRODUCTION

Dye tracer studies of subsurface water systems present a "black" box (literally and figuratively) problem in interpretation of the resulting data. The inputs and outputs are measured, and the researcher must attempt to reconstruct the invisible workings of the black box based on the changes in the output and any additional geologic and hydrologic information which may help to define the boundary conditions. The degree of success (or the amount of information gained) in interpretation of the groundwater flow system depends on 1) the complexity of the system under study, 2) the nature and quality of the tracer data, 3) the availability of geologic, topographic, speleologic, and hydrologic data for the study area, and 4) the researcher's familiarity with the study area and skill at working with the type of data available. As with the mechanics of performing the tracer tests, the interpretation of the results is often as much an art as a science. The main categories of tracer studies and data requirements are summarized in Table 1.

RECONNAISSANCE STUDIES

Data from this type of study are needed to provide the necessary background for more sophisticated (and more expensive) quantitative studies. A tracer is generally injected in a sinking stream or siphon in a cave, and passive detectors are left in all the possible resurgences. These qualitative studies are used to determine underground connections and to help delineate karstic watersheds. The main weaknesses in the type of study are that little insight is gained as to the exact route or nature of the subsurface flow paths and, without the ability to calculate the amount of dye recovered from each resurgence, no clue is available as to whether the tracer may also be emerging at additional resurgences.

Table 1.

Purpose of Study	Type of Study	Analysis	Type of Data	Additional Data Requirements
Reconnaissance	Tracer with passive detectors	Visual or Fluorometric	Location of resurgencies & general flow direction	Topographic maps & maps of known cave passages
Water Budget Travel Time	Dye with direct sampling	Fluorometric	Recovery concentration & time of travel	Discharge at each resurgence and sink
Internal Flow Characteristics of Aquifer	Dye with frequent direct sampling	Fluorometric	Breakthrough curves time-recovery concentrations	All of the above and geologic structure & water chemistry data

Several short tracer tests generally provide a more detailed picture of an area than one long test. As shown in the illustration in the introduction to this issue, successive tests are based on earlier results and, when combined with maps of area caves, can present a detailed view of the karst drainage network (White and Schmidt, 1966; Jones, 1973).

Changing flow conditions can alter the apparent flow network by distributing water into higher "flood overflow" routes which may discharge to completely different springs or drainage basins from the low flow routes. In some areas, tracer tests must be conducted under high flow conditions and be redone during base flow to get a more complete assessment of the drainage network. The travel time for the tracer, even over the same flow route, may vary by as much as an order of magnitude between high and low flow conditions.

QUANTITATIVE TRACER TESTS

Quantitative tests require a considerably higher level of effort, for all of the resurgences must be continuously sampled for the entire length of time the tracer is emerging. The samples must then be analyzed against known standards to determine the dye concentration. This recovery concentration versus time is plotted to give recovery (sometimes called breakthrough) curves. Note that the sampling interval may itself influence the shape of the recovery curve.

Although several researchers have attempted to devise methods of quantifying the dye recovered in the elutant from charcoal detectors (Thrailkill, 1983) or from direct readings of fluorescent intensity of cotton detectors (Smart, 1976), no satisfactory substitute for continuous water sampling has been found. Also, the natural variation in background fluorescence may seriously compromise the procedure.

If dye injected at one point is found to resurge at several different points, then flow (discharge) during the dye recovery period is needed along with the dye recovery concentrations to determine the "water budget" for the sink. The term "water budget" as used here, refers to determining the relative percentages of water which a karst flow system distributes to the various springs. Examples of different karst flow patterns are illustrated in Figure 1. If the karst drainage system is characterized by a few large springs which act as "collectors" for a number of sinks and caves, the water budget can probably be inferred from completely qualitative tests. However, more complex drainage systems which utilize different routes under different flow conditions should be studied using quantitative methods.

When using fluorescent tracers, a known quantity of a conservative dye is injected into the aquifer and water samples are collected at regular intervals at each resurgence (determined by previous qualitative tests). The samples are then analyzed using a fluorometer to determine dye concentration over the total recovery time (Figure 2). The discharge at each resurgence must be known for the entire dye recovery period. The total amount of dye recovered at each resurgence is found by integrating the area under the time concentration curve and multiplying by the discharge.

$$Wd = Q \int_0 C\, dt \qquad (1)$$

where Wd is the weight of the pure tracer and C is the dye concentration measured at the sampling site at time t (Figure 2). The percentage recovery (Rp) is computed as

$$Rp = 100 \bullet \frac{C \text{ observed}}{C \text{ conservative}} \qquad (2)$$

No tracer is 100% conservative. Some dye is always lost to adsorption, photochemical decay, and complexation and precipitation from solution. Tests conducted over short time periods (less than 2 days) and in relatively low sediment water should show minimal dye losses. Tests which take over a month or during which the discharge and sediment concentrations fluctuate greatly may be impossible to quantify. Data from surface water "time-of-travel" tests are often presented in a "normalized" form prepared by dividing the values from peak concentrations by values for the weight of the dye injected and adjusting the observed concentrations to conservative concentrations. The problem in karst tracer tests is that if some of the water flows to other resurgences with different travel times, only a rough estimate can be made of dye lost to adsorption versus the dye lost to other resurgences. The only result normalizing the data accomplishes in this case is to camouflage data which will not stand close inspection. Fortunately, time-of-travel can be determined from the general shape of the time-concentration curve, even if absolute values cannot be obtained.

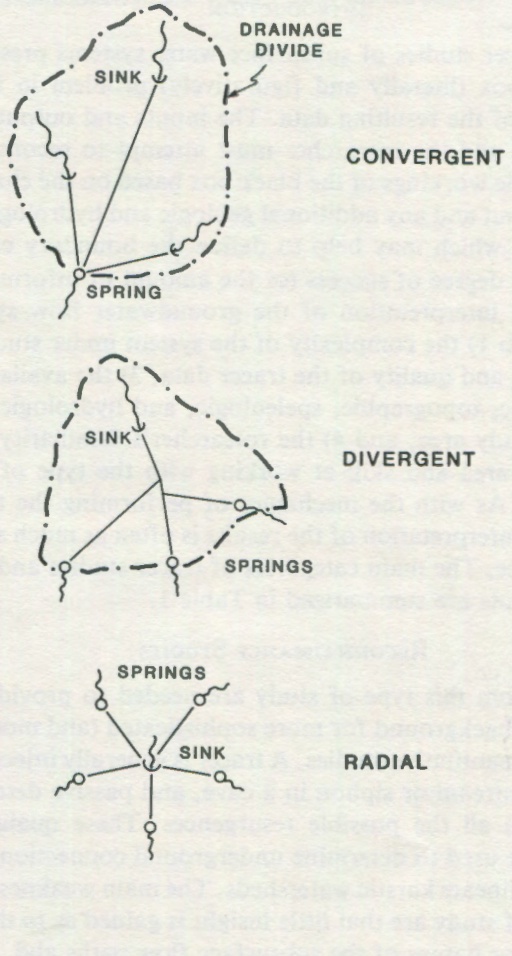

Figure 1. **Karst drainage patterns.**

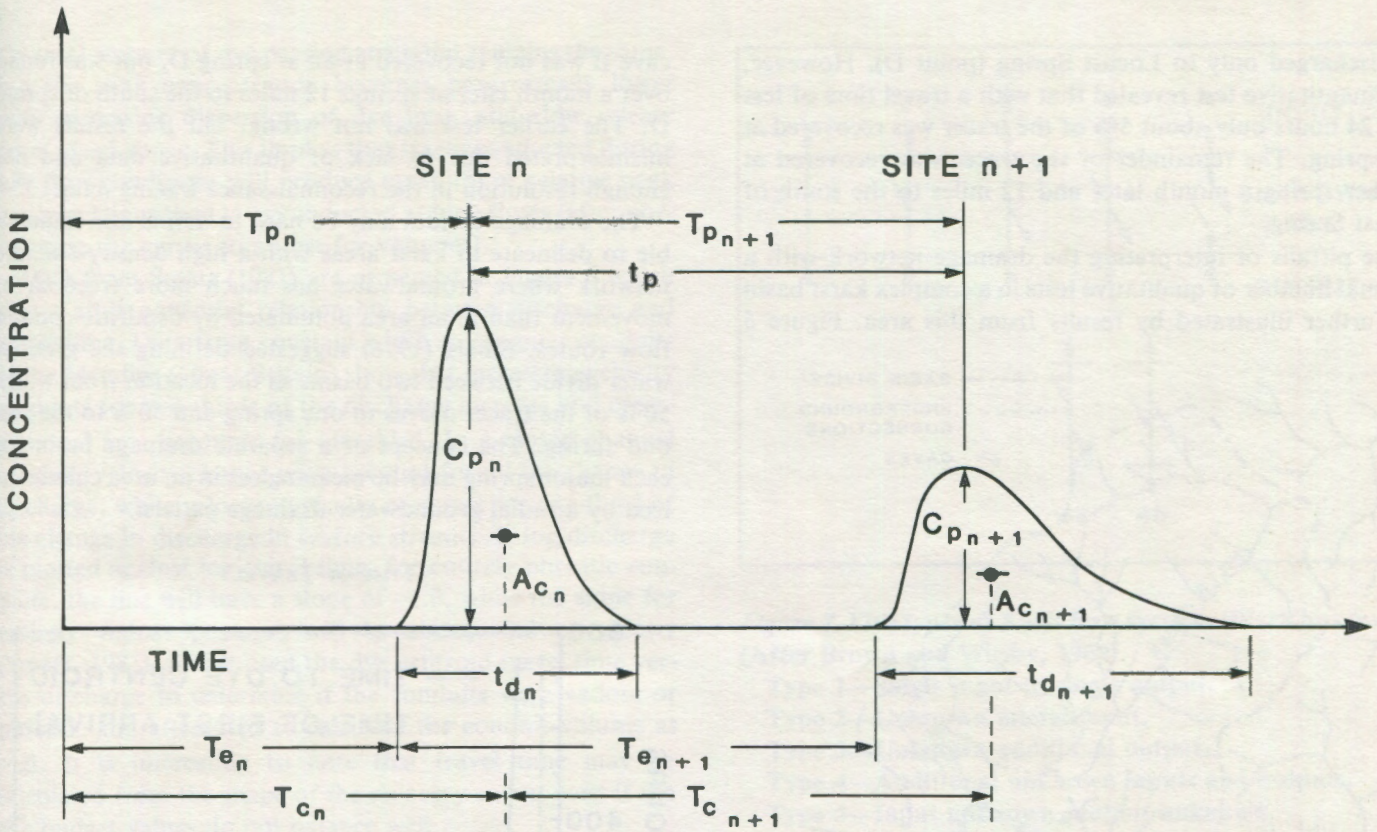

Figure 2. **Definition sketch of dye recovery curves resulting from instantaneous dye injection. Two sampling stations (site n and site n + 1) on an unbranched channel with no lateral inflows or outflows are shown. The principal components of the curves are: Time of first arrival (T_e), Time to peak concentration (T_p), Time to centroid (T_c), Total Time of dye passage (T_d), Peak (maximum) dye concentration (C_p), and point representing mean dye concentration (A_c). For a conservative tracer, the area under the curve at site n equals the area under the curve at site n + 1.**

DRAINAGE BASINS AND WATER BUDGETS

Discharge at the sinks and resurgences should be measured independently of the tracer. Figure 3 illustrates a simple cave passage which distributes water to three separate springs. The water emerging from the springs receives no lateral inputs past the point of divergence, so recovery data collected at one or two of the springs without independent discharge measurement will not yield any hint of the presence of additional springs for this distributory.

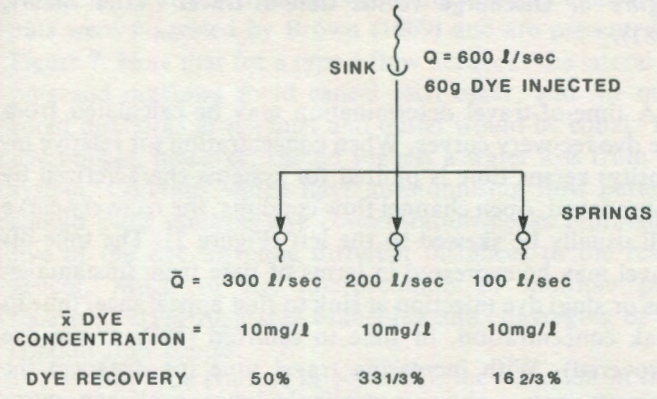

Figure 3. **Water budget calculation for a three-way distributory.**

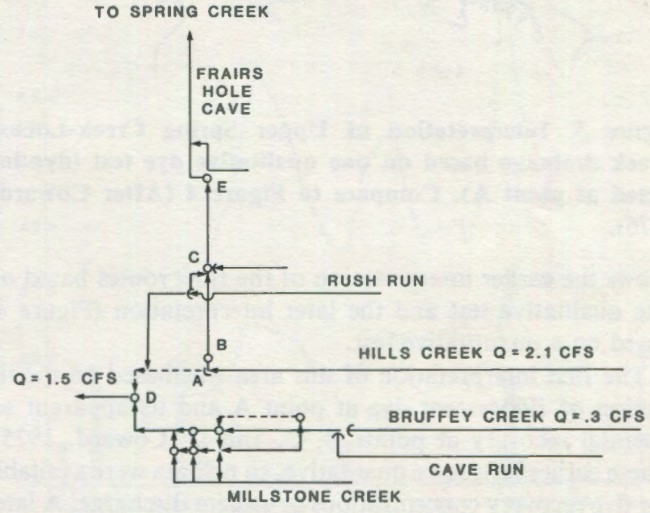

Figure 4. **Schematic drawing of drainage pattern of the upper Spring Creek-Locust Creek basins, West Virginia. Shown are Hills Creek Cave (A), Cutlip Cave (B), Clyde Cochran Cave (C), Locust Creek Cave (D), Upper Friars Hole Cave (E). (After Williams and Jones, 1983).**

The presence of additional springs is clearly shown in the data presented in Figure 4. In this example, it was long assumed from three qualitative tests that Hills Creek (point

A) discharged only to Locust Spring (point D). However, this quantitative test revealed that with a travel time of less than 24 hours only about 5% of the tracer was recovered at this spring. The remainder of the tracer was recovered at another spring a month later and 12 miles to the south of Locust Spring.

The pitfalls of interpreting the drainage network with a minimal number of qualitative tests in a complex karst basin are further illustrated by results from this area. Figure 5

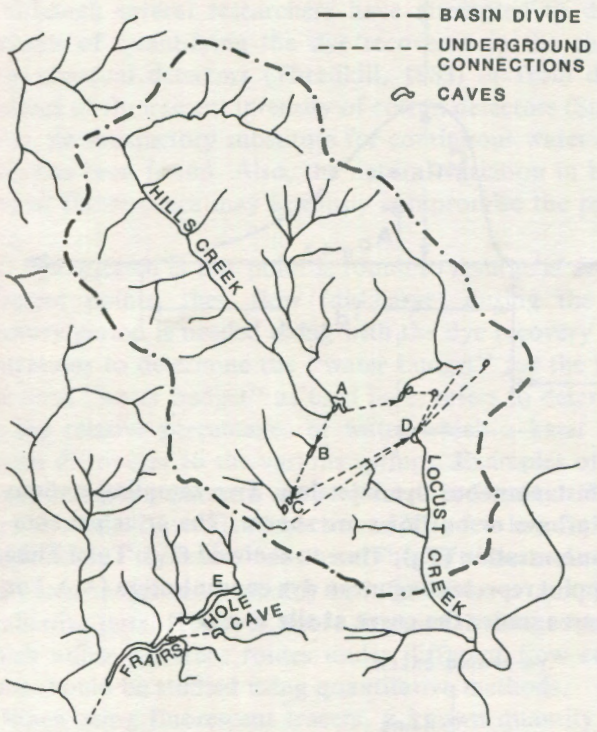

Figure 5. **Interpretation of Upper Spring Creek-Locust Creek drainage based on one qualitative dye test (dye injected at point A). Compare to Figure 4 (After Coward, 1975).**

shows the earlier interpretation of the flow routes based on one qualitative test and the later interpretation (Figure 4) based on a quantitative test.

The first interpretation of this area was based on the injection of fluorescent dye at point A and its apparent sequential recovery at points B, C, and D (Coward, 1975). These earlier tests were qualitative, so no data were available for dye recovery concentrations or stream discharge. A later quantitative test (Williams and Jones, 1983) involved simultaneous injection of three different tracer dyes, CWT at A, RWT at B, and FL at E. Discharge measurements indicated that, under base flow conditions, the stream flow at point A was greater than at D and therefore the spring at D could not account for all of the water from this sink. The water budget shows that at low flow only 5% of the water from sink A resurges at spring D. Furthermore, dye injected in cave B was not recovered at all at spring D, but was found over a month later at springs 12 miles to the south of spring D. The earlier test was not wrong, but the results were misinterpreted due to lack of quantitative data and not enough resolution in the reconnaissance tracing data.

The drainage divides may be hard to define and impossible to delineate in karst areas with a high density fracture network where groundwater has much more freedom of movement than in an area dominated by dendritic conduit flow routes. Smart (1976) suggested defining the groundwater divide between two basins as the location from which 50% of the tracer moves to one spring and 50% to the second spring. The concept of a separate drainage basin for each major spring may be meaningless in an area characterized by a radial groundwater drainage pattern.

TIME-OF-TRAVEL

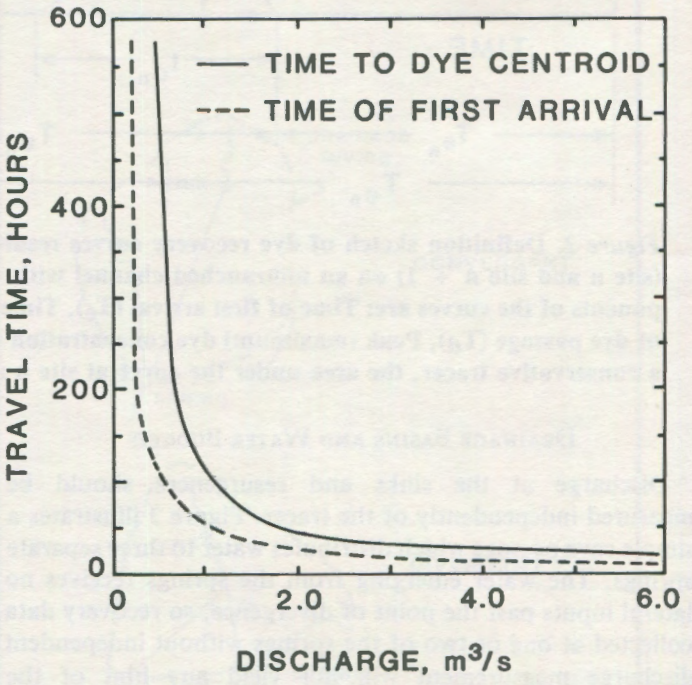

Figure 6. **Discharge versus time-of-travel (After Smart, 1981).**

A time-of-travel determination may be calculated from the dye recovery curves. When concentration (or relative intensity) versus time is plotted for systems characterized by unbranched, open channel flow conduits, the recovery curve will usually be skewed to the left (Figure 2). The time-of-travel may be expressed in terms of time from (instantaneous or slug) dye injection at sink to first appearance, time to peak concentration, or time to centroid (50% of the dye recovered). With increasing travel time (or distance) the recovery curves show increasingly lower peak concentrations and longer recession tails. With a conservative tracer,

the total amount of dye passing any point remains the same, but the peak concentration becomes progressively lower with increasing dispersion of dye into, and slow release from, dead zones. This implies that traces conducted during low flow conditions will produce much lower relative peak concentrations than tests conducted at high flows (after normalizing the curves to adjust for volumes).

Data from Smart (1981) are presented in Figure 6 which shows an exponential relationship between discharge and travel time. Data from constant width rectangular conduits in the Mendips (Great Britain) show that increasing velocity accounts for two-thirds of the discharge increase and cross-sectional area accounts for one-third. In a closed channel conduit, velocity change must account for all the change in discharge, while velocity typically accounts for one-third of the change in discharge in surface streams. If log discharge is plotted against log travel time, for entirely phreatic conduits, the line will have a slope of -1.0, while the slope for entirely vadose passages will be about -0.3 or greater (Smart, 1981). Smart used the dye centroid travel time versus discharge to determine if the conduits were vadose or phreatic and attempted to calculate the conduit volume as well. It is interesting to note that travel time may be calculated from the shape of the recovery curves even if the dye budget values do not balance well.

DYE RECOVERY PATTERNS

The typical dye recovery pattern from vadose conduits is similar to patterns from surface streams studies, but underground diverging and converging, lateral inputs and outflows, alternating vadose and phreatic flow conditions, and increased opportunities for adsorption and dispersion of the tracer alter the characteristic recovery patterns. The appearance of the dye recovery curve is determined by 1) the conduit network pattern itself, 2) the flow type, conditions, and variation during the test, and 3) the adsorption and dispersion characteristics of the tracer. The interpretation of multiple peak tests is especially subjective because it is often difficult to determine the exact causes of fluctuating dye recovery curves.

Five types of flow networks with discrete inputs and outputs were suggested by Brown (1969) and are presented in Figure 7. Note that for a type 4 flow network, the lateral inputs and outflows could cancel each other, and the measured discharge at the inlet and outlet would be equal. The dye budget, however, should suggest a water loss from the system. Conduits which have anastomosing flow patterns should show multiple dye concentration peaks representative of the dye traveling different distances to the resurgences. Much of the interpretation of multiple peak recovery curves suggested branching and converging of the conduit flow routes.

The flow type (vadose or phreatic), the variation in flow during the test (changes in discharge and velocity), the

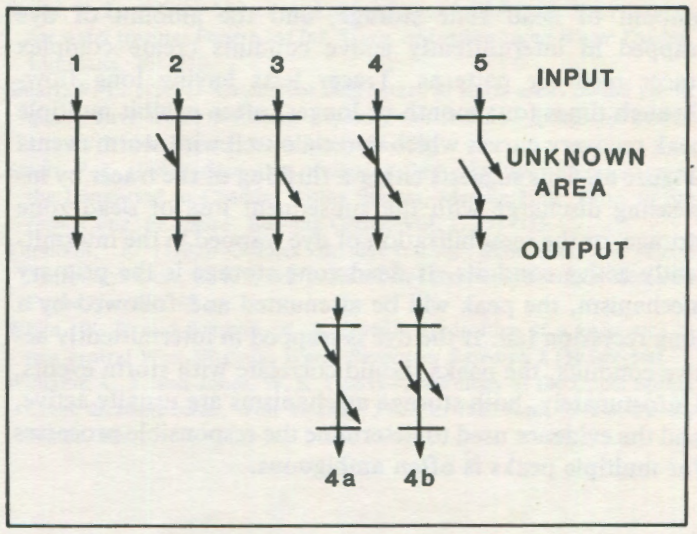

Figure 7. **Five types of Karst flow systems (Black box model) (After Brown and Wigley, 1969).**
Type 1—Single input to single output.
Type 2—Unknown lateral input.
Type 3—Unknown additional outputs.
Type 4—Additional unknown inputs and outputs.
Type 5—Input unknown, output unknown.

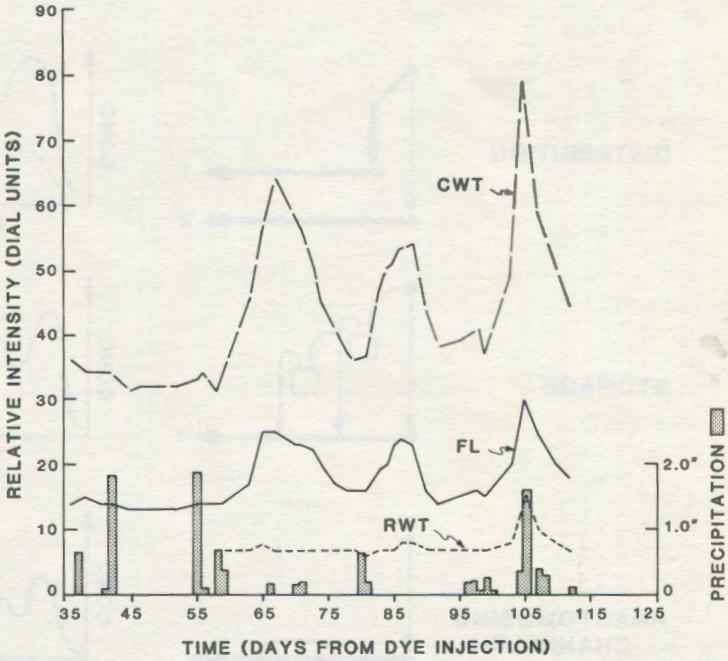

Figure 8. **Dye recovery curves and rainfall. JJ Spring, Spring Creek, West Virginia. Dye injection points are shown in Figure 4: Calcoph White (CWT) was injected at point A, fluorescein (FL) at point E, Rhodamine WT (RWT) in Rush Run (After Williams and Jones, 1983).**

amount of dead zone storage, and the amount of dye trapped in intermittently active conduits create complex tracer recovery patterns. Tracer tests having long flow-through times (one month or longer) often exhibit multiple peak recovery curves which correlate well with storm events (Figure 8). This suggests either a flushing of the tracer by increasing discharge with the subsequent loss of dead zone storage, or the remobilization of dye trapped in the intermittently active conduits. If dead zone storage is the primary mechanism, the peak will be attenuated and followed by a long recession tail. If the dye is trapped in intermittently active conduits, the peaks should correlate with storm events. Unfortunately, both storage mechanisms are usually active, and the evidence used to determine the responsible processes for multiple peaks is often ambiguous.

Smart and Ford (1982) suggested some simple shaft interpretations (Figure 9) of dye tracer experiments in a glacierized alpine karst area. These interpretations were not based on the dye recovery and discharge data alone, but also on a knowledge of the geology, morphology, and observations of the accessible caves in the region.

CONCLUSIONS

Water tracing and the interpretation of the data is often a somewhat subjective art, even if absolute values are available. The researcher must integrate the tracer test and hydrologic data with the study area's topographic and geomorphic patterns. As more quantitative data from different hydrogeologic regions becomes available, the interpretative techniques should become more sophisticated, if not more exact.

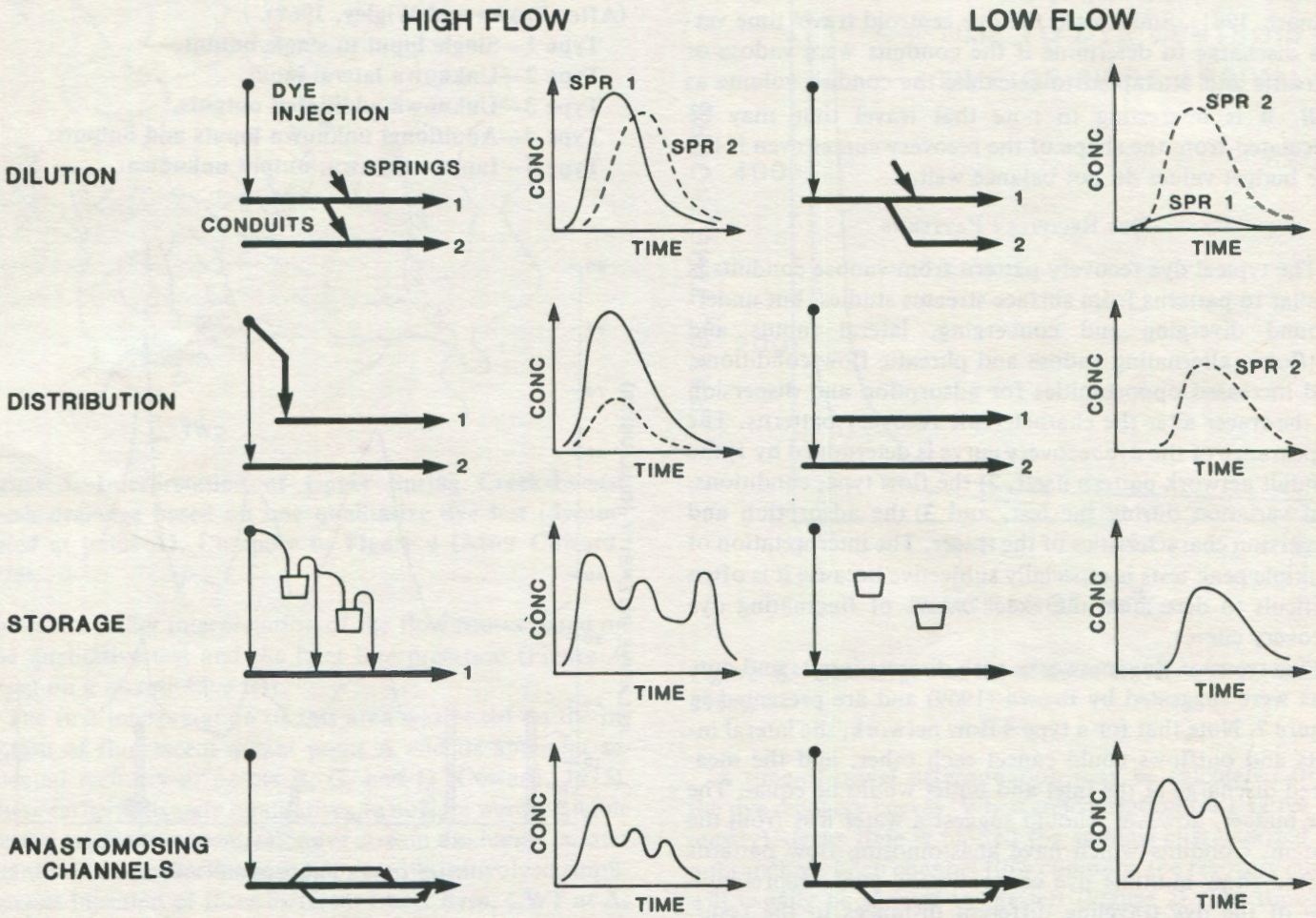

Figure 9. **Simple shaft-conduit interpretations of dye recovery patterns (After Smart and Ford, 1982). The expected dye recovery curves influenced by changing hydrologic conditions (high to low flows) and geometry of the conduit system is illustrated. Note that multiple peak recovery curves may be due to flushing of dye from dead zone storage or anastomosing conduit routes.**

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