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KARST GROUNDWATER INVESTIGATION RESEARCH PROCEDURES

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Introduction

Since 1970, the following research procedures have been utilized and developed by Crawford Hydrology Laboratory (CHL) to investigate the flow of groundwater in karst aquifers. This document describes the current standard procedures and criteria used by CHL in both the field and laboratory.

CHL can provide services for a full field investigation, consultation and project design, quality supplies, and laboratory analysis or any combination of these for your dye trace. A typical scope of work consists of a literature review, field survey for the karst hydrogeologic inventory, dye receptor deployment and retrieval, a background fluorescence study, dye injection, subsequent monitoring and fluorescent dye analysis. The scope of work may include all or portions of these components, outlined below, according to the needs of the particular investigation.

Delineation of Study Area

To ensure dye resurgence locations will not be missed during the investigation, the study area must extend in all directions away from the site until one of the following conditions is met:

1. The water table has been established by measurement to be higher than at the proposed dye injection location on site.

2. A definite discharge boundary has been reached, such as a large perennial stream.

Review of Literature

Prior to the initial the field survey, a desktop study is conducted to identify and review, as available, the following types of resources that pertain to karst features in the study area.

- Aerial photographs
- Geologic maps
- Flood maps
- Cave maps
- Topographic maps
- Soil surveys
- Storm water reports
- Previous reports about the site
- Maps showing locations of water and sewer pipes

References are sought from state and federal agencies, and local caving organizations. Pertinent information from these resources is transferred to a working base map to plan the subsequent field survey.

Karst Hydrogeologic Inventory

The field survey for the Karst Hydrogeologic Inventory (KHI) is conducted under conditions that range from moderate to high flow during a wet period so the dominant resurgence points are active. The survey is conducted by walking or floating all streams and associated impoundments (lakes, ponds) in the study area to visually identify karst features that include but are not limited to:

- Springs
- Soil springs
- Seeps
- Sinkholes
- Swallets
- Karst windows
- Sinking streams
- Caves

Each feature is plotted on topographic map, given a name, and a unique inventory number. General information and physical characteristics of the feature are recorded on the Karst Feature Inventory Form. The physical characteristics of each monitoring location include a measurement or estimate of the discharge volume, measurement of the discharge temperature, specific conductance, and pH. The feature is photographed and coordinates recorded from a hand-held Global Positioning System (GPS) receiver to complete the inventory record.

The equipment needed for the field survey includes the following:

1. Base maps and air photos for plotting features (7.5 minute topographic quadrangles)
2. Inventory forms
3. Meters for the measurement of temperature, pH, and specific conductivity.
4. Hand-held GPS Unit
5. Camera to capture two photos of each spring and surface stream location (one from about 10-15 feet and one from about 100 feet)
6. Flagging to mark features for surveying and/or monitoring
7. Steam gage equipment

Background Fluorescence Investigation

The background fluorescence investigation involves the monitoring of resurgence points and streams in the study area for possible background fluorescence from previous dye traces, man-made substances, and/or natural interference. The results from the monitoring locations are used to determine the appropriate dyes and dye concentrations to be used.

Dye receptors are typically deployed during of the Karst Hydrogeologic Inventory and for the same duration as proposed for post-injection receptor exchanges. Because of background fluctuation, particularly in urban and/or industrial areas, it may be necessary to measure the background weekly

over a two or three week period. All monitoring activities are conducted in accordance with Dye Receptor Deployment and Retrieval Procedures described later in this document.

The receptors consist of small packets constructed of fiberglass screen mesh filled with approximately three grams of activated coconut charcoal. Each receptor is secured in the main flow of the stream or resurgence point with the use of a system of floats and weights, as needed, with black nylon twine (for low visibility) or non-fluorescent monofilament line. In small springs or streams, the channel may be altered by moving rocks or by other minor means in order to maximize flow past the receptor. In shallow water, the receptors may be shielded to minimize photochemical decay of dyes in the sunlight. Receptors are secured so that they can be retrieved under high water conditions.

Upon retrieval, each dye receptor is washed off in the source water, placed in a clearly labeled, sealed plastic bag, and stored in a dark environment, such as a cooler to reduce exposure to sunlight. Each receptor is prepared in accordance with CHL procedures and analyzed for the presence of dyes that may be used during the investigation. The dyes used by Crawford Hydrology Laboratory include:

- **Fluorescein**
- **Sulphorhodamine B**
- **Rhodamine WT**
- **D&C Red 28**
- **Eosine**
- **Tinopal CBS-X**
- **FD&C Red #3**
- **D & C Green #8**

If the dye trace does not begin within two weeks of the collection of the background dye receptors, a second Background Fluorescence Investigation is recommended. Since background fluorescence can change, it is important to know the background fluorescence the week previous to dye injection.

Matrix Interference Investigation

A matrix interference investigation may be needed previous to selection of the dye or dyes to be used. This involves a bench-top investigation to measure the potential impact of chemicals in the groundwater on the dye concentration, dye peak wavelengths, the adsorption of the dye by the charcoal, or the release of dye from the charcoal during elution. The procedures used to conduct this investigation are outlined below:

MATRIX INTERFERENCE INVESTIGATION RESEARCH PROCEDURES

1. SAMPLE COLLECTION

A water sample is collected from each monitoring location and dye injection point for which a matrix interference test is desired. The selection of these locations is based on:

1. pH and conductivity results
2. Abnormal water color or odor
3. Results of previous analytical tests
4. The size and location of the hydrologic feature
5. The anticipated dye injection location(s) or dye recovery locations

A water sample volume of at least 500 ml is collected in a clean, labeled container. The label includes the name and inventory number of the hydrologic feature, the project name, date and time of sample collection, and initials of persons that collected the sample.

Along with water samples from selected monitoring locations, a 500 ml volume of de-ionized (provided by CHL) will be used as a control mechanism. This control sample is processed using the same procedures as the matrix interference samples. The control sample is used to quantify any effects the contaminants may have on the dyes that are tested.

2. SPIKED WATER SAMPLES AND BACKGROUND DYE LEVELS

Standard concentrations of dye are used to spike the water samples to achieve a target spike concentration of 10 ppb. 100 microliters of 10 ppm standard for each dye is added to 100 ml of sample water. For Tinopal CBS-X (optical brightener), 100 microliters of 100 ppm standard is used; the result is a concentration of 100 ppb in the sample jar. For fluorescein, 50 microliters of 10 ppm standard is used; the result is a concentration of 5 ppb in the sample jar. Two dyes are tested in each individual flask. The de-ionized water control, and a control sample of the water from each test sample, is also analyzed. This provides a background dye analysis for each sample submitted.

The sample flasks are agitated to ensure the dye is thoroughly mixed. Approximately three milliliter aliquots are removed from the sample jars and placed in labeled, borosilicate glass test tubes. The water samples are tested for the selected dyes by analysis in accordance with CHL Procedures.

Sample jars are stored in a dark place (not refrigerated) and analysis is repeated after at least 48 hours. Changes in dye concentration since the initial analysis demonstrate degradation of the dyes by sample constituents.

3. SPIKED CHARCOAL SAMPLES

Charcoal packets that contain equal amounts of activated coconut charcoal are placed in each spiked solution. The charcoal packets remain within the flasks for a minimum of 12 hours before they are individually removed, processed, and analyzed.

4. RESIDUAL DYE

An aliquot of water is removed from each of the spiked solutions and placed into labeled test tubes. These are analyzed to obtain the concentration of dyes that remain in the spiked solution after removal of the charcoal.

5. MATRIX INTERFERENCE REPORT

The results from each of the four analysis events are recorded and plotted. Results are analyzed to assess the impact of contaminants upon tested dyes. A short report is generated that addresses the impact of matrix interference on each of the dyes tested. Dye type and quantity may be determined, in part, by the information included in the report.

Dye Trace Notification or Permit Application

In Tennessee and Kentucky, the injection of a dye into subsurface waters requires that the Tennessee Division of Water Supply or the Kentucky Division of Water, respectively, be notified prior to injection. Therefore, the appropriate application will be made, and an acceptance notification received from that agency prior to the initiation of any injection of dye at a site in Tennessee or Kentucky. In other states, the State Division of Water will be contacted and permission obtained from the appropriate state agency previous to dye injection.

Dye Injection

After the analysis of the background dye receptors, and the placement of dye receptors in all monitoring locations that may include: springs, karst windows, cave streams, lakes, surface streams, monitoring wells, and selected water wells, dye is injected directly into a sinking stream, sinkhole, well, or excavated soil pit.

If a sinking stream or sinkhole can be located in the appropriate place for dye injection, it is utilized. However, it is sometimes necessary to either dig a dye injection pit with a backhoe or drill an injection well. If it is more than roughly 17 feet to bedrock, then an injection well is the only choice. It may not be necessary to drill a new well, however. Capacity tests can be performed on all existing monitoring wells, and if they will take water at a sufficient rate for dye injection and flushing, then it may be possible to use one or more of them for dye injection. If not, then it is necessary to drill an injection well (which can also serve as a monitoring well). The well or wells will be drilled at sites where there is a good chance of intersecting a karst conduit. Such sites are where lineaments intersect, or where geophysical techniques (microgravity or natural potential) have indicated significant conduits or fractures. Potable water is used to flush the dye past the soil into a bedrock crevice that leads to a cave stream. Usually approximately 500 gallons of priming water are injected into the hole or well to make sure that it drains sufficiently and to wet the soil so that less dye will be absorbed. The dye is then injected and flushed with approximately 2,000 gallons of water. Usually three or four dye traces can be performed simultaneously using different dyes.

Dye Receptor Deployment and Retrieval

Activated coconut charcoal dye receptors are typically collected and replaced on a weekly interval depending on study objectives, weather and other factors but longer deployment intervals may be appropriate. If time-of-travel data is necessary, samples may be exchanged more frequently during the first one to two weeks. Usually, dye receptors are exchanged weekly for at least two months after the first detection of dye at a spring. This is necessary to allow sufficient time for the dye to reach other receptor locations. If the karst aquifer has turbulent flow through well-developed bedrock conduits, the dye will usually resurge from a spring or springs rather quickly. However, if the dye must travel through a laminar flow, porous-media aquifer, even for a short distance, it could be several weeks to months before the first arrival of dye at a resurgence location. It is also recommended and CHL standard procedure to collect a water sample at the time of receptor collection. Water samples for quantitative dye tracing are processed and analyzed in accordance with CHL Procedures described later in this document. The field procedures below describe CHL protocol and are otherwise provided as guidance.

DYE RECEPTOR DEPLOYMENT AND RETRIEVAL PROCEDURES

1. RECEPTOR CONSTRUCTION

The receptors consist of small packets constructed of vinyl-coated fiberglass screen mesh approximately four inches long and two inches wide. The mesh is filled with three to four grams of activated coconut charcoal. Each receptor is prepared in a dye-free environment and individually packaged in sealed polyethylene bags.

2. GRAB SAMPLE VIALS

Grab sample vials are made of borosilicate glass suitable for fluorometric analysis. The caps used are PTFE lined to prevent contamination by fluorescent molecules that can leech out of standard rubber-lined caps. CHL tests 10% of all vials upon receipt from the supplier to ensure that they are dye-free. Each charcoal receptor provided by CHL is accompanied by a grab sample vial.

3. RECEPTOR PLACEMENT

Each receptor (along with a grab sample vial) is sealed in a polyethylene bag and transported in a cooler or other container under chain of custody procedures to the site. The receptors are inspected for signs of damage prior to deployment. Disposable nitrile gloves are worn when handling the receptors in order to avoid the transfer of dyes from clothing and other items. New gloves are used for the placement of each receptor.

Each receptor is deployed in the water flow of the monitoring location. The receptor is secured using a system of weights, floats, and tethers as necessary to secure the receptor in a location where flow past the receptor is maximized and exposure to sunlight is minimized. In areas accessible to the public, it may be necessary to make the receptor anchoring system inconspicuous in order to avoid tampering. In small springs or streams, the channel may be altered by rearranging rocks, or by other minor means, in order to maximize flow past the receptor. Each receptor is secured so that they can be retrieved under high water conditions.

Two dye receptors may be deployed at separate nearby locations at key resurgence and stream points and at any site accessible to the public. This provides a backup in the event that the primary receptor is lost or stolen.

4. RECEPTOR AND GRAB SAMPLE RETRIEVAL

During receptor retrieval, the condition of the stream or resurgence point is examined for the presence of dye or evidence of tampering or other disturbance. The receptor is retrieved from the monitoring location by means of its tether. Where wading is necessary, the receptor is approached from downstream. Disposable nitrile gloves are worn for each receptor when handled. The receptor is rinsed in the water from which it was removed to clean it of accumulated sediment. The receptor is placed in a labeled, sealed, polyethylene bag. A water grab sample is taken from the location the dye receptor occupies at the time of the receptor collection. The vial is then placed in the same bag as the dye receptor and stored in a closed container to shield it from sunlight. The receptor bag is labeled (with a black permanent ink marker) with the Project name, sample identification number, name of monitoring location, date and time of retrieval, and initials of staff who collected the receptor.

5. SAMPLE TRANSPORT

Dye adsorbed onto charcoal receptors is extremely stable at ambient temperatures. Retrieved receptors are transported under chain of custody procedures at ambient temperatures in a dark container, such as a sample cooler. If holding time of the receptors is more than 24 hours, they are refrigerated to prevent possible microbial growth.

Charcoal receptors and water samples collected for submission to CHL should be shipped promptly or stored under refrigeration until shipment. CHL recommends shipping samples in a cooler with or without a frozen ice pack. We do not recommend shipping samples with wet ice as this can create potential for cross contamination. Please ship samples using UPS or Fed Ex Services. The U.S. Postal Service does not deliver to the lab directly. CHL can only accept shipments Monday through Friday from 8:00 a.m. to 4:30 p.m.

Laboratory Procedures

Sample Custody and Storage

Water samples and charcoal dye receptors may be received by Crawford Hydrology Laboratory via courier or mail delivery. Chain-of-custody forms should accompany all samples submitted. Upon receipt the forms are verified, signed and filed in the appropriate project folder. The chain-of-custody form is added to the laboratory custody records and samples are stored in the lab refrigerator.

Sample Processing

Each dye receptor and grab sample vials are kept in the original, labeled, sealed, polyethylene, zip-lock bag until it is removed from the refrigerator. The bags are opened one at a time and the receptor removed. It is washed in a high-speed jet of tap water to remove excess sediment. The receptors can be washed in de-ionized upon request by the client. A typed laboratory identification tag containing the project name, lab identification number (lab ID) and collection date are stapled to the receptor. It is then placed on a tray in a drying oven and dried at 49^{o} C for a minimum of 12 hours.

The exterior of the accompanying water sample grab vial is bleached, rinsed, and dried. A label with the project name, lab ID, and collection date is attached to each vial. The vials are then placed in a vial tray in the refrigerator to await analysis. If samples are collected in containers other than those provided by CHL water sample aliquots are taken from each bottle with a disposable pipette, placed in borosilicate glass vials and given labels with the project name, date, and lab ID. They are placed in the refrigerator until analyzed. All pipettes, vials, and sample containers are discarded after one use.

Charcoal Preparation

1. Charcoal dye receptors are washed under a high-speed jet of tap water to remove as much sediment as possible.
2. A typed label containing the site location name, sample number, and date of collection is stapled to each receptor.
3. The receptors are placed in an oven and dried for 12 hours at 49^{o} C.
4. 1.0 grams of charcoal is weighed and placed into a disposable plastic container that is labeled with the sample identification number.
5. The remainder of the charcoal is returned to its original zip-lock bag and stored for six months past project completion (not refrigerated).
6. 5.0 ml of Smart solution (an eluent consisting of 1-propanol 100% assay, de-ionized water, and ammonium hydroxide 28-30% assay mixed at a ratio of 5:3:2) is added to the charcoal and the disposable sample container is capped.
7. After 30 minutes, the eluent is transferred into a borosilicate glass test tube that is then sealed with a polypropylene cap. The eluted charcoal is then discarded. Precautions are taken to avoid any charcoal from entering the sample vials during transfer but if this occurs removal is not attempted.
8. Unless analyzed immediately, eluted samples are placed in the refrigerator
9. Samples are placed in a constant temperature bath, covered to prevent photochemical decay and allowed to equilibrate to 30^{o} C just before analysis.

Water Sample Preparation

1. Water samples in CHL provided sample vials are bleached, rinsed and labeled upon receipt.
2. If a water sample is received in a non-CHL provided container, an aliquot is drawn from the bottle using a disposable polyethylene pipette and placed into a borosilicate glass test tube which is then sealed with a polypropylene cap.
3. Unless analyzed immediately, water samples are placed in the refrigerator

4. Water samples (amount remaining in the original containers received by Crawford Hydrology Laboratory) are stored for six months past project completion unrefrigerated.
6. Samples are placed in a constant temperature bath, covered to prevent photochemical decay and allowed to equilibrate to 30^{o} C just before analysis.

Analysis

Eluent and water samples are analyzed for dye by synchronous scanning on a Shimadzu Model RF 5301PC scanning spectrofluorophotometer. Our instrument was installed on-site by the manufacturer and has been operated and maintained under their guidelines. We carry a Shimadzu maintenance and insurance policy on our instrument which includes annual maintenance visits, and if needed repair, by a Shimadzu-authorized technician.

Analysis on a scanning spectrofluorophotometer provides low detection limits and reliable dye analysis. For a typical analysis, a synchronous scan is performed where the excitation and emission monochromators are kept at a fixed wavelength separation during the scan, 15 nanometers for eluted charcoal samples and 18nm for water samples. CHL uses an excitation scan of 350-625 nm that allows for the detection of all eight dyes that we commonly use. The scanning technique for water is similar to the analysis for eluted charcoal samples with the scanning parameters adjusted to compensate for shifts in the excitation and emission maximum wavelengths as well as differences in the Stoke's shift caused by the differences in pH and polarity of water as compared to eluent.

Typical Synchronous Scan Parameters for Charcoal Samples

Scan Speed: Fast

Sensitivity: High

Excitation Slit Width: 3.0

Emission Slit Width 5.0

Only the emission fluorescence from the synchronous scan is displayed on the monitor and plotted on a graph. The resulting printout has the sample identifier, scanning parameters, and calibration parameters at the bottom of the page. The proprietary software uses spectrum integration and calibration curves stored in the computer to determine the concentration of the dye in question. If the scan indicates positive results for fluorescent dye, a second printout is made to identify peak centers, again using proprietary software. Refer to Appendix I for emission peak centers of the fluorescent dyes used at CHL.

For samples with dye concentrations that meet or exceed the maximum threshold in the high sensitivity scan (fluorescent peak intensity and dye concentration cannot be accurately measured above a peak intensity of 1000), the samples are next analyzed using a low sensitivity scan. Serial dilutions are made with the appropriate matrix until peak intensity is within the measurable range. Typical dilutions are 100 fold (1:100) or 1000 (1:1000) fold. This involves combining one part of the test sample to 99 parts water or eluent or 999 parts for each dilution, respectively. All volume measurements for samples are made with an Acura Micropipette. All water and eluent measurements are made with a 5 or 10 mL Barnstead Thermolyne pump dispenser which will pump with 1.5% of the set value.

If the emission spectra from two or more dyes overlap, the spectra for each dye is separated by use of a non-linear curve-fitting computer program specifically designed for spectral separation. Spectrum integration and calibration curves stored in the computer are then used to determine the concentration of each individual dye present in the sample. All samples and standards analyzed on the Shimadzu RF 5301 are stored electronically with sample information. Sample processing and analysis is recorded in a laboratory logbook.

Dye Quantification

Dye concentrations are expressed in parts per billion (ppb) and are calculated by separating fluorescence peaks based on known emission ranges for each dye then calculating the fluorescence peak area. The area of the sample is proportional to the dye standard solution peak area. Dye standards are analyzed before and after each sample set. The dye standards run are according to the analysis needs of a specific project. For example, if a sample set needs to be analyzed for Fluorescein and Sulphorhodamine B the following standards would be run before and after the samples:

For Eluent:	0.005 ppb Fluorescein and Sulphorhodamine B
	0.100 ppb Fluorescein and Sulphorhodamine B
	10.00 ppb Fluorescein and Sulphorhodamine B
For Water:	0.010 ppb Fluorescein and Sulphorhodamine B
	0.100 ppb Fluorescein and Sulphorhodamine B
	10.00 ppb Fluorescein and Sulphorhodamine B

Also the lowest concentration standards for both dyes are run every 20 samples. If 20 or more samples must be analyzed in our low sensitivity scan then the highest concentration is run every 20th sample as well.

Although the dye concentration in water samples expressed in ppb is an accurate quantitative measurement of the amount of dye in the stream at the time the sample was collected, the same is not

true for the eluted charcoal samples. It is only semi-quantitative compared with the actual quantity of dye in the water passing over the receptor. The quantity of dye adsorbed by the charcoal is a function of the dye concentration in the water and the quantity, velocity, temperature and duration of exposure. Turbidity and the quantity and species of molecules competing with the dye for the charcoal adsorption sites can reduce the quantity of dye absorbed onto the charcoal. Also, the quantity of dye eluted from the charcoal is dependent on the amount of charcoal, the type of eluent, whether the charcoal was wet or dry before elution, and the length of time the charcoal is eluted before analysis. The laboratory procedures are standardized but the variables the receptor is exposed to in the stream cannot be standardized.

Dye concentrations for eluted samples are measured and recorded in ppb, however these values will virtually always be much higher than the dye concentrations reached in the stream. Also, because of several water exposure variables, the concentration of dye adsorbed by the charcoal does not necessarily represent the quantity of dye that flowed in the stream past the dye receptor. Analysis of two dye receptors placed in the same general area of the same stream for the same time period of time can result in differences in dye concentrations when expressed in ppb. Therefore, the following abbreviations are used to express the dye concentration in more general terms rather than ppb:

ND	Non-Detect
+	Positive
++	Very Positive
+++	Extremely Positive
B	Background
IB	Initial Background

Non-Detect Results (ND) - No dye detected at or above the determined quantitation limit.

Initial Background (IB) - Designation given to samples collected before dye injection. Initial background samples are the standard against which all post-injection samples from an individual site are compared in order to determine true positives (+).

Background Results (B) - Any sample which has a concentration greater than or equal to the quantitation limit, but less than 10 times the concentration of the highest initial background dye receptor analyzed shall be reported as Background (B) on the report sheet. Also included is any sample that does not meet the qualification for a positive result designation.

Positive Results (+, ++ or +++) - Any sample that is determined to be positive

Although dye concentrations obtained from charcoal dye receptors do not precisely reflect the concentration in the source water, detection of dye at a sufficient concentration above background levels does constitute a positive trace. If a quantitative dye trace is necessary, it must be based on dye analysis of water samples, not charcoal. The Crawford Hydrology Laboratory frequently performs quantitative traces by collecting water samples with an ISCO automatic water sampler. This method provides a dye breakthrough curve, which is an accurate measurement of the dye concentration in the stream as the dye cloud passes the monitoring site.

Preparation of Standards

The standards for analysis of water samples are prepared in de-ionized water. Standards for eluted charcoal sample analysis are prepared in Smart solution. Research by Crawford Hydrology Laboratory indicates that Smart solution elutes more dye from the charcoal than other eluents tested. The dye concentration in the dye sample used for standard preparation is based upon the dye assay figures provided by the dye manufacturer. The Crawford Hydrology Laboratory contacts the dye manufacturer and obtains the certificates of analysis on dyes used to make standards. Dye standards are made as follows:

1. A sample of the dye is weighed into a dye-free amber bottle. The sample is then diluted to make a 1% dye solution stock by weight. The 1 % stock solutions must set overnight to make sure all the dye is dissolved. A stock solution is prepared for each dye separately and is considered our long term standard.

2. A set of serial dilutions are then made using de-ionized water for water standards and Smart solution for eluent standards. We most commonly prepare 100 mL or 50 mL of each dilution. Standard dilutions used for calibration and analysis range from 0.005 ppb to 100 ppb for eluent and 0.010ppb to 100 ppb for water. These are considered short term standards and are made as needed.

3. All water standards are stored in amber bottles and placed in refrigeration. Eluent standards are made and stored in amber bottles at room temperature in a secure, dark lab cabinet.

Quality Control/ Quality Assurance

Field Duplicates - Duplicate dye receptors are placed at 10 percent or more of the sites to be monitored. The second receptor serves as a back-up in the event that the primary receptor is lost or stolen and/or can be analyzed as QA/QC duplicate.

Eluent Blank (for eluted samples only) - Each batch of Smart solution is analyzed for each dye before it is used to elute charcoal samples. Additionally, an eluent blank is analyzed at the beginning, end, and every 20th sample throughout the analysis.

De-ionized Water Blank (for water samples only) - Each batch of de-ionized water is analyzed for each dye before it is used. Additionally, a de-ionized water blank is analyzed at the beginning, end, and every 20th sample throughout analysis.

Charcoal Blank- A sample from each new sealed container of activated charcoal is eluted and analyzed for each dye before the remainder of the charcoal in the container is used. Charcoal receptors are also randomly tested on a monthly basis after construction so that all components used in manufacturing (charcoal, mesh bags, staples, paper clips, cable ties, gloves and polyethylene bags) are tested and confirmed dye-free.

Raman Scattering Sample- A Raman scattering pattern and signal-to-noise ratio check is performed on a de-ionized water blank at the beginning and end of each sample set analysis. This is the method recommended by the manufacturer for insuring that the instrument is working within specified parameters and for calibrating the instrument.

Laboratory Control Standards- Two low concentration standards and one high concentration standard for each of the dyes to be analyzed, is analyzed before and after each set of samples. A subset of these standards is also analyzed after each set of 20 samples. This demonstrates that the Shimadzu is capable of detection at the minimum detection limit and provides data that can be used to determine the accuracy and precision of the analysis.

Criteria for Interpreting Results of Synchronous Scanning

Interpretation of dye tracing data is not the same as interpreting the results of chemical analyses. Background levels of dye are often present above the quantitation limits of the fluorescent dyes used for tracing. One of the reasons for these background levels is due to the commercial use of the various dyes used for tracing. There are only a few non-toxic fluorescent dyes. For this reason, the dyes used for tracing can often be found in products ranging from food coloring to toilet bowl cleaners. Another reason for these background levels is the extremely low detection limits of fluorescent dyes. Virtually any tracer will have background levels if one can measure at very low concentrations. Background levels often fluctuate more in karst aquifers and to this end, Crawford Hydrology Laboratory developed a standard protocol to determine what constitutes background levels, what is positive, and what is negative (non-detect). This protocol is based upon the results of numerous dye traces that Dr. Crawford and the Crawford Hydrology Laboratory have performed since the 1970s. The protocol has been used for dye trace studies at numerous industrial sites, for federal and state projects and at EPA superfund sites.

Background Samples
In order for background fluorescence to be recorded, it must meet the following conditions:\

- The determined concentration for each dye must be greater than or equal to the lowest detection limit for that dye.

- The recorded peak of the emission curve must be + / - 5 nm for Fluorescein, Eosine, FD&C Red 3, D&C Red 28, Rhodamine WT, Tinopal CBS-X, and Sulphorhodamine B, and + or - 10 nm for D&C Green 8. The only times exceptions may be made are:

    - A water sample collected at the same location verifies the presence of the dye in question.

    - The emission spectrum from one dye overlaps the excitation spectra of another dye, causing a shift in peak position.

- The shape of the curve from the synchronous scanning must be the characteristic symmetrical shape of each particular dye as determined from its laboratory standard.

Post- Dye Injection Samples

Post-Dye Injection Samples must meet the following criteria for the determination of a positive trace:

- The determined concentration for each dye must be ten times greater than initial background concentrations or the lowest detection limit for that dye. This means that for a dye with a quantitation limit of 0.01 parts per billion (ppb), no sample can be designated Positive (+) unless its concentration is greater than or equal to 0.100 parts per billion.

- The recorded peak of the emission curve must be + / - 5 nm for Fluorescein, Eosine, FD&C Red 3, D&C Red 28, Rhodamine WT, Tinopal CBS-X (optical brightener), and Sulphorhodamine B, and + or - 10 nm for D&C Green 8. The only times exceptions may be made are:

    - A water sample collected at the same location verifies the presence of the dye in question.

    - The emission spectrum from one dye overlaps the excitation spectra of another dye, causing a shift in peak position.

- The shape of the curve from the synchronous scanning must be the characteristic symmetrical shape of each particular dye as determined from its laboratory standard.

- Two consecutive samples that meet the above criteria. The concentration of the dye eluted from the charcoal should display a rise and fall, similar to a dye breakthrough, over a period of time. Consequently, no location shall be called positive if there is only one occasion when the dye concentration met the above criteria. A minimum of two consecutive positives is needed in order to say that a particular location had a positive trace. If only one sample qualifies for a positive designation, then the location will either be designated as a potential positive, or the trace will be repeated.

- The presence of dye at a particular location must not be attributable to any source other than the dye injected for the purpose of conducting the dye trace.

APPENDIX I

Approximate Emission Wavelengths For Fluorescent Dyes

ELUTED CHARCOAL SAMPLES

Tinopal CBS-X	391.7 nm
D&C Green #8	494.5 nm
Fluorescein	516.1 nm
Eosine	540.2 nm
FD&C Red #3	549.8 nm
D&C Red #28	564.2 nm
Rhodamine WT	567.5 nm
Sulphorhodamine B	577.1 nm

WATER SAMPLES

Tinopal CBS-X	395.4 nm
D&C Green #8	491.3 nm
Fluorescein	510.0 nm
Eosine	534.9 nm
FD&C Red #3	546.0 nm
D&C Red #28	556.6 nm
Rhodamine WT	574.7 nm
Sulphorhodamine B	581.9 nm