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The Use of spectrofluorimetry and capillary electrophoresis/laser-induced fluorescence for the detection of fluorescent dyes in groundwater migration studies

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The Use of Spectrofluorimetry and Capillary Electrophoresis/Laser-Induced Fluorescence For The Detection of Fluorescent Dyes in Groundwater Migration Studies

A Thesis submitted in partial satisfaction
of the requirement for the degree of
Bachelor of Arts
in

Environmental Studies
UNIVERSITY OF NEVADA
Las Vegas

by

Patrick Ferguson

Fall 1997

Thesis Advisor: Dr. William Brumley
Research Chemist
U.S. Environmental Protection Agency

ABSTRACT

The Use of Spectrofluorimetry and Capillary Electrophoresis/Laser-Induced Fluorescence for the Determination of Fluorescent Dyes in Groundwater Migration Studies

by

Patrick Ferguson , B.A.

This thesis involves work that was accomplished during a two-year internship at the Environmental Protection Agency's (EPA), Environmental Chemistry Branch. The research is an application of trace level determination of fluorescein dyes used as groundwater tracers. The work was performed to determine whether groundwater could migrate from a Resource Conservation Recovery Act (RCRA) site to an adjacent Superfund site. The research involved using spectrofluorimetry and a new technique called capillary electrophoresis/laser induced fluorescence (CE/LIF) to determine tracer dyes that were injected at the RCRA site and monitored at the Superfund site. Results from spectrofluorimetry were compared to those of CE/LIF. CE provides required specificity because it is a high resolution separation technique that depends on ion mobility under free zone electrophoresis. LIF provides a sensitive detection technique for the capillary format of the separation. This study revealed fluorescein and tinopal at low parts per trillion (ppt) levels in the extracts taken from detector pads placed in the monitoring wells.

ACKNOWLEDGEMENTS

I could not have accomplished this thesis without the help of several people. I would like to first thank Paul Richitt. He got me the internship at the EPA and his help and guidance through the years has made this all possible. I would also like to thank Bill Brumley who has gone beyond anything I expected in helping, teaching, and giving me recognition in such a generous fashion.

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Introduction

Today, the cleanup of industrial properties is a complex and expensive undertaking. Several problems are encountered in these remediation projects. These include site characterization, sampling strategies, analytical protocols, and monitoring of the migration of pollutants through existing aquifers.

In this thesis, a new technique called capillary electrophoresis/laser induced fluorescence detection (CE/LIF) is used to monitor groundwater migration for the first time. CE/LIF is a high efficiency separation technique coupled to a high sensitivity detection technique.

The overall problem was one of determining whether groundwater migrated from a Resource Conservation and Recovery Act (RCRA) remediation site to an adjacent Superfund cleanup site. This possible migration could have the potential to carry pollutants from one site to the other. The monitoring approach is based on the injection of fluorescent dyes at injection wells, followed by sampling groundwater at monitoring wells. Usually, dye concentrations are determined by spectrofluorimetry. In this work, we compare spectrofluorimetry with the results from CE/LIF detection regarding specificity, detection limits, and background levels of fluorescent signals.

The hypothesis to be investigated is that groundwater migrates from the RCRA site to the Superfund site. This hypothesis will be tested by determining whether injected fluorescent dyes migrate from one site to the other.

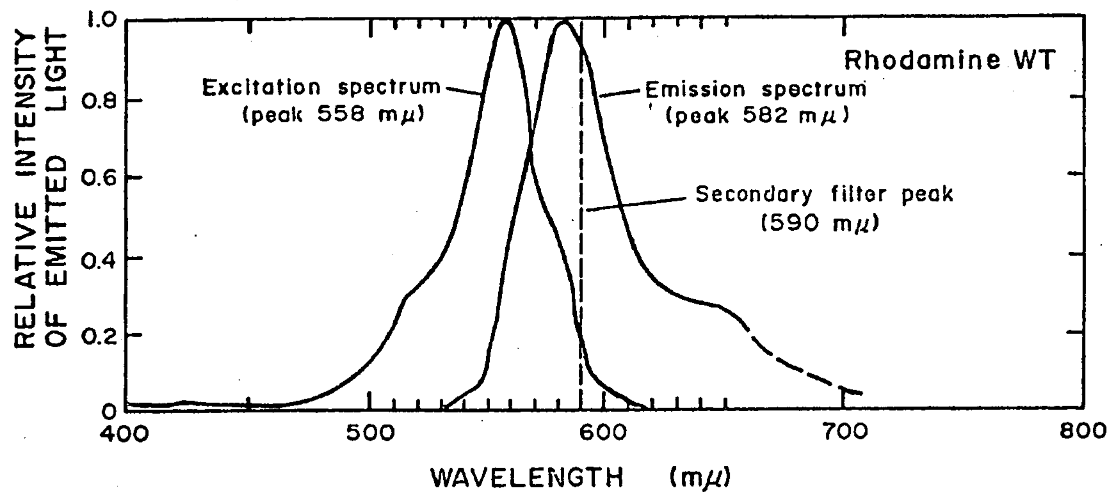
Background

Review of the relevant literature

Groundwater tracers.

Groundwater tracers are a useful and well-used method of determining the

Figure 1. Excitation and emission characteristics. (Reprinted from Davis et al, 1985)



Excitation and emission characteristics of rhodamine WT, a fluorescent dye commonly used as a tracer (source, Wilson, 1968).

movement of groundwater.(Davis, Campbell, Bentley, Flynn, 1985). Tracers can determine waterflow direction, velocity and dispersivity. Tracers can be ions, solid particles (yeast, bacteria, spores, ect.), organic acids, dyes or radioactive substances (Davis et al, 1985).

Various dyes have been used for surface water and ground-water tracing since

the 1800's. Fluorescent dyes are often preferred for use in groundwater studies (Davis et al, 1985) because of several advantages including high detectability, rapid field analysis, low cost and relatively low toxicity (Figure 1). There are, however, some disadvantages to using fluorescent dyes in groundwater studies. Certain environmental factors such as suspended sediment load, temperature, pH or salinity affects the reading of the amount of the dye in a sample. There are also problems of adsorption, photochemical decay, and reabsorbion of emitted fluorescent light (Davis et al, 1985).

In this study, green, orange, and ultra-violet (UV) fluorescent dyes were used as the tracers. Table 1 contains a list of several common dyes, including the green and orange dyes used in the present study. The generic names, alternative names, and the fluorescent properties are also included.

Table 1. Tracer dye characteristics (reprinted from Davis et al , 1985)

Generic and Alternative Names
And Chemical Structure of the Tracer Dyes

Name	Alternative Names	Maximum Excitation, nm	Maximum Emission, nm	Primary Filter	Mercury Line, nm	Secondary Filter
Blue Fluorescent Dyes						
Amino G acid	7-amino 1,3 napthalene disulphonic acid	355 (310)	445	7-37*	365	98**
Photine CU		345	435(455)			
Green Fluorescent Dyes						
Fluorescein	Fluorescein LT Uranine Sodium fluorescein	490	520	98**	436	55**
Lissamine FF	Lissamine yellow FF Brilliant sulpho flavine FF Brilliant acid yellow 8G	420	515			
Pyranine	Pyranine Conc. D & C green 8	455(405)	515			
Orange Fluorescent Dyes						
Rhodamine B		555	580	2x1-60*+61**	546	4-97+3-66*
Rhodamine WT		555	580			
Sulfo rhodamine B	Pontacyl brilliant pink B Lissamine red 4B Kiton rhodamine B Acid rhodamine B	565	590			

Figures in parentheses refer to secondary maxima. For all spectra, pH is 7.0.
* Corning filter.
** Kodak Wratten filter.

Source: Smart and Laidlaw (1977)

Spectrofluorimetry

Spectrofluorimetry is one of the oldest and most established analytical techniques. It was first observed by Monardes in 1565 from an extract of Ligram nephiticiem. Fluorescence, along with other methods, provide some of the most sensitive and selective means of chemical analysis in science today (Guilbault, 1973).

The fluoroscope was invented in France in 1901 by M. Trillat and perfected by M. Marboutin. The theory of fluorescence states that energy is absorbed by the molecule from an excitation source (eg. sunlight or an ultraviolet lamp), and an electronic transition to a higher, energy state takes place (Guilbault, 1973). The molecule then relaxes from the highest to the lowest vibrational energy of that electronic state, losing energy in the process. Next, if the excess energy does not dissipate by collisions with other molecules, the electrons will return to the ground state with an emission of light called fluorescence (Davis et al. , 1985).

Fluorescence that is normally observed in solutions is called Stokes fluorescence. Stokes fluorescence is the remission of lower energy photons, which have a longer wavelength or lower frequency, than the absorbed photons. Every molecule possesses a characteristic property described by a number called the quantum yield (Φ). The higher the values of the quantum yield, the greater the fluorescence of a compound (Guilbault, 1973).

Fluorescence instruments contain four principal components: a source of excitation energy; a sample cuvette; a detector to measure the photoluminescence; a pair of filters or monochromators for selecting the excitation and emission wavelengths

(Davis et al, 1985).

According to Davis et al, 1985 measurements for fluorescence are usually made by reference to some chosen standard. The standard is placed in the instrument and the circuit is balanced with the reading scale set at any reading. Then, without changing any circuit components, the standard is replaced by solutions of the material being tested, and the fluorescence of each sample recorded. Finally, the fluorescence of the solvent is measured to establish the zero or background concentration.

This method of detection provides the ability to quickly, easily, and accurately detect selected materials in trace amounts. For example, in Grange et al, 1996, a comparison was done contrasting spectrofluorimetry to CE/LIF. It was found that the spectrofluorimetric analysis could be completed accurately and quickly. In addition, the instrument was easy to use and a technician without prior experience could easily operate the equipment.

The ability to scan for multiple compounds reduces analysis time considerably, without a loss of quality. This is particularly useful when large numbers of samples must be analyzed and time is limited (Grange et al, 1996).

Capillary electrophoresis/laser induced fluorescence (CE/LIF)

Electrophoresis is the process of separating charged molecules based on their movement through a fluid under the influence of an electric field. The velocity of an ion can be given by $\mathbf{v} = \mu_e \mathbf{E}$, where $\mathbf{v}$ = ion velocity, μ_e = electrophoretic mobility, and $\mathbf{E}$ = applied electric field. The electric field is a function of the applied voltage and capillary length. The mobility, for a given ion and medium, is a constant which is characteristic of that ion. The mobility is determined by the electric force that the molecule experiences, balanced by its frictional drag through the medium (Heiger,1992). Electrophoresis and its related instrumentation have been developing over the last century. The first methods involved the use of a gel-medium. The gels provided physical support and mechanical stability. However, there were some problems associated with the use of the gels. The heat which occurred in the process induced convective movement of the electrolyte. This resulted in band broading that reduced the effectiveness of the separation. Also, gels had to be used that were viscous enough to provide physical support. Low-viscosity gels would flow if the apparatus was not held level. Later developments, like the arrival of high-performance capillary electrophoresis (HPCE),

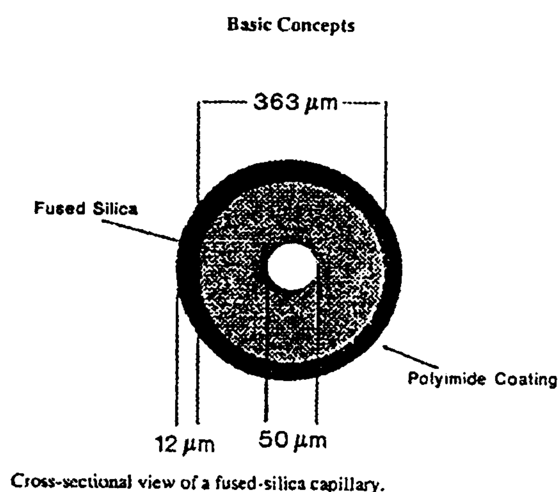


Figure 2 Capillary cutaway

(Reprinted from Weinberger, 1993)

solved many problems encountered with gels. (Weinberger, 1993).

Capillary electrophoresis is the process of conducting electrophoresis in a narrow diameter capillary column allowing efficient heat dissipation and permitting the use of high voltage to drive the separation. Fused-silica, polyimide-coated capillaries are the materials of choice for HPCE (See Figure 2). Capillaries are commercially available that range in size from 200 to 700 micrometers, with internal diameters ranging from 25 to 100 micrometers. Fused-silica is a good material because of its UV transparency and durability (Weinberger 1993). Instruments used today integrate the capillary column by either direct insertion into the unit or by using a cartridge holder (See table 2)

Table 2. HPCE Instumentation examples. (Reprinted from Weinberger, 1993)

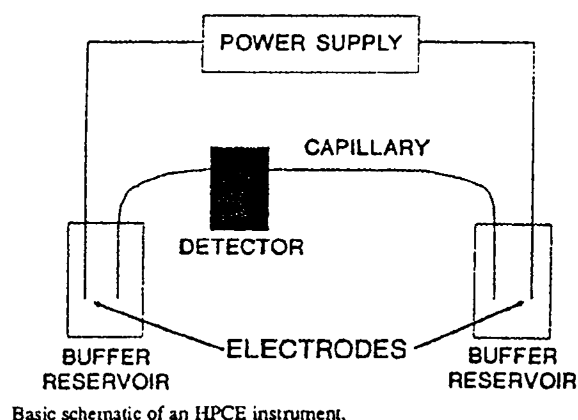
Instrumentation for HPCE

Instrument	Capillary	Detector UV/F	Sampler Positions	Cooling Capillary/ Sampler	Injection
Beckman P/ACE 2000	cartridge	filter/LIF	24	liquid[a]/none	P, E
Dionex CES 1	open	variable/variable	39	air[b]/none	P, E, G
ABI 270HT	open	variable/none	50	air/yes[a]	V, E
Isco 3140	open	variable/none	40	air[a]/none	V, E
Bio-Rad BioFocus 3000	cartridge	scanning/none	32	liquid[a]/yes[a]	P, E
Waters Quanta 4000	open	fixed/none	20	air[b]/none	G, E
Europhor IRIS 2000	cartridge	none/LIF	24	air[b]/none	P, G
SpectraPhysics 2000	cartridge	scanning/none	80	air[a]/none	V, E
Hewlett Packard HP^{3D}CE	cartridge	PDA[c]/none	48	air[a]/none	P, E

[a]Sub-ambient temperature operation included.
[b]Ambient temperature operation only.
[c]Photodiode array.
P = pressure; V = vacuum; E = electrokinetic; G = gravity.

In using the capillary, the required length needs to be determined. If you want to minimize thermal effects in the column, then you decrease the diameter and increase the length. Efficiency is constant at long lengths, >80 micrometers, but falls rapidly as the column length is shortened. This doesn't mean you can't operate with columns >80 micrometers, but that you must either reduce the voltage or decrease the ionic strength of the carrier electrolyte (Camilleri, 1993).

A carrier electrolyte is required for electrophoresis, this is also known as the running buffer. This solution maintains the required pH and provides the conductivity necessary for proper or effective separations. Often different materials, known as additives, are added to these buffers to adjust the selectivity of the separation. They can interact with a solution and modify its rate of movement or its electrophoretic migration (Weinberger, 1993).



Basic schematic of an HPCE instrument.

Figure 3. Schematic drawing of HPCE (Reprinted from Grange et al, 1996)

HPCE represents the merging of technologies derived from electrophoresis and high-performance liquid chromatography (HPLC) (Weinberger, 1993). Figure 3 shows a schematic drawing of an HPCE instrument. HPCE instrumentation for fluorescence detection, combined with the use of laser, can improve the limits of detection by several orders of magnitude compared to absorbance detection (Grange et al, 1996).

The high photon flux and focusing capability of laser light provide excellent properties for a fluorescence excitation source. These designs can be relatively simple and include state of the art technologies such as fiber optics. Selecting appropriate emission wavelengths avoids background interferences from scattering.

Site History

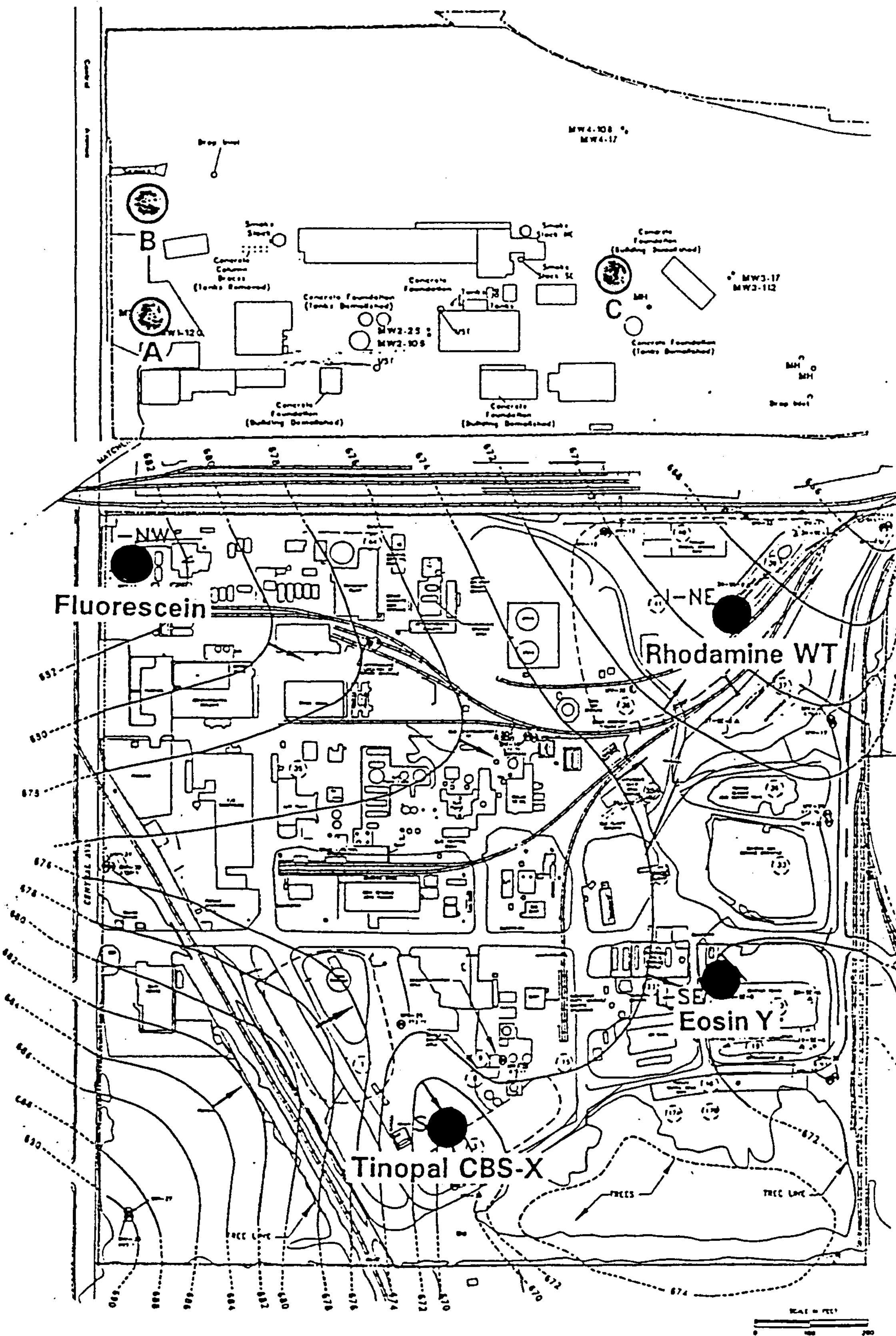
Past activities and pollution problems at both sites.

The two study sites investigated were found in an urban industrial and residential area of south Chattanooga, Tennessee. The first area is a Superfund site, and will be referred to as the Tennessee Products or TP. site. The second site falls under the Resource Conservation and Recovery Act (RCRA) and will be referred to as the RCRA site for the purpose of this study.

The Tennessee Products Site was placed on the National Priorities List, effective October 30, 1995, as reported in the Federal Register on September 29, 1995. Any contamination release associated with the RCRA site's presence could result in persons or companies being named a Potentially Responsible Party for the TP. site. Because of this, background information on the RCRA site is limited to a brief description.

Superfund Site (TP.)

This location had several coke plants occupying the property from 1918 until 1987 (Coke is a residue of carbonized coal used as a fuel in domestic and blast furnaces). From 1926 until 1964 it was operated by the Tennessee Products Company



Map of RCRA site (bottom) and adjacent Superfund site (top).

who owned the property for 38 of its 69-year operating history.

The TP site contains four distinct sources of contamination : a coke production plant, Hamill Road Dump #2, The Chattanooga Creek Tar Deposit Area, and approximately 2.5 miles of the Chattanooga Creek bed itself.

At the coke production plant most of the large piles of coke and coal have been removed, but two piles located in the eastern part of the site still remain. These piles are overgrown with vegetation. One noticeable feature of this site is the surface layer of thick coke dust that covers most of the site. The depth of this dust is not known. The only areas not covered with coke dust are building foundations and gravel roads where vehicles drive.

Most of the railroad tracks (in both areas) appear to have been removed; however, some may have been buried. There are also scattered piles of yard debris, such as timbers, located over the area.

The Hamill Road Dump #2 is a small area (less than one acre) located in the floodplain of the creek which contains large quantities of coal tar. The most notable features of this area are the mounds of coal tar waste (a black sludge) which constitute an area of about 1500 square feet. The depth of this waste is not known. There is an access road located within 500 feet of the site that leads to the bank of the creek. There is no fence around the dump.

The Chattanooga Creek Tar Deposit Area is also in the floodplain of the Chattanooga Creek. The deposit is approximately 3 feet in depth, covers an area approximately 30 feet by 40 feet wide, is covered by trees and light brush, and is very

unstable to stand on. A thin layer of soil and dried mud covers the deposit, which when disturbed looks like tar. If undisturbed, the area has an appearance that matches the surrounding area. A fence presently surrounds the tar deposit.

The 2.5 mile stretch of creek bed that is contaminated flows through some industrial areas and urban developments. It is barricaded by many fallen trees and sewage pipes which impede stream flow. Household litter has collected in the stream's pools. Oily sheens on top of the water have been observed. Heavy debris including metal structures, industrial containers, drums, cars, and animal carcasses are in the stream and along both banks.

Two types of visible tar deposits have been identified in the creek. The first type is stream sediment that is heavily contaminated with coal tar. It stretches for at least 11,900 feet. The second type exists primarily as large mounds of coal tar in the creek bed. Some of these deposits are estimated to be six to eight feet in depth. These deposits overlap the contaminated stream sediments by some 1800 feet (Law Environment Inc., 1994).

<u>RCRA Site</u>

The Velsicol Corporation owned this adjacent property and produced chemicals such as chlorinated toluene, benzonitrile, benzoic acid, benzyl alcohol, benzyl chloride, methyl 3-chlorobenzoate, benzaldehyde, benzoguanamine, lindane, dicamba, and benzoate esters. This area also contains recovered hydrochloric acid and has been used to produce coal tar products. Leaking drums containing unidentified liquids were

reportedly removed in 1975 (Donnelly,1996).

This entire site is located in a low lying area bordering the Chattanooga Creek floodplain. Two known underground storage tanks and the associated pipelines and conduits are still in place. One of the storage tanks is about half full, with two feet of water and two feet of gasoline The other tank is full of water, soil, coke and debris.

Site description

Land and water use.

Mixed in with the industrial facility are several public housing projects and private homes. Many schools and recreation centers exist in the area. There is a school approximately 0.2 miles east of the coke plant area. A second school is located about 0.75 miles northeast of this site. Additionally, there are four more schools, all within a two-mile radius.

Private drinking wells are not known to exist within a four-mile radius. The Tennessee-American Water Company supplies drinking water for the area. The water intake is located on the Tennessee River, which is four miles upstream. Groundwater is not known to be used for irrigation or livestock watering in this area. There are no known surface drinking water sources downstream from the immediate area. The closest downstream public water withdrawal intake is in South Pittsburgh, Tennessee, which is 30 miles away.

Although posted with warning signs, Chattanooga Creek is used for swimming, recreation, and fishing by both children and adults. Consumption of the fish caught from

the creek has been reported.

Surface water runoff is routed through a series of drainage ditches and storm drains to the city sewer system. During heavy rains the sewer system becomes overloaded and untreated water gets discharged into the adjacent tributaries. There are several springs and streams that feed into the creek.

Most of the Velsicol site and the adjacent area are underlain by hundreds of feet of gently dipping Paleozoic Era limestones that strike north to about 10 degrees west of north. The site is not on a well-developed karst terrain and passage through the aquifer is believed to be through small passages (Law Environment Inc, 1994).

Materials and Methods

Experimental design

Sampling.

Streamflow data was essential in solving the ground-water issue since seepage to or from streams is a major element of discharge or recharge of ground water (U.S. Department of the Interior, 1977). The flow rates in this area varied from 3 gallons per minute under normal conditions to 9 gallons per minute during rainfall events. (Law Environmental Inc, 1994).

Fluorescent dyes were injected in points 1-NW, 1-NE, 1-S, 1-SE at the RCRA site and monitored at locations A, B, C at the Superfund site (see map on page 10). Each of the dye injection points consisted of a 6-inch well drilled to bedrock. Four-inch PVC casing with several feet of slotted screen on the bottom was set on top of the bedrock and backfilled with sand and soil (Law Environmental Inc., 1994). The purpose of the screen was to stabilize the sides of the hole, to keep sand out of the well, and to ease flow into and within the well (U.S. Department of Interior, 1977). A 3 7/8-inch hole was drilled inside the PVC casing and 3 feet into the bedrock. One to four such wells were drilled at each of four dye injection sites. Each well was then evaluated by performing a percolation test. The desired rate was at least 4 gallons per minute. The well at each site that drained the closest to the desired rate was the one selected for dye injection (Law Environmental Inc., 1994). Once this was done, the actual injection process could be started.

The PVC casing with the slotted screening at the bottom was inserted into each borehole. Dye and water was then injected into the PVC casing. The dyes included: Eosine (Acid Red 87), Tinopal CBS-X (Fluorescent Whitening Agent 351), Rhodamine WT. (Acid Red 388), and Acid Yellow 73 (Fluorescein). There were two injections of these dyes using conventional methods employed for tracing ground water in karst aquifers.

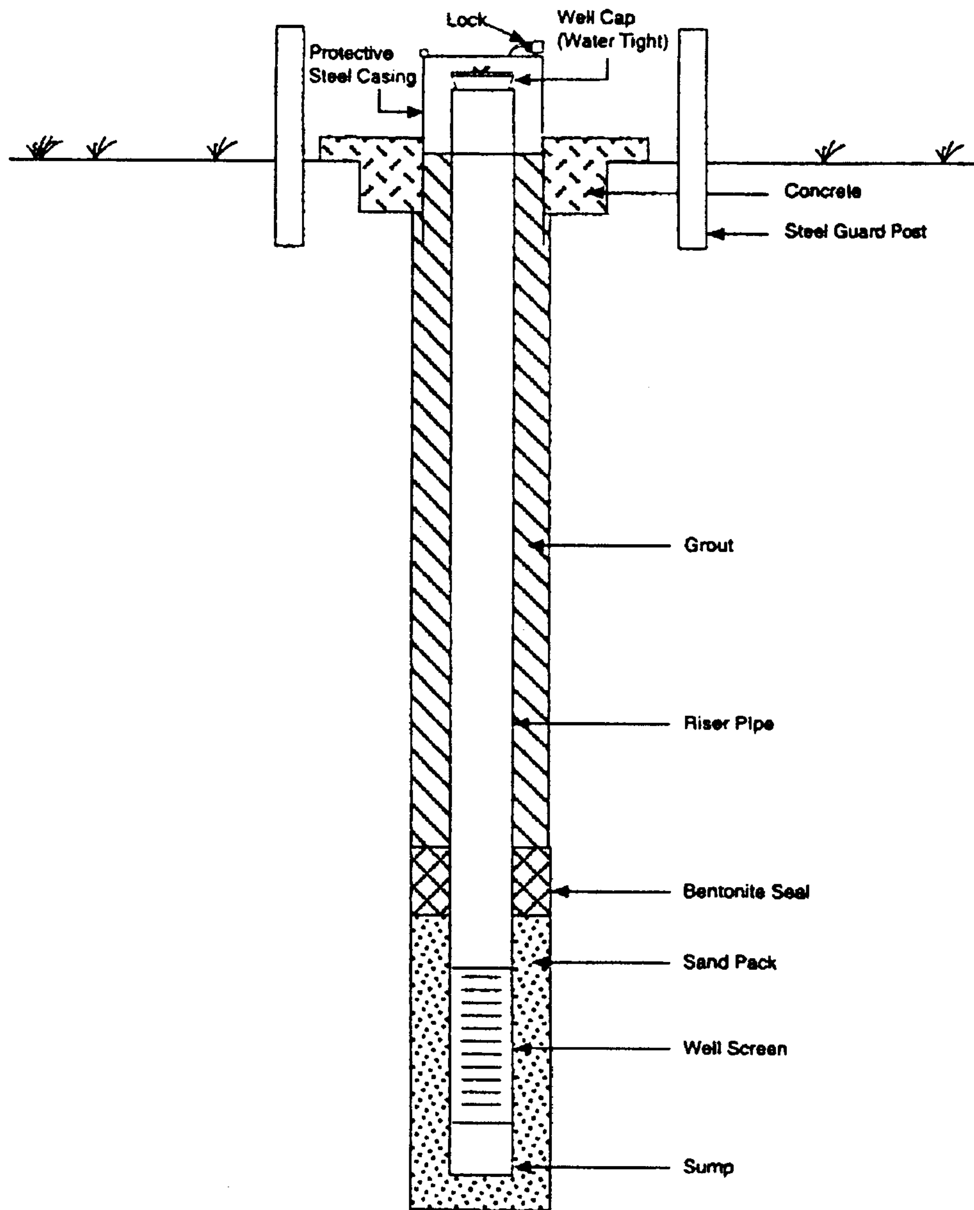


Figure 5. Well diagram (Reprinted from Dept. Interior, 1977)

Background concentrations were first determined 1 week before injection. Background monitoring was done for 3 consecutive one week periods, to include at least one major rainfall. A major rainfall was defined as rainfall > 0.2 inches. These samples were taken at springs, streams and wells down gradient (Law Environmental Inc, 1994).

The first injection consisted of 20 lbs. of Eosine (Acid Red 87) at location 1-SE; 30 lbs. of an optical brightener (Tinopal CBS- X, Fluorescent Whitening Agent 351) at location 1-S. The second injection was done 14 days after the first. It consisted of 18 lbs. of Rhodamine WT. (Acid Red 388) at location 1-NE; 10 lbs. of Fluorescein (Acid Yellow 73) at location 1-NW. The dyes were injected using the following steps:

1. Approximately 800 liters of potable " Primer" water was poured into the hole.
2. Next, the concentrated dye solution (approximately 10mM for each dye) was added.
3. Finally, about 8,000 liters of potable " chaser " water was added.

The day of the initial injection was designated Day 0. The day of the second injection event was designated Day 14. Sampling was done on days 0, 3, 7, 14, 21, 28 and every two weeks thereafter until the tracer sampling was terminated. Termination of sampling was a mutual agreement between Velsicol, the EPA, and the Tennessee Department of Environmental Compliance.

Detection.

Detection was accomplished by recovering the dyes on activated coconut charcoal and cotton dye detectors, both of which were placed in a small packet constructed from fiberglass screening. The cotton is used to detect and accumulate the

optical brightener. Activated charcoal is used to detect and accumulate the fluorescent dyes. Packages were rinsed with the well water to remove as much mud as possible. A water sample was also collected at the monitoring well sites whenever a dye detector was changed or was found to be missing. The water samples were collected:

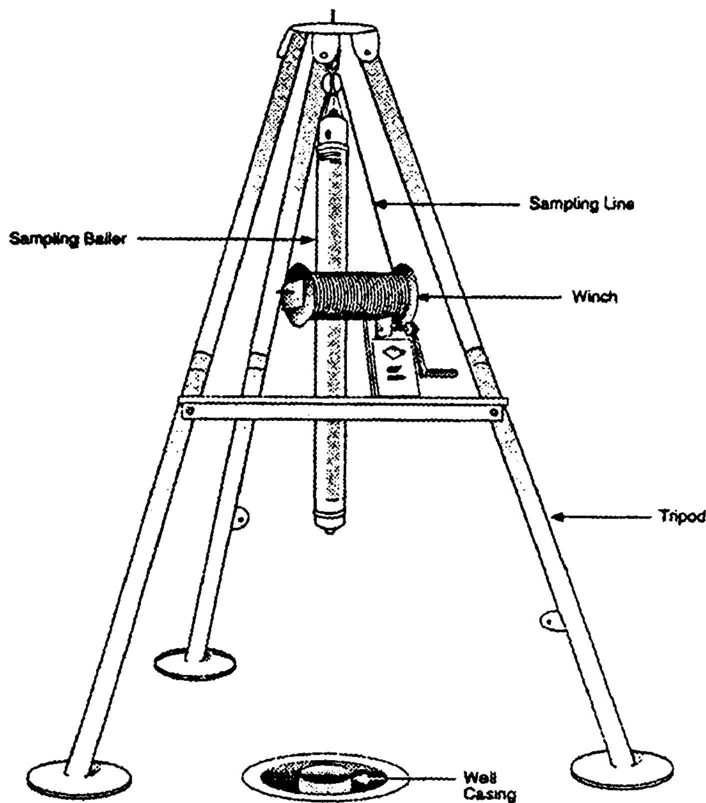
1. to establish the representiveness of the dye concentration measured on the detectors.
2. to check for effects from any potential tampering or vandalism.

All water samples were kept in a locked refrigerator in a locked laboratory to which no one but laboratory personnel have access to (Law Environmental Inc, 1994).

Detector packets and labeled water sample vials were then transported and stored in plastic Zip-lock Bags. These bags were labeled with the sample site identification number, site name, date of sample and the initials of the individual taking the sample (Law Environment Inc, 1994) . Additionally, this information was recorded in the field logbooks which were used to maintain records of all samples taken during this operation (Byrnes, 1994).

Pads.

As previously described the dyes were recovered on two recovery/sampling pads. The first pad is activated coconut charcoal. This is used to accumulate the fluorescent dyes. The second pad consists of cotton. This one is used to accumulate the optical brightener. Both pads are then placed into a final container or pad made of fiberglass



screening. The dye detectors were in monitoring wells A, B, C. The pads in the monitoring wells were suspended down into the well far enough to insure that as much water as possible flowed past it. Fishing line was used to suspend the pads (see Figure 6). Final pad rinsing using normal tap water was done at the laboratory.

Figure 6. Tripod (reprinted from Dept. Interior, 1977)

Analysis for the optical brightener was done by examining the washed cotton detectors. This was accomplished using a long wave ultraviolet lamp.(Note: this was done on site.) The charcoal detectors were analyzed for tinopal and visible fluorescent dyes by air drying in a fan dryer for 24 hours and extracting with a "smart" solution which is described below (Law Environment Inc, 1994).

Smart solutions.

After air drying for 24 hours, one gram of charcoal is removed from the detector pad. This one gram charcoal sample was placed into a 15mL screw top glass bottle, and eluted for 60 minutes with 10 ml solution of 1-propanol, de-ionized distilled water and

ammonium hydroxide mixed at a ratio of 5:3:2. This is called the smart solution. A 3 ml sample was then drawn using a disposable pipette and placed into a disposable rectangular cuvette. The sample was then analyzed by synchronous scan analysis using the spectrofluorimeter.

Spectrofluorimetry.

The analysis was done using a Jobin-Yvon Spex Flurolog II spectrofluorimeter. The following describes the method used in the Las Vegas laboratory:

1. The single scan setting was chosen starting at 350 nanometers (nm) and ending at 600nm. The increment was set at 0.5 nm, integration time was 0.5 seconds, emission mono offset was 15nm and the scan units were nanometers. 5 millimeter (mm) slits were used between the source monochtomator box and the sample holder box and an emission monochromator box.

2. A water or smart solution blank was analyzed daily. That sample was numbered and used as the Blank Subtraction File (BSF) setting. The BSF was used to subtract any background readings.

3. All samples were given the file designation TP. The number immediately following signified the number of the run. This designation was used to identify samples analyzed in the lab. These numbers were matched up to the sample numbers used by the field personnel during the actual process at the site.

4. Polyacrylate disposable cuvettes with disposable polyethylene caps were used to reduce interferences. The liquid was added until it covered the optical window area

of the cell holder in the instrument. Commonly, the cuvette was not all the way down in the holder. This helped to reduce the possibility of spilling the samples into the instrument while handling them.

5. The samples and all chemicals used in this work were stored out of light and kept refrigerated at 50 degrees F. All samples were analyzed within a four-week period. Since there was some degragration of the chemicals if left in the light unrefrigerated, this procedure prevented any degradation of these compounds.

6. The fluorescent brightener, Tinopal CBS-X was measured in the same analysis as the other three dyes. We did not analyze cotton pads with the " long wavelength UV lamp." Instead of measuring the Tinopal in this material, the smart solution extract of the charcoal was used.

7. Two types of method blanks were prepared for each batch of samples: one used distilled, deionized water to mimic the water sampling procedures; the other used muffle-furnace dried clean sand and smart solution to mimic the receptor sampling procedure. All blanks were found to be free of any dye contamination.

8. The charcoal and cotton receptors had the following physical characteristics when they were received: several had a dark discoloration; #17 had a noticeable odor. Others had some orange mud on the packages while the majority were reasonably clean.

9. Approximate detection limits for the four dyes were: Tinopal CBS-X, 20 parts per trillion (ppt.); Acid Yellow 73, 3ppt; Eosine and Rhodamine WT, 10ppt. We found that we could improve the detection limit for Tinopal to 10 ppt. if the Stoke shift was set at 25 nm. However, the background noise level in field samples doubled with

the 25 nm shift when compared to the original setting of 15nm. Because of this the 15 nm Stoke shift was used.

10. Because of software constraints, spectral integrations were not done on the Spex instrument. Capillary electrophoresis (CE) was used to confirm positive samples. Relative intensities were used to estimate concentrations with the Spex. It was felt that such estimations were adequate for reporting samples semiquantitatively, such as positive, very positive, ect.

11. We conducted CE/LIF confirmatory analysis on five samples that were found positive with the Spex instrument. This is explained in the next section.

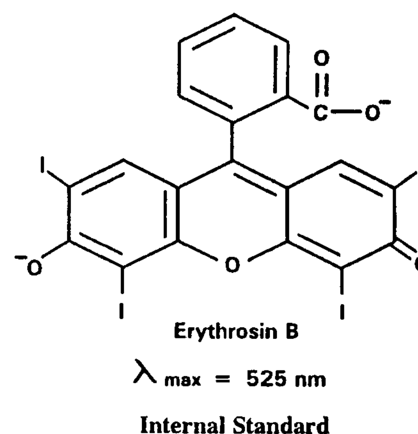


Figure 7. Internal Standard (Reprinted from Grange et al, 1996)

CE/LIF.

Capillary electrophoresis was conducted using Beckman P/ACE model 5000 equipped with the induced fluorescence (LIF) option. We used an argon ion laser with excitation at 488 nm and emission at 520 nm.

The capillary used was a fused-silica capillary manufactured by Polymicro Technologies, Phoenix, AZ, USA. It was 57 centimeters (cm) long, had a 75 micrometer (um) inside diameter (i.d.). There was a 50 cm distance to the detector window.

Temperature of the capillary was 25 degrees C. Runs were approximately 10 minutes long, at 25 kV using a running buffer of 50 mM sodium tetraborate with a pH

of 9.2.

The capillary was prepared at the start of each day by running a method called LIFRINS. This was a 15 minute wash. It consisted of a ten minute rinse with 0.1m of sodium hydroxide followed by a five minute rinse with de-ionized water. At the end of the day, the capillary was rinsed again using a method called ENDS. This was a two minute rinse with 0.01 m of sodium hydroxide. By following this procedure we extended the life of the capillary somewhat and the replacement of capillary cartridges was kept to a minimum.

Migration times, peak widths, and detection limits were calculated either directly from the monitor screen or from printouts of the data system.

We performed confirmatory analysis by CE/LIF on five samples that indicated the presence of dye. These samples were analyzed using the following methodology:

1. An internal standard which consisted of Erythrosin B at 10^{-5} Molar (M) in water, was prepared (see Figure 7). Initial runs were made to determine the migration time of Fluorescein. Erythrosin B migrated just before Fluorescein. This internal standard was used throughout the remainder of the work. Both of these chemicals migrated as anions.

2. A 5 mL vial was used for samples. The experiments were set up identically for each run. Each runs set up consisted of a lab blank, smart blank, internal standard, the water samples, followed by another internal standard/fluorescein standard.

3. Lab blanks consisted of 3 mL of de-ionized water, 1 mL of the internal standard, and 1 mL of the buffer. Again, this was the first vial placed in the instrument's

sample tray.

4. Smart blanks were received previously prepared from the contractor. This was the second vial in the instrument's sample tray holder.

5. The internal standard/fluorescein standard sample was made up of fluorescein at a concentration of 10^{-7}M and Erythrosin B at 10^{-5}M. 1 mL of each was placed into a vial along with 1 mL of the running buffer. The remainder, approximately 2 mL, consisted of de-ionized water. These samples were next in the instrument tray.

6. The last sample in the run was another internal standard. We injected samples for five seconds using the BILLFL10 method.

Printed copies of all runs were made and kept. In addition, all the files containing this experimental information were periodically downloaded onto computer disks, and maintained in a file.

Instruments Used

Spectrofluorimetry.

All samples were analyzed using a Spex Flurolog-2 series spectrofluorimeter, utilizing the Spex DM3000F spectroscopy computer software (version 3.2). The computer was a 486 DX2 that consisted of a color monitor, a main unit containing a hard drive, a 5.25 inch floppy disk drive, several Spex proprietary boards and a keyboard. A Hewlett-Packard Laser Jet II printer was used. The 486 DX2 computer uses Microsoft Disk Operating System (MS-DOS) version 3.2 This instrument was manufactured by SPEX Industries, Inc, Edison, NJ, USA.

CE/LIF.

For this work the P/ACE System 5000 was utilized. This is a high performance capillary electrophoresis (HPCE) instrument. The main components of this system include an autosampler (a rotating tray) that holds the vials of samples, a cartridge holder and interface, a high voltage power supply and electrodes, an optics module and detector, temperature control hardware, a sample injection mechanism, and a controller with front panel displays and various function switches. Input pressure to the instrument is provided by at least 80 psi of high purity grade nitrogen.

We used an IBM Personal System/2 Model 55SX with a Panasonic KX-P1091i printer equipped with Beckman System Gold software (version 8.1), Fullerton, CA, USA.

Chemicals used

All of the organic compounds were obtained from Aldrich Chemical Company, Milwaukee, WI, USA. Other chemicals were from standard sources of supply, and were used as we received them from the manufacturer. De-ionized water (ASTM Type II) was used in all aqueous solutions. Buffer solutions were prepared at least once a week. Solutions of the dye standards were prepared from solids and were diluted with de-ionized water. We followed all applicable safety precautions and proper storage and housekeeping in accordance with all local, state and federal directives.

Method of data analysis.

Spectrofluorimetry

A water or smart blank was analyzed first each day. (see Figure 8). This was used to subtract the normal

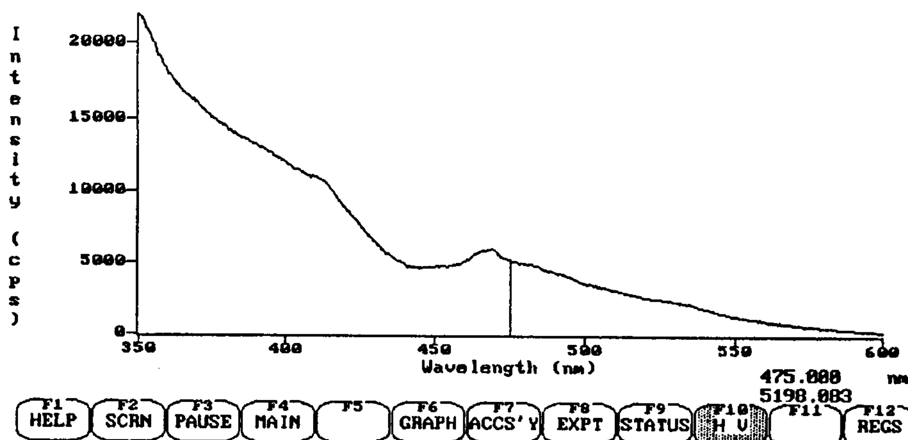


Figure 8. Water Blank

background readings. Mixed standards were analyzed at a concentration above the detection limit of (20-30ppt.). Dye standards were prepared using the activity figures supplied by the manufacturer for each particular lot number. We recorded

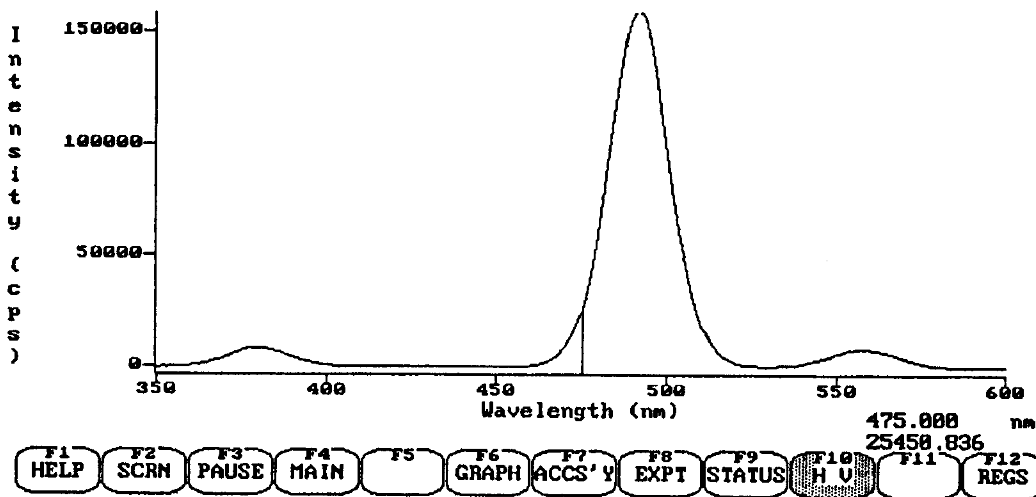


Figure 9. Tinapol, Rhodamine, Acid Yellow (try) Standard

the lambda max and intensity of each standard. Verification that the dyes were readily detected also

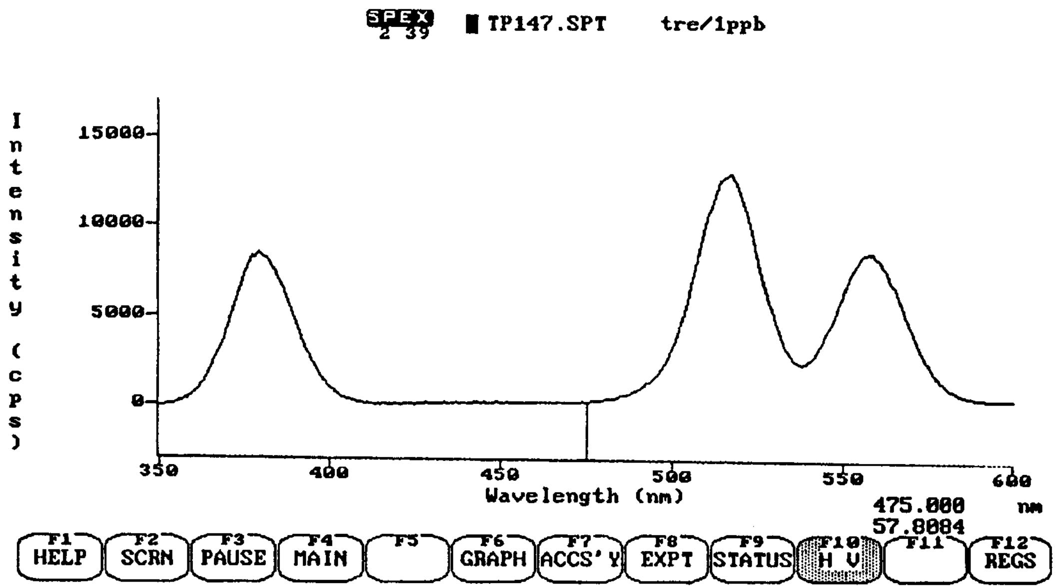


Figure 10. Tinopal,Rhodamine, Eosine (tre) Standard

insured that the instrument was working properly. This procedure was done each day prior to running samples (see Figures 9 and 10 for examples of the standards run.).

The lambda max differed for the two solvents. The differences for smart vs. water were small and are listed below:

approximately zero for Tinapol CBS-X

7 nm for Rhodamine W

9.5 nm for Acid Yellow 73

7.5 nm for Eosine

The lambda max values in water in earlier experiments were:

Tinapol, 379 nm

Acid Yellow 498 nm

Eosine, 516 nm

Rhodamine WT, 558 nm.

The values in freshly prepared smart solution were:

Tinopal, 379 nm

Acid Yellow, 498.5 nm

Eosine, 523.5 nm

Rhodamine WT, 551 nm

We reported no results for any sample having less than three times the

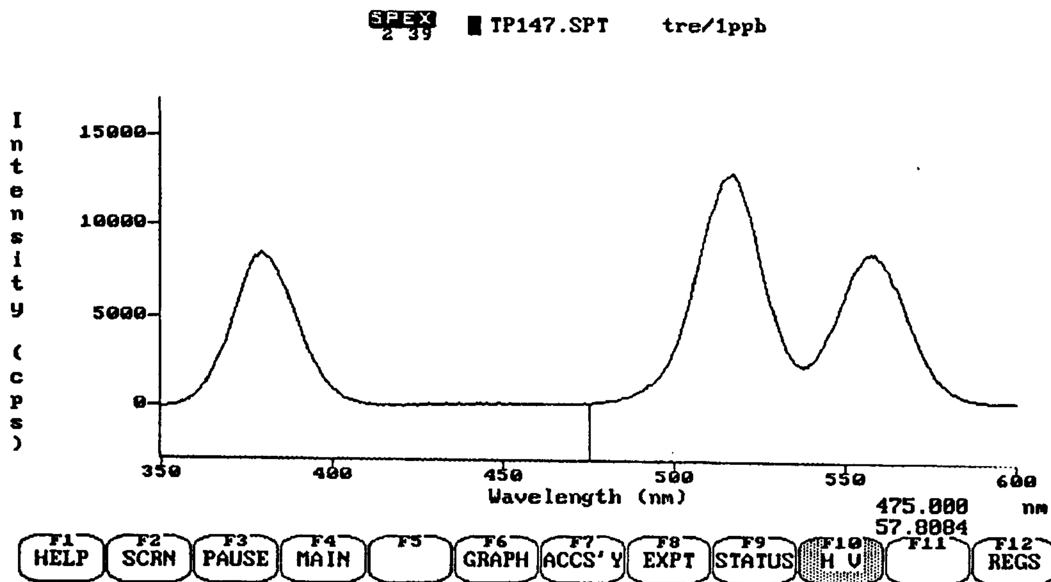
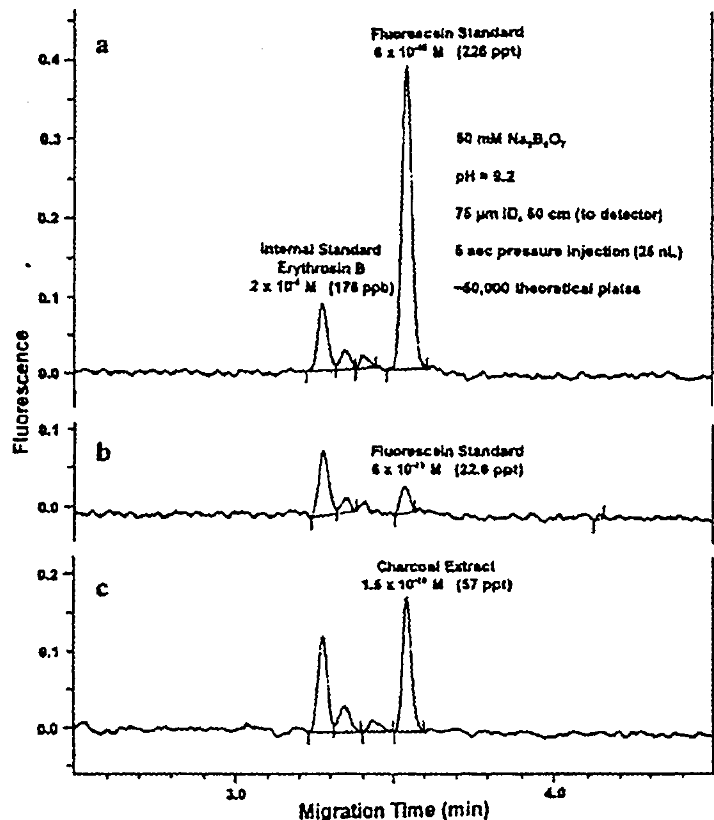


Figure 11. Example of synchronous scan for comparison

concentration of the highest or appropriate background dye receptor. For a sample to be considered positive, it would have to have a fluorescent intensity more than three times the highest background. At least three positives were needed to confirm the presence of the dye. Also, the shape of the synchronous scanning curve must match that of the particular dye (see Figure 11). Confirmatory analysis by capillary electrophoresis was done on all positive results.

CE/LIF.

Fluid levels in all buffer, water, sodium hydroxide and waste vials were checked daily. A daily rinse of the capillary was conducted. We ran standards before samples to insure that the instrument was working properly and detection levels were consistent.



Electropherograms: (a) 6×10^{-10} M Fluorescein standard, (b) 6×10^{-11} M Fluorescein standard, and (c) a charcoal extract. Erythrosin B (2×10^{-6} M) was the internal standard.

Figure 12. Electropherogram for standard solution(reprinted from Brumley, 1996)

Figure 12 shows electropherograms for internal standard solutions containing fluorescein and erythrosin B. The two extra peaks are due to impurities in the erythrosin B. Areas are corrected based on electrophoretic mobilities (area x velocity) and normalized to that of an internal standard, calibration curve regression, and calculations of the concentrations of unknowns. All calculations were done using Lotus 1-2-3 (version 5.0) spreadsheet.

Concentrations were determined by using a calibration curve plot of: area of the analyte/area of internal standard versus concentration of analyte/concentration of the internal standard. The linear regression yielded the best values for m and b in the equation $y=mx+b$, with $y=$ ratios of the areas and $x=$ the ratios of the concentrations.

The concentration of unknowns was determined by solving the equation : $x = y-b/m$ and substituting into the equation the known values of: the concentration of the internal standard, the amount of internal standard added, and the total sample volume. The areas of the known and unknown are printed out by the data system.

Since each analyte moves at a different velocity past the detector window in the column, we had to correct for this fact mathematically. This is done by using the formula: corrected area= the raw area X the velocity. This ratio is entered into the spreadsheet and is factored into the overall equations.

As was previously discussed a 10mL SMART solution was obtained from one gram of the charcoal in the pads. Parts per trillion were then determined from this. Calculations to determine parts per trillion were done by using the formula: concentration X molecular weight (of fluorescence) / 1×10^{-9}.

Results and Discussion

Determination of the tracer in the pads using Spectrofluorimetry.

Data was collected and analyzed from 22 sampling events. Background signals were much higher in the groundwater than in the blanks or reagent water. The background was, however, consistent from sample to sample (week to week collection) so we could identify the emergence of the signals due to the injected dyes on the synchronous fluorescence spectra (Donnelly, 1996).

Because the Spex spectrofluorimeter was repaired and reconditioned by a factory technician approximately three weeks after the samples were prepared, we observed some evaporation. Acid Yellow peaks found in these samples (charcoal extracted with smart solution) were observed to have lambda max values between those of the standards in water and those in freshly made smart solution. This result is to be expected because as the n-propanol and ammonia evaporate, the smart solution more closely approximates water (Donnelly, 1996).

Using a plus or minus 5 nm and a ratio of three to one signal to noise ratio, five samples were found to have dyes present. The lambda max values in these samples were the same as standards for Tinopal, and were between those of the standards in water and smart solution with Acid Yellow (fluorescein). These results showed that both the Tinopal CBS-X and Acid Yellow 73 (Fluorescence), that was injected into the wells, had migrated from the injection sites to the monitoring wells located on the Superfund site. The other two dyes were not found in these or any other water samples taken on the site

(Donnelly, 1996).

We detected fluorescein in three charcoal extracts from two monitoring wells. Tinopal was found in two charcoal extracts from one monitoring well. As previously mentioned we observed none of the other dyes in the samples when the packets were replaced (Grange et al, 1996).

Determination of the tracer in the pads using CE/LIF.

The spectrofluorimetic data provides evidence that two of the dyes had migrated through the groundwater from the RCRA site to the Superfund site. Confirmation by another analytical technique was desirable to provide additional evidence. Obviously, these findings were going to be required to withstand the scrutiny of any pending litigation.

Quanitations by CE/LIF agreed well with the concentrations estimated by peak height of synchronous fluorescent responses. Identifications and quantitations were made by comparison of the migration times and fluorescence intensities in comparison to the internal standard, erythrosin B.

Conclusion

Summary of the findings and the relationship to the hypothesis

Groundwater is migrating from the RCRA site into the Superfund site. This is based on the fact that we detected two of the tracer dyes in groundwater samples, specifically, fluorescein and tinopal were found at low parts per trillion (ppt.) Levels in extracts of the charcoal pads.

Since these dyes were found to be present in well water, the next step will be to make a determination of what, if any, pollutants are migrating from the RCRA site into the Superfund area. For many of the contaminants (not all) a full characterization of chemicals at one site needs to be accomplished, and compared to data from a full characterization of the other site. This information will be useful in determining if chemicals have migrated from the RCRA site to the Superfund site. This can be accomplished in exactly the same manner as the dyes. Large numbers of samples can be processed quickly and accurately using spectrofluorimetry. Precise analysis is obtainable using the CE/LIF method.

The use of CE/LIF in this process will be invaluable. Data compiled through this method is quantitative enough to withstand the scrutiny of the litigation process that will follow the discovery of any pollutants that have migrated.

Modeling of the contaminant plume may be necessary to determine the extent of any threat to the downstream aquifers, streams, or rivers. Further water flow and aquifer studies may be required to make these determinations. Knowing the exact size, shape

and range of any possible plumes will aid planners and decision makers in future cleanups.

The model will also show the impact of other factors such as storm events. A large storm event clearly has the potential of transporting pollutant chemical materials off the RCRA site. These pollutants not only have the potential to find their way into new areas but may recontaminate areas already remediated.

This potential movement of waterborne chemical contaminants could potentially impact the health of people residing in the area. In particular, the close proximity of the school to these cleanup sites may present a problem. It should be determined if it is possible for these materials to flow into playgrounds after major storm events. The concentrations of these chemicals may or may not be an issue, however, this needs to be assessed. Additional analysis of fish found in the contaminated waters should be done to determine possible health risks to consumers. Bioaccumulation of hydrophobic analytes could lead to orders of magnitude increase in concentrations of some contaminants relative to that for water.

Since it has been determined that groundwater does migrate from the RCRA site to the Superfund site the potential for pollutants to do the same exists. Further study of groundwater transport of contaminants is required in the areas I discussed above.

Comparative evaluation of spectrofluorimetry to CE/LIF

Spectrofluorimetry was able to analyze samples faster, with few preparation steps. The instrument was rugged and easy to use. No mechanical problems occurred

during the course of this work. In dealing with many samples this method proved to be an excellent first step in analyzing water samples (Grange et al, 1996).

CE/LIF detection offers a more sensitive, specific approach to analyzing ground water samples compared to spectrofluorimetry. The spectrofluorimetric limit for fluorescein was 3ppt in deionized water free of interferences and 60ppt using the Beckman P/ACE with a 5 second injection (Grange et al, 1996).

The high peak capacity of CE/LIF allowed the use of an internal standard that did not interfere with the analyte signal. Better data was obtainable by referencing the signal from the analyte to that of the internal standard. The signal obtained from CE had well defined peaks atop a level baseline that provided greater evidence for the presence of the dye than the small broad peaks superimposed on a very large and variable baseline caused by fluorescing interferences in the spectrofluorimetric data (Grange et al, 1996).

CE/LIF was an excellent tool to use, to evaluate the positive readings gotten with spectrofluorimetry. It provided a means of further analysis and eliminated other interferences that the spectrofluorimetry could not.

As discussed, CE/LIF detection is an accurate and sensitive approach to groundwater migration studies using fluorescent tracer dyes. It offers a greater specificity in the determination of these dyes and in eliminating background signals. This method showed itself to be a very good confirmatory technique. (Brumley et al, 1996).

The combination of these two methods of analysis should provide results that can withstand the scrutiny of litigation. This could be quite useful in situations where possible contamination sources pose serious risk and liability is to be determined.

(Grange et al, 1996).

In conclusion, this is the first example of a real environmental problem in groundwater migration that could show off the speed, specificity, and low detection limits of laser induced detection with capillary electrophoresis.

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