

AN ASSESSMENT OF THE POTENTIAL ADVERSE PROPERTIES OF FLUORESCENT TRACER DYES USED FOR GROUNDWATER TRACING *

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Abstract. The potential ecotoxicity of fluorescent dyes used in tracing, and their possible effects on human health, were evaluated by reviewing available toxicological information for 12 dyes – fluorescein, Lissamine Flavine FF, Rhodamine WT, Rhodamine B, Sulpho Rhodamine G, Sulpho Rhodamine B, eosin, pyranine, Phorwite BBH Pure, Tinopal 5BM GX, Tinopal CBS-X, and Diphenyl Brilliant Flavine 7GFF – and a dye-intermediate, amino G acid. This evaluation used available toxicological information, test data on analogous substances, and mathematical expressions for biological activity. Based on set criteria for human health and acute ecotoxicity, the evaluation indicated that these tracers have low to moderate levels of concern. The use of these tracers for the study of groundwater flow is appropriate if consideration is given to the overall human health and environmental effects. Their use in the environment requires tracer concentrations not exceeding $1\text{--}2\text{ mg l}^{-1}$ persisting for a period in excess of 24 h in the groundwater at the point of groundwater withdrawal or discharge. A simple calculated potential dose was used in a comparison of the estimated acute toxicity of Rhodamine WT in rats to the known acute oral toxic dose in humans for several known acutely toxic chemicals. This comparison showed that none of the fluorescent dyes evaluated would present an acutely toxic threat at or substantially above the recommended 2 mg l^{-1} concentration.

1. Introduction

The evaluation of subsurface flow regimes commonly requires more direct information than can be obtained by measurement of potentiometric levels and velocity calculations based on Darcy's law. In fact, potentiometric-surface measurements sometimes yield only minimally useful data, and Darcy's law may be totally inapplicable in various hydrologic settings. This is especially true for karst terranes where flow may be concentrated in subsurface conduits and in non-karst terranes where flow may be concentrated in discrete fractures. Fluorescent dyes are often used to overcome these difficulties by tracing the flow of groundwater from areas

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of recharge to areas of discharge. Some specific reasons for doing so are: (1) determination of flow paths and residence times; (2) measurement of aquifer properties; (3) mapping and characterization of conduit networks in karst terranes; (4) confirmation of sources of leachate contamination; (5) determination of site-specific hydrology; (6) design of monitoring networks; (7) determination of hydraulic properties of landfill materials; (8) prediction of leachate contamination and dilution; (9) determination of recharge areas for wellhead and springhead protection; and (10) planning for response to spills of hazardous materials (Smart, 1985; Quinlan, 1986, 1989; Quinlan *et al.*, 1988; Mull *et al.*, 1988).

Fluorescent dyes are commonly the first choice for tracer material used to label groundwater for tracing studies in rocks and sediments with low content of clay. Recent studies have shown fluorescent dyes to be useful not only in karst settings but also in fractured crystalline rocks and in water-saturated soils with low permeability such as clayey soils or glacial till soils. For example, the Thurston County Environmental Health Department (Olympia, Washington) is using fluorescein for detection of failing on-site septic systems (Hofstad, 1991, pp. 17–18).

Fluorescent dyes may be regarded as surrogates for pollutants, and they can provide useful information on the rate and direction of contaminant transport. However, to be useful as groundwater tracing agents the dyes should not: (a) change the hydraulic characteristics of the traced water; (b) cause contamination of the waters of springs or wells beyond permissible standards; (c) lead to the emergence of toxic or carcinogenic by-products following physical, chemical, or biological degradation; and (d) affect taste, odor, or color of the water (Milanović, 1981, p. 268; Gaspar, 1987, p. 46; Ford and Williams, 1989, p. 229). In addition, they should yield data that can be analyzed for distribution of tracer and pollutant residence times and provide a measure of longitudinal and transverse dispersion in the aquifer (Milanović, 1981, p. 268; Gaspar, 1987, p. 46). It should be noted here that although dyes have been successfully used in numerous unconsolidated formations (Smart *et al.*, 1988, citing 57 publications), most recent North American work in granular aquifers uses other tracers (Moltyaner, 1990) because of the typically smaller dilution factors involved and the exceptional detectability of dyes is not required.

Our objective is to address several of the issues related to toxicity and explain how the twelve fluorescent dyes and one dye-intermediate evaluated in this study may be used in a safe manner. A dye-intermediate is an organic compound produced during the manufacture of dyes; it is an intermediate product between the primary raw materials and the dyes themselves (Abrahart, 1968). Hereafter in this paper, the term *dyes*, when obviously used in the broad sense, includes the specific dye-intermediate, amino G acid. Smart (1984) reviewed the available toxicologic literature on all of the dyes we discuss except Tinopal 5BM GX. His excellent, useful review was limited to laboratory data on toxicity to various organisms, and it includes much useful data not cited here although considered in our toxicological

assessments. We have overcome this limitation by utilizing specialized chemical evaluation techniques developed by the U.S. Environmental Protection Agency (EPA) that consider effects on both human health and other biota. Determination of the amount of dye that can be safely used for groundwater tracing is discussed by Field *et al.* (1995).

The fluorescent tracers evaluated in this study and their structures are provided in Table I and Figure 1, respectively. These are the more common ones, and we estimate that 95% of dye traces are performed with one or more of them. Four of these dyes, when sold with certified high purity and having Drug and Cosmetic (D&C) designation, are used as colorants in cosmetics and other consumer products ranging from shampoo to toilet bowl cleaner. They are: fluorescein (D&C Yellow 8), Rhodamine B (D&C Red 19), eosin (D&C Red 22), and pyranine (D&C Green 8) (Marmion, 1991). Tracing, however, is done with industrial grade dyes, not certified grade dyes.

2. Important Fluorescent Dye Properties

Fluorescent dyes used as environmental water tracing agents should exhibit certain properties. To wit, they should be: (1) readily soluble in water; (2) adequately conservative, stable with time, and should not deteriorate on contact with the investigated medium (water, air, and aquifer material); (3) unambiguously and inexpensively measurable at large dilutions; (4) simply and easily detectable; (5) intrinsically low in toxicity and pose no significant health or environmental threat; and (6) readily available and inexpensive.

2.1. SOLUBILITY

Solubility of a tracer is an essential property that must be considered because high initial solubility allows for a greater detectability even after substantial dilution. Low solubility tracers may be affected by settling of tracer particles in the subsurface or by density separations (Milanović, 1981, p. 269). Miscible tracers are much more easily advected within the groundwater than are less soluble tracers because they tend to mix easily with the water. The solubility in water and alcohol of many of the tracer dyes is given by Green (1990).

The tracers considered in this study may all be regarded as miscible with water. Their solubility and fluorescence can be increased through the addition of alcohol and ammonia or sodium hydroxide. These additives are potentially toxic chemicals and may have synergistic effects on aquatic organisms; therefore, they are added to samples in the laboratory to enhance ease of analysis. It is neither practical nor necessary to add them to the tracer being injected.

TABLE I

Fluorescent dye types and dye names with their respective Colour Index and CAS numbers

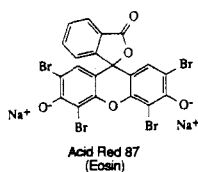
Dye type and common name	Colour Index generic name	CAS No.
<i>Xanthenes</i>		
fluorescein ^a	Acid Yellow 73	518-47-8
eosin	Acid Red 87	17372-87-1
<i>Rhodamines</i>		
Rhodamine B	Basic Violet 10	81-88-9
Rhodamine WT	Acid Red 388	37299-86-8
Sulpho Rhodamine G	Acid Red 50	5873-16-5
Sulpho Rhodamine B	Acid Red 52	3520-42-1
<i>Stilbenes</i>		
Tinopal CBS-X	Fluorescent Brightener 351	54351-85-8
Tinopal 5BM GX	Fluorescent Brightener 22	12224-01-0
Phorwite BBH Pure	Fluorescent Brightener 28	4404-43-7
Diphenyl Brilliant Flavine 7GFF	Direct Yellow 96	61725-08-4
<i>Functionalized Polycyclic</i>		
<i>Aromatic Hydrocarbons</i>		
Lissamine Flavine FF	Acid Yellow 7	2391-30-2
pyranine	Solvent Green 7	6358-69-6
amino G acid	—	86-65-7

^a There are two dyes called fluorescein; their nomenclature can be confusing. Fluorescein (C₂₀H₁₂O₅; C.I. Solvent Yellow 94) is very slightly soluble in water and not used for tracing. Sodium fluorescein (C₂₀H₁₀Na₂O₅; C.I. Acid Yellow 73) is highly soluble in water and used for tracing; in Europe it is known as uranine. American and British practice is to call Acid Yellow 73 *fluorescein* rather than its correct name, *sodium fluorescein*. We follow this convention: All dye that we call *fluorescein* is actually Acid Yellow 73, the dye highly soluble in water.

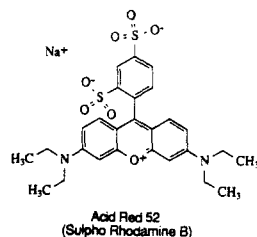
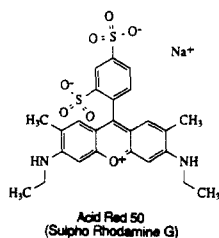
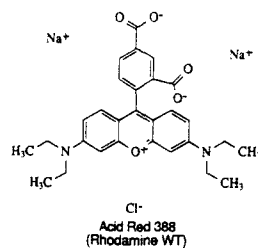
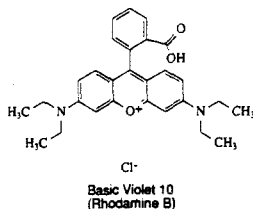
Fluorescein was sold in the U.S. under 17 names, at various strengths, and by 10 manufacturers in 1991. Similarly, four structurally different dyes were sold under 8 different names for Rhodamine, and 13 different Phorwite optical brighteners are sold by a single manufacturer (Source: *Textile Chemist and Colorist* **23**, (7), p. 88, 68, and 64, 1991). This is why we strongly recommend that investigators use caution in ordering dyes and, in their report, always specify the Color Index generic name, dye manufacturer, and dye manufacturer's name for any dye employed for tracing. Source: SDC & AATCC (1971-88); Abrahart (1968); Anliker and Müller (1975); Zollinger (1987).

CAS No. = Chemical Abstracts Service. A professional service of the American Chemical Society that registers substances from the chemical literature, publishes *Chemical Abstracts*, and serves as an internationally recognized authority on chemical nomenclature and identification.

XANTHENES



RHODAMINES



STILBENES

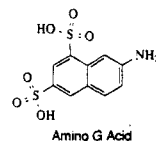
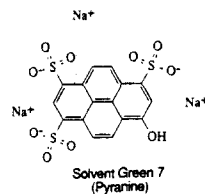
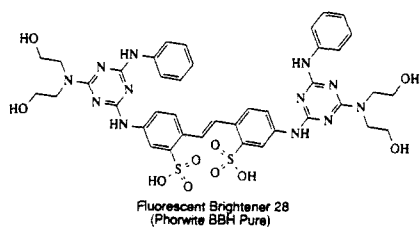
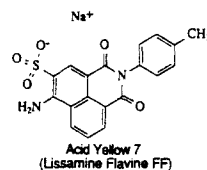
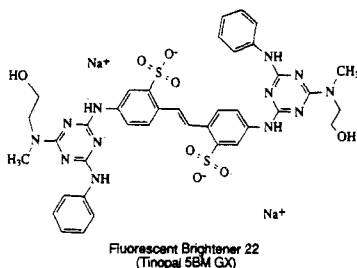
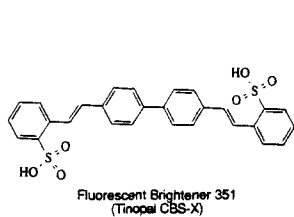


Fig. 1. Chemical structures for twelve of the thirteen fluorescent dyes evaluated for this study; the chemical structure for Direct Yellow 96 is unavailable.

2.2. STABILITY

The concentration of tracer employed is usually extremely small relative to the volume of subsurface water under investigation. Thus any loss of the tracer material along the groundwater flow route reduces the accuracy of some results and may interfere with their interpretation (Milanović, 1981, p. 269). Limestone, dolomite, sand, and clay are known to readily adsorb various dyes and clay may have a significant ion-exchange capacity. Although some dye types may have low sorption and ion-exchange capacities with limestone and dolomite, the surfaces of many conduits are coated with clay, iron oxide, and organic matter that may react significantly with the dyes considered in this study. Use of tracer materials susceptible to adsorption or ion exchange can result in a substantial loss of tracer material. Some dyes are also slightly biodegraded in some aquifers. Dyes can also be degraded by post-collection microbial activity (Behrens, 1986, pp. 130–131) which can be reduced by refrigeration of samples and detectors.

Desorption and/or reversible ion exchange may also have an adverse effect on tracer recovery. Continuous reactions of a tracer with the aquifer may yield inaccurate determinations of travel times and discharge locations. As a consequence, a tracer may be detected as an elongated wave rather than a sharp peak.

The fluorescent dyes considered in this study are likely to be only slightly sorbed by clays, charged solvent species, dissolved organic materials, and/or organic and inorganic particulates. More specifically, cationic dyes such as Rhodamine B are readily sorbed onto clays; the other dyes discussed herein are anionic and significantly less subject to sorption (Smart and Laidlaw, 1977; Trudgill, 1987; Sabatini and Austin, 1991). It is possible that the anionic nature of the other dyes facilitates their movement through an aquifer, for reasons advanced by Gvirtzman and Gorelich (1991).

In general, the dyes considered in this study may be regarded as structurally stable; they are not readily degraded. It has been suggested that photo-degradation of dyes is responsible for certain toxic effects (Smart, 1984), but other mechanisms (including photo-sensitized formation of singlet oxygen) are more plausible. More realistically, these dyes tend to photo-bleach when exposed to light because exposure to light allows an outer electron to freely migrate across the molecule. This heightened state of excitation is reversible however, which allows the dye to return to ground state (Charles Auer, Paul Bickart, and Russell Farris, Office of Toxic Substances, U.S. EPA, oral communication, 1991).

2.3. DETECTION

Detection of a tracer should be easy, inexpensive, and frequent enough to accurately characterize its concentration. One must balance the cost of the analysis against the need for accurate characterization. If sampling frequency is reduced in order to

lessen the cost, it is possible to miss the initial breakthrough of the tracer or obtain too crude an estimate of its concentration and residence time.

All of the dyes, except Tinopal 5BM GX and Diphenyl Brilliant Flavine 7GFF, react readily with a passive detector consisting of activated charcoal. Tinopal 5BM GX and Diphenyl Brilliant Flavine 7GFF can be detected with cotton. Both types of detectors can be used for inexpensive, continuous monitoring for tracer breakthrough, and they increase the detectability of the dyes by a factor ranging from 100 to 400.

The fluorescent dyes considered in this study are easily adsorbed onto activated charcoal or cotton, but reactive chemicals may displace them from the sorption sites. Packets of charcoal and/or cotton may be suspended in the flow of a stream, in the flow of a pumped well, or in a non-pumped well for continuous monitoring for tracer breakthrough. Adsorbed dye can then be eluted from the charcoal or cotton for the dye detection. An accurate quantitative determination of dye concentration can be made by analysis of water samples obtained from frequent grab samples or collected with automatic water samplers. Each water sample is analyzed for dye with a fluorometer or a scanning spectrofluorophotometer. The cost of analysis of fluorescent dyes with such instruments is inexpensive relative to the cost of analysis for other types of tracers that can be detected in the range from 0.005 to 100 $\mu\text{g l}^{-1}$.

2.4. DETECTABILITY

Detectability is the lowest concentration that can be reliably identified with a given analytical procedure. For example, the detectability of fluorescein in water, when analyzed with a scanning spectrofluorophotometer, is 5 ng l^{-1} (1 g of dye dissolved in 40 000 m^3 of water can be positively identified). Activated charcoal can be used to improve this detectability to about 0.03 ng l^{-1} . Use of activated charcoal does not increase the limit of detection *per se*, but sorption of dye by activated charcoal may increase concentration by a factor of 100 to 400, thus enabling detection of an inferred concentration of 0.03 ng l^{-1} . Larger concentrations of dye are desirable because of the added confidence gained by more clearly positive results.

2.5. TOXICITY

The dye being utilized for environmental tracing must not be toxic to people or to other organisms. Toxicity is relative, depending on the concentration present and the environment into which the dye is released. Accordingly, the level of toxicity of each dye chosen for environmental tracing must be considered. Three very important questions need to be answered prior to embarking on a tracing study. Can the chosen dyes be used effectively to trace the flow of groundwater at concentrations that are below their known or estimated toxicological concentrations? Is the area to be traced a known sensitive environment? Is the level of information to be

gained by dye tracing worth potentially impacting a sensitive environment? Our assessment provides relevant information necessary for answering these questions.

3. Toxicity Evaluation

For the purposes of this study, the twelve fluorescent dyes and one dye-intermediate shown in Table I were evaluated by the EPA's Office of Toxic Substances (OTS) for their ecotoxicity and human health effects (Wilhelm, 1989; Auer, 1989). The evaluation was conducted using structure activity relationships (SARs). This technique was developed to evaluate the toxicity of industrial chemicals for which detailed toxicological data are unavailable.

Essentially, the SAR technique is based on three components. Available toxicity data are used for a critical evaluation and interpretation of each chemical; test data on analogous or similar substances and/or potential metabolites are evaluated; and mathematical expressions for biological activity, known as quantitative structure activity relationships (QSARs), are derived and utilized. Presently, QSARs are limited to the estimation of physico-chemical properties, ecological toxicity, and fish bioconcentration factors (Auer and Gould, 1987; Auer *et al.*, 1990). A useful review of SAR methodology and applications has been made by Samiullah (1990, pp. 20–26) and by Osman (1990).

A limitation of the SAR technique is that EPA has no QSARs for either basic (cationic) dyes or acid (anionic) dyes or for many other types of dyes. However, unpublished data from OTS have made it possible to draw some tentative conclusions. In general, basic dyes – Rhodamine B is the only basic dye considered in this study – can be considered fairly toxic to sensitive organisms at relatively low concentrations. The toxicity of basic dyes has long been recognized (Little and Lamb, 1973, p. 17). Acid dyes and direct dyes, both of which are used in environmental tracing, have relatively low toxicity. Additional evaluation has shown that acid dyes possess low to moderate toxicity to green algae, but this is an indirect effect caused by light attenuation at high concentrations (hundreds to thousands of mg l^{-1}) of dye, not any intrinsic toxicity (Auer *et al.*, 1990).

The evaluation conducted by OTS and reported here has shown that none of the dyes is expected to present a significant health or environmental problem – if dye concentrations are maintained below the minimum easily visible to the naked eye, approximately $1\text{--}2 \text{ mg l}^{-1}$ for 24 h at the point of groundwater withdrawal or discharge. Furthermore, some dyes do not present significant toxicity concerns even at visible concentrations. It is important to note that the evaluation was based on both measured and estimated values. Therefore, although it is recommended that caution be exercised in the use of these dyes, especially in sensitive ecosystems, the guidelines stated in this paragraph constitute judicious exercise of such caution.

4. Concern Levels

As noted above; the toxicity assessment considered both human health effects and ecological toxicity (Auer, 1989). The assessment of human health effects specifically considered the following toxic endpoints: oncogenicity, mutagenicity, developmental toxicity, reproductive toxicity, acute toxicity, chronic toxicity, and neurotoxicity. Chronic toxicity evaluations included (but were not limited to) organ toxicity, blood effects, and immunotoxicity.

Ecotoxicity assessments included both acute and chronic effects for three indicator lifeforms: fish, Cladocera (water fleas, *Daphnia*), and algae. For both ecotoxicity and human health effects, the concern levels identified by use of the SAR technique are expressed as HIGH, MODERATE, and LOW according to the guidance below. These are toxicity values, not exposure values. The lack of test data suggests that the SAR results should not be considered the final word on these chemicals. SAR evaluations should be considered as screening techniques. Although toxicological effects could be missed, the use of SARs is generally regarded by EPA and the scientific community as being reliable and highly useful in estimating or predicting toxicity.

4.1. HUMAN HEALTH EFFECTS

Dyes, like other chemicals, received a HIGH concern if there was evidence of adverse effects in human populations or conclusive evidence of severe effects in animal studies for any of the toxic endpoints described above. Dyes providing suggestive evidence of any of the toxic endpoints listed above in animals or with a close analogy (structural, functional, and/or mechanistic) to chemicals with known toxicity received a MODERATE concern. Dyes with no evidence or suggestion of the toxic endpoints received a LOW concern.

4.2. ECOLOGICAL TOXICITY

Dyes with aquatic toxicity values below 1 mg l^{-1} received a HIGH concern level. Those dyes with toxicity values ranging from 1 to 100 mg l^{-1} received a MODERATE concern, while those with toxicity values greater than 100 mg l^{-1} received a LOW concern rating. Although this rating system was developed for the purpose of protecting surface water biota, not cave fauna and flora, it is consistent with the standard system used for evaluating the effects of other chemicals on human life.

None of the investigated dyes exhibited a HIGH concern level for either human health effects or ecotoxicity using this system (Table II). The majority of the dyes were assigned LOW to MODERATE ratings. Note, however, that MODERATE concerns issued for ecotoxicity were primarily based on indirect (shading) effects on algae. Shading only occurs if such large quantities of dye were injected that the point of subsurface discharge received sufficiently large quantities of dye (hundreds

TABLE II
Octanol-water partition coefficients and concern levels of fluorescent dyes

Colour Index generic name	log K_{ow} ^a	Concern level	
		Human	Ecological
Acid Yellow 73	-0.39	L	M
Acid Red 87	-1.33	L-M	L-M
Basic Violet 10	2.28	L-M	M
Acid Red 388	-1.33	L-M	M
Acid Red 50	-2.21	L-M	L-M
Acid Red 52	-2.02	L-M	L-M
Fluorescent Brightener 351	4.90 ^b	M	L-M
Fluorescent Brightener 22	2.70 ^b	M	L-M
Fluorescent Brightener 28	0.94 ^b	L-M	L-M
Direct Yellow 96	^c	L-M	L-M
Acid Yellow 7	2.60 ^b	L-M	L-M
Solvent Green 7	-0.68 ^b	L	L-M
amino G acid	-1.63 ^b	M	L-M

^a Values for log K_{ow} cited from Benoit-Guyod *et al.* (1979) except where otherwise indicated.

^b Estimates for log K_{ow} were made using the CLOGP program, version 3.32, developed at Pomona College, California. All estimates were based on the un-ionized form.

^c Unable to accurately estimate log K_{ow} because of the unavailability of structure.

Note: All of these dyes may be regarded as soluble in water.

K_{ow} = Octanol-water partition coefficient. This is the ratio of a chemical's solubility in *n*-octanol and water at equilibrium. The logarithm of K_{ow} is used as an indication of a chemical's propensity for bioconcentration by aquatic organisms and as an indicator of the chemical's mobility in the environment (Rand and Petrocelli, 1985, p. 654; Fetter, 1988, pp. 402-405). Low values for log K_{ow} indicate a low probability of food-chain effects (biomagnification) and a greater ease of mobility in water. The relation of the log K_{ow} for fluorescein and Rhodamine WT to the behavior of these dyes in granular media is discussed by Sabatini and Austin, 1991.

to thousands of mg l⁻¹) to greatly color the water for a period probably measured in days. Shading is irrelevant in groundwater-systems.

The values listed for the logarithm of the octanol-water partition coefficient, log k_{ow} , in Table II were estimated where necessary and possible to provide an indication of how readily a dye would be able to move across biological membranes and become absorbed by an organism. In solution, all these dyes equilibrate between two forms; a charged species and an uncharged species. Only the uncharged species will cross membrane boundaries. The dyes listed in this study are injected into the

subsurface as salts (charged species), but because of equilibrium effects in water, a small percentage will exist in the uncharged form. No discussion regarding food-chain effects is presented because nothing is known about the possibility of food-chain effects of dyes. However, since these dyes are not metabolized, or are only slightly metabolized (see Table II for $\log k_{ow}$ values), this is not expected to be a significant concern (Charles Auer, Office of Toxic Substances, U.S. EPA, oral communication, 1991).

Table III is a summary of the SAR concerns for human health and Table IV is a summary of the SAR concerns for ecological toxicity. A careful reading of Table III will show that the dyes considered in this study are of relatively little concern for human health for the most part although data are lacking in some instances. The specific values provided for ecological toxicity in Table IV indicate that fairly massive doses of the dyes evaluated in this are required to produce an effect on the test organisms.

5. Comparative Risk Assessment

In order to assess the environmental acceptability of a maximum of 24 h of 1–2 mg l^{-1} as a downgradient dye concentration in tracer experiments, it is appropriate to relate the potential toxicity of the thirteen fluorescent dyes considered in this study to that of other chemicals with known toxicities. This is accomplished by performing a simple dose calculation to yield an exposure level and by relating this value to known toxicity levels for the fluorescent dyes or for their “worst case” surrogate chemicals.

A potential dose via oral ingestion for any chemical can be calculated if the concentration of the chemical and the ingestion rate are known. The equation for potential dose (USEPA, 1989, p. 2-1; modified for acute dose estimation) is:

$$D_P = \frac{C_T I_R}{B_W} \quad (1)$$

where

- D_P = potential dose ($\text{mg kg}^{-1} \text{d}^{-1}$),
- C_T = contaminant concentration (mg l^{-1}),
- I_R = ingestion rate (l d^{-1}); 2 l d^{-1} is assumed worst case,
- B_W = body weight (kg); 70 kg is assumed for an adult male.

Using a maximum of 2 mg l^{-1} for downgradient dye concentration, we acquire a potential daily dose of 0.057 mg $\text{kg}^{-1} \text{d}^{-1}$ for a 70 kg adult. To estimate the potential for this dose value to elicit toxicity requires knowledge of the acute toxicity for whatever chemical is of interest. There are forms of toxicity other than acute oral toxicity that may be of significance, but given the normally short exposure duration associated with tracing experiments, only acute oral toxicity is considered here. In this section, we are primarily concerned with estimating the

TABLE III
Summary of human health SAR concerns

Dye type	Skin absorption	Oral uptake	Carcinogenicity	Oncogenic effects	Mutagenic effects	Additional concerns
fluorescein	None	Limited	Little concern ^a	None	Little concern	No other health concerns identified
eosin	None	Limited	Inadequate data	Some concern	Some concern	Little concern for liver effects based on brominated aromatic compounds as a class
Rhodamine B	None	Limited	Inadequate data	Little concern	Little concern	Little concern for chronic toxicity including liver and kidney effects
Rhodamine WT	None	Limited	Inadequate data	Little concern	Little concern	Little concern for chronic toxicity including liver and kidney effects
Sulpho Rhodamine G	None	None	Little concern	Little concern	Little concern	Little concern for chronic toxicity; uncertain concerns for developmental effects (based on Rhodamine G)
Sulpho Rhodamine B	None	None	Little concern	Little concern	Little concern	Has been proposed for chronic bioassay testing by the National Testing Program
Tinopal CBS-X	None	None	Little concern	Little concern	Little concern	Moderate concern for developmental effects based on structural analogs
Tinopal 5BM GX	None	None	Little concern	Little concern	Little concern	Moderate concern for developmental effects based on structural analogs

TABLE III
Continued.

Dye type	Skin absorption	Oral uptake	Carcinogenicity	Oncogenic effects	Mutagenic effects	Additional concerns
Phorwite BBH pure	None	None	Inadequate data	Little concern	Little concern	Concerns for developmental effects and sensitization are uncertain
Diphenyl Brilliant Flavine 7GFF ^b	None	None	Little concern	Little concern	Little concern	Moderate concern for developmental effects based on structural analogs
Lissamine Flavine FF	None	None	Little concern	Uncertain	Uncertain	Uncertain concerns for developmental effects
Pyranine	Negligible	Negligible	Little concern	Little concern	Little concern	No other health effects identified
amino G acid	None	None	Little concern	Some concern	Some concern	Concerns based on data from structural analogs

^a No adequate data available for carcinogenicity, but little concern based on mechanistic considerations.

^b Chemical structure is proprietary – assessment was based on Tinopal CBS-X.

TABLE IV
Summary of ecological toxicity SAR concerns

Dye type	Fish 96-h LC ₅₀	Cladocera 48-h LC ₅₀	Green algae 96-h EC ₅₀	Additional information
fluorescein	2200.0 mg l ^{-1a}	165.0 mg l ^{-1a}	<10.0 mg l ⁻¹	Algae EC ₅₀ based on a 7-day EC ₁₀₀ = 10 mg l ⁻¹
eosin	>1000.0 mg l ^{-1a}	>100.0 mg l ⁻¹	>10.0 mg l ⁻¹	Daphnid LC ₅₀ based on fish acute value. Algae EC ₅₀ based on 7-day NOEC = 10 mg l ⁻¹
Rhodamine B	314.0 mg l ^{-1a}	> 29.0 mg l ^{-1a}	<10.0 mg l ⁻¹	Algae EC ₅₀ based on a 7-day EC ₅₀ < 10.0 mg l ⁻¹
Rhodamine WT	> 320.0 mg l ^{-1a}	170.0 mg l ^{-1a}	20.0 mg l ⁻¹	Algae NEC = 0.200 mg l ⁻¹ based on acid dyes as a class
Sulpho Rhodamine G	> 500.0 mg l ^{-1a}	88.0 mg l ^{-1a}	20.0 mg l ⁻¹	Algae NEC = 0.200 mg l ⁻¹ based on acid dyes as a class
Sulpho Rhodamine B	1200.0 mg l ^{-1a}	139.0 mg l ^{-1a}	20.0 mg l ⁻¹	Algae NEC = 0.200 mg l ⁻¹ based on acid dyes as a class
Tinopal CBS-X	158.0 mg l ^{-1a}	>100.0 mg l ⁻¹	20.0 mg l ⁻¹	Daphnid LC ₅₀ based on fish acute value. Algae NEC = 0.200 mg l ⁻¹ based on acid dyes as a class
Tinopal 5BM GX	> 100.0 mg l ^{-1a}	>100.0 mg l ⁻¹	20.0 mg l ⁻¹	Algae NEC = 0.200 mg l ⁻¹ based on Acid Yellow 7 and acid dyes as a class
Phorwite BBH Pure	>1000.0 mg l ^{-1a}	>100.0 mg l ⁻¹	>10.0 mg l ⁻¹	Daphnid LC ₅₀ based on fish acute value. Algae EC ₅₀ based on 7-day NOEC = 10.0 mg l ⁻¹
Diphenyl Brilliant Flavine 7GFF ^b	158.0 mg l ^{-1a}	>100.0 mg l ⁻¹	20.0 mg l ⁻¹	Daphnid LC ₅₀ based on fish acute value. Algae NEC = 0.200 mg l ⁻¹ based on acid dyes as a class

TABLE IV
Continued.

Dye type	Fish 96.-h LC ₅₀	Cladocera 48-h LC ₅₀	Green algae 96-h EC ₅₀	Additional information
Lissamine Flavine FF pyranine	>1000.0 mg l ^{-1a} > 500.0 mg l ^{-1a}	>1000.0 mg l ^{-1a} > 100.0 mg l ⁻¹	20.0 mg l ⁻¹ 20.0 mg l ⁻¹	Algae NEC = 0.200 mg l ⁻¹ based on acid as a class Daphnid LC ₅₀ based on fish acute value. Algae NEC = 0.200 mg l ⁻¹ based on acid dyes as a class
amino G acid	1400.0 mg l ^{-1a}	386.0 mg l ^{-1a}	20.0 mg l ⁻¹	Algae NEC = 0.200 mg l ⁻¹ based on acid as a class

^a Measured effects; others unmarked were estimated.

^b Chemical structure proprietary; assessment based on Tinopal CBS-X.

EC₅₀ = Median effective concentration, expressed as a time-dependent value, usually 24, 48, or 96 h. This is the concentration of material in water (expressed as mg l⁻¹) to which test organisms are exposed that is estimated to be effective in producing some sublethal response in 50% of the test organisms (Rand and Petrocelli, 1985, p. 654).
EC₁₀₀ = Effective concentration, expressed as a time-dependent value, usually 24, 48, or 96 h. Concentration (mg l⁻¹) at which sublethal effects are observed in 100% of the test organisms.

LC₅₀ = Median lethal concentration, expressed as a time-dependent value, usually 24, 48, or 96 h. This is the concentration of material in water (mg l⁻¹) to which test organisms are exposed that is estimated to be lethal to 50% of the test organisms (Rand and Petrocelli, 1985, p. 654).
LD₅₀ = Median lethal dose. The chemical dose (expressed as mg kg⁻¹ d⁻¹) estimated to be lethal to 50% of the test organisms (e.g., mice, rats, and dogs).
It is not applicable to aquatic organisms because it indicates the quantity of a material introduced directly into the body by injection or ingestion rather than the concentration of the material in water to which aquatic organisms are exposed (Rand and Petrocelli, 1985, p. 654).
NEC = No effect concentration. Concentration at which no effects are expected.

NOEC = No observed effect concentration. This is the highest concentration of a material in a toxicity test that has no statistically significant adverse effect on the exposed population of test organisms as compared with the controls (Rand and Petrocelli, 1985, p. 654).

lethal dose of any one of the dyes to humans. No discussion regarding the amount of dye estimated to have a nonlethal but still toxic effect on humans is included.

Of the thirteen fluorescent dyes considered in this study, acute toxicity test results were available only for Rhodamine WT, which resulted in an estimated lethal dose for Rhodamine WT in the rat to be greater than $25\,000\text{ mg kg}^{-1}$ (no significant adverse effects were reported at this dose) (Haskell, 1976). No information is available on the acute human lethal dose for Rhodamine WT (or for any of the other fluorescent dyes considered in this study). For purposes of this discussion, we will assume an oral lethal human dose to be $25\,000\text{ mg kg}^{-1}\text{ d}^{-1}$.

In order to evaluate the potential acute toxicity of Rhodamine WT, the measured potential dose of $25\,000\text{ mg kg}^{-1}$ was compared to the known acutely toxic dose for other chemicals. For comparison purposes, four chemicals (with structures unrelated to any of the fluorescent dyes considered in his study) were chosen: two insecticides, one dye-intermediate, and hydrogen cyanide, all known to be acutely toxic at low doses. The two insecticides used for comparison purposes, aldicarb (CAS No. 116-06-3) and Paris Green (CAS No. 12002-03-8), were chosen because of their likelihood of being found in groundwater and surface water. Maxilon Red Intermediate Cake (CAS No. 26903-94-6) was chosen for comparison purposes because it is a dye-intermediate of known acute toxicity. Hydrogen cyanide (CAS No. 74-90-8) was chosen as an example of an extremely acutely toxic substance that is widely known. Each of these four chemicals has undergone extensive toxicity evaluations, the results of which have been reported in the literature.

Of the two insecticides and one dye-intermediate considered, each was found to be very toxic in low concentrations. In humans, aldicarb was estimated to have an acute lethal oral dose of less than 5 mg kg^{-1} (lower doses are known to have very serious acute effects on humans) (USEPA, 1988); Paris Green was estimated to have an acute oral lethal dose of $5\text{--}50\text{ mg kg}^{-1}$ (USEPA, 1988); and Maxilon Red Intermediate cake was reported to have an oral LD_{50} in rats of 13 mg kg^{-1} (USEPA, 1979). Finally, hydrogen cyanide was found to have an average lethal oral dose for humans of $50\text{--}60\text{ mg}$ ($0.71\text{--}0.86\text{ mg kg}^{-1}\text{ HCN}$) (USEPA, 1988). These values and that of Rhodamine WT are shown in Table V.

Relating the values shown in Table V with the previously calculated potential dose of $0.057\text{ mg kg}^{-1}\text{ d}^{-1}$ for a 70 kg adult shows that even if the chemical of concern was one of the acutely toxic chemicals described above, a downgradient peak concentration of 2 mg l^{-1} would not be lethal to humans. For example, an adult would have to ingest 2 l per day of water with a peak concentration for aldicarb of 87 mg l^{-1} in the water to reach the lethal dose (5 mg kg^{-1}) for humans; likewise the peak concentration for Paris Green would have to be between 87 and 877 mg l^{-1} in the water. The peak concentration of Maxilon Red Intermediate Cake would have to be 455 mg l^{-1} in the water for the oral LD_{50} in rats to be reached. If the chemical of concern was actually hydrogen cyanide, then the peak concentration of hydrogen cyanide in water would still have to be at least $25\text{--}30\text{ mg l}^{-1}$ to reach the average acute oral lethal dose in humans. This translates into an average acute

TABLE V

Toxicity and required peak concentration of Rhodamine WT and other selected compounds

Chemical compound	Toxicity level (mg kg ⁻¹)	Required concentration ^a (mg l ⁻¹)
Rhodamine WT ^b	> 25 000	870 000
aldicarb ^a	< 5	87
Paris Green ^c	5–50	87–870
Maxilon Red Interm. Cake ^b	13	455
hydrogen cyanide ^c	0.71–0.86	25–30

^a Based on an ingestion rate of 2 l d⁻¹ for a 70 kg adult.

^b LC₅₀ for the rat.

^c Estimated acute oral lethal dose for humans.

Source: Haskell (1976); USEPA (1979); USEPA (1988).

oral lethal dose that is 12 to 15 times higher for a one-day exposure than our maximum allowable 2 mg l⁻¹. In most tracing experiments in fractured-rock or karst aquifers, it is extremely uncommon to have consistent peak dye concentration levels to continue to be discharged over a period of several days. More commonly, after initial tracer breakthrough, concentrations tend to rapidly drop off (within a few hours to a few days). In relation to the toxic dose for Rhodamine WT, it would be necessary for an adult to ingest water with a much larger dye concentration in the discharge water (e.g., 875 000 mg l⁻¹) if a dose of 25 000 mg kg⁻¹ d⁻¹ were to be reached. Hence, even if it is assumed that an acute oral lethal dose for Rhodamine WT in humans (or any one of the other fluorescent dyes considered in this study) is much less than 25 000 mg kg⁻¹ d⁻¹, it is highly unlikely that the acute oral lethal dose would be achieved if the peak concentration of dye is maintained at or below 2 mg l⁻¹ in any given instance.

It must be reiterated, however, that this rough risk estimation only considered acute toxicity in the sense of a lethal dose. No other form of toxicity was evaluated in this risk calculation. This is important to note because other types of toxicological effects, such as developmental toxicity, can be significant for some compounds. Data regarding developmental toxicity is lacking and the SAR estimation of developmental toxicity was uncertain in most cases. Therefore, we would still recommend some caution in the use of the dyes, but if peak downgradient concentrations are maintained at or below 1–2 mg l⁻¹, it would be highly unlikely that any toxic effects on humans would be observed. Also, given the relatively high LC₅₀'s measured for these chemicals in aquatic organisms, it would seem unlikely that any serious effect on groundwater fauna result from a 1–2 mg l⁻¹ concentration in the water.

TABLE VI

LC₅₀ and EC₅₀ values (mg l⁻¹) for each fluorescent dye studied for fish, cladocera, and algae

Dye name	Fish LC ₅₀ (96-h)	Cladocera LC ₅₀ (48-h)	Algae EC ₅₀ (96-h)
fluorescein	2200	165	<10
eosin	>1000	> 100	>10
Rhodamine B	314	> 29	<10
Rhodamine WT	> 320	170	20
Sulpho Rhodamine G	> 500	88	20
Sulpho Rhodamine B	1200	139	20
Tinopal CBS-X	158	> 100	20
Tinopal 5BM GX	> 100	> 100	20
Phorwite BBH Pure	>1000	> 100	>10
Diphenyl Brilliant Flavine 7GFF	158	> 100	20
Lissamine Flavine FF	>1000	>1000	20
pyranine	> 500	> 100	20
amino G acid	1400	386	20

From SAR ecological toxicity summaries.

However, to assess the potential of a 2 mg l⁻¹ concentration level on aquatic groundwater biota, a comparison of the 2 mg l⁻¹ concentration level for any given dye to the LC₅₀ for selected aquatic organisms shows that 2 mg l⁻¹ is well below the LC₅₀'s for each dye. For example, the two most commonly used fluorescent dyes, fluorescein and Rhodamine WT, have LC₅₀'s of 2200 mg l⁻¹ and > 320 mg l⁻¹ for fish respectively. Table VI lists the LC₅₀ values for each of the thirteen dyes for both fish and daphnid. These values are substantially above the recommended maximum concentration of 2 mg l⁻¹. No appropriate toxicity studies have been conducted utilizing aquatic groundwater biota, but the values for surface water fauna are expected to provide conservative estimates for groundwater fauna. Groundwater fauna, such as troglobitic organisms found in some caves, typically have lower metabolic rates and lower birth rates than similar (but different species) animals found in surface waters. Acute toxicity for animals with low metabolic rates would be expected to occur at higher concentrations than would be the case for animals with higher metabolic rates. However, should a kill-off occur, the rate of recovery would be slower than for surface water environments.

6. Conclusions

Available toxicological on twelve fluorescent dyes and one dye-intermediate used in environmental tracing were reviewed for the purpose of evaluating the effects

on human health and the environment. The dyes – fluorescein, Lissamine Flavine FF, Rhodamine WT, Rhodamine B, Sulpho Rhodamine G, Sulpho Rhodamine B, eosin, pyranine, Phorwite BBH Pure, Tinopal 5BM GX, Tinopal CBS-X, Diphenyl Brilliant Flavine 7GFF – and the dye-intermediate, amino G acid, are those most commonly used throughout the world. The evaluation used available toxicological information, test data on analogous substances, and mathematical expressions for biological activity. Set criteria for human health and acute ecotoxicity were used to show that employment of these tracers was of low to moderate concern. Limitations on the amount of test data available made it necessary to characterize some modes of toxicity as “uncertain” effects, at least in part. This analysis did not consider any of the breakdown products that may develop from the dyes, cosolvation effects, or synergistic effects with any chemicals that might already be in the water. Whereas these three items were not directly assessed, we believe, based on the evaluations conducted, that none of the three items is likely to impart a significant toxicological threat to the environment if the dyes are used properly (e.g., appropriate concentrations given site conditions). We conclude that these fluorescent dyes can be usefully employed to study groundwater flow in karst or fractures-rock terranes if consideration has been given to the overall human health and environmental effects associated with their use.

Use of these fluorescent tracers in the environment requires that: (1) individuals doing the tracer work be experienced or well trained in their use and (2) tracer concentrations not exceed 1 to 2 mg l⁻¹ persisting for a period in excess of 24 h in groundwater at the point of groundwater withdrawal or discharge. This concentration is below known acute toxicity levels for these tracers. It is extremely close to the practical limit for easy recognition by the naked eye (1–2 mg l⁻¹ for most dyes) and is well above persistent dye concentrations traditionally recommended for tracer tests. The lower, traditional maximum concentrations of 10 µg l⁻¹ (Wilson *et al.*, 1986, p. 8; Mull *et al.*, 1988, pp. 28 and 37) have been selected primarily for aesthetic and public relations reasons, not as a result of comprehensive toxicological testing or evaluation of the dyes. A simple calculated potential dose was used in a comparison of the estimated acute toxicity of Rhodamine WT in rats to the known acute oral lethal dose in humans for several known acutely toxic chemicals. This comparison showed that it is unlikely that any of the fluorescent dyes evaluated in this study would present an acutely lethal threat to humans if concentrations are maintained at or below 2 mg l⁻¹. A concentration of 2 mg l⁻¹ is also below the reported acute toxic levels for the fluorescent dyes to some common aquatic organisms.

Although the evaluation of specific tracer properties (solubility, stability, detection, detectability, and toxicity) indicated that small amounts of fluorescent dye can be used to safely and successfully trace the flow of groundwater, some questions regarding toxicity of dyes in high concentrations may remain. It is neither necessary nor prudent to employ excessive amounts of dye. Therefore, the problem of determining the proper amount of dye to use, given a specific set of field condi-

tions, becomes important, and will be discussed in a later paper. Determination of the appropriate amount of dye to use in any tracing test may be a major difficulty that must be overcome if unambiguous positive results are to be achieved in an environmentally safe manner.

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