Assessment of the use of fluorescent tracers in a contaminated Chalk aquifer

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Abstract: Field tracer tests and parallel laboratory experiments were performed to establish tracer performance and solute transport velocity in a contaminant plume arising from an unleaded fuel spill in the Chalk aquifer of the southern UK. An initial forced gradient test between injection and pumped wells 24 m apart largely failed for practical reasons; subsequently other down-gradient wells were periodically monitored for tracer breakthrough under natural gradient conditions over the next 10 months, giving tracer velocities of 37–51 m a^{-1}, consistent with plume development and implying significant retardation of the tracer by fracture–matrix diffusion. Amino-G Acid concentrations fell rapidly during the field tests and laboratory experiments, and indicated that this tracer was susceptible to biodegradation under model plume conditions. Sodium Fluorescein was more strongly sorbed to aquifer materials than Amino-G Acid and exhibited some suppression of fluorescence in the presence of contaminants, but was resistant to biodegradation. Elevated background fluorescence produced an effective detection limit for Sodium Fluorescein of 1.8 μg l^{-1} in the most contaminated part of the plume.

Understanding the physical controls on solute migration in the subsurface is essential to predict the evolution and fate of groundwater contaminant plumes. Unless the physical processes of advection and dispersion that modify the distribution and concentration of contaminants in groundwater are properly constrained, it may be difficult or impossible to properly assess the role of other processes (adsorption, biodegradation, etc.) that may also contribute to the attenuation of contaminant migration. Failure to identify and quantify processes that are responsible for significant natural attenuation of contaminants can potentially lead to unnecessarily pessimistic predictions of contaminant behaviour and environmental risk. For aquifers dominated by intergranular porosity, groundwater flow and contaminant dispersion can be adequately deduced from standard site investigation, but in fractured porous (or 'dual-porosity') media, achieving this objective is a substantially more complex problem. In these settings, tracer tests are frequently undertaken to estimate fracture flow velocity, direction and solute travel times, quantify the extent of fracture–matrix solute exchange by diffusion, identify flow paths and deduce the connectivity of the fracture network (e.g. Ward *et al*. 1998; Atkinson *et al*. 2000; Hartmann *et al*. 2007). This information is often required to develop conceptual site models, design groundwater monitoring networks and implement remediation programmes at contaminated sites.

The Chalk aquifer is a major groundwater resource in southern and eastern England, and elsewhere in NW Europe, and is a classic example of a dual-porosity system. It accounts for 60% of the groundwater used in England and Wales and 20% of the total water used in England and Wales (UK Groundwater Forum 1998). The aquifer matrix is highly porous and the majority of groundwater exists as interstitial water in the matrix pore-space. However, groundwater is mobile only within a well-developed fracture system that contains only a small fraction of groundwater volume and it is this mobile fracture water that is recovered at abstraction or monitoring wells (MacDonald & Allen 2001). Solutes (e.g. tracers or contaminants) introduced into the saturated zone of the aquifer will initially enter the fracture system, where high flow velocities can increase the vulnerability of the aquifer to contaminant migration. However, tracer or contaminant migration in the aquifer will be affected by subsequent diffusional exchange of solutes in the fracture network with immobile, uncontaminated groundwater in the matrix (see, e.g. Bear *et al*. 1993, for a full description of such a dual-porosity system). A variety of local aquifer properties, such as pattern and intensity of fracturing, orientation of fracture network relative to groundwater head gradients, fracture spacing and effective size of matrix blocks, as well as the diffusion-exchange characteristics of the porous matrix, all influence the solute transport behaviour in a highly site-specific way.

Groundwater tracer tests are the only practical way to determine *in situ* the transport and matrix sorption of solutes for a specific site (e.g. Novakowski & Lapcevic 1994; Hartmann *et al*. 2007). This contrasts with indirect estimates of aquifer transport properties, obtained using pumping tests, for example, where results may be influenced by the volume of aquifer sampled with the hydraulic test, operational conditions for tests and assumptions used in their interpretation (Wealthall *et al*. 2001). A number of properties are required for an effective tracer of solute transport in dual-porosity

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media. In particular, the tracer must behave conservatively, have a near-zero background concentration and be readily detectable in the groundwater at high dilution factors (e.g. Ward *et al.* 1998). Such tracers generally fall into three categories: fluorescent dyestuffs, radioactive solutes and soluble halogenated solvent or gas molecules (e.g. SF_6). Fluorescent dyestuffs are the most frequently used tracers, as they have low toxicity and can be analysed easily (e.g. Smart & Laidlaw 1977). Furthermore, soluble halogenated solvent or gas molecules may not behave conservatively if they come into contact with a gas phase (e.g. in fractures at the interface of the saturated and unsaturated zone). Even under near-ideal field conditions such tests can be difficult to perform and to interpret (e.g. Riley *et al.* 2001). In an aquifer containing significant organic contamination a variety of additional factors may also confound the use of fluorescent (or other) tracers. The organic contaminants may produce a significant fluorescence at the analytical wavelengths used for tracer determination, contaminants may react with the tracers and quench or alter their fluorescence characteristics, and the tracer molecules may themselves be biodegraded where organic contaminants have stimulated biological activity within the aquifer. However, these problems cannot be avoided when tracer tests are undertaken at contaminated sites, as the test will usually need to be completed within the plume, to deduce the hydraulic properties of the aquifer along the plume flow path. In this study the application of two fluorescent dyes, which are commonly used as tracers of solute transport processes, is evaluated in the Chalk aquifer at a site that is contaminated with unleaded petroleum fuel hydrocarbons.

Site description

Background

The study site is a former retail petroleum filling station located on the Chalk aquifer in southern England. The Chalk aquifer is overlain by *c.* 11 m of Quaternary sediments, comprising predominantly sands and gravels. The upper part of the drift cover has been variably affected by urbanization and up to 4 m of made ground may be present. The aquifer is unconfined with an unsaturated zone thickness of *c.* 20 m, so the piezometric surface lies entirely within the Chalk (Wealthall *et al.* 2001). In February 1999 *c.* 525 000 l of unleaded petroleum fuel was accidentally released from an underground storage tank, contaminating the aquifer with petroleum hydrocarbons, composed mainly of benzene, toluene, ethylbenzene, xylenes (BTEX), and the ether oxygenate additives methyl *tert*-butyl ether (MTBE) and *tert*-amyl methyl ether (TAME). Upon recognition of a fuel leak, a groundwater management programme was established that included installation and routine monitoring of a network of wells at and down-gradient of the site. Treatment of the LNAPL source was carried out from 1999 to 2002 using a soil vapour extraction (SVE) system. Low vapour yields eventually led to discontinuation of the SVE system, although locally high BTEX and ether oxygenate concentrations in the unsaturated zone constitute a residual source of contaminated infiltration water to the saturated zone. In June 2002 the site was closed and in September 2002 six underground storage tanks were removed.

Groundwater flow at the site is toward the SSE in response to a regional-scale hydraulic gradient (*c.* 0.002), with water table fluctuations of ± 1.5 m over a 4 year period. Groundwater quality sampling using an extensive network of monitoring wells has identified dissolved phase contamination between 20 and 30 m depth in the saturated zone (Spence *et al.* 2005), with a mixed oxygenate–BTEX plume close to the site (<100 m) and an ether oxygenate-only plume further down-gradient (up to 300 m) (Fig. 1). Redox zones and biodegradation pathways within the plume have been characterized by a combination of chemical and isotopic techniques (Spence *et al.* 2005). Biodegradation of petroleum fuel hydrocarbons proceeds via a series of microbially mediated respiration reactions using different electron acceptors (oxidants) in the aquifer. These oxidants are often utilized during biodegradation in a sequence dictated by their relative energy yields per unit of organic carbon oxidized (e.g. Lyngkilde & Christensen 1992; Bottrell *et al.* 1995; Postma & Jakobsen 2000; Thornton *et al.* 2001), with the following general order: O_2, NO_3^-, Mn(IV), Fe(III), SO_4^{2-} (Table 1). The role of each electron acceptor in biodegradation of organic compounds depends on its availability (dissolved v. mineral-based form and quantity) in the aquifer and the ability (metabolic potential) of the indigenous micro-organisms to utilize it for biodegradation. In very reducing systems, where organic carbon loads are very high, fermentation of organic contaminants to methane can also occur. Close to the contaminant source (where the tracer tests were undertaken in this study) conditions are highly reducing: all dissolved oxygen and nitrate have been removed from the groundwater, sulphate reduction is active and free sulphide present, but methane concentrations are negligible (Spence *et al.* 2005).

Tracer injection, recovery and monitoring well network

Two monitoring well designs have been used in this tracer experiment. Their location at the site is shown in Figure 1. They consist of single-screen monitoring wells (labelled MW), where the screened interval extends from the water table *c.* 10 m into the saturated aquifer, and multi-level sampling wells (labelled MLS) (Einarson & Cherry 2002), fitted with seven monitoring ports, each containing a 10 cm screen. Each sample port on the

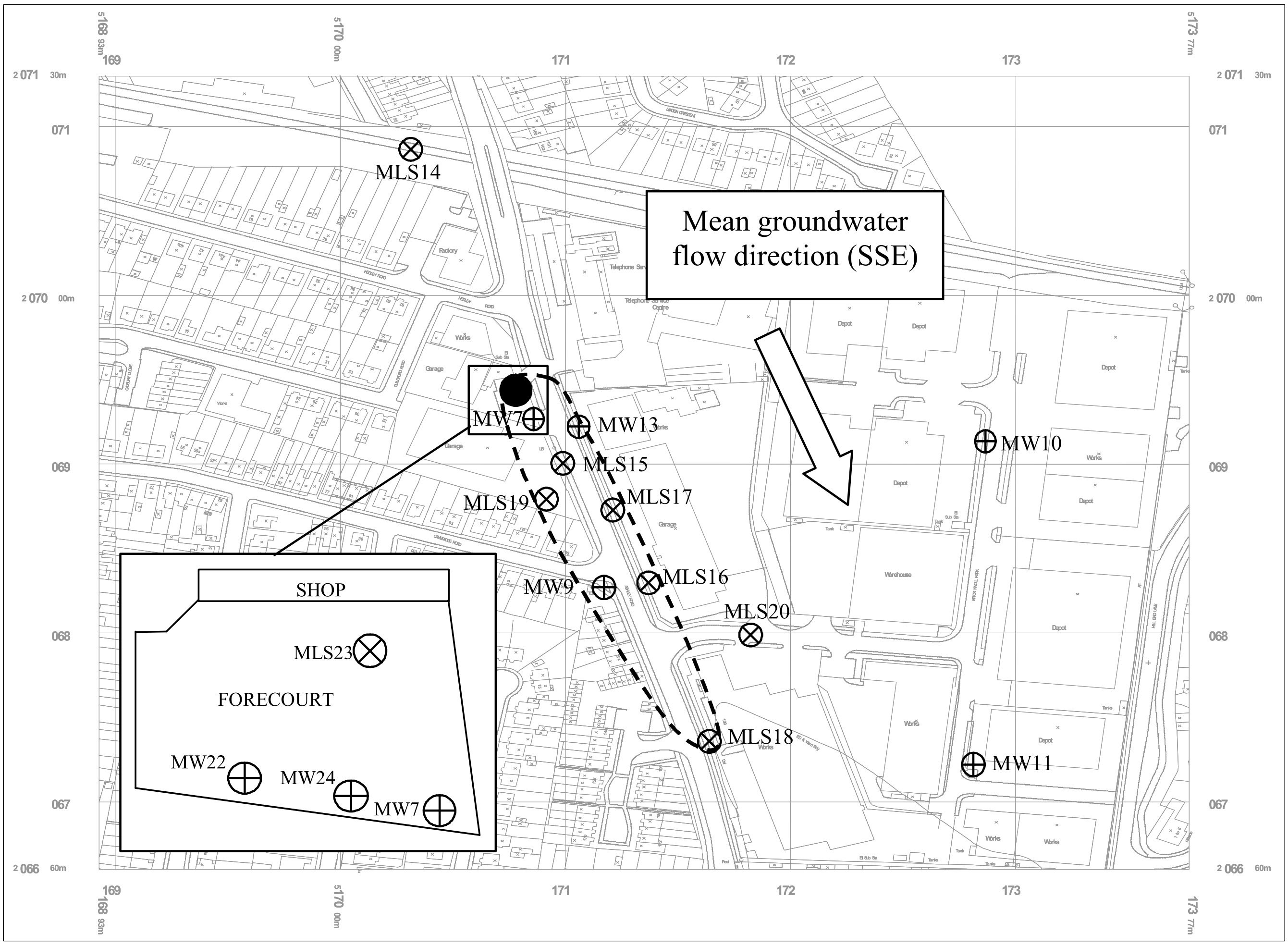

Fig. 1. Site map showing location of single screen monitoring wells (⊕), multilevel sampler (MLS) monitoring wells (⊗), the former filling station, mean groundwater flow direction and approximate location of the contaminant plume in June 2004.

Table 1. *Terminal electron accepting processes for toluene oxidation*

Microbial process	Degradation reactions for toluene (C_7H_8)	Reaction number
Aerobic degradation	$C_7H_8 + 9\mathbf{O_2} \rightarrow 7CO_2 + 4H_2O$	1
Nitrate reduction	$5C_7H_8 + 36\mathbf{NO_3}^- + H^+ \rightarrow 35HCO_3^- + 3H_2O + 18N_2$	2
Manganese(IV) reduction	$C_7H_8 + 18\mathbf{MnO_2} + 29H^+ \rightarrow 7HCO_3^- + 18Mn^{2+} + 15H_2O$	3
Fe(III) reduction	$C_7H_8 + 36\mathbf{FeOOH} + 65H^+ \rightarrow 7HCO_3^- + 36Fe^{2+} + 51H_2O$	4
Sulphate reduction	$2C_7H_8 + 9\mathbf{SO_4}^{2-} + 6H_2O \rightarrow 14HCO_3^- + 5H_2S + 4HS^-$	5
Methanogenesis	$2C_7H_8 + 10H_2O \rightarrow 5CO_2 + 9CH_4$	6

Electron acceptors are shown in bold.

MLS is located midway within a graded sand filter pack. Adjacent sample ports on the MLS are hydraulically isolated using bentonite seals, which allows level-discrete sampling from up to seven depth intervals in one borehole containing the MLS installation.

The tracer tests were completed using monitoring wells located broadly within the source area of the contaminant plume in the forecourt area of the former filling station (Fig. 1). MW7 was used as the pumped receiving well, which is directly down-gradient of MLS 23, which was used as the tracer injection well (Fig. 1). Additional measurements of tracer concentration were made in monitoring wells MW22, MW24, MW13, MLS15, MLS17 and MW9, which are located adjacent to, and down-gradient of, the pumped receiving well (MW7).

Materials and methods

Characterization of Chalk matrix

A number of core samples from the site were analysed for mineral and chemical constituents. Core samples were crushed to pass 150 mesh and dried at 105°C.

Accurately weighed samples were digested twice for 16 h (overnight) in excess 1M hydrochloric acid with continuous stirring, then repeatedly rinsed, dried at 105 °C and reweighed and rehomogenized. Weight loss on acid washing was assumed to represent the carbonate content of the samples. The insoluble residue was analysed for elemental composition (Carlo Erba 1106 elemental analyser for C (residual C is equivalent to organic C) and X-ray fluorescence analysis (XRF) for major elements) and mineralogical composition by X-ray diffraction (XRD). Details of these procedures have been given by Bottrell *et al.* (1999) and are summarized here.

X-ray fluorescence

Samples were prepared as fused beads and analysed using an ARL 9400 wavelength-dispersive sequential spectrometer calibrated against 49 certified reference materials. Fused bead preparation requires 0.3 g of material, a quantity that was not available for all insoluble residues. In these cases the available material was accurately diluted with spectroscopically pure silica and analyses were recalculated.

X-ray diffraction

A subsample was ground under acetone and pipetted onto the centre of a silicon holder and left to dry. The silicon slides are cut parallel to the (510) plane, as this contributes no background peaks in a normal Bragg–Brentano diffractometer. Diffractograms were acquired using Philips PW1050 goniometer with a Philips PW1730 generator and CuKα radiation. Minerals were identified from diffractogram peaks using data from the *Powder Diffraction File* search manuals (JCPDS 1980).

Fluorescence analysis

The fluorescence of groundwater samples collected during the pumped tracer test was determined on-site within 4 h of collection using a Perkin–Elmer LS-3 fluorescence spectrophotometer. Samples that were not analysed within a few minutes of collection were stored refrigerated at 4 °C. Samples from laboratory experiments were analysed immediately upon sampling. Only tracer samples from later sampling of monitoring wells when tracer tests were completed under natural gradient conditions were stored (chilled), for periods of up to 72 h.

Sodium Fluorescein and Amino-G Acid were chosen as tracers because of their reported low affinity for sorption and absence of overlap in their absorption and emission spectra (e.g. Smart & Laidlaw 1977). Initially, samples were analysed by constant offset scans, and separate scans with excitation and emission offsets appropriate to Sodium Fluorescein and Amino-G Acid were used for each sample. This allowed the fluorescence background of the sample at the analytical wavelengths of the tracers to be assessed and the size of the tracer peak above this background to be measured (see 'Background fluorescence' under Results below). Analyses were made at the peak analytical wavelengths and at one background offset once background fluorescence had stabilized. Dye standards were made up from aliquots of the injection or experimental solution and prepared by serial dilution in clean groundwater from a standpipe located upstream of the field site (which was checked for background fluorescence).

Background fluorescence of contaminated groundwater at tracer analytical wavelengths

The background fluorescence of contaminated groundwater (as a result of fluorescence of organic contaminant species present in samples) from MW7 was tested (as were distilled water and clean Chalk aquifer groundwater) by determination of the fluorescence spectrum of the sample either side of the wavelengths used for analysis of the tracer dyes. Scans performed were of the 'constant offset' or 'constant $\Delta\lambda$' type, where the excitation and emission wavelengths remain at a constant separation (that used for analysis of dye fluorescence). Samples were scanned at equal rate from below to above the normal analytical wavelength. Subsequently, separate aliquots of the same MW7 groundwater sample were spiked with very small volumes of a high-concentration solution of each tracer dye and identical constant $\Delta\lambda$ scans were performed.

Sorption of tracers to the aquifer matrix

Chalk rock core from an uncontaminated location in the aquifer was crushed and sieved to three grain-size ranges (0.5–1.0 mm, 1.0–2.0 mm and 2.0–6.0 mm), then washed to remove fines and sterilized by autoclaving. The tracer dyes Sodium Fluorescein and Amino-G Acid were added to fragments of Chalk rock core from each size range in separate experiments. Core material (100 g) was placed in a 400 ml sterilized glass jar and 50 ml of dye solution (*c.* 0.1 mg l^{-1} dye in distilled water equilibrated with clean Chalk fines) added and allowed to equilibrate for 72 h. Experiments showed that sorption was completed after this interval. The jars were closed with modified pierced lids containing a Whatman filter, so they could be drained by inversion whilst retaining the solids. The jars were then drained and the solution replaced on a 24 h cycle with distilled water that had previously been equilibrated with clean Chalk fines. Each solution drained from the equilibrated core material was analysed for dye fluorescence.

Chemical interference and biodegradation of tracer dyes in the contaminant plume

To investigate the possible interaction between the selected tracer dyes and either the contaminants present in the plume or the indigenous microbial population, two sets of microcosm experiments were performed. Microcosm Set A comprised two experiments with a common control to evaluate chemical interactions in an abiotic (sterile) system. The control consisted of boiled distilled water that was equilibrated with sterile Chalk slurry for 72 h before being placed in a sterile glass bottle. Experiments 1 ('contaminants') and 2 ('contaminants + sulphide') both consisted of filter-sterilized (0.2 μm nylon) MW7 groundwater in a sterile glass bottle, but experiment 2 had sufficient solid Na_2S added to yield a final concentration of $50\,mg\,l^{-1}\,HS^{-}$. Experiment 1 investigated any direct influence of contaminants present on the fluorescence analysis of tracer dyes. Experiment 2 was used to simulate SO_4-reducing conditions in the plume and assess any additional effect of dissolved sulphide produced from this biological process on tracer detection. All experiments consisted of the same volume of liquid. Equal spikes of both Sodium Fluorescein and Amino-G Acid were added to all three bottles and thoroughly mixed. Samples were taken, filtered and immediately analysed for dye fluorescence after various time intervals.

Microcosm Set B comprised a triplicate active experiment with duplicate sterile control to investigate possible biodegradation of the dye compounds in the aquifer during the tracer tests. Each microcosm comprised a sterilized glass bottle with an equal mass of sterilized Chalk slurry and equal spikes of both Sodium Fluorescein and Amino-G Acid, to which the same volume of either unfiltered MW7 groundwater (experiments) or boiled distilled water (controls) was added. Water samples were taken immediately after preparing the experiments and at approximately weekly intervals for 10 weeks thereafter. The experiments were maintained at 10 °C, close to *in situ* groundwater temperature (Spence *et al.* 2005). Samples were filter-sterilized (0.2 μm) and stored overnight at 2 °C prior to analysis for dye fluorescence.

Tracer test geometry, tracer injection and groundwater sampling

The tracer test was conducted in two phases. In the first phase, a forced gradient tracer test was attempted, using two monitoring wells (MW7 and MLS 23), whereas in the second phase, a natural gradient tracer test was performed, immediately after the first phase and using a larger number of monitoring wells for groundwater sampling. These phases are described separately.

For the forced gradient test the single screen (19–29 m below ground level (b.g.l.)) monitoring well (MW7) was pumped for 6 h prior to dye injection. During this time drawdown at the injection well, MLS23, in sampling ports covering the screen interval of MW7 reached 0.05 m. Two tracer dye solutions were injected at shallow depth into the sampling ports of MLS23: Sodium Fluorescein into sampling port 1 at 23.00 m b.g.l. and Amino-G Acid into sampling port 2 at 24.75 m b.g.l. The two dye solutions were injected simultaneously as a pulse at the sampling port level using a dual-channel peristaltic pump. The tracer was contained in an initial 2 l solution and a further 8 l of clean groundwater was then injected to purge all tracer from the filter pack surrounding the sample port into the aquifer. All calculations of volumetric tracer recovery are based on the 10 l total injection volume. Injection of dye and purge volumes took 30 min; during injection, drawdown at MLS 23 (monitored at a sampling port below those used for injection) decreased by 0.008 m.

Well MW7 was unable to sustain the target pumping rate of $0.60\,m^3\,h^{-1}$ and the drawdown in MW7 varied considerably during the tracer test. During this period the discharge from MW7 was sampled at 10 min intervals. For logistical reasons the tracer test could not continue over weekends, so pumping was ceased for 69 h. In the second week of the tracer test MW7 was only pumped during the day (*c.* 9 $h\,day^{-1}$) for 3.5 days and sampled every 15 min during pumping. At the end of the pumped test a number of monitoring wells (MW9, MW13, MW22 and MW24; Fig. 1) were sampled for tracer analysis. Following the end of the forced gradient test the experiment continued as a natural gradient tracer test. MW7 was pumped again for 9 h and sampled 3 weeks later (28 July 2003) along with other monitoring wells adjacent to, and down-gradient of, MW7. Subsequently, a number of source area and downstream monitoring wells in the plume were sampled at intervals up to 10 months after the initial test.

Results and discussion

Chemical and mineralogical composition of Chalk core samples

Proximate and elemental analyses of 12 Chalk samples from cores recovered during site investigation are given in Table 2. All samples are dominated by calcium carbonate (all >85% $CaCO_3$, 11/12 > 90% $CaCO_3$, 7/12 > 95% $CaCO_3$). Organic content is very low; the organic carbon content of all samples falls in the range 620–2000 $mg\,kg^{-1}$. The mineralogy of the non-carbonate fraction of the 12 Chalk samples was determined by XRD. All samples contained major quartz with variable quantities of illite–muscovite, heulandite and feldspars (albite and microcline). Four samples also contained trace tridymite.

Background fluorescence of contaminated groundwater at tracer analytical wavelengths and correction for its effects

Scans of sample fluorescence for contaminated groundwater from MW7 and distilled water are presented in Figure 2. Scans were performed close to the analytical wavelengths for Sodium Fluorescein and Amino-G Acid

Table 2. *Proximate and elemental analysis of Chalk samples*

ID:	R1	R10	R26	R30	20A	20B	20C	20D	20E	20F	20G	20H
Depth (m):					35.3	51.1	40.0	43.1	33.5	27.5	49.1	31.8
$CaCO_3$	96.88	97.19	93.05	96.29	96.95	93.67	95.59	96.01	90.36	96.68	85.07	93.84
SiO_2	1.79	1.50	4.18	1.97	1.70	4.40	2.57	2.31	5.08	1.60	9.48	3.27
Al_2O_3	0.33	0.40	0.93	0.52	0.43	0.54	0.59	0.55	1.26	0.46	1.17	0.85
Fe_2O_3	0.17	0.13	0.22	0.16	0.12	0.14	0.21	0.15	0.34	0.14	0.27	0.26
TiO_2*	139	159	458	211	210	275	290	292	641	176	569	400
Mn_3O_4*	3	3	5	4	3	4	4	3	8	3	10	6
MgO*	314	284	697	374	307	634	443	401	966	335	1494	618
CaO*	49	69	1045	201	150	379	274	269	2761	151	4882	894
Na_2O*	686	626	523	665	669	766	734	694	1386	817	1356	1124
K_2O*	885	833	1849	1088	877	1209	1251	1140	2399	866	2404	1591
P_2O_5*	4	3	7	4	3	4	4	6	21	7	15	9
Cr_2O_3*	4	4	9	6	5	6	6	5	13	5	12	8
C_{org}*	655	683	334	935	320	639	622	622	1031	1962	702	1016
Total	99.58	99.63	99.00	99.50	99.55	99.25	99.47	99.50	98.23	99.67	97.25	99.03

Samples A–H are from the same core recovered from MW20. All components are reported in weight per cent of original dried sample mass except those indicated with an asterisk.

* Components reported as parts per million by mass of the original dried sample.

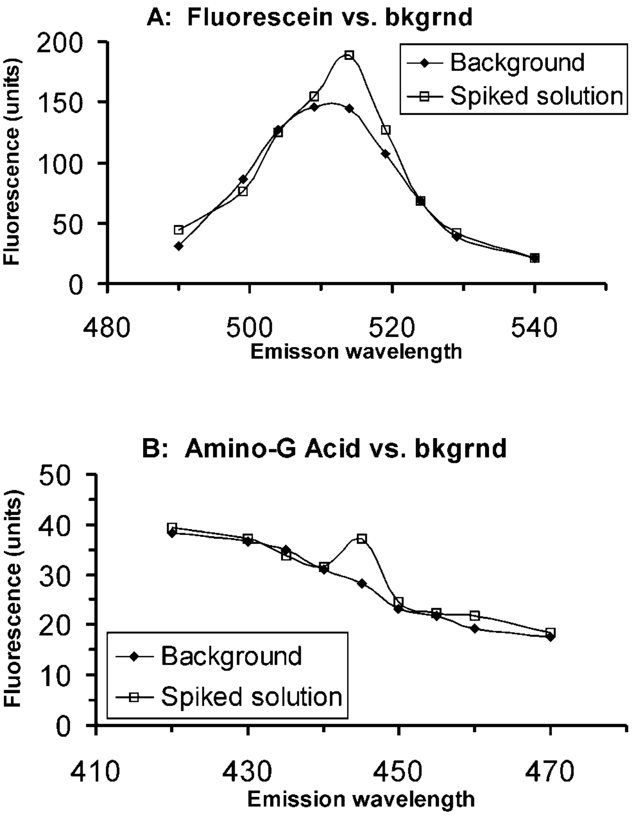

Fig. 2. A constant Δλ plot comparing background fluorescence of contaminated groundwater (‘Background’ trace) and a similar groundwater sample containing a tracer dye (‘Spiked solution’ trace) close to the analytical wavelengths. (**A**) Sodium Fluorescein; (**B**) Amino-G Acid. (Note different fluorescence scales between the two traces).

and at constant Δλ (excitation, emission). There is clearly a fluorescence background imparted by the groundwater contaminants in both cases. This background is much higher for Sodium Fluorescein, probably because of the smaller Δλ used (23 nm, v. 90 nm for Amino-G Acid). Figure 2 also shows scans for the same sample spiked with low concentrations of Sodium Fluorescein or Amino-G Acid. The excess fluorescence associated with the tracer dye is clearly visible as a narrow peak superimposed on the broader ‘hump’ of the background fluorescence. To account for the effect of background fluorescence in groundwater samples taken during the tracer test, a significant number of samples were scanned for fluorescence intensity rather than simply analysed at the standard analytical wavelengths. This allowed the background fluorescence intensity to be measured (where no tracer dye was present) to: (1) ascertain the presence or absence of tracer dye; (2) quantify tracer dye concentration, where present; (3) establish the variation in fluorescence background during the tracer test for treatment of the full dataset.

The last of these demonstrated that the background fluorescence for Sodium Fluorescein fell pseudo-exponentially during the period of (semi-)continuous pumping in the first week of the tracer test, the initial rapid exponential fall becoming linear with time (Fig. 3, R^2 for a linear fit to background fluorescence with time after 29.5 h is 0.998). The rapid initial fall in background fluorescence indicates that progressively more clean groundwater was drawn into the cone of influence of MW7 in the early stages of the tracer test, and that this mixture more or less stabilized after 29.5 h. Although the continued presence of a high background fluorescence in these groundwater samples prohibits the detection of very low concentrations of tracer (<0.35 μl tracer solution l^{-1}, equivalent to 1.8 μg l^{-1} Sodium Fluorescein), proper treatment of the data allows successful detection and realistic quantification of concentrations higher than this. The lower background fluorescence at the analytical wavelengths for Amino-G

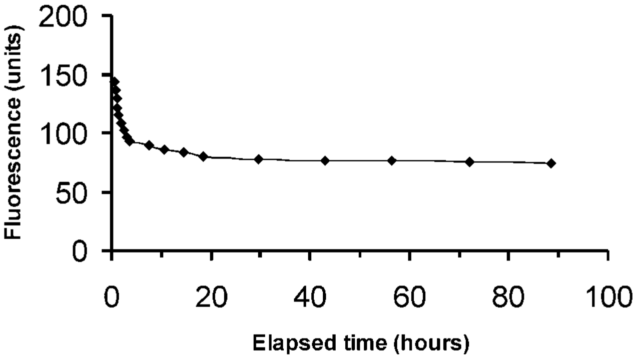

Fig. 3. Change in fluorescence background at Sodium Fluorescein analytical wavelengths during week 1 of the tracer tests.

Acid gives a detection limit of 0.08 µl tracer solution l^{-1}, equivalent to 0.38 µg L^{-1}. Uncertainties (1σ) in the background corrected fluorescence values are <2 units for Sodium Fluorescein (equivalent to ± 0.14 µl tracer solution l^{-1} or 0.7 µg l^{-1} Sodium Fluorescein) and *c.* 1 unit for Amino-G Acid (equivalent to ± 0.03 µl tracer solution l^{-1} or 0.15 µg l^{-1} Amino-G Acid). Calibration of fluorescence response v. concentration for both Sodium Fluorescein and Amino-G Acid was made using clean groundwater standards (as described above) and from the spiked additions to contaminated groundwater. On short time scales the two approaches give identical responses. For simplicity, it was decided to use clean groundwater standards to calibrate laboratory analyses and to make subsequent allowance for the longer-term fluorescence quenching observed in the laboratory experiments (see below).

Sorption of tracers to the aquifer matrix

The sorption characteristics of the dyes were investigated experimentally to determine whether adsorption of tracer dye to mineral and organic components of the Chalk aquifer matrix could significantly retard tracer migration, such that sorption could 'mimic' storage of tracer by diffusion into matrix pore-water. The sorption characteristics of the dyes intended for use as tracers were thus investigated experimentally. It is not possible to deduce the contribution of tracer sorption by different mineral and organic sorbents in the matrix, but instead we can evaluate the effect of sorption to the aquifer matrix surface in general. The sorption experiments were carried out at different matrix 'grain sizes' to investigate the effect of variable surface area:volume ratios. This approach is broadly analogous to assessing the effect of tracer sorption at different matrix block sizes in the aquifer, considering the variation in fracture and microfracture intensity that is often found (Spence *et al.* 2005). At higher ratios (smaller grain sizes) the effect of sorption to the matrix surface should be more pronounced, whereas with larger grain sizes diffusion of tracer into the porous grains will become more important in determining tracer behaviour. There was no significant sorption of Amino-G Acid in either experiment 1 or 2 (0.5–1.0 and 1.0–2.0 mm size range), but there was reduced recovery of this tracer in experiment 3 (2.0–6.0 mm size range) (Table 3). This is consistent with minimal sorption of Amino-G Acid to the matrix surface and retention of this tracer within the larger porous grains in experiment 3. In contrast, there was low recovery of Sodium Fluorescein in experiment 1, indicating a greater affinity for surface sorption than Amino-G Acid (Table 3). However, neither dye is strongly attenuated by sorption, as would be expected for the Upper Chalk aquifer, which contains only low proportions of clays and organic matter (see Table 2). The relative affinities of Sodium Fluorescein and Amino-G Acid with regard to surface sorption are consistent with those found by Riley *et al.* (2001) for limestone materials.

Chemical interference and biodegradation of tracer dyes in the contaminant plume

The fluorescence of both Sodium Fluorescein and Amino-G Acid in subsamples from microcosm Set A (abiotic experiments) is given in Table 4. Experiments containing contaminants had a background fluorescence at the analytical wavelength for both dyes, which was corrected for the analysis of true tracer concentration as discussed above. In both 'Contaminants' and 'Contaminants + sulphide' experiments, the fluorescence of Amino-G Acid was always within 3% of that in the clean water control. Given the likely error introduced by background correction of the experiments, there is no evidence to support any effect of contaminants or contaminants + sulphide on the fluorescence of this dye. In the 'Contaminants' experiment, Sodium Fluorescein fluorescence was close to the control (97%) after 1 h but declined progressively to 88 and 89% of blank fluorescence at 168 and 336 h, respectively. This progressive decline indicates that the difference is not simply a result of the background correction procedure, but a real effect of interaction between Sodium Fluorescein and the contaminant mixture. There is a similar, but more marked, effect on fluorescence of Sodium Fluorescein in the 'Contaminants + sulphide' experiment, with fluorescence at 91% of the control value at 1 h falling to 79 and 78% at 168 and 336 h, respectively. This stronger interaction may result from 'fluorescence quenching' of Sodium Fluorescein by sulphide (see e.g. Axelrod *et al.* 1969).

Dye fluorescence data for the microcosm Set B experiments that investigated potential loss of tracer by biodegradation are shown in Table 5. Experiments containing contaminants had background fluorescence at the analytical wavelength for both dyes, which was corrected for as discussed above. In these experiments, Sodium Fluorescein exhibits very similar behaviour to that seen in the analogous Set A 'Contaminants' experiment, falling to 93%, 93% and 89% of the control value

Table 3. *Recovery of Sodium Fluorescein and Amino-G acid in solution from dye sorption experiments*

Experiment number:	1 (0.5–1.0 mm)		2 (1.0–2.0 mm)		3 (2.0–6.0 mm)	
Dye:	Flcn	AG	Flcn	AG	Flcn	AG
% recovery, leach A	36.4	48.9	39.2	41.3	35.5	37.1
% recovery, leach B	25.3	27.9	27.7	29.9	25.0	27.0
% recovery, leach C	16.1	14.1	16.1	15.9	13.8	13.9
% recovery, leach D	9.2	6.9	9.0	8.4	7.0	7.1
% recovery, leach E	4.7	3.3	4.4	3.9	3.8	3.5
Cumulative recovery	91.7	101.1	96.4	99.4	85.1	88.6

Flcn, Sodium Fluorescein; AG, Amino-G Acid.

Table 4. *Fluorescence intensity of Sodium Fluorescein and Amino-G Acid in experiments to investigate interaction of dyes with groundwater contaminants at the field site*

Time elapsed (h)	Dye	Fluorescence				
		Clean	Contaminants		Contaminants + sulphide	
		Units	Units	% of ‘clean’	Units	% of ‘clean’
1	Fluorescein	117	113	97	106	91
	Amino-G Acid	96	98	102	97	101
24	Fluorescein	114	108	95	95	83
	Amino-G Acid	91	94	103	92	101
96	Fluorescein	121	110	91	96	79
	Amino-G Acid	97	98	101	95	98
168	Fluorescein	112	99	88	86	77
	Amino-G Acid	90	90	100	88	98
336	Fluorescein	109	97	89	85	78
	Amino-G Acid	88	86	98	85	97

Fluorescence is given in units, corrected for background fluorescence in the experiments with contaminants added. For the ‘contaminants’ and ‘contaminants + sulphide’ experiments the fluorescence of each dye is expressed as a percentage of the clean water blank experiment of the same duration.

Table 5. *Fluorescence intensity of Sodium Fluorescein and Amino-G Acid in microcosm experiments to investigate potential biodegradation of dyes under groundwater conditions at the field site*

Elapsed time (days)	Control 1		Control 2		Exp. 1		Exp. 2		Exp. 3	
	Flcn	AG	Flcn	AG	Flcn	AG	Flcn	AG	Flcn	AG
0	138	102	130	97	136	100	133	94	133	98
3	133	97	125	94	132	98	128	91	129	91
10	129	98	127	92	129	91	128	90	128	84
17	131	95	125	95	131	81	124	86	123	71
24	129	95	124	91	123	67	125	71	120	50
31	125	96	120	93	122	33	120	55	119	29
38	128	93	123	92	119	21	118	46	118	13
45	128	95	121	90	120	14	120	38	113	7
52	123	94	122	94	117	11	115	35	115	6
59	126	92	125	91	115	12	117	32	113	7
67	124	94	121	91	116	10	113	28	108	4

Fluorescence is given in units, corrected for background fluorescence in the experiments with contaminants added. Flcn, Sodium Fluorescein; AG, Amino-G Acid.

in Experiments 1, 2 and 3, respectively. The presence of aquifer micro-organisms in the unfiltered MW7 groundwater thus had no discernible additional effect on Sodium Fluorescein dye. In contrast, in the same experiments the fluorescence of Amino-G Acid fell very markedly compared with the controls; fluorescence fell to 11, 30 and 4% of the control value in Experiments 1, 2 and 3, respectively. In the sterile microcosm Set A experiments there was no significant fall in fluorescence (Table 4). This demonstrates that the indigenous

Table 6. *Dye recovery at MW7 during forced gradient tracer test*

Dye recovered and time	Recovery
Sodium Fluorescein	
Week 1	First recovery at 970 min Peak concentration 6 μl l^{-1} of dye solution at 1380 min Concentrations erratic but generally fall to end of continuous monitoring
Week 2	Dye concentrations erratic but increase after 165 h to a peak of 3 μl l^{-1} of dye solution at 212 h
Week 5	Concentrations very erratic but maximum concentration observed (72 μl l^{-1} of dye solution) at 838 h
Amino-G Acid	
Week 1	First recovery at 2070 min Peak concentration 2.2 μl l^{-1} of dye solution at 2070 min Concentrations decline erratically to zero at 354 min
Week 2	Dye concentrations erratic but increase after 163 h to a peak of 3 μl l^{-1} of dye solution at 188.5 h
Week 5	Concentrations zero or very low (0.7 μl l^{-1})

microbial populations introduced in unfiltered MW7 groundwater had the potential to very significantly degrade Amino-G Acid on time scales of tens of days.

Tracer test results

Tracer recovery is reported as excess fluorescence. Equivalent concentrations of recovered tracers are reported as microlitres of the original 10 l dye solution injected per litre of sample, at this stage uncorrected for any fluorescence quenching effects. Dye breakthrough curves (as background-corrected fluorescence) for week 1 of the test (continuous monitoring) and week 2 (semi-continuous monitoring) are given in Figure 4. Time of recovery and concentration of the two dyes during the forced gradient test is summarized in Table 6. Groundwater samples from a number of monitoring wells were subsequently analysed for fluorescence of Sodium Fluorescein only, and these data are reported in Table 7.

Tracer test interpretation

It was initially intended to apply a radial flow solution to the forced gradient phase of the tracer test (e.g. Atkinson *et al.* 2000; Hartmann *et al.* 2007), but the observed asymmetric evolution of the tracer plume and failure to maintain a constant pump rate mean that only qualitative interpretations can be made. The initial breakthrough times of tracer dye indicate rapid transport, 29.7 m day^{-1} for Sodium Fluorescein injected at MW23-level 1 and 13.9 m day^{-1} for Amino-G injected at MW23-level 2. The rapid transport velocities are characteristic of a well-developed interconnected fracture flow system contributing to groundwater transport at these shallow depths below the water table. The total tracer recovered during the initial breakthrough phase in week 1 of the test is, however, a very small fraction (*c.* 15 ml of the 10 l Sodium Fluorescein solution (0.15%) and only *c.* 2.5 ml of the 10 l Amino-G Acid solution (0.025%)) originally injected. The effects of fluorescence quenching will not significantly increase these recoveries. Rapid flow through the fracture flow system is thus probably not the most important mechanism responsible for tracer migration between MLS23 and MW7.

When pumping recommenced after the weekend break there was a significant delay before either tracer was detected in the MW7 effluent (Fig. 4). This is attributed to poor connection of the inflow to MW7 to the fracture pathways used by the majority of the tracer. Thus tracer-free groundwater surrounded MW7 immediately after the break. In the second week of the pumping test there was a rather erratic time-series of concentrations for both dyes, but at similar low concentration and recovery to the first week of pumping. At the end of the second week of pumping, groundwater samples from a number of other monitoring wells were analysed for dye fluorescence (3 July 2003 data, Table 7). At this time Sodium Fluorescein was also found in MW22 (at 75% of the excess fluorescence in MW7), but not in MW24, located between MW22 and MW7 (Fig. 1). No Amino-G Acid was detected in either MW22 or MW24. This indicates that the flow field during pumping must be extremely anisotropic at the scale of the experiment, probably as a result of the orientation of open fractures within the Chalk. Unfortunately, it was anticipated that the flow field during pumping would have been of a more or less radial geometry, so MW22 and MW24 were not sampled during the first (forced gradient) phase of the test.

On 28 July 2003 MW7 was pumped again for 9 h and sampled four times, with MW22, MW24 and MW13 also being sampled (Tables 6 and 7). During this time the maximum Sodium Fluorescein fluorescence observed at MW7 was far higher than during the earlier forced gradient test (corresponding to a concentration of 72 μl l^{-1} of injected dye solution). Sodium Fluorescein fluorescence also increased at MW22 and MW24 (corresponding to 11.2 and 5.3 μl l^{-1} of injected dye solution, respectively). Clearly, the migration of tracer dye in a southwesterly direction from MLS23 had continued to occur under natural gradient conditions. However, despite showing rather similar behaviour to Sodium Fluorescein during the earlier parts of the test, Amino-G Acid was barely detectable in MW7 at this time (and was not detected in MW22 or MW24). This is attributed to attenuation of this tracer by biodegradation, as

Table 7. *Tracer recovery during the natural gradient phase of the tracer test*

Borehole: Distance:	MW7 24 m	MW22 24 m	MW24 19.5 m	MW13 38 m	MLS15 52 m	MLS17 82 m	MW9 100 m	23(inj) 0
Date								
End test 3/7/03	24	18	n.d.	n.d.	n.a.	n.a.	n.d.	n.a.
28/7/03	654*	102	48	n.d.	n.d.	n.a.	n.a.	n.d.
28/8/03	526	n.a.	n.a.	n.d.	n.d.	n.a.	n.d.	n.a.
27/10/03	317	196	94	47	n.d.	n.d.	n.d.	n.d.
26/11/03	221	n.a.	n.a.	320	n.d.	n.a.	n.d.	n.a.
16/1/04	74	300	158	650	n.d.	n.d.	n.d.	n.a.
22/2/04	64	n.a.	n.a.	497	n.d.	n.a.	n.d.	n.a.
24/3/04	52	n.a.	n.a.	162	n.d.	n.d.	n.d.	n.d.
24/4/04	45	159	60	59	3.5 in 15-5 only	n.d.	n.d.	n.d.

Values are excess fluorescence at Sodium Fluorescein wavelength. n.a., no sample analysed; n.d., sample analysed but tracer not detected.
* Highest concentration obtained during 8 h pumping.

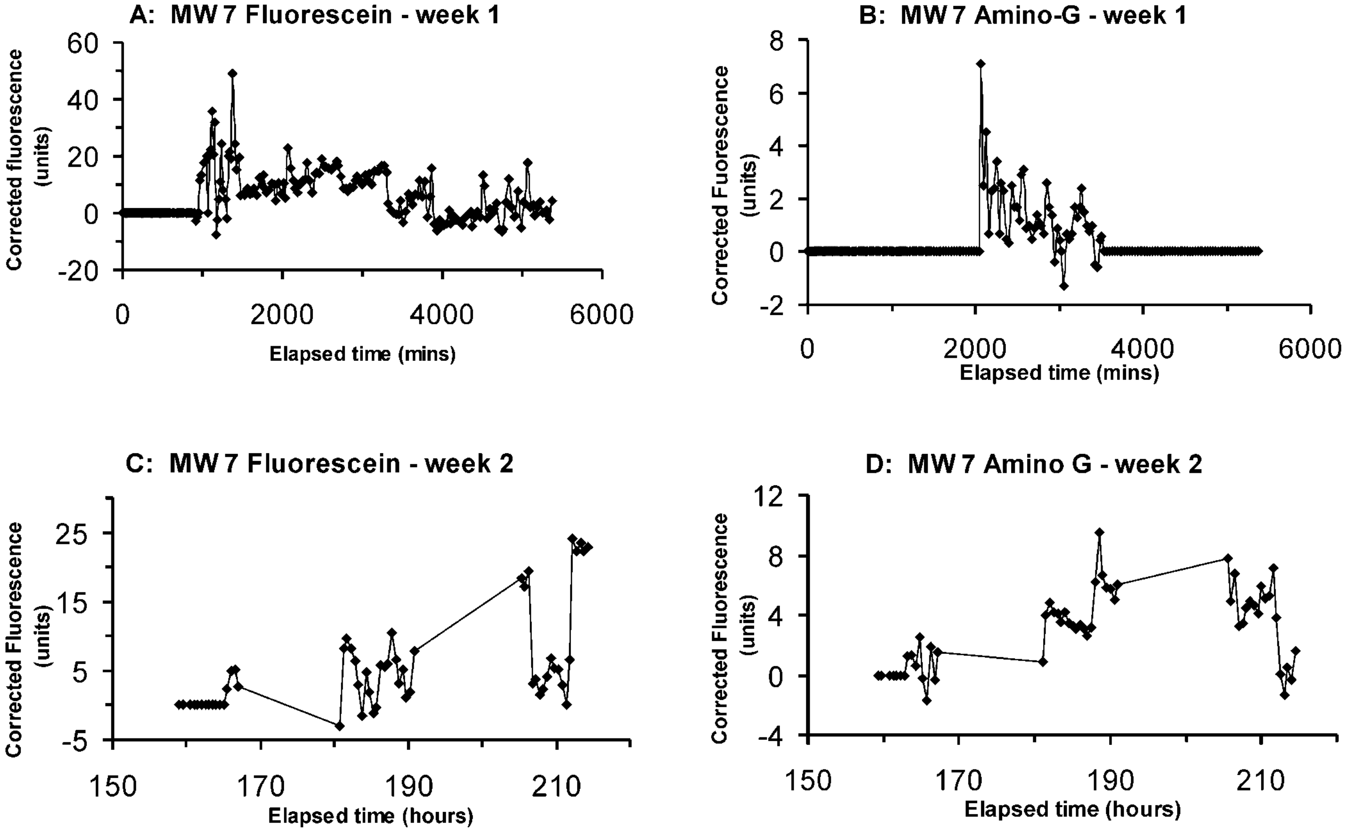

Fig. 4. Tracer breakthrough curves at MW7. All times are relative to tracer injection at MLS23. (**A**) Sodium Fluorescein, during week 1; (**B**) Amino-G Acid, during week 1; (**C**) Sodium Fluorescein, during week 2; (**D**) Amino-G Acid, during week 2.

observed in the live aquifer microcosm experiments (see above).

The evolution of the Sodium Fluorescein tracer 'cloud' in the groundwater was subsequently monitored as an extension of regular groundwater quality sampling at the site (Table 7). Over the subsequent 9 months, Sodium Fluorescein fluorescence gradually decreased at MW7, whereas it peaked at MW22 and MW24 in the mid-January sample, 7 months after initial injection of the tracer. First breakthrough of tracer at MW13 (transverse to MW7) occurred in the late October sampling, peaking in mid-January, and first breakthrough of tracer at sampling port 5 of MLS15 (downgradient of MW7) occurred in late April 2004. The continued spread of tracer from MLS23 toward MW22 and MW24 under the SSE natural hydraulic gradient also attests to the strong anisotropy caused by fractures in the Chalk aquifer at a scale of *c.* 10 m.

Tracer velocities during the natural gradient phase of the tracer test are calculated assuming: (1) MW7 and 3 July 2003 as the starting point for first arrival calculations (the tracer cloud had certainly reached this point at this time and should not have passed it, so this is a good start point for first arrival calculations); (2) MW7 and

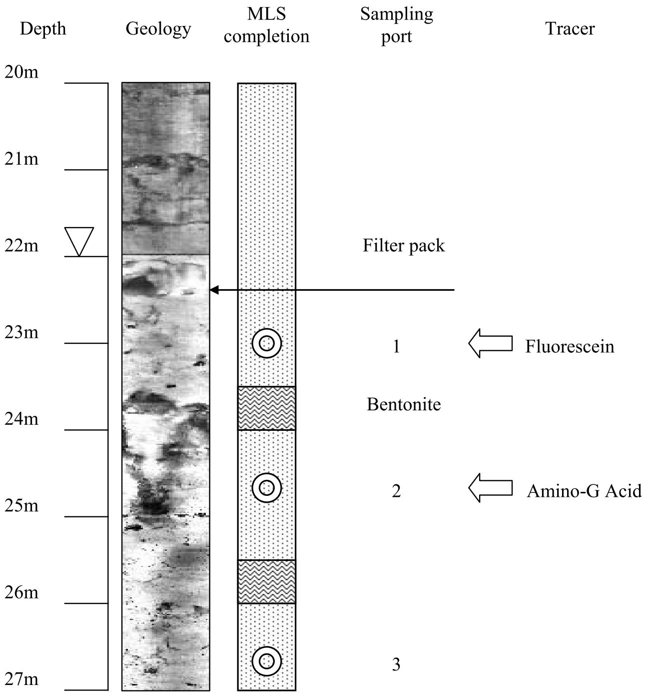

Fig. 5. Composite schematic illustration comparing fracture locations identified by optical televiewer log with location of sampling ports and tracer injection on MLS completion used as the injection well (MLS23).

28 July 2003 (the observed peak at MW7) for the peak of the tracer cloud. This gives average groundwater velocities of 0.137 m day^{-1} and 0.101 m day^{-1} for first arrival of the tracer at MW13 and MLS15, respectively, and 0.083 m day^{-1} for the migration of the tracer peak between MW7 and MW13.

Synthesis

The strong anisotropy in tracer migration indicated by the results of the tracer test presumably results from the development of fissures with different orientations in the Chalk aquifer, with the best developed and interconnected fissures being almost transverse to the natural hydraulic gradient at this site. A similar deviation from radial flow was observed in fractured limestones by Riley *et al.* (2001). The source of this anisotropy in permeability and influence on tracer migration may be the location and orientation of fractures relative to the tracer injection points used in the tests. Figure 5 is an optical televiewer (OPTV) image of the open borehole at MW23, prior to the installation of the MLS monitoring well. The image shows that there are significant fissures in the formation located within the zone (between MLS ports 1 and 2) used to inject the tracers. Previous investigation at this site indicated that there are strong ENE–WSW-trending vertical fractures and shallow SSE-dipping bedding plane fractures (Wealthall *et al.* 2001, 2002). If the high-angle fractures in the OPTV image of MLS23 are part of the ENE–WSW-trending set then they will be oblique to the general groundwater flow direction (SSE). This would either transport the tracer along a preferential flow path that bypasses MW7 or transport large volumes of tracer-free groundwater to MW7 if the tracer is not injected into a connecting fracture. In each case, there would be a mechanism to carry the tracer plume away from MW7 and account for the field observations. Over longer distances the tracer will migrate along a circuitous route within this fracture network, in the direction of the mean groundwater flow. This explains the detection of tracer in several down-gradient monitoring wells during the natural gradient experiment, several months after the injection in MLS23, where the effect of the local-scale anisotropy is averaged out during transport over greater distances.

Whereas Amino-G Acid proved to be useless as a long-term tracer because of its susceptibility for biodegradation by aquifer micro-organisms, Sodium Fluorescein continued to function as a conservative tracer during subsequent monitoring under natural gradient conditions. The observed long-term tracer velocities (*c.* 0.1 m day^{-1} or 35–40 m a^{-1}) are consistent with the observed size of the MTBE plume at the time of sampling and retardation–attenuation only by diffusional exchange with matrix pore-water. The smaller size of the BTEX plume is clear evidence for highly effective natural attenuation of BTEX components by biodegradation as described by Spence *et al.* (2005).

Conclusions

Tracer tests with fluorescent dye tracers can yield useful results on aquifer hydraulic properties, even in aquifers heavily contaminated with organic compounds, if care is taken in tracer selection, detection and analysis. Amino-G Acid seemed to be a good choice of tracer, because of its low sorption to the Chalk aquifer matrix and low interference owing to contaminants, but it proved to be severely affected by biodegradation by aquifer micro-organisms on a time scale of tens of days. This implies that concentrations of Amino-G Acid can be attenuated more by biodegradation process in plumes than by physical processes (e.g. advection, dispersion, diffusion) when this dye is used in long-term tracer tests for the characterization of contaminated sites. The use of Amino-G Acid as a ‘conservative’ tracer in these circumstances could lead to overestimation of tracer attenuation by physical processes in the aquifer and consequently an underestimation of solute transport velocity. In contrast, Sodium Fluorescein sorbed more strongly in laboratory tests using aquifer materials under representative conditions, but proved to be an effective tracer, as it was still readily detected in groundwater after 300 days, even though it was found to undergo some quenching of fluorescence in the contaminated groundwater matrix. These observations indicate that tracer properties, groundwater chemistry and duration of the measurement period should be considered when tracer tests are undertaken at sites contaminated with petroleum hydrocarbons.

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