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**Bacteriophage response characterization  
in highly permeable  
porous media aquifers (Switzerland)**

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by  
Keith Gordon Kennedy

M. Sc. (Univ. of Waterloo, Canada – Earth Sciences-Hydrogeology)  
B. Sc. Honors (Univ. of Manitoba, Canada - Geology)

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# IMPRIMATUR POUR LA THESE

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La Faculté des sciences de l'Université de  
Neuchâtel sur le rapport des membres du jury,

MM. F. Zwahlen (directeur de thèse), I. Müller,  
M. Aragno, J. Buffle (Genève), H. Hoetzi (Karlsruhe D)  
et R.W. Harvey (Boulder, Colorado, USA)

autorise l'impression de la présente thèse.

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F. Zwahlen

# Abstract

Breakthrough responses of biocolloid particles, specifically bacteriophages (phages), were compared to those of more classical tracer solutes, salt and uranine, during transport in heterogeneous, highly permeable granular porous media. Research focused on phage performance and characterization in a 6 m laboratory tank model and at three ground water field sites in gravel aquifer settings (Switzerland).

The MARCEAUS tank facility (Univ. Louis Pasteur, Inst. Fluid Mechanics, Strasbourg) with dimensions of 1 m by 1 m by 6 m was used to distinguish flow path variability and rank mass transport rate/recovery of six different bacteriophages (Marine-H4, H6, H40, Non-marine-T7, Psf2, MS2) in a moderately heterogeneous sandy porous media. Phages were co-mingled with salt and uranine and input with a consistent (~4 h) step signal. Testing was done under controllable gradient, temperature and chemistry conditions. Salt levels were monitored in-situ in real time with 353 conductivity probes. Phage and uranine samples were taken from six internal and the outflow locations. Salt and uranine transport responses were similar. Phage recovery, normalized to the solutes' recovery (relative breakthrough - RB) changed according to phage type, location within the tank's heterogeneity and the test water's ionic strength. RB decreased consistently when EC reduced from 3700 to 700  $\mu\text{S cm}^{-1}$ . RB ranged from 5 to 94% (H40), 3 to 59% (MS2), 4 to 38% (H4), 0 to 33% (Psf2) and 0.3 to 11% (H6). Total recovery varying at the outflow was 28 to 51% (H40-5 tests), 25 to 31% (MS2-2 tests), 24% (H4), 2 to 4% (H6-2 tests), and 1% (Psf2). T7 was never detected. Velocity, based on first detection and normalized to flow rate varied with path heterogeneity from 0.17 to 0.27 in lower permeability multiple paths and was 0.32 to 0.37 in channel paths. Double solute peaks reflected multiple contributions along longer and differing pathways but the phages had either no, or a subdued double, response illustrating higher attachment and more restrictive pathways.

In Bois de Finges (BdF), six tracer tests were done under high, moderate and low water level conditions using two injection points and five monitoring wells to 22 m distances. Contaminant migration in this highly permeable gravel setting along the Rhone River Valley was documented using three phages and uranine so as to be able to evaluate particle transport and ground water protection rationale. Solute and biocolloid transport rates to 22 m were from about 100 to 500  $\text{m d}^{-1}$ , up to 40-fold faster than predicted using the conventional flow/effective porosity calculation approach. In one well, a 1 m vertical heterogeneity dominated transport in 12 m of gravel-dominated aquifer. Biocolloids were consistently detected earlier likely due to lower than solute detection limits. Maximum biocolloid concentrations (pseudo-peaks) commonly occurred prior to solute peaks with colloid removal processes rather than media properties control particle breakthrough responses and patterns. Phage H40 RBs were typically 1.5 to 4%, commonly 10-fold greater than MS2. In a thin lens situation, solute maximum occurred marginally before H4 phage maximum, the only instance of this occurring during this research. As well, phage H4 RB exceeded 72% illustrating negligible particle attenuation to 10 m distance. Detected transport directions were up to 90 degrees from measured gradient estimates. This degree of evident heterogeneity, the high particulate RB and uncertainty in different flow system locations demonstrated detailed field testing is critical to realistic ground water protection strategies for particulate contaminants.

At Wilerwald (WW), two colloid types (bacteriophages H40, MS2, Psf2 and 1  $\mu\text{m}$  fluorescent microsphere) were injected with uranine. The site's well-channeled heterogeneity had previously been documented with surface geophysics. Injection was done into a preferential flow layer from 4 to 6 m deep with sampling in three wells to 64 m. Prototype instruments were successfully applied under field conditions to provide direct inline measurement of the microspheres (laser detection) and uranine (spectrofluorometry) allowing an optimized sample strategy and minimized test duration and analytical scope. Tracer resolutions were 0.1 microspheres  $\text{mL}^{-1}$ , 0.5 to 1 ppb (uranine) and 0.5 pfu  $\text{mL}^{-1}$  (phages). The four colloids had consistent initial detection at two 'channel' and one 'off-channel' well and particulate velocity was 165 to 285  $\text{m d}^{-1}$ . There was no conclusive evidence of preferential colloid advection. Solute velocity (peak value) in the channel decreased from 135  $\text{m d}^{-1}$  at 14 m to 80  $\text{m d}^{-1}$  at 64 m. To the off channel well at 34 m, it was 40  $\text{m d}^{-1}$ . Colloid maximums (pseudo-peaks) occurred similarly at each well, 1.25- to 2.5-fold earlier than uranine peaks. Phage RBs were high at 14 m (16 to 41%) reflecting relatively open, large and clay-free pores and pathways. They decreased to 0.2 to 0.3% (MS2, Psf2) and 3% (H40) at both 34 and 64 m. Microsphere RBs were 9% at both 14 and 64 m. Phage H40 had 2- to 10-fold higher RB than MS2 and Psf2. Colloid maximum

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dimensionless concentrations (MDC) were typically 2.5 to 3 orders of magnitude lower than uranine. A dormancy effect for suspended sediment-attached H40 was unexpectedly observed two years after its last use. A numerical interpretation of the response at 64 m was done by matching the data using a 1-D finite difference code (CoTrans), a model based on solute dual porosity zones (mobile/immobile) with particle reversible sorption mechanisms. By matching the colloid breakthrough, the wider range of dispersivity determined by matching the solute data was limited to 2.5 to 3.5 m.

Characterization at Kappelen (KAP) involved development and testing of a new well field installed in a 15 to 20 m deep gravel-dominated deposit located in the prolific Seeland aquifer. Initially, reconnaissance surface geophysics (VLF-EM, RMT) was used to define the nature and extent of the fluvial-glacial gravel aquifer over a 6 km<sup>2</sup> area. Localized RMT work further identified the preferred site in a flat forested area with an underlying silty aquitard. Two piezometers and 16 wells were installed over a 120 by 120 m area at depths in both lower (~10 to 15 m) and upper (~4 to 9 m) zones where the relatively low hydraulic gradient ranged from 0.0005 to 0.001, the hydraulic conductivity varied from 1e<sup>-04</sup> to 1e<sup>-02</sup> m s<sup>-1</sup> and there were partially confined conditions documented in the lower zone. Seven tracer tests were done over 15 months. Bacteriophages and uranine responses were well characterized to 20 m although both zones responded with dissimilar transport direction and velocity. To 60 m, the H40 phage and uranine were only detected at one well in each zone well enough to characterize the component behavior. Phage and solute initial detection times were not sufficiently different to distinguish if particles exhibited preferential advective transport. Solute velocity (peak time) ranged from about 14 to 21 and 3 to 7 m d<sup>-1</sup> in the lower and upper zones, respectively. Phage transport velocities (first detection/arrival) were 3- to 8-fold higher. Phage retardation factors were from 0.2 to 0.9 reflecting that phage maximum values commonly occurred 2- to 5-fold earlier than uranine consistent with a diminishing mass attenuated along transport paths. Phage H40 RB was from 0.005% (lower zone - 60 m) to 0.01 to 0.02% (upper and lower zone - 20 m). REDOX conditions in the aquifer caused well bacterial biofouling and likely account for the phage H40 RB at KAP being 10- to 100- fold lower than at BdF and WW.

Biocolloid particle response varied with setting heterogeneity, site geochemistry, matrix mineralogy and microbiology in addition to individual biocolloid properties. Off channel locations exhibited 3- to 10-fold lower recovery than channels. Bacteriophage recovery (RB) ranking in the tank was H40 = or > MS2 > H4 >> H6 >>> Psf2 >>>> T7 and in the field was H40 >> MS2 = Psf2 ~ or > H4. H40 RBs were 4- to 16-fold higher than for MS2. Where concurrently injected, each bacteriophage was typically detected in the same initial sample implying equal phage advective rates despite their wide recovery range. Phages were not always detected ahead of solutes but in any case, earlier phage detection may directly result from their lower detection levels than solutes rather than from preferential advective flow. Times at which phage maximum concentrations (pseudo-peak) occur at a sampling point can be strongly influenced by attenuation processes along their pathways and therefore, particle maximum concentration should not be comparable to the peaks for solutes. With one exception, phage maximums occurred earlier than solute peaks. However, the phage pseudo-peak timing, as reflected by calculated RF values, was not indicative of the wider variability in recovery/attenuation. On the other hand, RB and MDC values for phage responses were consistent. These parameters should be used for comparative recovery evaluations. Internal circulation provided a continuously mixed system opposite contributing intervals and an effective sampling approach with undisturbed flow dynamics. Real-time on-line solute and microsphere measurement methods and the reduced laboratory phage results feedback greatly improved field execution. Combined use of both solute and particles as tracers increased subsurface definition particularly in strongly heterogeneous settings and provided a consistent basis for evaluating intra-phage behavior when concurrent phage injection cannot be carried out. Particle migration velocity based on particle initial detection time (10 to 45 m d<sup>-1</sup> at KAP, 175 to 300 m d<sup>-1</sup> at WW, BdF) were 3- to 8-fold higher than the corresponding velocity calculated by applying the conventional approach of using the solute tracer breakthrough peak time. The coherent analytical results together with appropriately applied recovery evaluation methods illustrate that phages, when concurrently injected and sampled appropriately, can be well characterized despite 20-fold or higher RB ranges. This characterization, which can distinguish among and reflect viral transport processes, is recommended in more large-scale aquifer settings to continue development and validation of effective predictive particle transport modeling and related tools.

# Résumé

Les courbes de restitution de particules biocolloïdales, en particulier de bactériophages, ont été comparées à celles de traceurs sous forme de soluté (sel de cuisine et uranine), plus classiques, pour des transports en milieux poreux meubles très perméables et hétérogènes. La recherche a porté sur la caractérisation et la performance de phages, d'une part en laboratoire au moyen d'un modèle physique (réservoir de 6 m de long), d'autre part dans trois aquifères graveleux de Suisse.

Le réservoir MARCEAUS (Université Louis Pasteur, Institut de Mécanique des Fluides, Strasbourg), de dimensions 1 m x 1 m x 6 m, a été utilisé pour étudier la variabilité des voies d'écoulement et le taux de restitution du transport massif de six bactériophages différents (bactériophages marins-H4, H6, H40, et non-marins-T7, Psf2, MS2) en milieu poreux sableux moyennement hétérogène. Les phages ont été mélangés à du sel et de l'uranine et injectés selon un signal uniforme de 4 heures environ. Les essais ont été réalisés sous des conditions de gradient, de température et de chimisme contrôlées. Les concentrations en sel ont été enregistrées en temps réel au moyen de 353 senseurs de conductivité répartis dans le réservoir. Les échantillons pour l'analyse des phages et de l'uranine ont été prélevés à six emplacements différents du réservoir et à sa sortie. Les réponses du transport du sel et de l'uranine se sont révélées similaires. La restitution des phages, normée à celle des solutés (restitution relative RB – "Relative Breakthrough") s'est révélée différente selon le type de phage, l'emplacement par rapport à l'hétérogénéité du réservoir, et la minéralisation de l'eau utilisée. RB décroît sensiblement quand la conductivité électrique passe de 3700 à 700  $\mu\text{S cm}^{-1}$ . Elle varie pour les différents phages de 5 à 94 % (H40), de 3 à 59 % (MS2), de 4 à 38 % (H4), de 0 à 33 % (Psf2) et de 0.3 à 11 % (H6). La restitution totale à la sortie varie de 28 à 51 % pour H40 (5 essais), de 25 à 31 % pour MS2 (2 essais), de 2 à 4 % pour H6 (2 essais) ; elle est de 24 % pour H4 (1 essai) et de 1 % pour Psf2 (1 essai). Le phage T7 n'a jamais été détecté. La vitesse de transport, basée sur la première apparition et normée à la vitesse d'écoulement de l'eau, varie selon l'hétérogénéité du chemin de 0.17 à 0.27 sur les trajets à perméabilité réduite, et de 0.32 à 0.37 sur les trajets à perméabilité élevée. On a observé un double pic des solutés, reflétant un transport dominé par de longs trajets différenciés, mais les phages ne l'ont pas révélé, ou alors de manière très atténuée, ce qui montre une plus grande rétention et des cheminements indépendants plus restreints.

Au Bois de Finges (BdF), six essais de traçage ont été effectués en conditions de hautes, moyennes et basses eaux, avec deux points d'injection et cinq puits de surveillance à des distances jusqu'à 22 m. La migration de contaminants sur ce site graveleux très perméable le long du Rhône a été étudiée au moyen de trois phages et de l'uranine, afin de pouvoir évaluer le transport de particules et les paramètres de protection des eaux souterraines. Les vitesses de transport du soluté et des biocolloïdes à 22 m de distance varient de ca.100 à 500 m/jour, soit jusqu'à 40 fois les vitesses prédites par les calculs conventionnels d'écoulement prenant en compte la porosité efficace. Dans un des puits, le transport a été dominé par un niveau perméable de 1 m d'épaisseur dans un aquifère essentiellement graveleux de 12 m d'épaisseur. Les biocolloïdes ont été détectés systématiquement plus tôt que le soluté, probablement en raison de leur limite de détection plus faible. Les concentrations maximales de biocolloïdes (pseudo-pics) sont apparues généralement avant les pics du soluté, avec une allure de restitution conditionnée plus par des processus de capture des colloïdes que par les propriétés du milieu. Le RB (restitution relative) du phage H40 se situait généralement entre 1.5 et 4 %, soit à un taux 10 fois plus élevé que celui de MS2. A une seule occasion au cours de cette recherche, en présence d'une mince lentille, le maximum du soluté est apparu avant celui du phage H4. Dans ce cas, le RB du phage H4 a dépassé 72 %, montrant une capture négligeable des particules sur 10 m de distance. Les directions de transport identifiées s'écartaient jusqu'à 90 degrés des directions déduites des gradients. Avec un tel degré d'hétérogénéité, un RB élevé pour les particules et une incertitude à différents endroits du système d'écoulement, les essais détaillés de terrain se sont révélés déterminants pour établir des stratégies réalistes de protection des eaux souterraines vis à vis de contaminants solides.

A Wilerwald (WW), deux types de colloïdes ont été injectés avec de l'uranine, d'une part les bactériophages H40, MS2, Psf2, d'autre part des microsphères fluorescentes de 1  $\mu$  de diamètre. L'hétérogénéité de ce site aux sillons développés a préalablement été reconnue par géophysique en surface. L'injection a été réalisée dans une couche d'écoulement préférentiel entre 4 et 6 m de profondeur, et l'échantillonnage dans trois puits jusqu'à une distance de 64 m. On a appliqué avec succès sur le terrain des instruments prototypes pour la mesure directe des microsphères (détection au laser) et de l'uranine (spectrofluorimètre), afin d'optimiser la stratégie d'échantillonnage et de minimiser la durée de l'essai et le volet analytique. Les limites de détection des traceurs étaient de 0.1

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microsphère  $\text{ml}^{-1}$ , 0.5 à 1 ppb (uranine) et 0.5 pfu  $\text{ml}^{-1}$  (phages). La première apparition, aux deux puits dans les sillons et au puits hors sillon, était cohérente pour les 4 colloïdes. La vitesse des particules allait de 165 à 285 m/jour. Un transport préférentiel des colloïdes par convection n'a pas été détecté de manière évidente. La vitesse du pic du soluté dans le sillon a décru de 135 m/jour à 14 m de distance à 80 m/jour à 64 m de distance. La vitesse vers le puits hors sillon, à 34 m de distance, était de 40 m/jour. Le maximum de concentration des colloïdes (pseudo-pic) est apparu de manière semblable à chaque puits 1.25 à 2.5 fois plus tôt que celui de l'uranine. A 14 m de distance, les RB des phages étaient élevés (de 16 à 41 %), reflétant des pores et des voies d'écoulement relativement ouverts, larges et libres d'argile. A 34 et 64 m de distance, les RB étaient réduits à 0.2 à 0.3 % (MS2, Psf2) et 3 % (H40). Les RB des microsphères s'élevaient à 9 % à 14 et 64 m de distance. Le phage H40 présentait un RB de 2 à 10 fois plus élevé que ceux de MS2 et Psf2. La concentration maximale normée (MDC, sans dimension) des colloïdes était en général de 2.5 à 3 ordres de grandeur plus faible que celle de l'uranine. De manière inattendue, on a observé un effet de latence de H40 lié aux sédiments en suspension ; H40 a été détecté deux ans après sa dernière utilisation. On a effectué une interprétation numérique de la réponse à 64 m en utilisant un modèle numérique unidimensionnel à différences finies (CoTrans), à double porosité pour le soluté (mobile/immobile) et à mécanismes d'adsorption réversible des particules. La large fourchette de dispersivité obtenue par calage du soluté a été réduite entre 2.5 et 3.5 m par calage de la restitution des colloïdes.

A Kappelen (KAP), on a mis en place et testé un nouveau champ de puits dans des dépôts de 15 à 20 m de profondeur essentiellement graveleux situés dans l'aquifère exploité du Seeland. Au départ, on a effectué une reconnaissance géophysique en surface (VLF-EM, RMT) pour explorer la nature et l'étendue de l'aquifère fluvioglaciaire sur plus de 6  $\text{km}^2$ . Ensuite une investigation RMT locale a permis d'identifier un site adéquat comprenant un aquitard limoneux séparant 2 aquifères, dans une forêt en terrain plat. Dans une zone de 120 m sur 120, on a installé 2 piézomètres et 16 puits recoupant l'aquifère supérieur (4 à 9 m de profondeur) et l'aquifère inférieur (10 à 15 m de profondeur). Les gradients hydrauliques sont compris entre 0.0005 et 0.001 et les conductivités hydrauliques  $1\text{e}^{-04} \text{ ms}^{-1}$  et  $1\text{e}^{-02} \text{ ms}^{-1}$ . L'aquifère inférieur est partiellement captif. Sept essais de traçage ont été effectués en 15 mois. Les bactériophages et l'uranine ont donné des réponses bien caractérisées jusqu'à une distance de 20 m, bien que les directions et vitesses de transport soient différentes dans les deux aquifères. A 60 m de distance, seuls le phage H40 et l'uranine ont été détectés dans un puits de chaque aquifère, mais suffisamment bien pour caractériser le comportement du traceur. Les temps de première apparition du phage et du soluté n'étaient pas suffisamment différents pour identifier un transport convectif préférentiel des particules. La vitesse du pic du soluté a été de 14 à 21 m/jour dans l'aquifère inférieur, et de 3 à 7 m/jour dans l'aquifère supérieur. La vitesse de transport du phage (première détection) s'est montrée 3 à 8 fois supérieure. Les facteurs de retardement du phage ont varié de 0.2 à 0.9. Ceci montre que le pseudo-pic des phages apparaît en général 2 à 5 fois plus tôt que celui de l'uranine, en raison d'une réduction de la masse, une partie des phages étant retenue le long des voies de transport. Le taux de restitution relatif (RB) du phage H40 était de 0.005 % à 0.01 % dans l'aquifère inférieur (à 60 m), et de 0.02 % dans l'aquifère supérieur (à 20 m). Les conditions REDOX de l'aquifère ont provoqué un encrassement bactérien des puits, ce qui peut expliquer que le RB du phage H40 était à KAP 10 à 100 fois inférieur à ce qu'il était à BdF et à WW.

La réponse des biocolloïdes a varié en fonction de l'hétérogénéité et de la géochimie du site, de la minéralogie et de la microbiologie de la matrice, en plus des variations dues aux propriétés individuelles des biocolloïdes. Le taux de restitution était de 3 à 10 fois inférieur hors sillon que dans les sillons perméables. Le taux de restitution relatif (RB), classé par ordre décroissant, est dans le réservoir en laboratoire de H40 = ou > MS2 > H4 >> H6 >>> Psf2 >>>> T7, et sur le terrain de H40 >> MS2 = Psf2 ~ ou > H4. Les RB de H40 sont 4 à 16 fois plus élevés que ceux de MS2. Les bactériophages injectés simultanément ont été en général détectés pour la première fois dans le même échantillon, ce qui indique une même vitesse de convection des phages en dépit de leur taux de restitution très variable. Les phages n'ont pas toujours été détectés avant les solutés mais en tous cas une détection précoce des phages peut être due à leur seuil de détection plus bas plutôt qu'à un transport convectif préférentiel. Le moment d'apparition du maximum des phages (pseudo-pic) en un point donné peut être fortement influencé par des processus d'atténuation le long des voies d'écoulement, c'est pourquoi on ne devrait pas comparer les pseudo-pics des particules (concentrations maximales) avec les pics des solutés. A une exception près, le maximum des phages est apparu avant le pic des solutés. Mais il n'y a pas de corrélation entre les temps d'apparition des pseudo-pics des phages, exprimés par les valeurs RF, et la grande variabilité de la restitution/atténuation. D'autre part, les valeurs RB (taux de restitution relatif) et MDC (concentration

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maximale normée) des phages sont cohérentes. Ces paramètres devraient être utilisés pour comparer les restitutions. La circulation interne a produit dans le système un mélange continu entre intervalles contributeurs opposés et nécessite une approche d'échantillonnage efficace pour une dynamique d'écoulement non perturbée. Les méthodes de mesure en ligne et en temps réel de solutés et de microsphères, ainsi que les résultats normés du transport des phages en laboratoire, ont grandement amélioré l'exécution sur le terrain. L'utilisation combinée de solutés et de particules comme traceurs a permis d'améliorer la définition du sous-sol, particulièrement sur les sites fortement hétérogènes, et a fourni une base cohérente pour la comparaison du comportement entre phages lorsqu'une injection simultanée ne peut être réalisée. La vitesse de migration des particules basée sur le temps de la première apparition (10 à 45 m/jour à KAP, 175 à 300 m/jour à WW et BdF) s'est révélée 3 à 8 fois plus grande que la vitesse calculée par l'approche conventionnelle utilisant le temps d'apparition des pics de solutés. Des résultats analytiques cohérents, conjointement à des méthodes d'évaluation de la restitution appliquées de manière appropriée, montrent que les phages, s'ils sont injectés simultanément et échantillonnés de manière appropriée, peuvent être bien caractérisés en dépit de valeurs RB variant d'un facteur 20 ou plus. Cette caractérisation, qui permet de distinguer et de refléter des processus de transport de virus, est recommandée dans le cadre de plus grands aquifères pour poursuivre le développement et la validation des outils et de la modélisation du transport de particules appliqués à une prédiction efficace<sup>1</sup>.

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## **CHAPTER 1**

### ***Introduction, Results Summary and Conclusions***

The purpose of this section is to:

- describe the rationale and objectives of the scientific work that was done,
- present the background associated with previous research related to the scope of research,
- describe the thesis research activities and how the results of these undertakings have been organized and presented in this document, and,
- Summarize the results and findings at the four principal sites where the research was done.

#### ***1.1 Rationale and Objectives***

The goals of the research were to compare the response of biological tracers, i.e. bacteriophages, with classical dyes in granular porous media and to identify how to develop an effective approach to consistently compare their different breakthrough responses. This research was done as a collaborative effort between the Dept. of Microbiology and the Centre of Hydrogeology, University of Neuchatel (CHYN). Three preceding completed activities related to bacteriophages used as surface and ground water tracers had been undertaken in a similar manner. First, detailed analysis and method development to produce strains of relatively resilient marine bacteriophages had been undertaken by P. Rossi (1992, 1994). Second, P. Rossi and other colleagues from Univ. of Neuchatel undertook preliminary testing to refine tracer techniques using bacteriophages in both surface water and ground water environments (A. Carvalho Dill, 1993, P. Rossi *et al.* 1998). Finally, quantitative analyses based on the applying and comparing phages with typical fluorescent solute tracers in karst terrain was done by N. Doerfliger (1996).

Artificial tracers have been typically used in hydrogeological research with injection points and measurements at downgradient springs or in wells to delineate catchment basins, determine water transit velocity commonly used to establish ground water protection areas, and, determine aquifer transport parameters (convection, dispersivity). Fluorescent, salt-based and to a lesser extent radioactive dyes have historically been used as tracers in subsurface research to define ground water flow and transport paths. Bacteriophages (phages) are non-toxic and their flow rates in fissured aquifers are now known to be comparable to chemical tracers. Different phage species can be injected simultaneously, co-mingled or used at individual points to conduct multiple tracer tests simultaneously. Their persistency is generally believed to be short, on the order of months, allowing relatively quick repetition of tracer tests without the persistence of residual background levels typical of solute tracers. Phages also can mimic the behavior of viral particles and can be considered as surrogates of these types of water contaminants that represent a risk to human health and the environment. Therefore, developing a better understanding of how phages responded when used as tracers has multiple benefits in terms of defining transport paths in aquifers but also the degree to which there is attenuation of these particles within different flow regimes.

We decided to pursue what we thought were the logical next steps having concluded the three previous research stages and given the historical use of tracers in a variety of applied research areas related to ground water protection and contaminant transport. The approach was based on the fundamental viewpoint of an experimentalist focussing on enhanced field methods and data reliability. It involved the following four steps addressing bacteriophage response characterization in aquifers by:

- 1) development of a better understanding of the behavior patterns of various phages in different hydrogeologic settings,
- 2) establishing field protocols for sampling and to the extent possible more efficient and reliable on site real time analysis methods,
- 3) determine how to best characterize the transport performance and response of bacteriophages in porous media aquifers typical of those found in Switzerland, and
- 4) assess how to quantitatively compare these responses consistently with those from classical fluorescent tracers.

The research was supplemented partially through the original schedule by collaboration with colleagues from IHW at ETH in Zurich. Together, we were able to benefit from combining resources to compare not just bacteriophage with solute responses but also those of a colloid-sized fluorescent microsphere. We further evaluated a prototype detection counter developed for on site real time analysis of the particles as we were then measuring the fluorescent tracer solute responses in situ.

### 1.2 Background

This research work related principally to biocolloids being used to investigate transport patterns in the subsurface in relatively high permeability aquifer settings. Comparing the behavior of colloids and solutes as tracers in heterogeneous porous media aquifer environments is complicated by a variety of factors related to the aquifer materials, water chemistry, and tracer interactions with both the solid and liquid phases with which they come into contact. Laboratory experiments tend to show idealized responses from isolated variables being evaluated rather than the composite effects that are inherent in any real world set of conditions. Field tests are the basis for documenting actual transport conditions and providing a base to better simulate and calibrate predictive transport models.

#### 1.2.1 Biocolloid characterization in subsurface settings

Since the early 1970s, advances in the fields of fluid flow and mass transport in homogeneous and heterogeneous media have been made in response to the emergence of concern, legislation and funding provided to deal with the formerly unregulated and now controlled disposal and remediation of wastes. Well-documented and thorough contributions have been made internationally by individuals, groups and institutions working in academic, regulatory and practical settings. Today, there are sound established basic principles of both flow and mass transport that can be applied to a variety of, including heterogeneous, hydrogeologic environments. Despite this, there are no lack of viable and variable theories for many laboratory and most field research projects when it comes to explaining the relationships among factors that specifically and uniquely control solute and particle mass transport in the subsurface. However, there appears to be, likely due to the paucity of experience with and historical concern over subsurface biocolloid migration and documentation, little consistency for standardized approaches to field site particle data characterization compared to standardized approaches for sampling and characterization of dissolved chemical transport patterns.

Column tests, essentially depicting a 1D physical model, have been repeatedly used to evaluate and demonstrate unique factors that influence solute and particle migration. A myriad of column migration tests have been done using single or groups of chemicals moving through either natural, reconstituted or artificial soils or idealized, in most cases porous, media. Particles of various nature (microspheres, virus, bacteria, humic acids), size and physico-chemico-electric character also are evaluated in this manner frequently with changing liquid (solute) chemistry and hydrodynamic conditions (e.g. R. Bales *et al.*, 1991; R. Bales *et al.*, 1993; G. Bengtsson and R. Lindqvist, 1995; M. Corapcioglu and S. Jaing, 1993; S. Dowd *et al.*, 1998; D. Fontes *et al.*, 1991; S. Funderburg *et al.* 1981; C. Gerba (1984); T. Harter *et al.*, 2000; K. Herbold-Paschke *et al.*, 1991; G. Hornberger *et al.*, 1992; B. Harmen and M. Sardin, 1994; J. Loveland *et al.*, 1996; O. Jacobsen *et al.*, 1997; Y. Jin *et al.*, 1997; W. Johnson *et al.*, 1995; P. Johnson *et al.*, 1996a, 1996b; R. Kretzschmar *et al.*, 1997; J. Loveland *et al.*, 1996; J. Nocito-Goebel and J. Tobiasson, 1996; D. Powelson and C. Gerba, 1994; S. Penrod *et al.*, 1996; J. Redman *et al.*, 1997; S. Roy & D. Dzombach, 1996, 1997; J. Seaman *et al.*, 1997; T. Weiss *et al.*, 1995). The colloidal particle parameters such as hydrophobicity, electrophoretic mobility, size and shape have been reviewed in these and other literature references and have been identified as relevant for the migration of particles in porous media. However, despite these claims, there has been little actual field effort involved in attempts to relate these parameters to real world settings. According to S. Penrod *et al.* (1996) electrostatic and non-electrostatic (i.e. hydrophobicity) repulsive forces can influence the filtration of the MS2 bacteriophage. The filtration rate of MS2 is correlated to both acidity and position of the exterior charge groups. The role of the electrostatic interactions in the migration of MS2 and a recombinant Norwalk virus were surveyed in details by J. Redman *et al.* (1997) in quartz sand. These authors have showed that not only electrostatic interactions influence the migration but also probably other parameters such as non-electrostatic repulsive forces play an important role in virus migration. D. McCaulou and R. Bales (1995) illustrated temperature impacts on bacteria and microspheres in saturated media. L. McDowell-Boyer *et al.* (1986) and L. McDowell-Boyer (1992) address particle transport through porous media and chemical changes on particle migration, respectively. Therefore, there is some knowledge of the physico-chemical characteristics of some of the particles that have been used in laboratory studies. However, these are done commonly in isolation and without differences in other environmental setting

variables. Alone, unique factors may assist explain differences in response behavior in laboratory environments, but such is rarely the case in field settings.

Column tests can fulfill a need to evaluate trends in behavior as they can be used with better control of independent variables that may influence mass transport behavior. Despite their documenting and being able to segregate independent influences on basic behavior patterns, however, difficulties arise in directly extrapolating the results from column tests to real world environments because first their environmental setting and conditions are too simplistic compared to aquifer settings, and second, the results cannot be well scaled to aquifer dimensions. Bacterial migration work has recently involved pore-related physical observations and measurements using Confocal Laser Microscopy (J. Lawrence & M. Hendry, 1996). A particular area of interest, referred to as 'colloid-facilitated migration', has been the subject of contaminant studies directed at the potential of naturally occurring colloids to 'piggyback' contaminants. Much of this work has been done with radio nuclides, where observed transport has occurred at rates on occasion several orders of magnitude greater than accounted for by ground water flow and advective/dispersive theory.

Larger experimental models such as tanks have fewer physical restrictions. Their increased size and capability for monitoring flow and transport in three dimensions with variable heterogeneity provides further flexibility and dimensionality to migration phenomena as demonstrated by J. Wendling (1994), M. Ruch (1992), and P. Siegel (1995) based on their experiments and mass transport modeling in a nine layer heterogeneous porous media structure created in the MARCEAUS tank (IMF, Strasbourg). However, there are few such facilities such as MARCEAUS and thus there are few real scale changes between column tests and field experiments. Indeed, the MARCEAUS tank facility represents the longitudinal scale of several highly-referenced field experiments. It was within this tank that I was able to carry out extensive phage response characterization and evaluation (K. Kennedy *et al.*, 2001d).

Field experiments have extended the scale of research from columns and tanks to both experimental and real world environments. There is an increasingly large body of knowledge related to dissolved solute, multiphase and organic wastes migration in a wide variety of heterogeneous environments at disposal site undergoing characterization and remediation. To date, however, the focus of data has been on remediation. Many of the early landfill related and even recent superfund-type contamination studies have focussed on data related principally to chemistry and risk assessment. Unlike the work at the field test research sites noted below, the nature of a site being contaminated restricts the amount of related detailed geologic, hydrogeologic and interpretive geophysics that is done. Thus, many of these sites are commonly limited in terms of other than basic geochemical information and water level measurements. Ultimately, this contaminant-related data will undoubtedly advance the state of the art in understanding mass transfer in hydrogeology. R. Harvey (1997), a well-known researcher in the field of subsurface microbiology, recently stated:

"there is a growing realization that processes controlling subsurface microbial transport behavior are inter-related and can be operative on spatial and temporal scales not conducive to lab study. This is leading to more field-scale experiments and to research programs that involve a combination of field and laboratory studies"

According to L. Sinton *et al.* (1997), viral transport in the saturated subsurface is affected principally by virus type, adsorption rate and inactivation. The effects of filtration and sedimentation which can dramatically reduce larger particle concentrations (e.g. bacteria) are commonly negligible for these smaller particles. Adsorption rates tend to increase in as pH, flow rate and grain size is reduced. They also repeat the commonly-held theories that increased removal occurs in high cation concentrations and the presence of dissolved organics and percolating rainwater can increase mobility by remobilizing previously adsorbed biocolloids. Sinton showed transport of the MS2 phage and E. Coli J6-2 in a New Zealand gravel aquifer extending over distances to 400 m. The data showed transport rates based on initial detection of between 190 and 230 m/d. High attenuation occurred to these distances with maximum  $C/M_0$  values on the order of about 2 to  $5e^{-10}$ . Matching 2D and 3D model results provided reasonable fits to parts of the data according to the author although there was a second peak in both cases which could not be explained or modeled.

Currently, there are only a handful of well documented porous media field research sites at which fundamental mass transport and tracer migration experiments have been undertaken by university and environmental protection agencies and that have significantly added to our understanding of dissolved and particle mass transport in different heterogeneous settings. These include work done in dune sand environments at the Camp Borden/University of Waterloo field site, Canada (E. Sudicky, 1986; D. Mackay *et al.*, 1986; D. Mackay *et al.*, 1994; R. Bales *et al.*, 1997) and the Kiwa/Waterworks

Haarlem/NIPHE Castricum field site in Holland (J. Schijven *et al.*, 1995, 1999) at the US Geological Survey Cape Cod sand and gravel field site (L. Gelhar and M. Celia, 1991; R. Harvey and S. Garabedian, 1991; R. Harvey *et al.*, 1989; R. Harvey *et al.*, 1995; G. Hornberger *et al.*, 1992; D. LeBlanc *et al.*, 1991; A. Pieper *et al.*, 1997), in South Carolina (J. McCarthy *et al.*, 1996) and at Horkheimer Insel (T. Ptak and G. Teutsch, 1994). Work by the Dutch public health agency studying migration patterns in both laboratory experiments and at other field sites with both virus and conventional tracers began Holland almost a decade ago and they, in Europe, seem the most active and concerned about viral migration. Publications by A. Havelaar (1993), A. Havelaar *et al.* (1993), A. Havelaar *et al.* (1995) and J. Schijven *et al.* (1995, 1999) address the concern of viral migration as it relates to ground water contamination and the use of bacteriophages as models for virus. Their works at a variety of field sites and with column tests have shown a strong relationship of soil and geochemical factors to migration patterns. They have relied principally on the MS2 and PRD1 phages as well as *E. Coli*, two biocolloids that are no longer allowed for use in Switzerland.

Perhaps there are less well-known sites that have had extensive testing and only a minor amount of the data has been published. For the sites where the data is extensive, the responses seen at Cape Cod, Camp Borden and most recently, Castricum, indicate that there is a potential for using recovery and diagnostic characterization techniques to quantify migration behavior of different particles in variable settings. At Castricum, the homogenous nature of the dune sand setting was relatively ideal for the direct downgradient modeling of viral transport as it had been released continuously from a water treatment lagoon. The largest difficulty appears, however, in segregating the variable environmental influences on the different colloidal particles with any degree of certainty in the field settings being evaluated. Accordingly, with my experimentalist bent, and given the available resources at hand for the work, my approach was to focus not on the potential variability of the micro-specific factors, but on undertaking adequate breadth and depth in sampling. In this way, I believed that I could better precisely document, characterize and quantify the phage responses in three real aquifer settings of similar geologic provenance. By quantifying and depicting the variability in real systems, albeit with diverse heterogeneity, then we can begin to build the base for wider predictions and input to model validation of particle transport.

In Switzerland, prior work in porous media has been done to evaluate migration potential at two locations where data is available. At St. Ursanne near Delemont (Canton Jura), bacteriophages as well as solute tracers were injected up gradient of a gravel aquifer located over about 200 m along the edge of the Doubs River plain. Sampling took place in a variety of wells of varying construction and efficacy. Results were generally poor and inconclusive other than to determine there were or were not phages detected. No phages were detected in the pumping well although they were detected in irregular patterns at selected locations in the aquifer. The second Swiss site where phages have been evaluated in porous media is the Wilerwald (WW) test site near Utzenstorf in Canton Bern (A. Carvalho Dill, 1993; P. Rossi, 1994; K. Kennedy *et al.*, 2001c). At WW, three tests prior to the work as part of this research showed that migration of both solute and phages in the aquifer at this site occurred irregularly and without predictability. Further work as part of my thesis research was continued at WW as well as at Kappelen near Lyss in Canton Bern (E. Oyono, 1996; K. Kennedy *et al.*, 2001b) and at the Bois de Finges Site near Sion in Canton Valais (K. Kennedy *et al.*, 2001a).

Migration studies in fractured granite rock domains have been extensively carried out at Stripa (Sweden), Pinawa (Canada - e.g. P. Vilks and D. Bachinski (1996)) and Grimsel (Switzerland - e.g. E. Hoehn *et al.* (1994)), in basalt at Hanford (USA) and in tuff at the Nevada test site (USA) as part of nuclear waste disposal studies. L. McKay *et al.* (1993) have studied particulate transport in fractured clay tills. Work related to mass transport, as typified by extensive studies of tracer hydrology in Karst terrain has emerged and has continuing scientific development in Europe (H. Hötzel and A. Werner, 1992; F. Cucchi *et al.*, 1997; N. Doerfliger, 1996; D. Drew *et al.*, 1997).

### **1.2.2 Bacteriophages – development as biologic tracers**

Artificial tracers have been used for some time to estimate and quantify the nature, direction and rate of flow. Biological tracers differ from chemical tracer solutions that have been more commonly employed in the past. They are present in water as suspended particles, of microscopic size (colloids), rather than as dissolved chemicals. The particular biological materials used as tracers (spores, bacteria, viruses) are composed of organisms, living or dead, which range in size from tens to several hundreds of nanometers (nm). They have no adverse impact on humans or on the environment. Following an early discussion by J. Wimpenny (1972) on microbes used as tracers, co-operation between the Microbiology Laboratory and the Hydrogeology Center at the University of Neuchâtel. A

successful tracer test was conducted in the early 1980s with bacteriophages in the Swiss Karst Jura (M. Aragno and I. Müller, 1982). This was followed by the development of a systematic experimental approach using microbiological tracers, notably, bacteriophages. This produced in-depth research into the use of bacteriophages as biological tracers in hydrogeology (A. Carvalho Dill, 1993; P. Rossi, 1994; N. Dörfli, 1996). Such research at Neuchâtel resulted in the development of an efficient means of analyzing phage tracer test data quantitatively. It was shown to be effective in karst terrain, with preliminary testing in surface water and at one porous media site, Wilerwald (Canton Bern).

Bacteriophages, or simply, phages, means “that which feeds on bacteria”. This term represents a virus that uniquely invades specific bacterial cells. It has no effect on human, animal or vegetal cells. Like all viruses, bacteriophages are incapable of multiplying in an independent manner. Viruses have to be integrated physically within a specific host cell. When a phage enters its specific bacterial host, it takes control of it and starts the production of new phages that will be released at the end of the infection cycle. Phages are ubiquitous in the environment and occur in widely diverse locations. Concentrations in both fresh and sea water are reported to vary from  $10^3$  to  $10^7$  per ml and can reach concentrations of about  $5 \times 10^8$  viral particles per ml with the highest recorded level from the Gulf Stream (K. Børsheim (1993), O. Bergh *et al.*, 1989; G. Bratbak *et al.* 1990, respectively in P. Rossi *et al.* (1998). Phages range in size from ten to several hundred nanometers. They are composed of a complex protein structure that varies considerably among them. All phages have a capsid that encloses their genetic material. The capsid is an assemblage of a number of protein substructures called capsomeres that together form a complex geometric structure (usually an icosahedron or a filament). This structure is sometimes also covered with a lipid coating. Some phages possess a tail that can retract. Various fibers and spikes complete the structure in most cases (H.-V. Ackerman and M. DuBow, 1987a, 1987b). Bacteriophages seem useful as organisms well suited to hydrogeology from the available biological tracers. Their size range is similar to viruses of Eucaryotes. However, as noted above, the bacteriophages available for work such as was undertaken in this study are neither pathogenic nor toxic for human, animals or plants. Furthermore, by careful and appropriate choice of a phage/bacterial-host system, there will be no risk to the aquifer microflora. The phages, even when used as tracers at high concentrations, are not visible. Therefore, ground and surface water flow paths can be tested and analyzed even in inhabited urban and rural areas.

Another benefit of using phages as tracers comes from the specific affinity of a particular bacteriophage to its unique bacterial host. Because of this biological phenomenon, several different bacteriophages can be injected at the same time in the same aquifer. Their enumeration/counting is done without any interference between the phages as different bacterial hosts are used for the analysis (see P. Rossi & W. Käss, 1998, for more details on the analytical method). One liter of laboratory culture can contain from about  $10^{12}$  to  $10^{14}$  phages. This quantity of organic material, weighing about 100g, is small and presents no possible risk of organic pollution. Analytical detection methods have sensitivity on the order of 1 to 2 phages per ml under routine analysis conditions. This degree of absolute accuracy is better than the best chemical tracer or spectrofluorometric analysis capability suggesting that they can be relied upon for detection at lower levels of concentration.

Phages are believed to be stable over periods of from several weeks to months, depending on the phage and the in-situ conditions. This means phages will be effective during tracer tests in most surface water and karst environments, in discrete or well connected fracture studies and in moderate to high permeability porous media settings. In nature, injected phage concentrations degrade exponentially in aquifer or surface water effectively leaving no residual background levels. Detailed laboratory studies have shown that the degradation (or inactivation) rates of phages are influenced by diverse physical and chemical factors (temperature and ionic charge) and by the presence of colloidal particles, minerals and organic matter (clays and humic acids) (P. Rossi, 1994). One surprising feature of the continuing work at WW was our detection, before the test began, of the H40 phage in samples taken from pumping of the B4 injection as well as two down gradient wells. The site had last had active testing with the H40 phage two years earlier. Therefore, we did not expect to detect residual levels of this particle in the ground water. It appears that the H40 phage may have been inactivated by attachment to sand or smaller grain sized material and that the material to which the phage was attached may have been mobilized by high rate pumping. Therefore, empirical evidence now exists suggesting that the length of ‘temporary’ inactivation by attachment can be, at least in this setting and for the H40 phage, on the order of years.

| THESIS RESEARCH ACTIVITIES                         | RESEARCH LOCATION                 |                    |                            |                     |
|----------------------------------------------------|-----------------------------------|--------------------|----------------------------|---------------------|
| RESEARCH                                           | LABORATORY                        | FIELD SITES        |                            |                     |
|                                                    | MARCEAUS<br>Univ. Strasbourg (FR) | KAPPELEN<br>(Bern) | BOIS de FINGES<br>(Valais) | WILERWALD<br>(Bern) |
| <b>METHOD DEVELOPMENT &amp; DATA RELIABILITY</b>   |                                   |                    |                            |                     |
| Solute sampling & equipment                        |                                   |                    |                            |                     |
| Solute in situ analysis                            |                                   |                    |                            |                     |
| Phage sampling                                     |                                   |                    |                            |                     |
| Colloid on site analysis                           |                                   |                    |                            |                     |
| <b>COMPARATIVE RESPONSE &amp; CHARACTERIZATION</b> |                                   |                    |                            |                     |
| Solute and phage                                   |                                   |                    |                            |                     |
| Intra-phage                                        |                                   |                    |                            |                     |
| Phage and microsphere                              |                                   |                    |                            |                     |
| Solute phage and micropsheres                      |                                   |                    |                            |                     |
| Aquifer heterogeneity                              |                                   |                    |                            |                     |
| ACTIVITY RELEVANCE                                 |                                   | PRINCIPAL          |                            |                     |
|                                                    |                                   | SUPPORTING         |                            |                     |
|                                                    |                                   | RELATED            |                            |                     |
|                                                    |                                   | NONE               |                            |                     |

Figure 1-1 Thesis activity by type, location and relevance.

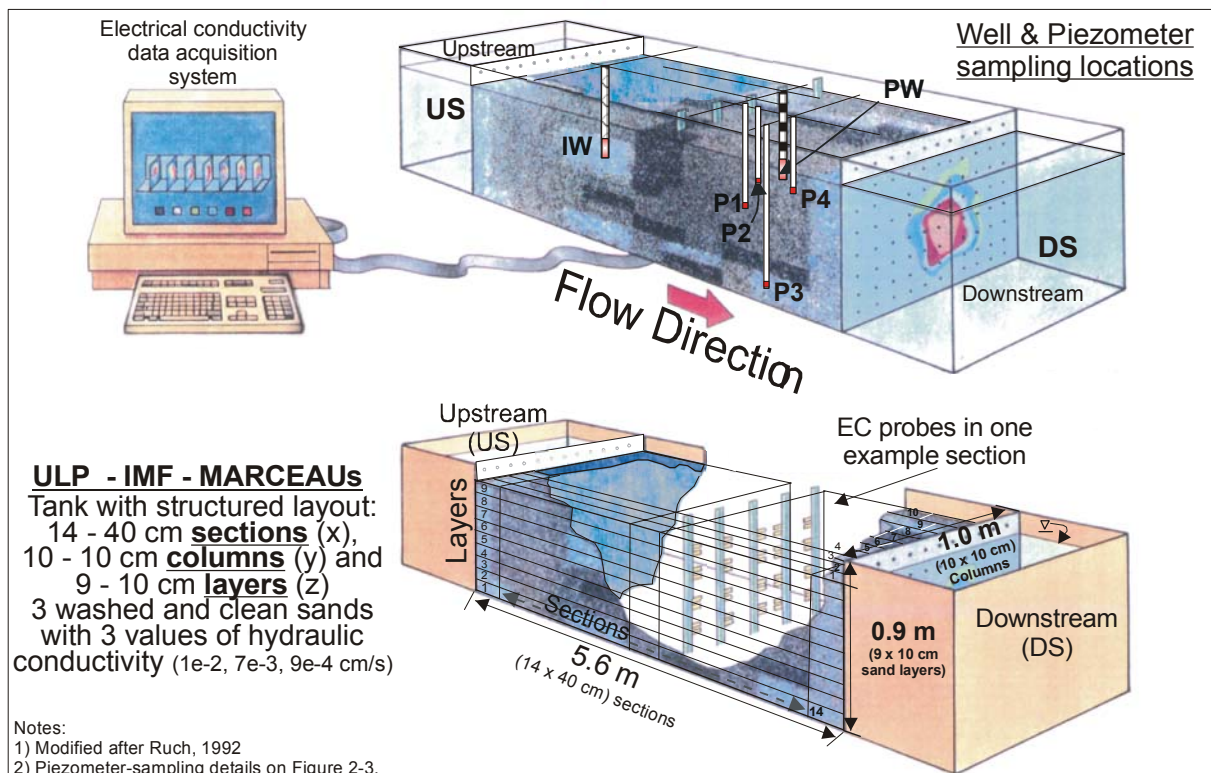


Figure 1-2 Schematic layout of Strasbourg IMF laboratory testing model (MARCEAUS).

Combining the historical laboratory characterization with the use of the phages seemed perhaps ideal at the beginning of the work. However, there is detailed characterization of the properties of only a few phages that could be used for the testing and consequently, some early plans to compare performance of the phages to their properties other than size and shape were not undertaken. In contrast, we were able to characterize the response of six different phages and compare their behavior to two conservative solute tracers, salt and uranine. In addition, we were able to compare the responses of the phages to another non-biologic colloid in the field with success.

### **1.2.3 Evaluating heterogeneity in the highly permeable gravel aquifers**

All three field sites where I undertook research are located in areas of expected vertical and lateral heterogeneity associated with glacio-fluvial deposits. Sedimentology studies in Switzerland indicate these high-energy and rapid deposition environments can quickly change both horizontally and laterally. Geophysics was a key in demonstrating the extent of such high and sudden variability at the WW site as manifest by the work done by A. Carvalho Dill (1993). We also undertook similar geophysical characterization at Kappelen as did colleagues working at Bois de Finges. Clearly, both surface and downhole geophysics provides critical insight into the nature and extent of subsurface heterogeneity that may be expected in these gravel-dominated aquifers. However, the vertical resolution is currently inadequate to predict the observed important changes that must significantly influence transport patterns in this type of aquifer. The results from both WW and BdF showed lenses of about 1 m thickness dominating, probably controlling the transport phenomena in the well vicinity. At both sites, this apparent lenticular flow continued over the testing distances to 22 and 64 m. Field observations done vertically in the boreholes using ground water velocity meters could be done as a matter of course to more routinely evaluate and document these features. The impact of this heterogeneity can be significant in ground water protection studies. Commonly, regional or site specific flow models are used for these types of studies. Limited field characterization may be carried out to document potential contaminant transport rates in the subsurface. However, such approaches may significantly underestimate potential migration velocity where these dominating structures occur.

### **1.3 Thesis Research Activities and Document Organization**

My fundamental thesis work was undertaken beginning in the late summer of 1996 and ended in the early fall of 1999. Fig 1-1 illustrates the activity components of the work that were involved in the research. Research was done related to method development of equipment and procedures and sample data reliability in a laboratory environment in a sand tank model at the Univ. of Strasbourg (Fig 1-2) and initially at the Kappelen (KAP) ground Water research Site (Fig 1-3). The laboratory work helped confirm the phage analysis reliability and evaluations in terms of ranking for best recovery. In the field in particular, the development of the Kappelen ground water research site was undertaken in the summer and fall of 1996. By successfully applying surface geophysical methods, we were able to rapidly narrow the site selection process within available areas of the Seeland aquifer and then to optimize the locations at which well clusters were oriented and installed at the preferred site. The initial tracer component evaluation at Kappelen was augmented during five intense periods of tracer testing at the Bois de Finges (BDF) and Wilerwald (WW) test sites.

As an experimentalist, the approach naturally followed a trend biased towards improving data reliability and methods of assuring that the data being obtained were representative of the media and setting being sampled. Highly permeable porous media aquifers are typically found in the foothill and pre-alpine regions of Switzerland where we were to undertake our testing and the rapidity with which migration takes places necessitates additional precautions and planning that otherwise could perhaps be forgiven in lower energy environments. The improvement of down hole sampling and online measurements of the tracer concentrations was also key to further improvements documenting the transport conditions. Resources for analysis of the phages in the laboratory were not unlimited. Trying to eliminate unnecessary analyses did maximize the effort in the lab. The focus was on acquiring the important tracer component data in such a way that it addressed obtaining both the critical and typical parts of the particle and solute breakthrough curves and the entirety of the response curves for both component types so as to calculate total relative recovery.

In conducting work such as tracer tests in dynamic field settings, the opportunity for sampling is unique in most cases particularly where a prolonged period of waiting may be required for background tracer levels to reduce before being able to repeat the field trial. From the outset it was evident that real-time documentation of the tracer component data would dramatically improve both the data quality and the efficacy of the field work. On line sample characterization methods were developed for

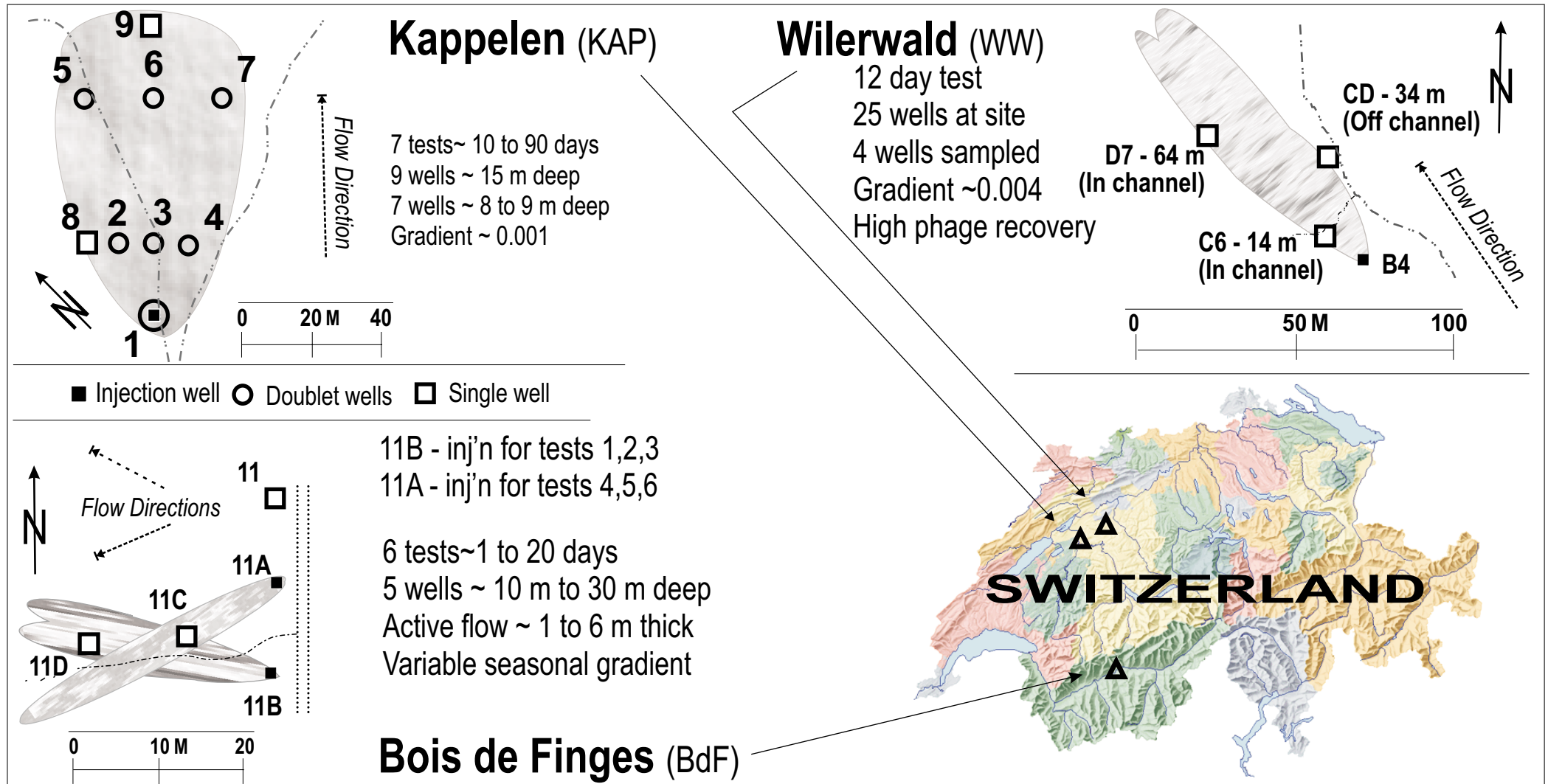


Figure 1-3 Location of research activity field sites.

the solutes soon into the program and this was followed up with input from using a prototype for on-site colloidal analyses. Given the differences in the breakthrough curve characteristics of the solutes and the particles, the solute data alone could be relied upon to predict particle concentration patterns.

Estimating the recovery of the particles and being able to consistently compare it to the solutes anywhere in the flow field required obtaining a complete component record. Methods developed by the United States Geological Survey (e.g. R. Harvey *et al.* 1989) for studies of bacterial and microsphere transport in sand and gravel aquifers in Cape Cod were principally relied upon but could only be used when the data records were complete. Comparison of the solute-based techniques of diagnosing and characterizing transport in flow systems based on times to maximum concentration were not found reliable or uniform when applied to particle responses. Recent approaches by D. Gromilund & M. Borovec (1999) and P. Zhang *et al.* (in review) look carefully at comparative early time diagnostic plots and the extent to which the retardation of the particles influences the shift in the shape of the recovery curve – in particular the time of maximum concentration. Further work developing these methods and applying them to well documented concentration profiles appears to be appropriate.

Modeling of the response of the three bacteriophages, a microsphere and the solute uranine at the Wilerwald site was attempted using a 1-D dual-region code containing additional colloid transport features. It was carried out as part of the joint effort with ETH involving their prototype colloid counter and showed that the results from the field data could be approximated using reasonable values of dispersivity and attachment for the colloidal particles.

Transport rates for the environments in which testing took place were relatively high varying from 20 to over 400 m per day in the gravel aquifer settings. Heterogeneity in layered gravel was pronounced and in two settings we believe that flow through fully screened wells with standing columns of water of from 10 to 15 m was principally occurring in layers of about 1 m or less in thickness. Recovery of the bacteriophages at two of the three field sites were high in contrast to much of the data published in the literature which commonly shows levels of attenuation over 99 percent. At both Bois de Finges and Wilerwald phage relative recoveries were high and varied both by phage species and by the distance from injection. They were as high as 70 % at 10 m in the fastest setting at BDF and to about 4 % at 20 m in the initial three tests with a different injection configuration. At WW, three phage recoveries were from 16 to 40 % at 14 m and from 0.3 to 3.4 % at 34 and 64 m. At KAP, the influence of reduced oxygen (REDOX) conditions appears to have had a major influence on the tracer testing results including reduced recoveries. This has lead to further work which is being continued by CHYN and LAMUN research scientists.

The results from the laboratory and field site work has resulted in an improved set of field methods for injection, sampling and where applicable, on site real time analyses. The follow up response characterization developed allows for consistent documentation and distinction to compare among the particles themselves as well as correlated solute concentrations. These results are presented in the four principal chapters of the thesis as is outlined below.

The organization of this thesis is as follows:

- Chapter 1 Introduction and results summary
- Chapter 2 Laboratory tank model
- Chapter 3 Bois de Finges field site
- Chapter 4 Kappelen field site
- Chapter 5 Wilerwald field site
- Chapter 6 Bibliography
- Appendices

There are minor variations in text, table reference and figure formats in this document. This reflects publication specifics of the individual chapters that can differ somewhat in this integrated document. The reader is encouraged to view the different published articles as referenced where appropriate in the individual chapters.

Chapter 1 Introduction, results summary and conclusions - explains the purpose and rationale associated with this research work; the background associated with related studies of bacteriophage and biocolloid particle migration in subsurface environments, the methods of response characterization and how the work was undertaken and presented in this document and finally, a

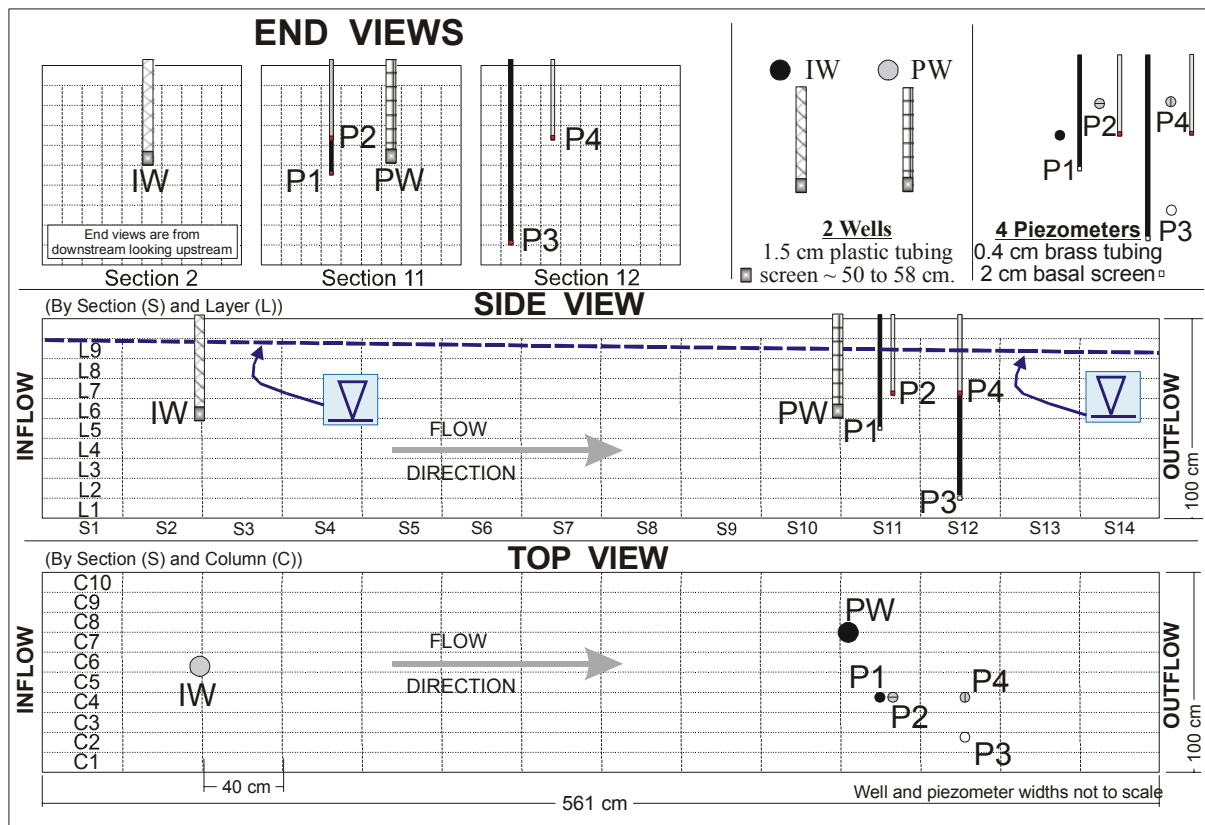


Figure 1-4 Sampling locations within Strasbourg tank.

summary of the results and conclusions based on the findings in the tank laboratory model and at three field sites.

**Chapter 2 Laboratory Sand Tank Model** - presents the biocolloid transport documentation that was done in the laboratory part of the research work. A physical model with known hydraulic conductivity heterogeneity was used to demonstrate the impact of how documenting variability in pathways, some of which are clearly preferential, can well define the movement of both the solutes and particles in the subsurface. Six different phages were compared to each other and the two solutes under variable ionic strength conditions and evaluated in terms of relative breakthrough and attenuation at different locations within the tanks' porous media. This chapter forms the basis of K. Kennedy *et al.* (2001d)

**Chapter 3 Bois de Finges** - summarizes the work done during three migration testing activities at a field site in the upper Rhone River Valley. Water level conditions and transport phenomena in the highly permeable buried river gravel varied seasonally. Contaminant velocity results from an aquifer protection standpoint differed between the field-documented conditions and those derived from typical conventionally-used hydraulically-based interpretations. Migration direction could not be correlated with hydraulic gradient. Breakthrough curve-derived dispersivity values for the solute tracer were similar to published values at distances of 10 to 25 m. This chapter forms the basis of K. Kennedy *et al.* (2001a) as well as additional paper under review)

**Chapter 4 Kappelen** - describes the site and aquifer characterization work using surface geophysical and hydrogeological techniques that were undertaken at a new ground water research test field in the Seeland Aquifer. Reduced oxygen conditions and aquifer heterogeneity caused irregular response patterns for both the particle and conventional solute tracer components. Variability in REDOX geochemical parameters was similar at 10 to 100 m scales, as had been previously determined in km-scale investigations. Lower and upper parts of a 15 m deep aquifer displayed similar but unequal responses in terms of transport velocity and direction. This chapter is the basis of K. Kennedy *et al.* (2001b)

**Chapter 5 Wilerwald** - illustrates the current online, real-time instrumentation that can be used under typical field conditions to accurately portray tracer component response. Microspheres, bacteriophage and uranine responses were documented in detail to distances of about 65 m. Both particle and solute tracer components were defined in the field, as testing was ongoing simplifying and making

more effective the sampling and consequent lab analyses for three different bacteriophages. As at Bois de Finges, an irregular thin layer within the 10 m aquifer dominated the response suggesting the importance of preferential pathways in these highly permeable glacio-fluvial deposited settings. This chapter forms the basis of K. Kennedy *et al.* (2001c)

Chapter 6 Bibliography - lists the references cited in all chapters and appendices. The manner of referencing may differ somewhat among the chapters as Chapters 2,3,4 and 5 all have been prepared for different publications whose manner of referencing are not consistent. I apologize for any inconvenience that this may cause to the reader.

Five appendices contain a list of topic-related papers and presentations that I have given and participated in with other colleagues during the course of this research (Appendix A1) as well as the key tracer-related data from the work in the MARCEAUS sand tank (Appendix A2) and at the three field sites (Appendices A3-Bois de Finges, A4-Kappelen and A5-Wilerwald). A CD containing this publication in PDF format is the basis of this document's distribution according to UNINE procedures that became effective in 2001.

### **1.4 Results Summary**

The purpose of this section is to summarize the research findings. Initially the principal findings are presented for the research carried out at the Institute of Fluid Mechanics (Univ. Louis Pasteur - Strasbourg) in its 5.6 m<sup>3</sup> layered sand tank model (MARCEAUS). Following this, the results and conclusions associated with bacteriophage responses at each of the three experimental field sites are summarized.

#### **1.4.1 Laboratory tank model**

The tank research at the Univ. of Strasbourg, Institute of Fluid Mechanics' MARCEAUS facility (Fig 1-2) was done to: a) further develop sampling and on-line equipment and verify bioparticles and solute tracer component compatibility, b) improve sampling and biocolloid analysis efficacy given the varied nature of the timing, number of components and samples generated, c) rank the performance of six bacteriophages transported through different pathways in the tank's heterogeneity to select promising biocolloid candidates for field use and d) evaluate different methods of consistent recovery and colloid response evaluation and characterization. Results are summarized below and presented in Table 1-1.

##### Equipment/analyses/environment applicability

Over 1500 samples were obtained with an average of about 250 samples per test during this research. Relatively constant flow rate, gradient and background water quality were achieved during all tests. At each of eight sampling locations, continuous pumping of a small volume proved effective to obtain samples consistently from the silt and clay-free porous sandy media. Each set of test samples was analyzed for at least two phages and uranine and/or EC. In total about 10,500 analytical/counting results were made for the phage concentrations. The approach provided a solid base with reproducible and reliable results according to the analytical data. The two most logistically-difficult parts of the work were first, acquiring the samples at the correct time for the different locations in the tank while continuing this effort over a concentrated 24 h period (no automation was possible), and b) managing the large phage sample volume per test (compared to the typical 50 sample/day capacity). Despite 350 in-tank points for EC values, solute data could not be relied upon to compare phage arrival or peak concentration times. Not all in-tank sampling points were in close enough proximity to in-tank EC probes to yield representative data compared to samples. Performing consistent experiments reduced timing uncertainty and optimized analytical procedures and sample processing. Solute results from same sample aliquots allowed effective comparison with phage data.

The tank environment provided the opportunity to evaluate and develop a consistent approach to rank phage performance and compare particle and conservative solute responses. Despite slight differences in the two solutes' response character, they had a generally similar behavior. Therefore, we concluded that the field use of uranine would be analogous to the salt tracer and that, within the expected relatively clean gravel settings in which the phages were to be used; combining the phage and uranine in the tracer cocktail would be appropriate and adequate.

##### Tracer component arrival/detection and maximum concentration characterization

Initial detection of the particles represented a more reliable indicator of the true arrival of the initial breakthrough than that of the solutes. Particle detection limits are several orders of magnitude lower (i.e. better) than for the solutes (molar quantification). This detection limit difference alone could

# Chapter 1- Introduction, Results Summary and Conclusions

**Table 1-1. Summary of tracer test results in the MARCEAUS sand tank model <sup>(1)</sup>.**

| SOLUTES           |                       |      |                                  |                      |                       |         |                      |                       |         | BACTERIOPHAGES       |                       |     |         |        |           |                                 |                       |     |         |        |           |             |
|-------------------|-----------------------|------|----------------------------------|----------------------|-----------------------|---------|----------------------|-----------------------|---------|----------------------|-----------------------|-----|---------|--------|-----------|---------------------------------|-----------------------|-----|---------|--------|-----------|-------------|
| Sampling Location | Distance from US (cm) | Test | Flow rate (L min <sup>-1</sup> ) | SALT (EC)            |                       |         | URANINE              |                       |         | Phage H40            |                       |     |         |        |           | 2nd phage (according to Test #) |                       |     |         |        |           |             |
|                   |                       |      |                                  | T <sub>arr</sub> (h) | T <sub>peak</sub> (h) | MDC (%) | T <sub>arr</sub> (h) | T <sub>peak</sub> (h) | MDC (%) | T <sub>arr</sub> (h) | T <sub>peak</sub> (h) | RF  | MDC (%) | RB (%) | Att'n (%) | T <sub>arr</sub> (h)            | T <sub>peak</sub> (h) | RF  | MDC (%) | RB (%) | Att'n (%) | 2nd Phase   |
| IW                | 79                    | 1    | 4.7                              | 1.0                  | 3.0                   | 100     |                      |                       |         | 0.3                  | 1.3                   |     | 42      | 94     | 6         | 0.3                             | 1.0                   | na  | 18      | 38     | 62        | H4          |
|                   |                       | 2    | 4.3                              | 1.0                  | 3.5                   | 98      |                      |                       | none    | 0.5                  | 3.8                   | 1.1 | 46      | 45     | 55        | 0.5                             | 3.2                   | 0.9 | 6       | 8      | 92        | H6          |
|                   |                       | 3    | 7.6                              | 0.8                  | 3.2                   | 99      | 0.8                  | 3.2                   | 98      | 0.3                  | 3.0                   | 0.9 | 79      | 85     | 15        | 0.5                             | 3.0                   | 0.9 | 10      | 11     | 89        | H6          |
| PW                | 399                   | 1    | 4.7                              | 2.0                  | 4.5                   | 101     |                      |                       |         | 1.7                  | 2.0                   |     | 41      | 73     | 27        | 1.7                             | 2.0                   | na  | 19      | 31     | 69        | H4          |
|                   |                       | 2    | 4.3                              | 2.0                  | 4.0                   | 100     |                      |                       |         | 1.7                  | 4.0                   | 1.0 | 40      | 46     | 54        | 1.7                             | 3.7                   | 0.9 | 2       | 3      | 97        | H6          |
|                   |                       | 3    | 7.6                              | 1.7                  | 4.7                   | 99      | 1.7                  | 4.7                   | 98      | 1.7                  | 5.0                   | 1.1 | 72      | 78     | 22        | 1.7                             | 5.0                   | 1.1 | 6       | 6      | 94        | H6          |
|                   |                       | 4    | 7.2                              | 1.5                  | 4.3                   | 99      | 1.7                  | 3.9                   | 97      | 1.5                  | 4.7                   | 1.1 | 84      | 76     | 24        | 2.0                             | 3.9                   | 0.9 | 40      | 33     | 67        | Psf2        |
|                   |                       | 5    | 4.8                              | 2.5                  | 6.5                   | 102     | 2.5                  | 6.0                   | 98      | 2.5                  | 7.0                   | 1.1 | 71      | 66     | 34        | 2.5                             | 5.5                   | 0.8 | 61      | 59     | 41        | MS2-salt    |
|                   |                       | 6    | 4.9                              |                      | none                  |         | 2.5                  | 5.0                   | 94      | 2.5                  | 4.5                   | 0.9 | 42      | 45     | 55        | 2.5                             | 5.0                   | 1.0 | 43      | 44     | 56        | MS2-no salt |
| P1                | 420                   | 1    | 4.7                              | 3.7                  | 8.0                   | 78      |                      |                       |         | 3.0                  | 5.5                   |     | 4       | 29     | 71        | 3.3                             | 5.5                   | na  | 4       | 21     | 79        | H4          |
|                   |                       | 2    | 4.3                              | 3.3                  | 8.0                   | 82      |                      |                       | none    | 3.3                  | 9.0                   | 1.1 | 20      | 27     | 73        | 3.3                             | 8.5                   | 1.1 | 2       | 2      | 98        | H6          |
|                   |                       | 3    | 7.6                              | 3.0                  | 9.0                   | 81      | 3.7                  | 9.0                   | 80      | 3.3                  | 9.0                   | 1.0 | 33      | 41     | 59        | 3.3                             | 9.0                   | 1.0 | 4       | 4      | 96        | H6          |
| P2                | 427                   | 1    | 4.7                              | 4.0                  | 10.0                  | 82      |                      |                       |         | 4.0                  | 7.0                   |     | 3       | 20     | 80        | 4.0                             | 7.0                   | na  | 1       | 8      | 92        | H4          |
|                   |                       | 2    | 4.3                              | 4.0                  | 10.5                  | 80      |                      |                       | none    | 4.0                  | 8.5                   | 0.8 | 6       | 11     | 89        | 4.3                             | 9.0                   | 0.9 | 0.3     | 0.5    | 99.5      | H6          |
|                   |                       | 3    | 7.6                              | 3.9                  | 9.5                   | 78      | 3.9                  | 9.5                   | 71      | 3.9                  | 8.0                   | 0.8 | 29      | 30     | 70        | 3.9                             | 8.0                   | 0.8 | 3       | 3      | 97        | H6          |
|                   |                       | 4    | 7.2                              | 3.3                  | 9.0                   | 84      | 3.7                  | 9.0                   | 82      | 3.0                  | 7.0                   | 0.8 | 34      | 29     | 71        | 7.0                             | 7.5                   | 0.8 | 0.2     | 0      | 100       | Psf2        |
|                   |                       | 5    | 4.8                              | 4.5                  | 11.0                  | 65      | 4.5                  | 11.5                  | 55      | 3.9                  | 9.5                   | 0.9 | 10      | 24     | 76        | 4.1                             | 9.5                   | 0.9 | 6.1     | 11     | 89        | MS2-salt    |
|                   |                       | 6    | 4.9                              |                      | none                  |         | 5.5                  | 12.5                  | 56      | 5.0                  | 12.0                  | 1.0 | 7       | 13     | 87        | 5.0                             | 12.0                  | 1.0 | 2       | 3      | 97        | MS2-no salt |
| P3                | 460                   | 1    | 4.7                              | 2.7                  | 5.7                   | 98      |                      |                       |         | 2.3                  | 3.0                   |     | 12      | 29     | 71        | 2.3                             | 3.0                   | na  | 4       | 8      | 92        | H4          |
|                   |                       | 2    | 4.3                              | 2.7                  | 5.4                   | 95      |                      |                       | none    | 2.7                  | 5.1                   | 0.9 | 16      | 21     | 79        | 2.7                             | 4.9                   | 0.9 | 0.3     | 0      | 99        | H6          |
|                   |                       | 3    | 7.6                              | 2.7                  | 5.8                   | 96      | 2.7                  | 5.8                   | 94      | 2.7                  | 5.0                   | 0.9 | 39      | 41     | 59        | 2.7                             | 5.0                   | 0.9 | 2       | 2      | 98        | H6          |
| P4                | 460                   | 1    | 4.7                              | 3.0                  | 11.5                  | 68      |                      |                       |         | 4.0                  | 6.0                   |     | 2       | 12     | 88        | 4.0                             | 6.5                   | na  | 0.4     | 4      | 96        | H4          |
|                   |                       | 2    | 4.3                              | 3.7                  | 12.0                  | 66      |                      |                       | none    | 4.7                  | 8.5                   | 0.7 | 3       | 5      | 95        | 4.3                             | 8.5                   | 0.7 | 0.1     | 0.3    | 99.7      | H6          |
|                   |                       | 3    | 7.6                              | 3.7                  | 11.0                  | 50      | 3.7                  | 11.0                  | 42      | 3.7                  | 7.5                   | 0.7 | 11      | 15     | 85        | 3.7                             | 8.5                   | 0.8 | 1       | 2      | 98        | H6          |
|                   |                       | 4    | 7.2                              | 3.3                  | 10.0                  | 57      | 3.0                  | 10.0                  | 40      | 3.0                  | 7.0                   | 0.7 | 13      | 19     | 81        | none                            | none                  | -   | none    | 0      | 100       | Psf2        |
|                   |                       | 5    | 4.8                              | 4.5                  | 10.5                  | 45      | 4.5                  | 11.0                  | 38      | 3.5                  | 9.0                   | 0.9 | 13      | 28     | 72        | 3.9                             | 7.5                   | 0.7 | 6       | 11     | 89        | MS2-salt    |
|                   |                       | 6    | 4.9                              |                      | none                  |         | 3.9                  | 12.0                  | 27      | 3.5                  | 8.5                   | 0.7 | 8       | 16     | 84        | 3.5                             | 8.5                   | 0.7 | 5       | 8      | 92        | MS2-no salt |
| DS                | 560                   | 1    | 4.7                              | 3.0                  | 7.2                   | 55      |                      |                       |         | 3.0                  | 4.0                   |     | 9       | 43     | 57        | 3.0                             | 3.0                   | na  | 3       | 24     | 76        | H4          |
|                   |                       | 2    | 4.3                              | 2.7                  | 6.5                   | 92      |                      |                       | none    | 2.7                  | 6.0                   | 0.9 | 25      | 31     | 69        | 2.7                             | 6.5                   | 1.0 | 2       | 2      | 98        | H6          |
|                   |                       | 3    | 7.6                              | 2.7                  | 7.0                   | 76      | 2.7                  | 7.0                   | 69      | 2.3                  | 7.0                   | 1.0 | 28      | 51     | 49        | 3.0                             | 7.0                   | 1.0 | 3       | 4      | 96        | H6          |
|                   |                       | 4    | 7.2                              | 2.7                  | 7.0                   | 77      | 2.7                  | 7.0                   | 70      | 2.3                  | 7.0                   | 1.0 | 37      | 42     | 58        | 3.0                             | 4.3                   | 0.6 | 1       | 1      | 99        | Psf2        |
|                   |                       | 5    | 4.8                              | 4.1                  | 8.0                   | 70      | 4.1                  | 8.0                   | 66      | 3.5                  | 9.0                   | 1.1 | 29      | 48     | 52        | 3.5                             | 8.5                   | 1.1 | 25      | 31     | 69        | MS2-salt    |
|                   |                       | 6    | 4.9                              |                      | none                  |         | 3.5                  | 8.0                   | 66      | 3.5                  | 7.5                   | 0.9 | 25      | 28     | 72        | 3.5                             | 7.0                   | 0.9 | 22      | 25     | 75        | MS2-no salt |

Footnote: 1) Terminology: MDC-Maximum dimensionless concentration; RB-Relative Breakthrough; RF-Recovery Factor; Att'n-Attenuation (see descriptions in Chapter 2)

explain the consistent earlier or no-later-than-the-uranine detection of the H40 phage at all 7 downstream sampling locations in all six tests. The other possibility is that there was preferential particle advective transport that occurred during some of the testing to some of the locations. However, the detection of the solute uranine and the phage particle was in the same sample about 30 to 40 % of the cases at all downstream locations and occurred at all but the IW and P1 locations when both the uranine and the EC are considered. The other factor that could have influenced the results is the sampling frequency. With such rapid migration, it is also possible that there were not enough samples to adequately distinguish initial detection. The last three tests in the tank had sampling densities that better discriminated between intra-phage and solute-phage differences for both initial detection and maximum values. In these tests, the two component types were still detected about 33 % of the time in the same sample. We conclude that there is not adequate evidence to support preferential advection based solely on the initial detection of the two types of tracers. Additional analyses could be undertaken using approaches recently described in D. Grolimund and M. Borkovec (1999) and P. Zhang *et al.* (in review).

Initial H40 and second phage (H4, H6, MS2, Psf2) detection occurred in the same sample in 21 of the 33 sampling situations. T7 was never detected down gradient (Test 4). Psf2 was always initially detected after H40 but it was not detected at P4. H4 was detected in the same sample as H40 at 6 of the 7 locations sampled (Test 1). H6 was initially detected in the same sample as H40 in 9 of the 14 cases, later in 4 cases, and earlier at P4 (a unique situation where the second phage was detected earlier than H40 in all the testing). MS2 was initially detected in the same sample as H40 in 6 of the 8 cases. H40 was detected earlier than MS2 in samples in the two cases (P2, P4 Test 5 - higher ionic strength).

Solute maximum concentrations occurred at essentially equal or quite similar times for the uranine and the EC (11 of the 15 cases). Twice, EC or uranine maximum values occurred earlier than the other. In these two cases, the maximum concentration was for the previous sample only separated by 0.5 h. Solute and phage maximums could be reasonably compared for all except Test 1. In the four channel locations (IW, P1, P3, PW) and at the outlet, DS, the timing of the two component type maximum concentrations were similar. Ranges in Retardation Factor (RF) consistently at each of these five locations were from 0.9 to 1.1. At the P2 and P4 locations, the RF values reflected earlier times at which the phage maximums occurred with value ranges averaging about 0.84 at P2 and 0.74 at P4. The intra-phage comparison to H40 was less conclusive. Maximum concentration times were equal for H40 and the 2<sup>nd</sup> phage in 14 of 33 cases, earlier in 12 cases, later in 6 cases and did not occur for Psf2 at P4 (Test 3). Accepting the premise that the earlier time to maximum concentration is influenced at least in part by the increased removal or attenuation and these patterns are relatively consistent with RB values. RB values indicate higher attenuation of the 2<sup>nd</sup> phage compared to Phage H40 in most cases. In 18 of the 33 cases, the 2<sup>nd</sup> phage's maximum was similar or earlier than H40. This suggests that comparing times of maximum value among particles by itself would not be a conclusive removal characteristic as it is not always consistent with the relative breakthrough characterization. Therefore, RF values seem of less diagnostic significance when assessing attenuation among particles or even to solutes. That being the case, it also suggests that the processes causing attenuation along pathways are not always reflected in differences in the shape and occurrence of the particles and co-relative solute maximum values alone (pseudo-peak or peak). Increased contrasts in solute and particle maximum times generally reflected longer pathway and lower hydraulic conductivity settings, at least in this laboratory sandy environment. However, the similarity in the maximum timing in the channeled environments did not reflect the relatively large changes in the RB values seen at these same locations among the phages. The similarity in the shapes of the breakthrough curves in terms including the timing of their peaks therefore suggests that the removal process is acting in a similar manner but with a first-order difference in the attenuation.

### Environmental heterogeneity

The four in-tank piezometers plus the two sampling wells at IW and PW provided an adequate base to demonstrate the influence of heterogeneity on particle behavior within the system. The difference in both solute and particle transport at the six sampling locations within the tank was considerable. The tank is filled with relatively clean sands that have about 1½ order of magnitude range in hydraulic conductivity. Despite this, there were marked changes in the patterns of not only the arrival/detection times at similar distances from the injection point, but also in the presence, concentrations and duration and shape of phage breakthrough curves. Clear distinctions are evident for the solutes reflecting channel or preferential pathway flow areas compared to areas of slower migration. Phages illustrated similar patterns. In the case of Psf2, it was for the most part not detected outside the channeled areas yet was readily there. Part of this result may have been due to an inadequate source mass for Psf2 that was strongly attenuated. Variability in magnitude and extent of the migration

response with time in the spatial environment of the tank clearly leads to a greater awareness of the need to establish reliable and adequate sampling density in heterogeneous flow systems. This has been demonstrated when there was only relatively minor heterogeneity in hydraulic conductivity.

Within the heterogeneity, the particles had substantially lower recovery in the pathways with lower permeability than in the channeled areas. This was to some extent surprising given the absence of silt and clay fractions within the sand environment. It suggests that the presence of these smaller grained materials are not always required to reduce the particle concentrations along the flow path but that the combined longer residence and path lengths can also have similar influences with flow in fine grained sand conditions.

### Tracer component response characterization

At final collection or total recovery points in a flow field, the comparison of the total recovery of particle and solute tracers is readily accomplished using the integrated product of flow rate and sample concentration with time. These data yield a component mass recovered which can be directly contrasted among components. At individual in-field or along pathway locations, the flow rate through the pathway point is not known and will vary. Here it is useful to combine the data graphically so as to evaluate the component responses and to depict the plotted features that are evident and can characterize either the component or the location response. Using different diagnostic plots facilitates this comparison. Such diagnostic plots illustrate time versus component concentration with the concentration data depicted a) normalized to their maximum concentration, b) normalized to their original injected concentration and c) normalized to their original injected mass. The maximum dimensionless concentration (MDC or  $C_{\max}/C_0$ ) are the values obtained when the maximum sample concentration is divided by the components' originally injected concentration. This number expressed as a percent or an absolute value is a diagnostic feature of the response when comparing solute and phage responses at different locations throughout the flow field.

Component recovery comparison can be quantified at individual sampling points by comparing the time-based concentration product normalized to each component's originally injected mass or concentration. To characterize and compare the response at different locations throughout the flow field, the timing of the response pattern as well as the total breakthrough at those points can be quantified when related to the response of a solute at each location assuming that the solute response is conservative throughout the flow field. This approach results in the quantity termed Relative Breakthrough (RB) (Harvey *et al.*, 1989) which provides a consistent approach for evaluation of the relevant components' - in our case biocolloids' - total recovery anywhere within a flow field. Comparison of the pattern of changes in RB with time also can be quantified relatively and illustrates where differences occur among particles and solutes.

### Intra-phage response characterization and ranking

The tank tracer activities allowed us to compare the efficacy of different phages as they migrated throughout the flow system. This approach was the basis for the planning of field experiments to follow and continue at three field sites. Each field site was expected to have relatively clean and highly permeable gravel-dominated porous media making up the aquifer being tested. It was important therefore to rank the phage responses and to use this ranking to a) select those that were the most resilient, i.e. least attenuated, for the related field studies and b) provide a basis of comparison with the eventual field trial performances. The results of ranking of the six phages indicated the same relative persistence in the tank in almost all test cases and at most sampling points. The relative ranking, reflecting increased removal (higher attenuation) within the tank-testing environment as based on the MDC and RB results from all sampling locations, was:

$$H40 = \text{or} > MS2 > H4 >> H6 >>> Psf2 >>>> T7$$

There were similarities in the response of MS2 and H40 in Tests 5 and 6 at the PW and DS locations. MS2 MDC and RB values were equal and to 1.4-fold lower than H40. At the P2 and P4 locations in the non-channel areas, MS2 values were from about 1.6- to 4.3-fold lower than H40. In general, the MS2 response more closely resembled the H40 response values in Test 6 which was done with a lower ionic strength. H4 was also similar to H40 during Test 1 at P1 with MDC and RB values equal and 1.8-fold lower than H40. At the other 6 locations, these values were from 1.8- to 3.9-fold lower than H40. The H6 phage was attenuated more than the H4 phage. MDC and RB were about 6- to 60-fold lower than H40. Phage Psf2 was attenuated more than the H6 phage. Psf2 MDC and RB values were about 30-to 3000-fold lower than H40 except at PW where it was 2-fold lower. Phage T7 was completely attenuated, as it was not detected in the tank.

Table 1-2 Field site testing results

| $K^1$<br>(m s <sup>-1</sup> )                | Test<br>no.          | Hydraulic<br>gradient | Wells:       |                |              |              | Uranine<br>response:       |                  |                               | Bacteriophage response: |                         |                  |     |                             |           |              |  |  |
|----------------------------------------------|----------------------|-----------------------|--------------|----------------|--------------|--------------|----------------------------|------------------|-------------------------------|-------------------------|-------------------------|------------------|-----|-----------------------------|-----------|--------------|--|--|
|                                              |                      |                       | Depth<br>(m) | Injec-<br>tion | Moni-<br>tor | Apart<br>(m) | $t_{arr}$<br>(h)           | $t_{max}$<br>(h) | $\frac{MDC}{(Tr_{max}/Tr_0)}$ | Phage                   | $t_{arr}$<br>(h)        | $t_{max}$<br>(h) | RF  | $\frac{MDC}{(C_{max}/C_0)}$ | RB<br>(%) | Att'n<br>(%) |  |  |
| Bois de Finges <sup>(fn-BdF)</sup>           |                      |                       |              |                |              |              |                            |                  |                               |                         |                         |                  |     |                             |           |              |  |  |
| 3e <sup>-03</sup><br>to<br>>1e <sup>-2</sup> | 1                    | 0.002 to<br>0.003     | 6 to<br>12   | 11B            | 11C          | 11           | 3.7                        | 13.5             | 6e <sup>-06</sup>             | H40                     | 2.0                     | 4                | 0.3 | 5e <sup>-07</sup>           | 0.6       | 99.4         |  |  |
|                                              |                      |                       |              |                | 11D          | 22           | 2.5                        | 6.5              | 3e <sup>-04</sup>             |                         | 2.0                     | 4                | 0.7 | 3e <sup>-05</sup>           | 3.9       | 96.1         |  |  |
|                                              | 2                    | 0.001                 | 6 to<br>12   | 11B            | 11C          | 11           | 4.3                        | 24.1             | 3e <sup>-05</sup>             | H40                     | 3.5                     | 6                | 0.3 | 8e <sup>-07</sup>           | 0.2       | 99.8         |  |  |
|                                              |                      |                       |              |                |              |              |                            |                  |                               | MS2                     | 3.3                     | 6                | 0.3 | 1e <sup>-07</sup>           | 0.03      | 99.97        |  |  |
|                                              |                      |                       |              |                | 11D          | 22           | 4.1                        | 11.6             | 1e <sup>-03</sup>             | H40                     | ~3                      | 9                | 0.7 | 5e <sup>-05</sup>           | 1.3       | 98.7         |  |  |
|                                              |                      |                       |              |                |              |              |                            |                  |                               | MS2                     | ~3                      | 9                | 0.8 | 6e <sup>-06</sup>           | 0.1       | 99.9         |  |  |
|                                              | 3                    | 0.003 to<br>0.005     | 6 to<br>12   | 11B            | 11C          | 11           | 2.1                        | 6.8              | 2e <sup>-04</sup>             | H40                     | 1.0                     | 4                | 0.6 | 2e <sup>-05</sup>           | 1.4       | 98.6         |  |  |
|                                              |                      |                       |              |                | 11D          | 22           | 1.7                        | 4.7              | 4e <sup>-03</sup>             |                         | 1.5                     | 3                | 0.7 | 4e <sup>-04</sup>           | 4.4       | 95.6         |  |  |
|                                              | 4                    | 0.002                 | 5 to 6       | 11A            | 11C          | 10           | 0.5                        | 1.4              | 4e <sup>-04</sup>             | H4                      | <0.75                   | 1.7              | 1.2 | 4e <sup>-04</sup>           | >72       | <28          |  |  |
|                                              | 5                    | 0.002                 | 5 to 6       | 11A            | 11C          | 10           | 0.6                        | 1.7              | 6e <sup>-04</sup>             |                         |                         |                  |     |                             |           |              |  |  |
|                                              | 6                    | 0.002                 | 5 to 6       | 11A            | 11C          | 10           | 0.6                        | 2.0              | 1e <sup>-03</sup>             |                         |                         |                  |     |                             |           |              |  |  |
| Kappelen <sup>(fn-KAP)</sup>                 |                      |                       |              |                |              |              |                            |                  |                               |                         |                         |                  |     |                             |           |              |  |  |
| 5e <sup>-04</sup><br>to<br>8e <sup>-03</sup> | 3<br>(lower<br>zone) | 0.0005 to<br>0.001    | 10 to<br>15  | K1.1           | K2.1         | 21           | 15                         | 42               | 2e <sup>-03</sup>             |                         | 13                      | 38               | 0.9 | 9e <sup>-07</sup>           | 0.02      | 99.98        |  |  |
|                                              |                      |                       |              |                | K3.1         | 20           | 11                         | 29               | 2e <sup>-03</sup>             |                         | 11                      | 16               | 0.6 | 1e <sup>-06</sup>           | 0.02      | 99.98        |  |  |
|                                              |                      |                       |              |                | K4.1         | 21           | 22                         | 36               | 2e <sup>-05</sup>             |                         | not adequately sampled  |                  |     |                             |           |              |  |  |
|                                              |                      |                       |              |                | K5.1         | 63           | present, not<br>quantified |                  |                               | H40                     | none detected           |                  |     |                             |           |              |  |  |
|                                              |                      |                       |              |                | K6.1         | 60           | <44                        | 129              | 2e <sup>-04</sup>             |                         | <44                     | 62               | 0.5 | 3e <sup>-08</sup>           | 0.005     | 99.995       |  |  |
|                                              |                      |                       |              |                | K7.1         | 63           | present, not<br>quantified |                  |                               |                         | present, not quantified |                  |     |                             |           |              |  |  |
|                                              | 7<br>(upper<br>zone) | 0.0005 to<br>0.001    | 4 to 9       | K1.2           | K2.2         | 21           | none detected              |                  |                               |                         | 18                      | 30               | na  | 3e <sup>-10</sup>           | na        | na           |  |  |
|                                              |                      |                       |              |                | K3.2         | 20           | 13                         | 83               | 1e <sup>-03</sup>             | H40                     | 12                      | 27               | 0.3 | 2e <sup>-06</sup>           | 0.01      | 99.99        |  |  |
|                                              |                      |                       |              |                | K4.2         | 21           | 14                         | 89               | 1e <sup>-05</sup>             |                         | 11                      | 17               | 0.2 | 6e <sup>-08</sup>           | 0.01      | 99.99        |  |  |
|                                              |                      |                       |              |                | K7.2         | 63           | ~144                       | ~600             | 5e <sup>-06</sup>             |                         | none detected           |                  |     |                             |           |              |  |  |
|                                              |                      |                       |              |                |              |              |                            |                  |                               |                         |                         |                  |     |                             |           |              |  |  |
| Wilerwald <sup>(fn-WW)</sup>                 |                      |                       |              |                |              |              |                            |                  |                               |                         |                         |                  |     |                             |           |              |  |  |
| 4e <sup>-04</sup><br>to<br>>1e <sup>-2</sup> | 4                    | ~0.004                | 4 to 7       | B4             |              |              |                            |                  |                               | H40                     | 1.1                     | 2                | 0.9 | 9e <sup>-05</sup>           | 41        | 59           |  |  |
|                                              |                      |                       |              |                |              |              |                            |                  |                               | Psf2                    | 1.3                     | 2                | 0.9 | 4e <sup>-05</sup>           | 16        | 84           |  |  |
|                                              |                      |                       |              |                |              |              |                            |                  |                               | MS2                     | 1.3                     | 2                | 0.9 | 5e <sup>-05</sup>           | 17        | 83           |  |  |
|                                              |                      |                       |              |                |              |              |                            |                  |                               | M-Sph                   | 1.4                     | 2.2,2.8          | 0.9 | 2.5e <sup>-05</sup>         | 9         | 91           |  |  |
|                                              |                      |                       |              |                |              |              |                            |                  |                               |                         |                         |                  |     |                             |           |              |  |  |
|                                              |                      |                       |              |                |              |              |                            |                  |                               | H40                     | 4.9                     | 9                | 0.5 | 8e <sup>-06</sup>           | 3.4       | 96.6         |  |  |
|                                              |                      |                       |              |                |              |              |                            |                  |                               | Psf2                    | 4.9                     | 8.4,11           | 0.5 | 7e <sup>-07</sup>           | 0.3       | 99.7         |  |  |
|                                              |                      |                       |              |                |              |              |                            |                  |                               | MS2                     | 4.4                     | 8                | 0.4 | 9e <sup>-07</sup>           | 0.2       | 99.8         |  |  |
|                                              |                      |                       |              |                |              |              |                            |                  |                               |                         |                         |                  |     |                             |           |              |  |  |
|                                              |                      |                       |              |                |              |              |                            |                  |                               | H40                     | 6.9                     | 12               | 0.6 | 1e <sup>-05</sup>           | 3.3       | 96.7         |  |  |
|                                              |                      |                       |              |                |              |              |                            |                  |                               | Psf2                    | 6.9                     | 11               | 0.6 | 8e <sup>-07</sup>           | 0.2       | 99.8         |  |  |
|                                              |                      |                       |              |                | MS2          | 6.9          | 11                         | 0.6              | 6e <sup>-07</sup>             | 0.2                     | 99.8                    |                  |     |                             |           |              |  |  |
|                                              |                      |                       |              |                | M-Sph        | nd           | 11.3                       | 0.6              | 2e <sup>-05</sup>             | 8.2                     | 91.8                    |                  |     |                             |           |              |  |  |

fn-1: Values approximate based on field characterization, testing done by others and author (Kappelen) over the past 2 to 10 y.

fn-KAP: Tests 3, 7 used one injection, six monitoring wells. If no well given, no detection.

fn-BdF: Tests 1, 2, 3: injection (11B), sampling (11, 11A, 11C, 11D). No tracers detected at 11, 11A. Test 4: injection (11A), sampling (11, 11C, 11D). No tracers detected at 11 or 11D.

fn-WW: Test-new microsphere (M-Sph) & 3 phages, on-line, real time colloid analysis, comparative behavior (4 colloids, 3 wells).

### Phage response under variable ionic-strength conditions

H40 was observed under variable ionic strength conditions. Except at P4 in Test 5, the migration or transport resilience generally increased after a value of about  $2500 \mu\text{S cm}^{-1}$  as EC increases. The increase was not expected compared to current theoretical predictions and many laboratory column studies that suggest that as ionic strength increases, increased adsorption of the colloids would occur onto the matrix materials thereby leading to a decrease in both parameter values. We cannot explain this apparent contradiction at this time. Originally, we thought that it could relate directly to the marine nature of the H40 phage. Specifically, MS2 and H40 responses were compared under salt and no salt conditions in Tests 5 and 6 that were conducted one after the other. In these tests the MS2 and H40 MDC and RB values were consistently and significantly higher in the higher ionic strength environment at all four downstream locations sampled. These consistent results during these two tests illustrate that both MS2 and H40 attenuation decreased with higher salt content in the water albeit that MS2 attenuation was greater than H40 attenuation as the salt content increased.

### **1.4.2 Field testing sites**

Comparative tracer testing was carried out at three field sites (Bois de Finges, Kappelen and Wilerwald, Figure 1-3). The subsurface setting at each location was a gravel-dominated aquifer but there were differences in objectives of the testing as well as the hydrogeological situation at each site. The hydraulic gradient and geochemical conditions varied which also influenced the tracer components' behavior. Results related to the tracer transport characterization during the testing at each of the field research sites are summarized in Table 1-2.

Biocolloid response characterization methodology for the field testing results was done in a consistent and similar manner to the laboratory tank work. The same characteristic parameters (MDC, RB, first detection and RF) were calculated and compared among the colloids as well as to the solute used which in all cases was uranine. MDC and RB were found to be the most consistent in characterizing the differences in the solute and biocolloid responses as well as among the phages themselves.

#### Bois de Finges

The Bois de Finges field tracer testing trials took place at a site where there are ground water research activities ongoing along part of the floodplain of the upper Rhone River valley near Sierre (Canton Valais). The trials focused on a) identifying comparative responses of different phages with the solute uranine, b) determining if seasonal changes in the hydraulic gradient observed regionally could be detected at a local ~25 m scale, c) demonstrating the influence and impact on ground water protection strategies of vertical heterogeneity and particle and solute transport rates, and d) colloid response characterization in a highly permeable gravel aquifer. Two series of tests were carried out, each series of three tests done using a different injection well believed to be up gradient from the same monitoring wells (Figure 1-3).

Direction of transport depends on scale. It does not always occur along regional or even local flow lines. The illustrations from the gravel aquifer responses seen at BdF showed anomalies compared to predictions from more-traditionally-interpreted hydrodynamic results. Dominant responses occurred in a direction, likely along preferential pathway channels, which were as much as 90 degrees different from what was anticipated from the flow direction determined from nearby water level data. Despite the local 10 to 25 m scale investigated in this work, the conventional gradient data would have been relied upon in most situations to reflect general and site specific transport direction. Our work shows that in that case, the direction suggested for investigation would have been wrong. It further points out the need to have a well spacing that is adequate to define migration paths. For contaminant and protection studies in heterogeneous gravel aquifers, well spacing cannot be effective on the typically relied upon hundreds of m scale. Rather it needs to be targeted to site conditions, including a close understanding of the depositional setting and can likely be likely no more than 10 m near important release point scenarios.

Vertical segregation of pathways in individual wells and the aquifer setting was also observed with the tracer testing and had a marked influence on the results in 2<sup>nd</sup> test series. An expected 6 m contributing interval was defined by conventional borehole geophysical surveys. It was defined as less than 1 m under the tracer test conditions. A detection down gradient at 24 m unexpectedly did not occur. The type and degree of heterogeneity in such a setting is hard to define with precision except within individual wells. Detailed borehole flow logging and related tracer work is valuable and should always be considered in this type of setting.

Tracer injection times lasted over periods from 10 to 75 min. No significant influence of the injection length was observed on transport rates or migration conditions. The recovery rate, however, seemed to increase with increasing injection time length.

Four of the six tests in the two series were undertaken with bacteriophages as well as uranine as the conservative solute tracer. The MDC values in preferential pathways were from  $3e^{-04}$  to  $4e^{-03}$  for solutes and  $6e^{-06}$  to  $4e^{-04}$  for biocolloids. These values are relatively high for the biocolloids given the investigated flow distances of from 10 to 22m. The RB was also high in the channeled areas from ~ 1.4 to 4 percent in the first test series. In the test series 2, an extreme value of about 70 % was obtained over a distance of 10 m with phage H4. Therefore, it was clear from these tests that there was a strong potential for significantly less attenuation in flow systems in these types of gravel environments than typically considered in many particle transport evaluations. Characterization using RB and MDC values was able to be calculated based on detailed sampling and full characterization of the breakthrough curves over the entire testing intervals. Except during Test 4 when there was a much faster than expected response, recovery was likely in excess of 90 percent in all testing cases.

Both the MS2 and H40 phage were used in Test 2 of the three series 1 trials. Times of arrival and maximum concentration were similar for both phages. However, the MS2 recovery response was significantly lower than H40 with both MDC and RB values in the channel about 10-fold lower than those of H40. Off channel at well 11C, H40 recovery was from 6 to 8 times greater than for MS2. H4 was used in Test 4 without comparison to H40. In MARCEAUs, H4 had responded in a manner most similar to MS2 in some of the travel pathways. These results from the tank work would suggest that the recovery response of H40 and MS2 would be even higher than what was documented for H4.

Solute arrival detection was consistently well after that of the biocolloids in the three series one tests. Times for solute detection were from 13 to 36 percent later in the channeled areas and from 21 to 200 percent later off channel. This difference could in part be related to the large differences in the analytical detection limit, rather than preferential advective flow. However, it seems reasonable, given the size of difference observed between the channel and off channel environments, that some preferential advection of the biocolloids may be taking place in the channeled areas compared to the off channel regions where there are more dispersive effects.

Solute maximum concentrations consistently occurred after the particle maximums in the series 1 tests consistent with diminishing mass source along the pathway. RF values ranged from 0.7 to 0.8 in the channeled areas. Off channel, the timing of the maximal differences were much greater reflected by RF values from 0.3 to 0.6. For the rapid response in the Series 2 test, the phage maximum occurred slightly after the solute maximum, the only instance of that occurring in the entire field testing undertaken in this research work. The RB value for this test was the highest observed at 72 percent and in this instance the diminishing mass source along the pathway apparently did not influence the maximum concentration timing differences of the solute and the particles. Perhaps the influences of solute dispersion likely being greater than particle dispersion in this type of setting provide a false RB value.

We believe that the facts upon which ground water protection studies should be based is field testing and tracer data involving multiple types of materials used as tracers. The applicability of factual input to such strategies is considerable compared to theoretical models that rely on conventional flow and widely averaged gradient data. Conventional geologic information derived from the understanding of sediment deposition patterns and settings should be relied upon to define the scale and types of experiments that need to be conducted to best characterize the subsurface environments. Using the results from a correct experimental approach will then allow a logical extrapolation from the field-derived conceptual transport model to a more predictive larger scale model whose results have been validated and are consistent with field observations.

Particle arrival times within the gravel-dominated preferential pathways indicated transport velocities of from about 200 to 500 m/d. Measured arrival times for both solutes and particles were from two to six times faster to well 11c off channel and about 10 times faster to well 11d in the channel than estimated from conventional flow-related and aquifer pump testing data. For our series 2 tests, arrival was from 35 to 45 times faster than had been predicted based on the physical characterization of the aquifer by pump testing. The presence of extremely low concentrations of harmful material, particularly biocolloid materials such as virus or bacteria can represent a health threat. Therefore, estimates of when a contaminant will arrive and its predicted concentrations should not be based solely on average pore water velocity or dispersion and transit velocities based solely on times to reach maximum concentrations. Flow system migration studies should be undertaken to document performance and

behavior for actual setting and input should be based on actual field testing at a site, particularly if the site is providing a public water supply and ground water protection zones need to be established.

In considering how to best protect wells and other sources of water supply coming from aquifers situated in highly permeable gravel settings, the heterogeneity of preferential pathways, transverse directional gradients, rapid and low attenuation transport phenomena should be well understood and defined on a site specific basis. Failing this, no effective ground water protection strategy can be assured in this type of hydrogeological setting. It is inadequate to rely solely upon applying regional models that do not include the possibility to depict potential selective transport paths.

### Kappelen

Field research activities at the Kappelen site (Fig 1-3) were oriented to: a) geophysical characterization of the Seeland aquifer and selection of a preferred location to install a ground water research site, b) development of improved injection and sampling techniques to obtain representative and reproducible results using in line well circulation and on line down hole solute concentration characterization, c) definition of the reduced oxygen environment at the site, and d) comparative biocolloid response comparisons in the upper and lower (stronger and weaker REDOX conditions) parts of the aquifer at the site.

Characterization of the ground water environment at the Kappelen site began by using surface geophysical exploration methods. Results from VLF-EM and RMT techniques allowed development of a conceptualized geologic model depicting the nature and extent of the relatively prolific gravel-dominated aquifer found beneath the site. This model suggested a highly permeable aquifer extended over the investigated 100 by 100 m area, that it had a high degree of continuity and that its thickness varied principally from about 15 to 18 m. Underlying this was a lower permeability zone - an aquitard that at this site did not have the dramatic changes in relief that had been observed at other fluvial-glacial settings nearby in the region. Drilling for well installation confirmed that the actual depths to the aquitard were generally within one meter of the geophysical interpretation. The good correlation between the geophysics and the geologic data illustrates that these methods can be relied upon in this setting to distinguish between the two contrasting lithologic types and arrive at a reasonable estimate of the aquifer thickness.

Hydrogeologic characterization started with updating the regional and local water levels to document the aquifer's potentiometric surface. A relatively stable and flat gradient of about 0.0005 to 0.001 has existed during the site research. Confirming the ground water flow direction and magnitude was followed by the installation of two piezometers and 16 wells in the upper and lower parts of the aquifer. The wells were aligned along the expected northwesterly gradient over a length of 90 m and a width of 40 m. Localized pumping in each well was principally relied upon to arrive at estimates of the screened-thickness hydraulic conductivity ranging from  $1\text{e}^{-04}$  to  $1\text{e}^{-02}$  m/s. Travel times observed in the tracer testing imply that there may be preferential pathway lenses in the gravel structure that are more transmissive than the highest value in this range, consistent with findings at the two other field sites where tracer testing was done.

Biogeochemical characterization of the ground water began by defining the REDOX conditions as they existed particularly in the lower part of the aquifer at the site. Site wells may have inadvertently provided a path for oxygen to migrate by diffusion to the base of the aquifer. This oxygen likely has led to the extensive bacterial biofouling that has been documented. These conditions may continue to have an impact and if so could affect the ability to successfully undertake tracer tests in the lower zone. High rate pumping and possible treatment or specialized well and cleaning of the well screen and surrounding gravel pack will likely be required for future testing in this lower zone at the site to be successful.

Bacteriophage H4 response has not been able to be well defined and it may not be suitable for use at this site. However, bacteriophage H40 could be defined and its response, along with uranine have been well characterized to distances of about 20 m (row 1). Both the particle and solute tracer component responses in the two zones exhibited differences in transport direction and velocity characteristics. To a distance of 60 m, there was less detection of either phages or uranine and precise arrival times have not been well documented. Only one well in each zone to date has had a response adequate to characterize tracer component behavior over this distance. The lack of a greater detection of at least the solute uranine in row 2 is surprising unless a) there are preferential pathways between the wells and the dispersion expected is not occurring effectively outside these channels or b) the geochemical conditions are removing the uranine. Either of these conditions could explain why a wider plume of solute at that distance has not been detected. However, based on

experience in these types of aquifers elsewhere suggests that narrow channels in the aquifer are likely transmitting most of the flow and with it the tracer. Regardless, longer injection times on the order of days should be anticipated along with increased phage mass in order to be able to observe and better characterize the phage responses to distances greater than 20 m in this setting.

Phage and solute initial detection times were not sufficiently different to conclude whether or not there was clear evidence of phages undergoing preferential advection compared to the solute. Solute velocities based on the time of maximum concentration ranged from about 14 to 21 m/d and 3 to 7 m/d in the lower and upper zones, respectively. Transport velocities based on arrival times were 2- to 4-fold higher. MDCs for the phages ( $3\text{e}^{-10}$  to  $2\text{e}^{-06}$ ) were from 2- to 4- orders of magnitude lower than for the solute uranine. Phage maximum concentrations consistently occurred substantially earlier than those of uranine consistent with diminishing mass source along the transport pathway due to particle adsorption/attenuation. Retardation Factor values were from 0.2 to 0.9. Phage H40 recovery, based on relative breakthrough normalized to uranine recovery was about 0.02 % and 0.005% in the lower zone at 20 and 60 m distances, respectively. In the upper zone, it was 0.01 % at the two locations where it was detected in the first row at about 20 m. Accordingly, the phages have been attenuated to in excess of 99.9 %. Despite large attenuation, the H40 phage responses in KAP 3 and 7 have illustrated that bacteriophages do have consistent and continuous breakthrough patterns. With adequate planning and resources, even in difficult field settings, these responses can be well characterized and defined to provide a quantitative comparison to solute responses. This suggests that the Kappelen site's use, given that the REDOX conditions remain understood and controlled, could likely be expanded to conduct further more detailed biocolloid/particulate migration and adsorption process research in its special subsurface environment.

### Wilerwald

The objectives of the research at the Wilerwald field site (Fig 1-3) were similar to the work at Kappelen except that there was a) no geophysical activity as it had been done previously and b) there were no REDOX conditions at the site. The focus was on: a) determining realistic transport rates of three phages (H40, Psf2, MS2) also comparing them with those of a larger diameter fluorescent microsphere, b) evaluating the efficacy of on line real time detection of the microspheres as surrogates for colloid particles and uranine as a dissolved solute tracer, and, c) conducting quantified colloid response characterization with detailed sampling at three selected monitoring wells at the site. The geophysical characterization done previously at the site had indicated, from an aerial perspective, the likelihood of preferential pathways in channeled areas. These could dominate transport patterns in the gravel aquifer. Prior tracing activities had not been adequate to fully characterize the detailed breakthrough curves for particles as well as for solutes. Therefore there was less confidence in the manner in which different biocolloids could be quantified compared to others and to solute responses.

We approached this work principally from an experimentalist viewpoint with objectives targeted principally at two areas – field equipment verification and particle and solute efficacy evaluation. Injection conditions influence the test results and should not be minimized either in their importance or in their detailed presentation for any tracer work done. The higher the degree of subsurface heterogeneity, the more critical are the precise definitions of the actions taken during this phase and in the down gradient sampling locations. Comparison of test data from one series of investigators to another is typically complicated and without clear presentation of the field approach and results, it at best provides a database from which conclusions can be quite erroneous. This is particularly true in Wilerwald with its high degree of heterogeneity, lack of consistent documentation and poor geologic record.

Injection was done at Well B4. During the first few minutes of injection, it became apparent that lateral migration was taking place away from the well within a discrete and relatively thin lens/layer situated between the depths of 4 and 6 m. This was similar to the condition subsequently documented at the Bois de Finges field site. This finding suggested that hydraulic conductivity used in the transport scenarios and modeling attempts may be significantly underestimated. The effective zone contributing to lateral migration in this aquifer may be preferentially occurring in a limited zone on the order of 0.5 m to 1 m thick rather than the fully-screened 10 m interval reported and relied upon in earlier analytical attempts. This same restricted and limited preferential flow layer was also apparent down gradient at C6 and D7 where our sample concentrations were higher than historical tests that had used the same injection wells and tracer components and concentrations. There had been prior tracer testing at Wilerwald with both solutes and phages albeit that some of it was done using the full-screened zone for sampling rather than the likely effectively limited contributing thickness. However, the previous tracer testing results proved were more reliable than the hydraulic data to depict the initial response timing at the three down gradient wells. These prior-testing results greatly enhanced the ability to

undertake the work with the appropriate scope in terms of how to better define the response of the solutes as well as the biocolloids.

'Arrival' times based on the first sample in which there was detection were similar for all three phages. At well C6, the phage detection was able to be compared to the time of the microsphere detection and all occurred at about the same time. Solute detection occurred consistently later at C6 and D7 but was similar at the CD well. Wells C6 and D7, based on both the tracer response and geophysical interpretation, are located along the longitudinal flow direction of the channel down gradient from the B4 injection point. Well CD is off the main channel and its results indicate more transverse dispersion than seen at C6 or D7. There was no indication of preferential colloidal advection at C6. At CD and D7, Phage H40 exhibited a partial earlier response than uranine for periods of 30 min and 2 h, respectively, during the rising limb, but this is not considered conclusive of earlier colloid arrival due to preferential advection.

Transport velocity values pertinent to viral surrogates, such as the colloids we used, may be more relevant when based on the first detection or arrival time rather than the time of maximum concentration. At C6 and D7, phages had 'first detection' migration rates of 285 (colloid average) and 223 m/d, respectively. The phage-based arrival velocity to CD off the main channel was 167 m/d. These values are from 2- to 4-fold higher than the average velocities of 135, 80 and 40 m/d calculated from the observed peak uranine concentration times at the same wells. Using colloid (pseudo-peak) maximums and solute peak time to determine, which "arrived" earlier, is not an appropriate approach given the high colloid adsorption along the pathway. The along pathway reduction can provide a false maximum in the particle breakthrough curve. Using this timing for transport characterization does not reflect the same meaning as it relates to a conservative solute. Important considerations for particles in contaminant transport scenarios are more importantly based on the first detection rather than maximum concentrations.

Both H40 and the microspheres were significantly less attenuated compared to MS2 and Psf2. The relative breakthrough (recovery) of each colloid at a distance of 14 m (C6) ranged from 9 to 41 %. At C6, biocolloid MDC values ranged from about  $4e^{-05}$  to  $5e^{-05}$  for Psf2 and MS2 and  $9e^{-05}$ . The microspheres value was  $3e^{-05}$  and the solute value was  $1e^{-04}$ . At CD (off channel), MDC values were from  $7e^{-07}$  and  $9e^{-07}$  for Psf2 and MS2,  $8e^{-06}$  for H40 and  $3e^{-05}$  for uranine. At D7 in the channel about 64 m down gradient, MDC values were from  $6e^{-07}$  and  $8e^{-07}$  for Psf2 and MS2,  $1e^{-05}$  for H40 and  $2e^{-05}$  for the microspheres and  $5e^{-05}$  for the uranine. Differences in H40 and MS2 and Psf2 recovery were only about 2.5 fold those at C6, but their differences increased to about 10- to 17-fold at CD and D7. Similar results occur when comparing MDC values. Having similar recovery results at both 34 and 64 m but in two different subsurface settings illustrates the non-uniqueness of solutions suggested for the variations in the colloid behavior. There was, however, little visual difference in the slopes and patterns of their individual responses in the falling limb and tailing section at each well suggesting the processes acting on the individual phages, prior to their arrival may be scalable and linearly proportional, at least among these three phages. Only at CD were the shapes of the phage responses after about 50 h less similar. This may have been related more to the MS2 and Psf2 concentrations approaching their detection limits than to real differences in breakthrough responses. The dispersive characteristic of each phage appears similar based on the consistent breakthrough shapes at each well. However, the degree of attenuation differs dramatically.

Recovery of the colloids was significantly higher than has been reported for other similar-sized tracer components in sand and gravel porous aquifers. However, there is little data in the literature that presents comparisons in similar environments. Only the work reported from New Zealand is perhaps valid to compare to the settings tested in Switzerland. The high values in these subsurface settings suggest little colloid filtration is occurring over 14 to 64 m distances at this site.

Matching the response of H40, microspheres with the solute uranine at well D7 was done using CoTrans, a 1-D numerical code. The initial approach with attempts solely to match the uranine data had indicated the solute response could be approximated with a longitudinal dispersivity range of from 2 to 8 m. By matching the particle data at well D7, a longitudinal dispersivity of about 3 m was determined. Using this input from the colloid data matching, the dispersivity values of all three components were able to be limited to a smaller range while matching both the solute and biocolloid component data. The presence of both colloid and solute tracer helped reach consistent model results using a double porosity (two-region) model for solute migration and variable sorption conditions for the colloids.

The prototype instrumentation was able to be successfully deployed under typical field conditions. Microspheres and uranine were well documented continuously in the field and rapid analyses were possible of the discrete samples for uranine analysis as well. The inline equipment provided real time assessment of both colloid and uranine tracers that allowed site sampling to be effectively modified and optimized. This was particularly important when considering when and with what frequency it was important to sampling for phages. Relying on solute arrival and maximum concentrations would not have provided the first colloid detection and maximum concentrations characteristics. Relying on solute responses to sample for colloids has therefore been conclusively shown as not to be appropriate. The phage data based on extensive laboratory analyses were also consistent and shown as particularly reliable for these three phages. An exceptionally high density of biocolloid sampling during this test allowed definition of precise breakthrough curves and determination of response characteristics. These response characterization techniques, given the adequate field data, allow quantification to compare the biocolloid recovery to the solutes and to be contrasted among the phages themselves. Despite large losses in all colloid quantities along their flow paths, the extensive and detailed sampling illustrated that they all had relatively regular breakthrough patterns. The results show that they can be relied upon to be persistent and with this consistency, they can be effective to evaluate and portray viral migration patterns. The mass injected and environmental settings need to be appropriate and compatible.

Additional characterization of individual biocolloids used for field tests is needed in order to improve the detailed understanding of which particle parameters and in situ processes lead to the observed differences in their transport and adsorption behavior along flow paths. Consistent and extensive field testing using viral surrogates such as bacteriophages is needed in specific settings. These studies need also to be supported by laboratory testing programs and model scoping studies. The composite of these efforts is justified where the importance of the transport phenomena can potentially impact human health and critical aquifer environmental settings whose protection is to be guaranteed.

### **1.5 Conclusions**

The purpose of this section is first to broadly summarize what I believe is reasonable to conclude from the scientific activities of this research work. I would particularly like to address the context of my originally intended objectives and rationale that was specifically related to the use of bacteriophages in porous media for ground water transport and contamination studies. Second, I will take this opportunity to outline what I believe could be useful and pertinent steps for future scientific activities to address improving methods and reducing uncertainty in biocolloid and related migration studies.

#### **1.5.1 Biocolloid/Phage response variability**

The following five conclusions relate to intra-phage aspects of such work where differences in the response are phage and setting dependent:

- Setting definition
- Ranking
- First detection/Arrival
- Maximum concentrations/Pseudo-Peaks
- Recovery and pertinent performance indicators

##### Setting definition

Biocolloid particle transport behavior is dependent on diverse factors beyond just the nature of the particle. Characteristics of the subsurface setting in which the migration is being investigated need to be well defined. These include the geochemical, mineralogical and biological considerations. Defining these aspects is important as they relate to both the solids (soil/rock matrix) and the liquids filling the actively hydrodynamic and diffusion-related pore spaces. Geochemistry is not yet well enough understood to be able to apply any global, over-riding or rule-of-thumb characterizations for all settings. This was evident from the disproportionate responses of phage recovery as ionic strength increased in trails in the tank and from the differences in recovery at Kappelen with its low oxygen condition compared to WW and BdF. Mineralogical aspects and the degree of finer grained materials

present can preferentially affect reactions between the particles and both the matrix and the liquid. None of the field or laboratory testing sites had strong clay contents so there was not believed to be any basic influence of this factor in the results presented. Interference and co-generative considerations from the presence of biological entities can also affect responses from biocolloid particles. The extent to which there was increased attenuation at Kappelen arising from the near-well bore effects of iron bacteria clogging the well screens and being present in the aquifer are not yet documented. However, iron has been clearly shown in related laboratory work to influence particle attenuation so such an effect from biological entities is possible from the biochemical conditions in this setting. Critical also is the definition of the hydrodynamic setting (heterogeneity) of the work being done as we have clearly shown that heterogeneity in pathways can dramatically influence field concentrations and influence findings and conclusions based on the interpreted results.

### Ranking

Surrogate work related to viral migration can rely on the phage used. In some instances, regulatory authorities have attempted to use the least attenuated phage as the one to consider for transport definitions and evaluations. Therefore, the higher efficacy phages are typically sought after for field and experimental programs. The work in the MARCEAUs tank setting over distances of about 5 m illustrated the following ranking for the six phages used:

$$H40 = \text{or} > MS2 > H4 >> H6 >>> Psf2 >>>> T7$$

The research work at the three field sites to distances of about 65 m depicted the following ranking for three phages used:

$$H40 >> MS2 = Psf2 \sim = H4$$

In all field and laboratory cases, H40 was less attenuated than any other phage and in the field had from about 4 to 16 times higher relative recovery than MS2 at the two field sites where they were used. MS2 responded in a similar manner as Psf2 at Wilerwald although it had a 50% higher RB value at one of the three WW locations. H4 responded strongly during Test 4 at BdF and from this observation alone it is ranked highly relative to other field observations although it was not used comparatively at any sites. Without question, it is clearly evident that the phage with the least attenuation and highest efficacy under all test external conditions and hydrodynamic settings is H40.

### First detection/arrival

Comparative bacteriophage tests demonstrated, for the most part, that the phages are initially detected (present) in the same test samples. It also should be stated that the initial times of phage detection were only rarely inconsistent with the subsequent applicable increases in concentrations removing the likelihood of cross contamination or laboratory error. This similarity implies that the advective rate for at least the initially arriving particles at each of the sampling points, regardless of the phage, was similar. It further suggests that despite the large losses occurring along the pathways from attenuation, first detection was consistent among at least four of the six phages. Two notable exceptions occurred during the MARCEAUs testing. T7 was never detected within the tank and we conclude T7 was not transported in the system likely being affected by the high ionic strength setting. Psf2 was not present or its presence was detected significantly later at some of the locations where other phages were observed. This is not consistent with the comparative testing at Wilerwald where Psf2 was detected in about the same sample at all downstream locations as were MS2 and H40. Seemingly, the initial Psf2 mass used in the tank was not adequate for saturation of the pore space and related pathways. Overall, however, in the quite rapid velocity settings in which the field and even laboratory testing were carried out, it should also be acknowledged that more discrete sampling and repeated attempts would be required to verify this conclusion adequately. Certainly at this point, the initial detection's consistencies strongly suggest that the time for first particle detection can be relied upon to represent a common feature among different biocolloid particles with large variations in degree of attenuation and documented size, shape and surface properties.

### Maximum concentrations/pseudo-peaks

I have used the term pseudo-peak for the particle BTC feature that depicts the maximum phage concentrations ( $C_{\text{max-phage or particle}}$ ) as it occurs at time -  $t_{\text{max}}$ . Distinguishing the BTC feature for particles from a solute's BTC maximum concentration (peak) should be done. The effects of processes that contribute to the shape of the particle BTC including its maximum, at any given point in the flow system, are substantively different than those that occur on conservative non-reactive or even sorptive

tracers. Particles typically exhibit such high degrees of attenuation/adsorption along their flow paths that their relative concentrations compared to solutes are two to five orders of magnitude lower or in some instances removed altogether. Much of the injected particle mass can be effectively, in some instances exponentially, removed with distance along the flow path. The scale of the reduction in particle mass is so high compared to what is associated with most solute tracers even those that are considered reactive, that it is not reasonable to conceptualize a BTC maximum for a particle in the same manner as it is manifest for a solute. The time at which the particle maximum occurs on the BTC curve can be strongly influenced by the continual mass reduction along the flow path. On one hand, particles that remain unattached are funneled into and along the advective channels. They do not have the same potential for concentration reduction diffusing into dead end pores that may occur with solutes in some flow systems. On the other hand, large particle attenuation can result, and this was observed at all locations where we tested, in maximum concentrations on the BTCs to occur earlier than for a non-reactive solute. Others workers have reported particle maximums occurring later than for solutes but the particles used in these studies also exhibited high attenuation. Therefore, the complexity of the particle-water-media interactive processes along transport path can cause inconsistent times for their BTC maximum concentrations. It is not always clear what processes will dominate in the subsurface setting to produce the maximum concentration timing. A convention for solutes used in tracer studies is to use the time elapsed from injection to a BTC peak at a given point as input to calculating the transport velocity between the injection and detection well. However, given the context of the transport process differences between solutes and particles, it does not seem justified to use the time of the particle pseudo-peak as input to calculate or compare a particle velocity with the results commonly arrived at for solutes.

During the trials when phages were compared, the times to maximum concentrations, in addition to the first detection times, were relatively similar both during laboratory and field tests (except as noted for the T7 and Psf2 conditions in MARCEAUs). The largest intra-phage variations occurred in the tank and were as much as 30 percent different occurring at IW (the closest sampling point to injection) and at several locations when the H40 and MS2 trials in the high and low ionic strength settings were carried out. At IW, the differences were between subsequent sampling events. In the other tests and in the field, there was less than 10 percent difference in the times of their maximum concentration and in most cases the differences were in subsequent sampling events. This consistency among time to maximum concentration of the phages suggests this time-maximum breakthrough curve feature (commonly used with solutes, for example) does not seemingly reflect, at least in a direct or obvious manner, the wide variations in relative recovery or retardation and attenuation among the phages compared. Its reference as a peak is therefore largely misleading within the context of common hydrogeological applications and more appropriately could be termed 'pseudo-peak'.

### Recovery and pertinent performance indicators

To date there have been numerous laboratory and a few well-documented field programs that address transport efficacy of bacteriophages and some other types of small colloid-sized particles. These laboratory and field research activities have consistently shown that there are two methods (RB and concentration-normalized data/maxima) that allow an effective evaluation or ranking of the efficacy of individual particles' and therefore their performance. Relative breakthrough (RB) is a calculated recovery that integrates the total response of the particle over the duration of its presence at an observation point. It is more useful than using the Recovery Factor (RF) which compares the times of maximum concentration for individual particles to the time of the solute maximum. Concentration-normalized data illustrate the degree of attenuation occurring in the system with time at each monitored location. Graphs that portray the phage response versus time by taking into consideration the initial phage and/or initial solute mass or concentration at the injection point allow a rapid evaluation that also reflects, and agrees well with, the RB calculations. C-normalized maxima ( $(C_{\max}/C_0)$  or  $(C_{\max}/M_0)$ ) values also were consistent and reliable during testing and in most cases agreed well with RB relative values. The two calculations (RB,  $(C_{\max}/C_0 - \text{MDC}, C_{\max}/M_0)$ ) can therefore be relied upon to characterize the response or provide a meaningful ranking among phages thereby defining their relative efficacy. The differences in RB and C-normalized maxima that have been documented among the phages and at different locations within the subsurface environment at each site substantially verify changes in degrees and patterns of phage relative recovery. These changes, when documented consistently, should be able to be relied upon as significant interpretive performance and response features related to migration potential with given setting environments.

### **1.5.2 Tracer-related injection, sampling and analysis methods**

The purpose of this section is to outline four important considerations arising from the more operational, but still directly pertinent, aspects of site and experimental practice, analyses and methodology. Those are:

- Injection conditions
- Internal well circulation
- Real-time, onsite data acquisition
- Rapid bacteriophage analysis feedback

#### Injection conditions

We undertook this work with the assumption that a step function injection approach could be more readily achieved in the research settings and at wells typically found in monitoring or pumping situations. The use of instantaneous injection conditions in well-field conditions is frequently problematic and in some cases not feasible. On the other hand, the hydraulic conditions and geochemical concentrations during the entire injection duration with its initial buildup, constant-level plateau and final falloff period of the step function are able to be well-documented using inline detectors or closely spaced samples. These data are instrumental to the deconvolution activity required for the subsequent detailed analysis and interpretation. In two cases, the initial conditions expected were not found and there was in effect a direct injection of the material into a thinner and more permeable than predicted layer opposite the interval. In both cases, the injection continued with the mass injected well able to be documented but with the average initial concentration only able to be estimated. Down gradient location sampling protocols, however, could be adjusted to reflect the observed preferential injection pathways and satisfactory results were obtained. In some cases, the injection step function, related to the total response within the system, was short enough to be considered instantaneous. This was not the case in the tank where the 4-h injection, needed to saturate all the down gradient tank pathways, was followed by a period of from 24 to 36 h for complete recovery. In the field however, the typical 10- to 90-min injection periods were followed by several days to, in some instances, weeks of recovery down gradient and in those cases, the relative injection period could be considered instantaneous for a preliminary analysis.

#### Internal well circulation

An existing but relatively rarely applied approach, using a depth/thickness-integrated injection and related sampling conditions at down gradient locations, was further refined and developed. It proved a pertinent and appropriate method for both injection and sampling activities in wells that otherwise could not be easily sampled. Reliance on internal fluid circulating inside a well opposite the expected contributing interval thickness is an effective and readily reliable technique that yields consistent long-term data. It can be particularly effective when the possibility exists of higher hydraulic conductivity (preferential pathway) layers within the porous medium's contributing thickness (relative to a well's screened zone) or the hydraulic conductivity is relatively high as were the cases in the gravel-dominated aquifers at all the field sites. It is also useful, when using stationary down-hole analysis equipment that may not be directly opposite the contributing interval and there is no effective internal mixing within the well as was the case at KAP and BdF. Three important criteria critical for the method's effectiveness include a) well-defined (geophysical) validation of contributing thickness, b) internal circulation greater than cross flow, and c) no-impact sampling equipment, down-hole measurement and circulation equipment.

#### Real-time, onsite data acquisition

It is becoming more and more evident that being able to obtain correct and meaningful data in real time during any kind of highly demanding and active subsurface field and detailed laboratory testing dramatically improves the data quality, the testing efficiency and maximizes the possibility of experiment success. At the same time, it considerably lowers both time and costs required to carry out the work. The over 350 on-line electrical conductivity measurements in the MARCEAUs tank allowed immediate tracking of the conjunctive salt/urinine plume as it migrated. We achieved substantial improvements in both quality and rate for the on-site and real-time measuring of urinine values in discrete samples and from down-hole borehole or with in-line sensors of circulating systems. Sample urinine content could be determined on-site within a few minutes of sampling. In-line data provided readings every 10 s or every 4 min. As seen at BdF, this was critical. This as-it-happens approach permitted the research work to be done with direct solute-analysis that optimized tracer-testing efforts. The on-line real-time microsphere detection analysis directly contributed to the

effective colloid sampling strategy for the other three biocolloids being evaluated at WW. It is now more evident that sampling strategy based on solute-derived breakthrough curve data can be of no technical merit under certain rapid-response conditions.

### Rapid bacteriophage analysis feedback

Despite the clear advantages of on-line real-time analyses, it is not always possible to achieve. At this point in microbiological research, real-time or on-line analyses with an adequate quantification capability seem to remain an apparent far-reaching goal as far as it pertains to biocolloids (phages). However, the rapid feedback we have developed at LAMUN within our research program was significantly shorter than common turnaround time for phage analyses. Typically, indicator-level analyses were available within 36 to 48 h of sampling and definitive concentrations were determined with a lag time of no more than 96 h. Preliminary results from the samples taken for bacteriophage analysis were instrumental in conducting the subsequent fieldwork with little need for either duplicity or excessive sampling periods extending over weeks or longer. This allowed a greater focus on the important sample verifications and prevented dilution of the manpower effort for extraneous analyses – a significant advantage in any intensive field-sampling program. In reduced sampling situations when ground water flow rates are low, this rate of turnaround may not be as critical. However, in the situations where flow, as was the case for our three sites, is on the order of several 10s to 100s of m/d and where sampling activities may need to be taking place every 10 to 60 min, then this relatively rapid response was critical. Without the capability for such rapid feedback, field programs in these types of fast-acting settings could not likely be well conducted or they would be so expensive that it would be prohibitive (even with graduate student labor fulfillment) to carry them out.

### **1.5.3 Biocolloid versus classical solute tracer responses**

One key objective of this research was to examine and compare the important characteristics of solute and particle responses when used together in tracer test and related transport studies in porous media. The four aspects addressed below which seem most pertinent based on the work include:

- Breakthrough curve features
- Detection limits
- Time to maximum concentrations – Solute 'Peak' versus Biocolloid 'Pseudo peak'
- Relative breakthrough and solute-normalized data

#### Breakthrough curve features

These curves are typically relied upon to develop input values for solute-type contaminant studies with or without a considered retardation factor. Therefore, a frequent question that arises is what parts of a solute and particle breakthrough curve can be compared and when this is done, what can we learn from the comparison. Unfortunately, it is unlikely that there is any simple result to be derived from comparing the variation of distinct features in the response curves of the two types of materials being transported. We have seen the differences in the pathway responses in the tank and in the field between solutes and particles by location and by setting. The transport and attenuation processes of the particles with their large degree of removal compared to the solute do not occur in a similar manner to solutes. As discussed below, it is unlikely that comparison of, or cross-application of the solute-related diagnostic feature to the particle for either the time of initial detection or maximum concentration of the two responses are justified. Therefore, the traditional solute-based point or even fixed percentage-related comparisons appear of little meaning if cross-applied to particle responses.

#### Detection limits

Solutes appear to have a significantly higher (molar-based) detection limit than particles, even given today's advanced part per trillion (ppt) or better level of analytical resolution for fluorescent tracers such as uranine. In this instance, it therefore seems unlikely that comparisons of the time of first detection for particles and solutes can be always justified. Therefore a simple result such as detecting particle concentrations on-line or in samples earlier than solutes cannot be uniquely relied upon as evidence of preferential advection. The detection limit of each analytical method needs to be well documented and clearly specified for any comparative evaluation of this type. While this may be more evident to workers in some disciplines than others, there is little consistency or evaluation of this potential discrepancy in the literature when such comparisons are being made. Recently developed

diagnostic plots using graphical comparisons of the early time of concentration- or mass-normalized data may provide a more consistent approach to identifying preferential advection of particles. Documenting the time of first detection of a particle is however, a valuable and useful exercise as it directly provides and identifies the realistic migration rate of the particle. Defining well this time of occurrence is specifically important in situations where very low concentrations of for example, harmful viruses, are possibly being transported along flow paths. In cases of biocolloid contaminant-related protection studies, then particle first detection is a more useful rate-dependent indicator than any solute presence of percentage detection.

### Time to maximum concentrations – solute ‘peak’ versus biocolloid ‘pseudo peak’

The strong attenuation that occurs with particles along the flow path means there is an ever-reducing mass of particles available for transport down gradient. Using even a simple interpretive approach, the result will be that properties of concentration versus time curves at any point distant from the injection or constant source will be dissimilar to those for a conservative solute. The shape of the particle response curve and in particular the time at which the maximum concentration occurs are going to be highly dependent on the nature and extent of the processes resulting in particle attenuation along the flow path. The typical contaminant-related significance for a conservative solute-peak or its 50 % mass is therefore not going to have the same meaning for a particle response curve. The peak-related times for solutes are values that are used as common input to basic transport rate calculations and imply a non-sorptive behavior. Time of the particle maximum concentration is strongly dependent on attenuation extent in all cases and accordingly, use of this time-based feature of the breakthrough curve, is a pseudo-peak. The time of maximum concentration of a particle response cannot be meaningfully used to calculate a transport velocity for those particles. Thus, it should not be used as an input value compared to migration evaluation results commonly developed from ‘conservative’ solute peak times. As discussed below (Migration Rates), indicative particle transport velocity would more appropriately be based on the initial particle detection time (using an analysis method with reasonably low detection limits), not its breakthrough curve maximum concentration time.

### Relative breakthrough and solute-normalized data

‘Conservative’ solutes should be used together with a particle in any contaminant or setting characterization study. The integrated response over time provides the needed data to contrast the relative recovery of one or more particles with the solute recovery. Applying early and late time diagnostic plots appear to be useful to distinguish preferential advection and attachment/detachment patterns of the particles compared to conventional solute tracer responses. The benefits are of sufficient importance to particle-based contaminant studies that no- or low-retardation solutes should be included together with particles in detailed investigative programs to further our base for documenting and understanding biocolloid response, recovery and attenuation variability. Combined use of both solutes and particles can also provide greater distinction of the setting being tested particularly when there are important heterogeneity features in the subsurface. Particles will tend to migrate along the most highly advective channels with less lateral dispersion expected than for the solutes. Therefore, the differences in the patterns and relative recovery of the material types within the tested area seem, at least qualitatively at this point, to help us better understand or conceptualize differences in migration pathway characteristics.

### **1.5.4 Gravel aquifer environment – biocolloid transport characterization**

The setting in which the work is done is clearly critical. There are transport and performance factors for bacteriophages specifically associated with gravel aquifers, perhaps also pertinent to those found in fluvio-glacial settings as seen here in Switzerland that have to be taken into consideration for this work. Three such key aspects are:

- Bacteriophage distinction and applicability
- Relative recovery and attenuation
- Migration rates

### Bacteriophage distinction and applicability

The ability of bacteriophages to be effectively used in contaminant transport and particle migration studies, in the gravel aquifer environments that were tested, has been successfully demonstrated.

Despite large and substantial losses in the subsurface along the flow paths, the breakthrough patterns of the phages were clear and distinct and a highly consistent pattern for each phage's response was obtained at most sampling locations. The response among different phage types could be clearly shown and distinguished at most of the field sampling sites despite the up to 20 fold differences in relative recovery. From this viewpoint, the field data strongly reflects the consistent laboratory tank model preparatory evaluation data for at least four of the phages used (H40, MS2, H4, Psf2).

There is a further large benefit from being able to use multiple phages at the same time in these settings for tracer studies. They do not interfere with each other's migration and they can be clearly separated when they are concurrent at a down gradient location. This research did not involve multiple point entry tests. However, it seems evident from the extent and reliability of field-test data generated that this type of positive result from porous media field-testing is readily achievable and that the same degree of distinction among the phages would be readily achievable.

An unexpected result was the detection of the H40 bacteriophage at WW over two years after it had been injected in a prior testing campaign. The concentrations measured were small in comparison to what had been observed during the prior work. Levels were from samples taken after high rate pumping. The discriminatory centrifuge preparation suggested the phages were likely attached and therefore transported on suspended materials during pumping. This situation, however, seems to have documented that phage presence can be measured after a 'dormancy' or inactivity period when attached that lasts on the order of years, at least for H40 in the WW setting conditions. Therefore, under conditions where sampling could result in suspended materials being mobilized during testing, we should be aware that a persistence or background level may occur. The extent to which this applies to the wider number of the phages used for particle transport may also be worth further evaluation in important field settings.

### Relative recovery and attenuation

The phage recovery results obtained for the aquifers were able to be directly and clearly quantified and compared among the bacteriophages and to the solute used. Both RB and MDC values varied by up to 40-fold for the six phages tested in the tank and by up to 16-fold for the four phages evaluated at the three field sites. Both in the tank and at field sites, the impact of heterogeneity and flow path length was evident. In the tank, total H40 recovery at the downstream location varied over six tests from 28 to 47 % and these variations were likely ionic-strength related. Relative recovery varied at individual sampling points during the test by up to 9-fold for all phages. The large differences were heterogeneity dependent and not solely distance related. At the BdF field site, RB values were relatively high for particles at such distances of 10 to 22 m distance. For H40 they ranged from 0.1 to 1.4 % off channel and from 1.4 to 4.4 % on channel. They were consistently about one order of magnitude higher than for MS2. Lateral dispersive effects likely accounted for the evident channel location values from 3- to 10-fold higher than off-channel. The atypically high 72 % RB for H4 in BdF Test 4 demonstrates the rapid channeling and exceptionally limited attenuation possible in this type of gravel aquifer system. The WW site demonstrated distance-related reduction in RB with values in the down gradient channel locations decreasing by 15-fold from 14 to 64 m. Here also, H40 and MS2/Psf2 had relatively high RB values for these distances that decreased from 41 to 3 % and 16 to 0.2 %, respectively. Off channel at 34 m, lateral dispersion was evident but even in this expected lower-permeability setting, the recovery was still comparable to that found in the channel at 64m. This demonstrated the efficacy of transport with lateral dispersion. However, rather than the transport occurring in silt or clayey materials, it suggests that the migration at WW occurred laterally in interlayer or lens-like deposits of higher permeable materials extending, with continuity, away from the principal channel deposits. At BdF and WW, the recovery values obtained in these settings were relatively high and show the extent of reduced particle attenuation that can occur in such gravel-dominated subsurface environments.

KAP had the lowest RBs from about 0.005 to 0.2 %, more consistent with the levels reported for the extensive testing done in sand and gravel deposits at the Cape Cod site. At KAP, however, the differences with the low recovery compared to BdF/WW, are believed more related to the geochemical or biological conditions linked to the REDOX environment at the site than could attributable to differences in the hydrodynamics of the KAP and BdF/WW subsurface flow regimes. Clearly, however, the degree of attenuation differed at each of the three field sites further highlighting the need to concurrently develop detailed understanding the other site setting variables in order to evaluate and eventually develop tools to forecast particle migration intra-dependencies.

### Migration rates

The transport rates of both particles and solutes in the gravel aquifers we tested in Switzerland are on the order of several 10s to 100s of m/d based on the times of documented initial detection of particles. These rates were confirmed by multiple tests at three different settings. The flow rates in the Rhone River valley gravel were as high as 450 m/d and otherwise ranged from about 175 to 350 m/d in the channel settings. These values reflect a high velocity setting occurring in some instances in large preferential pathways in the near-surface boulder and gravel aquifer materials. At KAP and WW, the particle transport rates ranged from about 30 to 45 m/d and 170 to 300 m/d, respectively. For uranine, the velocities calculated based on the time of the first solute detection were not appreciably different than for those of the particles. Obviously, however, the velocities derived from conventional rule-of-thumb approach using the time of the solute maximum were less. Typically, the comparisons showed they were from 3- to 8-fold lower than the particle detection derived documented transport rates. Site-specifically documented ranges in particle migration rates are those that should, conservatively, be given consideration in associated contaminant studies and ground water protection evaluation studies. They are likely going to be higher than expected or predicted in many aquifer regions.

### **1.5.5 Future research – bacteriophages/biocolloids to characterize contaminant migration**

We are in the early days of knowledge about particle transport in a wide variety of subsurface settings. Historically, public concern has been low, as obtaining 'clean' ground water for drinking or agricultural uses, was rarely an issue. Today, however, that has ceased to be the case in most countries. Therefore, I am certain that work related to protection of aquifer environments and public health will continue to be viewed globally with a high priority and that funding shall be allocated for key areas of related research. These appropriately would result in extending programs to follow from what other workers and we have conclusively shown are the benefits of using bacteriophages in ground water environments. I believe that applied research in the five areas described below would be valuable. First, it could enhance our ability for bioparticles, in particular bacteriophages, to be used more effectively as indicators of subsurface migration behavior. Second, it could illustrate how improved particle/biocolloid migration work can increase the safety and quality of public water supplies. Bacteriophages, after all, are the prime candidates for surrogates of viral contaminants. Therefore, they should be strongly considered contenders for associated ground water protection and drinking water supply studies in a wide variety of subsurface settings. Several aspects significant to future work are summarized under the following headings:

- Standardization of methods and integration of inter-disciplinary analysis
- Phage characterization and variability
- Correlation column studies
- Aquifer setting variability
- Emphasis on field studies

### Standardization of methods and integration of inter-disciplinary analysis

Consistent approaches to undertaking essential applied and specialty research work in the subsurface have to be developed for particle migration studies. They are different than the solute-related requirements and undoubtedly more complex. The field of particle migration in ground water and the subsurface is still relatively new and there are important considerations being developed in a variety of co-relative disciplines. Each of these disciplines has specific methods and standards that do not seem to be able to readily address cross-disciplinary technical boundaries. Funding sources seem to be currently pigeonholing viral- and microbiologic research scientific efforts into narrow health-related channels that are failing to address the bigger aspects of public water supply and quality concerns which need to be addressed on a broader and systematic scale. As a result, there is much research being carried out but little of it seems focused beyond each discipline's specialized research area. Despite research progress, there is no clear advocacy entity for standardizing multi-disciplinary approaches inherent to the use and integration of the data related to protection of public water supplies and colloid particle contaminant migration issues. A strong advocacy group is needed to provide the basis for integrating the combined resources of several of these key and inter-related disciplines to work closely together to develop pertinent databases and constructive new approaches to drinking water and water resource management/protection studies. Given the variety of objectives and applications in each discipline, it is critical that basic approaches and standard methods be established and relied upon in order that comprehensive results will emerge and be applicable over the long term. The active commitment of researchers to work closely together in this field is needed

hand in hand with providing the funding to support this work. The focal point of such a group around the recently formed “International Society of Subsurface Microbiology” could be a formative positive step in this direction. Regulatory authorities could also help by requiring the development, implementation and publicizing of ISO/ASTM-like protocols and procedures to be adhered to on ongoing and future public water supply programs which principally rely on ground water or its conjunctive use with lakes or rivers.

### Phage characterization and variability

Although perhaps self-evident, the basic nature of the particles themselves used in such studies must be completely defined before a better understanding of their performance in various settings can begin to be coherently developed. It is not likely that using biocolloid or inert particles, even those demonstrating a high efficacy in different subsurface environments, will further our understanding of co-relational effects in contaminant transport unless the particles themselves are characterized in an elementary manner. This would include describing their shape, size, weight, electrical charge(s) and degree of sensitivity and changes in performance (attachment/detachment) according to variations in bio- and geo-chemical and hydrodynamic settings. Given the diversity and number of bacteriophages, and their demonstrated utility as surrogates for viral studies to date, it seems clear that microbiologists should focus on developing and applying consistent and efficient prime characterization methods for these particles. Furthermore, focus should be given to developing direct or indirect methods of real-time or increasingly rapid turn around in concentration levels for these biocolloids. The emphasis in regulatory environments should not be solely related to worst-case scenarios with the highest efficacy performer given the predominant center of attention. Given the diversity of phages able to act as surrogates for a wide variety of viral entities of concern, it would be pertinent to develop more individual phages for use where they can be applied to demonstrate the attenuation achieved in given aquifer field settings.

### Correlation column studies

The ability to obtain representative environmental subsurface samples is increasing, albeit costly and time consuming. Nevertheless, there are strong benefits to being able to work with relatively large-scale subsurface samples in the laboratory or to try to set up pilot-scale column work in well-designed field studies. The controlled conditions and increased operational flexibility in these settings can commonly be used to isolate critical dependencies and relational factors using screening and blank/surrogate trials. There are standard and now more advanced microbiologically based techniques that rely on cm-scale column work, much of which can address viral/phage characterization in specific and well-contained environments. Some researchers working in a variety of disciplines have also employed short columns with a variety of artificial and simulated porous matrix materials and effects to characterize and evaluate relative performance of virus and phages. However, to scale up the application of the results from these data to field sites should be recognized as needing at least significant intermediate steps involving larger columns or tanks using real aquifer sediment. It is equally important that these settings be able to be regulated so that there is a realistic and maintainable geochemical environment that reasonably reflects in-situ conditions. Clearly there is a need and benefit from conducting the more basic studies in controlled settings until such time as behavioral patterns in different settings have been conducted. What is too often the case, however, is that interdependencies that seem evident and well founded in such laboratory environments are either not tested in real-world conditions or when they are, the results differ. When there are major differences, the apparent contradiction to the lab data are frequently assigned to irregularities in the environment being tested. It would seem that at some point, the importance of developing and understanding greater details of, and therefore better protecting, the actual aquifer settings could become more highly prioritized and the needed up scaling work will be seen as equally justified to laboratory evaluations.

### Aquifer setting variability

Evaluating the multiple factors that affect phage performance in site-specific settings is the larger programmatic goal. This will require detailed delineation of the geochemistry and biological setting of aquifers – both their waters and matrix - where interactions take place. A rigorous systematic approach is needed. There are few established rules-of-thumb that are either universal or guaranteed to predict particulate responses under variable water quality and sediment mineralogy. The differences noted particularly with Psf2, H40 and MS2 phages demonstrated that there are likely more than simply ionic strength or temperature variability that may dramatically influence their efficacy and recovery/transport responses. Recognizing that a high reliability and consistency of data can be achieved with appropriate injection and sampling protocols, the next steps should address multiple sites and repetitive testing of aquifers with key phages. Given their innocuous nature and clear

distinction capability, focused testing of representative aquifers that target significant public water supply sources could be tested with a set of candidate phages deemed pertinent for viral-surrogate purposes. Developing a widely ranging database of aquifer conditions of particulate responses in well-characterized and important aquifers would be a beginning to provide the results to address more in-depth understanding of the important variable factors that influence and control their transport behavior. Repetitive testing programs would also be included, as they are critical to verify consistency and reliability. Priority would be given to the aquifer settings that are critical to public water supplies, particularly those where there is an acknowledged potential risk of contaminant release combined with large uncertainty in the characterization of the aquifer transport conditions.

### Emphasis on field studies

I certainly cannot hide a personal bias that when weighing the benefit from reliance on empirical data or theoretical small scale lab column tests and predictive modeling, the balance should commonly tip on the side of undertaking actual case studies. Only these site-specific actions will provide the needed first hand knowledge that can document the concrete responses pertinent to the subsurface environments that need to be characterized and the performance of the colloidal materials within those settings. A common misleading approach from theoreticians and micro-scale disciplines is that the field settings are too logistically and technically difficult to test, are commonly only unique to that setting and therefore of little global significance or value. Also, inappropriate up scaling of laboratory results and extrapolation of simplistic or screening-level predictive flow or transport models to aquifer settings are conducted without results verification included as an integrated part of the environmental protection process. There is a certain utility for supportive studies but there is considerably larger uncertainty without site-specific validation. Today, the type of important field studies required are able to be done with significantly less time and costs using the modern equipment and techniques and optimization methods we have demonstrated, many of which were not available as little as five years ago. These studies are operationally simpler to undertake with the advent of remote sampling, in-situ and real time analyses and data telecommunication. Real-time detection from downhole or in-line analyses is critical and can dramatically improve the success of many field-based programs and research. Public water supply and aquifer protection programs need these kinds of precise definition and justify such aquifer-specific site-oriented work.

## CHAPTER 2

### ***Comparative Bacteriophage Transport in a 5.6 m<sup>3</sup> Sand-filled Heterogeneous Experimental Tank Model (MARCEAUS)<sup>1</sup>***

#### Abstract

Our research in this laboratory environment focussed on distinguishing flow path and mass transport rates of biocolloids and dissolved solutes in heterogeneous porous media. The clear objective was to better understand particulate and dissolved constituent contaminant migrations responses and evaluate how to compare and characterise them. Conducting several tracer tests in a physical tank model with a well-defined physical heterogeneity (MARCEAUS) did this. Six different biocolloids (marine-H4, H6, H40, non-marine-T7, Psf2, MS2 bacteriophages) were variably co-mingled with both salt and uranine to begin the experiments with a uniform input signal. The 1 m x 1 m x 6 m tank with three different-permeability sands arranged discretely in 1260 "boxes" (10 cm x 10 cm x 40 cm) has scale and pore-size conditions similar to some field sites currently used for viral migration studies. The three sand materials were washed and therefore the environment did not contain noticeable silt or clay size fractions. MARCEAUS' advantage was a controllable gradient, temperature and chemistry. Salt, a "conventional" tracer, was monitored on-line and continuously at about 353 nodes giving a fundamental solute signal to compare among bacteriophages and uranine responses. Phages and uranine were measured from samples taken at six locations reflecting heterogeneity in the tank transport. Results showed consistent responses of salt and uranine transport in the tank. Relative breakthrough (RB) decreased consistently with electrical conductivity reduction from 3700 to 700  $\mu\text{S cm}^{-1}$  for Phage H40 (32 to 46%) and MS2 (22 to 73%). Biocolloid recovery was lower than the solutes according to principally phage type, sampling location within the tank's heterogeneity and ionic strength. Within the tank, RB values for H40, MS2 and H4 were generally high from 5 to 94%, 3 to 59% and 4 to 38%, respectively. They were zero to 33% for Psf2 and from 0.3 to 11% for H6. Total recovery, ranked for at the outflow, was H40 (28 to 51% - 5 tests), MS2 (25 to 31% - 2 tests), H4 (24%), H6 (2 to 4% - 2 tests), and Psf2 (1%). T7 was not detected in, or at the outflow of, the tank. Velocity calculated from first detection and normalised to account for variable flow rates during the tests varied according to heterogeneity in the system. It was from about 0.17 to 0.27 in the lower permeability multiple paths and from 0.32 to 0.37 in the preferential channel pathways. Double solute peaks reflected multiple route contributions along longer and differing pathways. Phages had either no, or a subdued, double response illustrating the high degree of attachment and more restrictive pathway routing for the biocolloid particles compared to the solutes. Results have provided a start point for biocolloid transport modelling and migration processes evaluation and ranking.

#### Keywords

Tracer test, biocolloids, bacteriophages, uranine, laboratory tank models, sand, porous medium, particulate transport

<sup>1</sup> This chapter forms the basis of a paper (Keith Kennedy <sup>a,b</sup>, Robert Mose <sup>c</sup>, Pierre Rossi <sup>d</sup>, Philippe Ackerer <sup>c</sup> and Imre Müller <sup>a</sup>, 2001) published in the Univ. of Neuchâtel, Hydrogeology Center Bulletin, Year 2001, Vol. 19, pp. 49-105. (Ref. Official - Bulletin d'Hydrogéologie No 19 (2001): 49-105, Centre d'Hydrogéologie, Univ. de Neuchâtel Eds: PETER LANG). We are extremely grateful to the CHYN Bulletin Editor, Dr. Francois Vuataz, for his diligence and patience in helping us work through this lengthy manuscript and his translation of the abstract into French and to Carine Brayek for her careful and thorough preparation of the final version used for publication in the CHYN Bulletin. We appreciate the opportunity CHYN provided us with to publish this article in their journal.

Author contacts: a) Centre d'hydrogéologie, Univ. Neuchâtel, Rue Emile-Argand 11, CH-2007 Neuchâtel, b) Actual address: NAGRA/CEDRA, Hardstr. 73, CH-5430 Wettingen; c) Institut de mécanique des fluides, Univ. Louis Pasteur, Rue Boussingault 2; F-67000 Strasbourg, France; d) Laboratoire de microbiologie, Univ. Neuchâtel, Rue Emile-Argand 11, CH-2007 Neuchâtel.

## Résumé

Notre recherche dans un environnement de laboratoire s'est concentrée sur la quantification de l'écoulement et du transport biocolloïdes et de substances dissoutes dans des milieux poreux hétérogènes. L'objectif principal était d'une part de mieux comprendre la réponse de substances contaminantes migrant sous forme de particules et de solutés, et d'autre part essayer de les comparer et de les caractériser. La mise en oeuvre de plusieurs essais de traçage dans un modèle physique de réservoir avec une hétérogénéité bien définie (MARCEAUS) a permis d'atteindre cet objectif. Six biocolloïdes différents (bactériophages marins-H4, H6, H40, et non-marins-T7, Psf2, MS2) ont été mélangés en proportion variable avec du sel et de l'uranine afin débiter les expériences avec un signal d'entrée uniforme. Le réservoir de 1 m x 1 m x 6 m, avec trois sables de perméabilité différente arrangés de manière discrète dans 1260 cellules (10 cm x 10 cm x 40 cm), se trouve dans des conditions d'échelle et de porosité semblables à quelques sites sur le terrain actuellement utilisés pour l'étude de la migration des virus. Les trois types de sable ont été lavés et donc le milieu utilisé ne contient pas de manière significative de particules de la taille du limon ou de l'argile. Un des avantages de MARCEAUS est de pouvoir contrôler le gradient hydraulique, la température et la chimie.

Du sel, un traceur conventionnel, a été surveillé en continu à quelques 353 nœuds, donnant ainsi le signal fondamental d'une substance dissoute, pouvant être comparé aux réponses des bactériophages et de l'uranine. Les phages et l'uranine ont été mesurés sur des échantillons prélevés à six emplacements différents reflétant l'hétérogénéité du transport dans le réservoir. Les résultats ont montré des réponses cohérentes du transport de sel et d'uranine dans le réservoir. La percée relative a diminué régulièrement avec la réduction de conductivité électrique de 3700 à 700  $\mu\text{S cm}^{-1}$  pour les phages H40 (32 à 46%) et MS2 (22 à 73%). La récupération des biocolloïdes était inférieure à celle des substances dissoutes, principalement en raison du type de bactériophage, du lieu d'échantillonnage selon l'hétérogénéité du réservoir et de la force ionique. Dans le réservoir, les valeurs de percée relative pour H40, les MS2 et les H4 étaient généralement élevées de 5 à 94%, de 3 à 59% et de 4 à 38%, respectivement. Elles étaient de 0 à 33% pour Psf2 et de 0.3 à 11% pour H6. La récupération totale à la sortie du réservoir atteint les valeurs suivantes: H40 (28 à 51% - 5 tests), MS2 (25 à 31% - 2 tests), H4 (24%), H6 (2 à 4% - 2 tests) et Psf2 (1%). T7 n'a été détecté ni dans le réservoir ni à sa sortie.

La vitesse calculée à partir de la première détection et normalisée pour tenir compte de débits variables pendant les essais a changé en raison de l'hétérogénéité du système. Elle était d'environ 0.17 à 0.27 dans les cheminements multiples de faible perméabilité, et de 0.32 à 0.37 dans les trajectoires préférentielles. Les doubles pics des substances dissoutes reflètent de multiples contributions d'itinéraires plus longs et différents. Les bactériophages n'ont pas montré, ou de manière limitée, un double comportement illustrant pour les particules de biocolloïdes leur haut degré d'attachement et des cheminements plus restreints. Les résultats de cette étude ont fourni un point de départ pour modéliser le transport des biocolloïdes, ainsi qu'évaluer et classer les processus de migration.

## Mots-clés

Test de traçage, biocolloïdes, bactériophages, uranine, modèle de réservoir en laboratoire, sable, milieu poreux, transport de particules

## 2.1 Introduction

The purpose of carrying out this work was to document the differences in behaviour between three marine bacteriophages (phages), three other phages and two conservative solute tracers in a porous media over a medium scale (6 m) in a controlled and well-hydraulically-portrayed laboratory tank environment. The three marine phages had been developed for use as bioparticle tracers and were used in Switzerland for tracer experiments carried out extensively in both karst and river settings and in one porous media test field (N. DOERFLIGER 1996, A. CARVALHO DILL 1993, P. ROSSI 1994). The experimental tank model had been used for the previous development, evaluation and validation of a variety of computational transport models in porous media and had therefore been well characterized with respect to its heterogeneity (M. RUCH 1992, P. SIEGEL 1995). There were advantages in using an already well-characterized tank environment under stable flow conditions compared to a field terrain setting. First, it allowed an evaluation of comparable migration responses of different phages to be carried out in a relatively short period of time, with a high certainty of results and with less costly instrumentation than needed in field settings. Second, interactions taking place among the phages, porous media and its associated fluid chemistry could be better documented, establishing a data base to be subsequently evaluated related to the parameter variability.

Phages have been used as indicators or surrogates in the study of viral migration as well as indicators of differences in pathways followed by variable tracer types in heterogeneous environments. Typically, biocolloid particles undergo large percentage reduction over short distances and unless they are injected in a relatively continuous manner, their detection in many subsurface environments is difficult. We had the ability to control the mixing process and the time of injection into the tank as well as to observe the changes in migration response at different discrete locations within the tank's variable flow paths and compare these biocolloid responses with conservative chemical solutes. The scale of the work was over distances up to 5.6 m with the heterogeneity allowing the influence of the preferential and slower transport paths to be seen in a series of tests with variable ionic strength and flow rates.

The scope of work involved carrying out six tests with six different phages and two conservative solute tracers. The tank instrumentation to document salt transport with electrical conductivity probes was supplemented to allow discrete samples to be taken from specific locations within the tank. The results of the samples were compared for all different materials that were injected. Selected test data are in Appendix 2.

The work in the tank at IMF Strasbourg was related to broader biocolloid research focussing on the migration behavior and response characterization of bacteriophages in porous media aquifers in Switzerland. This larger body of field work was undertaken and has been published in a Ph.D. thesis (K. KENNEDY 2001) as well as in several subject-related articles (K. KENNEDY 2000, K. KENNEDY et al. 2001a, K. KENNEDY et al. 2001b, and K. KENNEDY et al. 2001c).

## 2.2 Materials and Methods

The purpose of this section is to describe the materials and methods that were used to undertake the experimental research. It consists of describing the:

- experimental setting and materials injected into the system;
- tracer testing approach and methodology;
- laboratory methods for analysis;
- basis of how the comparative transport and phage evaluation were performed.

### 2.2.1 Experimental setting

We adapted the instrumentation and facilities of an existing experimental laboratory tank model to our needs for comparative particle migration assessment. We installed additional sampling points and conducted on site monitoring and analysis of both electrical conductivity and uranine as well as taking samples for off-site microbiological analyses.

### 2.2.2 The tank MARCEAUS

The Institute of Fluid Mechanics at Louis Pasteur University, Strasbourg has an experimental physical tank model facility referred to as the MARCEAUS tank laboratory model. Details of the tank and its construction, as reported below, are taken from one of the original workers in the tank (M. RUCH 1992) and related publications referenced by him, subsequent co-workers and updates from our work.

Physical characterization of the flow within the tank, as well as salt solute migration under different viscosity and density conditions, were the basis of mathematical model development and validation using MARCEAUS since about 1990 (M. RUCH 1992, P. SIEGEL 1995).

The schematic of the experimental set up is shown on figure 2-1. The MARCEAUS facility consists of four basic components:

- an external vat from which the mixed tracers are conveyed to the tank using a high-precision peristaltic pump;
- the tank model with its associated piping and hydraulic controls;
- an enclosure to maintain the tank environment at a constant temperature;
- a dedicated data acquisition system for monitoring electrical conductivity at 353 locations in the porous media.

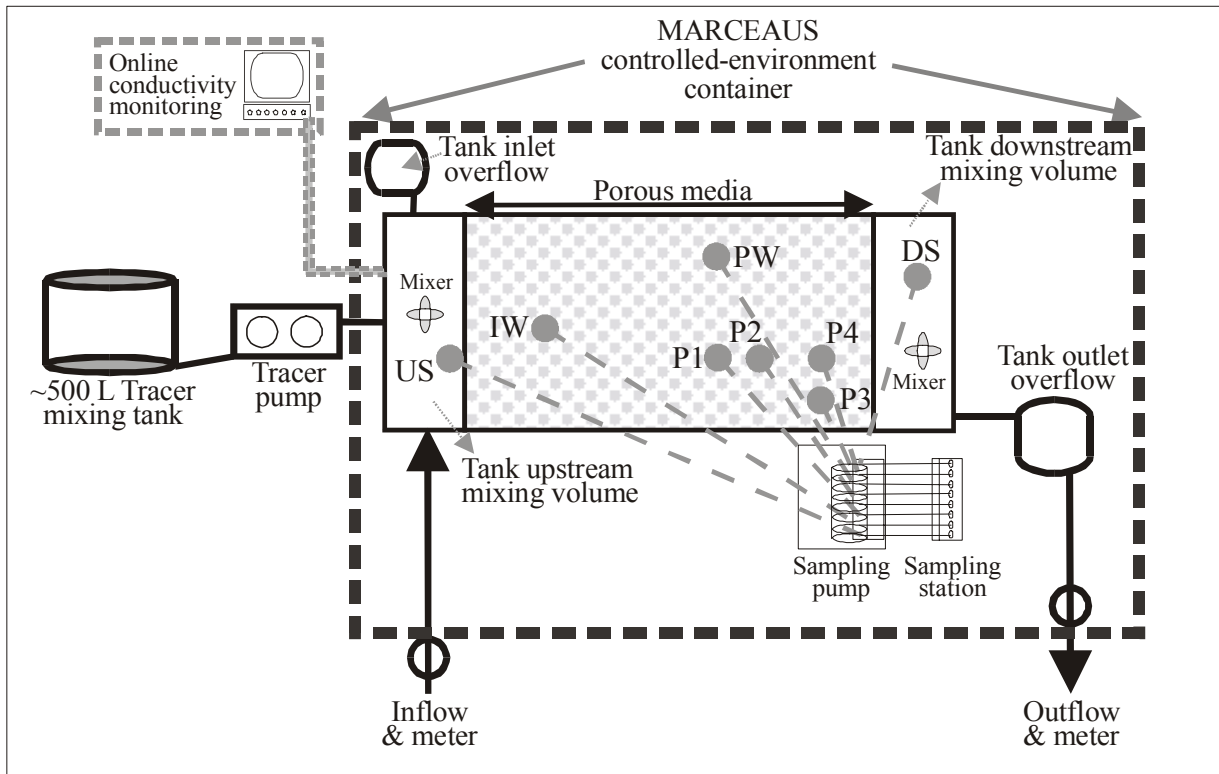


Figure 2-1. MARCEAUS facility instrumentation schematic diagram.

### Tracer mixing vat

The external mixing vat is a large circular polypropylene container with an open top that can be used to mix up to about 500 L of solution using either manual stirring or motor-driven propeller device for mixing. This tank has a spigot near its bottom that is connected with a hose to a highly accurate peristaltic pump. The pump delivers the tracer solution by hose at an adjustable, but constant rate, to an injection line with multiple tips situated about 10 cm above the water level in the tank's upstream mixing area.

### Tank

The physical tank model itself (figure 2-2) is about 6.4 m long and consists of three separate volumetric compartments, two filled with water at either end and the centre one containing the porous medium, sand. The first water-filled volume we refer to as the upstream location (US). It is about 40 cm long and 1 m wide and is where the tracers arrive and are mixed with make-up water coming from the City of Strasbourg's public drinking water supply. The make-up water and tracer are mechanically stirred in this area to achieve a well-mixed input source to the tank's porous media. The solution in the tank, in part, flows into the porous media across a highly permeable membrane. The supplemental, and for the experiments, unused volume of mixed solution that does not flow into the porous media goes out through an overflow system control which is maintained at a constant height during testing. The water flow rate from the city's supply and the height of the water level in the upstream location, can be regulated using inline valves and by adjusting the height of the overflow lip, respectively. A flow meter is used to measure the overflow from the upstream part of the tank. The height of the water in the upstream location is kept at about 90 cm so that the input water level is kept close to the top level

of the sand. It is from within this circulating water volume that the US samples are collected and the concentrations are used to characterize the input signal to the model's porous media.

The downstream compartment is the same size as the upstream location. It collects all the flow and associated tracer that has migrated through the tank's centre. We refer to this as the downstream location (DS). Its hydraulic level is also regulated and kept constant by means of a fixed overflow apparatus as the water level height is controlled by the elevation of the outflow overflow lip. Differences between the two overflow lip elevations control the variations in flow rate due to the gradient along the length of the tank. A flow meter is used to measure the overflow from the downstream part of the tank which is considered the total system flux. It is from within this circulating water volume that the DS samples are collected. The concentrations from these samples are used to characterize the output arriving from all the tracer pathways through the model's porous media. Flow rates in the tank can be varied. During testing they were from about 4 to 8 L min<sup>-1</sup>.

The middle part of the tank model is where the porous media is located. It is within a volume of about 1 m high by 1 m wide by 5.6 m long. The porous media was emplaced in nine vertical layers, each of 10 cm height. In each of the nine layers, there are 140, 40 cm long (longitudinally) by 10 cm wide (horizontally) sections. The total saturated system can consist of 1260 individual sections. Washed sands of three different hydraulic conductivity values were carefully emplaced within each of the individual sections. Water volume in the saturated media of the tank, based on an estimated 25% porosity for the sand, is about 1300 L. The initial (upstream-side) and end (downstream-side) groupings each resemble a homogeneous block of sand. These entry and exit blocks consist of their ten lateral and nine vertical sections where the porous media is homogeneous. The remaining 12 sections between the start and end blocks have differing heterogeneity patterns which are a function of how the three types of sand have been emplaced in the sections. The three sand hydraulic conductivity values are reported to have values from  $9.0 \text{ e}^{-4}$ ,  $7.13 \text{ e}^{-3}$  and  $1.02 \text{ e}^{-2} \text{ m s}^{-1}$ . The layout of the sand distribution in the cells of the nine layers is in M. RUCH (1992).

#### Constant environment container

The entire tank and its inflow and outlet components are enclosed in a container-like structure that has an air-conditioned environment kept at about 12 to 13°C with a dedicated air-conditioning supply.

#### Electrical conductivity data acquisition system

Different flow paths develop in the tank sediments as a function of its heterogeneity. Being able to make electrical conductivity measurements facilitates defining and illustrating the variability of solute pathways through the tank. Every six minutes EC values are recorded from each of 353 conductivity probes installed systematically in the tank. All probe data are recorded.

### **2.2.3 Tank sampling adaptations and instrumentation**

For our biocolloid and solute transport-related research, it was necessary to: first, rejuvenate the tank; second, install more sampling points in order to take discrete samples for subsequent analyses; and third, establish a sampling program that allowed an adequate volume of sample to be taken from the system without disturbing the flow through the system at the points of interest. Neither uranine nor any of the phages could be automatically tracked in-place like the concentration of salt using the EC probes. We expected that the uranine would mimic the behaviour of the salt, and in fact, for the most part it did. However, we chose to focus our efforts principally on:

- sampling at discrete points in the flow system that were of interest based on the patterns of solute transport documented during pre-trials;
- comparing test results for all materials injected based on individual concentrations determined for the samples taken from only the discrete sampling points in the flow system.

#### Tank rejuvenation

The tank had not been used for over 12 months prior to starting our experimental program. The tank pathways can typically become affected with temporary build up of iron and bacterial growth in the media as well as on the entry screen when it sits idle for prolonged periods without flow. First, the tank was flushed with a dilute acid wash for about 24 h to remove the build up. Second, the flow was initialised and left to continue for a period of about two weeks during which time the standard operating conditions and flow regime of the tank were restored. Finally, four pre-trials using salt were undertaken and results compared with those from previous testing to document the conditions of flow at locations throughout the tank and confirm that their data were similar to the "standard" that had been observed in previous MARCEAUS testing programs.

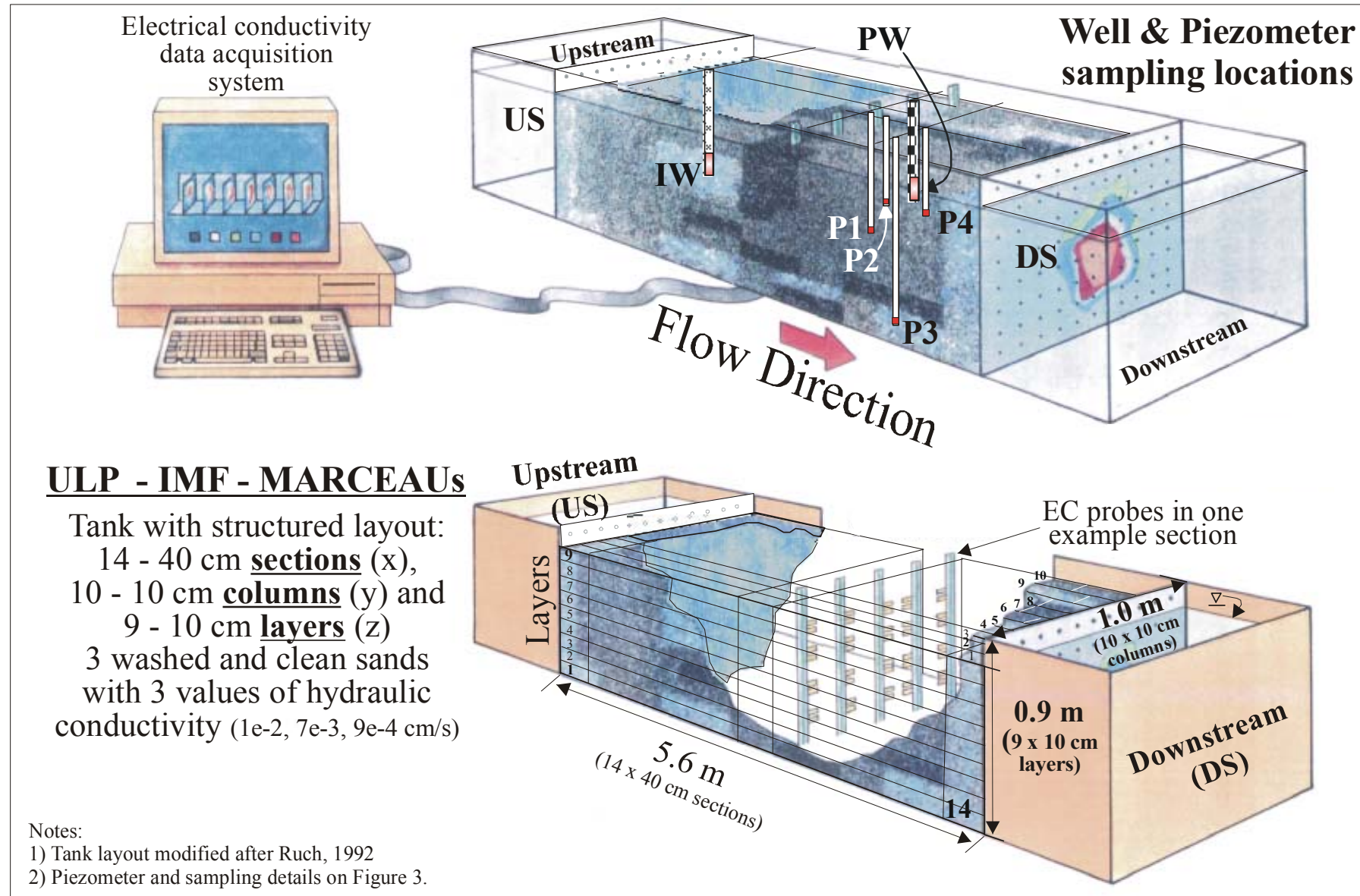


Figure 2-2. Tank flow system, cell organization and sampling location layout.

### Supplemental sampling points

Based on the preliminary salt-related trials after rejuvenation, we installed 4 mm wide by 20 mm high piezometer-tip type points (P1, P2, P3, P4) for sampling at four locations within the tank's porous media (figure 2-2). Their locations were believed to be representative of both the faster and slower pathways that had been observed in prior tracer testing throughout the system. These supplemented the two existing larger, 15 mm diameter, 70 mm high "well points" (IW, PW), that were part of the tank's original monitoring facilities that were located in the faster flow pattern regions of the tank. In this manner, we were able to sample at up to six individual points in the tank as well as at the injection (US) source and in the outflow (DS) locations. Table 2-1 summarizes the characteristics and locations of the sampling points within the system. Figure 2-3 schematically represents the sampling locations in a cut away cross section of the tank and illustrates the specific sampling locations from the lateral (side) cross section and top views. The relative location of these sampling points is important to understanding the data and comparative response of the different materials injected during our experiments.

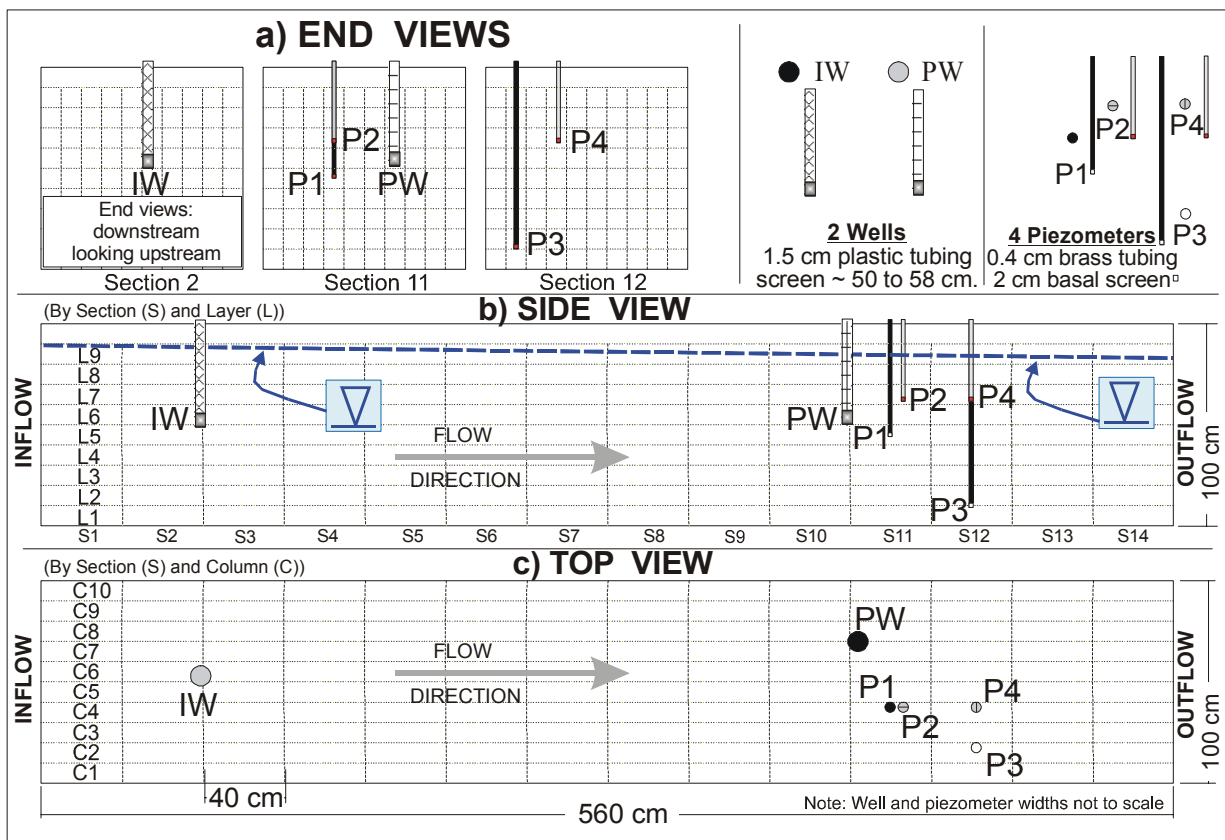


Figure 2-3. Tank structure with cell layout and sampling locations.

### Sampling strategy and equipment

Online EC data provided us with the ability to continuously monitor the solute transport through the tank during the tests. However, EC concentrations were determined from the individual samples taken at these locations so as to compare the uranine, bacteriophage and EC values from the exact same point. Samples from the eight points (US, IW, PW, P1, P2, P3, P4, DS) were obtained using an eight (8) channel peristaltic pump (Ismatec Model IPC 8 (equiv.)) connected with 1.6 mm ID tubing (Nalgene 180 pvc tubing, 1/16 ID, 3/16OD) to each of the eight sampling locations. Rates of withdrawal were continuous over the testing at about 20 to 30 ml min<sup>-1</sup> per location. Flow from the tubing was directed from the peristaltic pump a distance of about 25 cm to an 8-position sampling stand located adjacent to the tank. Samples were taken in 100 ml dark brown coloured polyvinyl chloride bottles on a timed basis routinely during each test. When not sampling, the volume of flow from the pump drained into a separate metal overflow compartment and left the controlled temperature container through an outflow line in the bottom of the compartment.

**Table 2-1. Sampling point specifications <sup>(1)</sup>.**

| Sampling point |                   |                                 |               | Location in tank   |                      |                     | Screen location details |        |      |
|----------------|-------------------|---------------------------------|---------------|--------------------|----------------------|---------------------|-------------------------|--------|------|
| Point          | Name              | Section location <sup>(3)</sup> | Screen height | Along gradient (x) | Lateral (corner) (y) | Vertical (base) (z) | Bottom                  | Middle | Top  |
| 1              | US <sup>(2)</sup> | Upstream                        | 100           | 0                  | 0 to 100             | 0                   | 0                       | 50     | 100  |
| 2              | IW <sup>(2)</sup> | 2                               | 7             | 79                 | 53                   | 50                  | 50                      | 53.5   | 57   |
| 3              | PW <sup>(2)</sup> | 11                              | 7             | 399                | 65                   | 51                  | 51                      | 54.5   | 58   |
| 4              | P1                | 11                              | 2             | 420                | 35                   | 45                  | 45                      | 46     | 47   |
| 5              | P2                | 11                              | 2             | 427                | 35                   | 62.5                | 62.5                    | 63.5   | 64.5 |
| 6              | P3                | 12                              | 2             | 460                | 15                   | 10                  | 10                      | 11     | 12   |
| 7              | P4                | 12                              | 2             | 460                | 35                   | 62.5                | 62.5                    | 63.5   | 64.5 |
| 8              | DS <sup>(2)</sup> | Downstream                      | 100           | 560                | 0 to 100             | 0                   | 0                       | 50     | 100  |

<sup>(1)</sup> All dimensions in cm unless otherwise noted.

<sup>(2)</sup> Location specifications from M. Ruch (1992). P1, P2, P3 and P4 installed and are as measured.

<sup>(3)</sup> Layout in figure 2-2.

### 2.2.4 Materials used as tracers

The solute tracers used were salt (NaCl) and the fluorescent material uranine ( $C_{20}H_{10}O_5Na_2$ ). The biocolloid particle materials injected consisted of different bacteriophages. All tracers were injected into the tank simultaneously. The use of both salt and uranine in both laboratory and field studies is extensively documented. Both are considered as conservative tracers meaning that they well reflect the actual pathways and apportioned mass of contaminants dissolved in water as they migrate through the subsurface geologic media. Uranine has been observed in some studies to be subject to adsorption and thus being retarded relative to non-sorptive tracers. Most of the occurrences suggesting a non-conservative behaviour of uranine, however, were documented in situations where there were large amounts of clay and silt-sized materials in the transport pathways. In the case of our testing in MARCEAUS, we believe that the washed nature of the emplaced clean sands would not result in any measurable removal of uranine from the flow pathways by adsorption. Upstream, injected tracer changes in the initial concentration of electrical conductivity ( $dEC_0$ ) ranged from 0 to 3700  $\mu S\ cm^{-1}$  and uranine initial concentrations ( $UR_0$ ) were from 250 to 900 ppb.

The characteristics of the bacteriophages are perhaps the least well know and documented properties of the materials are in table 2-2. Among colloids, bacteriophages present several advantages. Their small size (about 20 to 350 nm) puts them on the same scale as pathogenic viruses of eucaryotic organisms. They are non-toxic and contrary to their "bacterial cousins" they are non-pathogenic. Furthermore, by choosing an appropriate bacteriophage (B) /host bacterium (HB) (B/HB) system, the risk of influencing an aquifer's micro-flora can be reduced. They are commonly injected with high concentrations ( $>1^{e10}$  plate forming units (pfu) per  $ml^{-1}$ ), can have a large percentage removal in the subsurface, yet are useful due to their low detection level ( $0.5\ pfu\ ml^{-1}$ ) and the uniqueness in analytical discrimination. Upstream, phage initial concentrations ( $C_0$ ) ranged from about 600 to 11,000  $pfu\ ml^{-1}$ .

### 2.2.5 Experimental approach and tracer testing methodology

The purpose of this section is to describe the rationale used to develop the original approach to comparing the intra-particle and solute responses and how the program evolved in response to the observed results that occurred during the six tests. The original purpose of the laboratory testing program at the MARCEAUS facility was to evaluate the efficacy of different phages to migrate over distances of up to about 6 m. This testing was in part preparation for field testing programs about to be undertaken over distances of up to 100 m in relatively permeable glacial outwash and river deposits. The objectives of this upcoming work involved comparison of how different biocolloid particles and solute tracers were transported in porous media. Therefore, the results from the tank were to be instructive to both field test design and biocolloid selection for these programs.

Six tests were carried out in the MARCEAUS facility. The first three tests included sampling at all eight of the locations as described above in Section 2.1. The last three tests focussed on increased sampling density at the four points further down gradient from the injection location. Table 2-3 summarizes the characteristic of the tests in terms of materials used and testing approach for the six tests. All tests involved the use of a time step injection duration of about four hours. A step function, rather than an instantaneous "dirac" was used because prior experience from testing showed that the entire volume of the media was not subjected to mass being transported if injection time was shorter.

**Table 2-2. Bacteriophage characteristics.**

| <b>Bacteriophage</b>                | <b>T7</b>        | <b>Psf2</b>       | <b>MS2</b>                                           | <b>H40/1</b>                | <b>H4</b>                  | <b>H6</b>                  |
|-------------------------------------|------------------|-------------------|------------------------------------------------------|-----------------------------|----------------------------|----------------------------|
| <b>Description (morphotype)</b>     | Podoviridae (C1) | Siphoviridae (B1) | Leviviridae (E1)                                     | Siphoviridae (B1)           | Siphoviridae (B1)          | Leviviridae (E1)           |
| <b>Size</b>                         | 60*17 nm         | ND                | 24 nm                                                | 85 *44 nm                   | ND                         | 30 nm                      |
| <b>Isoelectric point</b>            | ND               | ND                | 3.5<br>+/- 0.08 <sup>(1)</sup><br>3.9 <sup>(2)</sup> | ND                          | ND                         | ND                         |
| <b>Zeta potential (at pH 7.4)</b>   | -31,7 mV         | -36 mV            | ND                                                   | -42 mV                      | ND                         | -50,1 mV                   |
| <b>Weight (gm/cc)<sup>(3)</sup></b> | ND               | ND                | ND                                                   | ND                          | ND                         | ND                         |
| <b>Bacterial host</b>               | E. coli B        | P. fluorescens    | E. coli K12                                          | H40 (Unknown marine strain) | H4 (Unknown marine strain) | H6 (Unknown marine strain) |
| <b>Reference</b>                    | Rossi (1994)     | Rossi (1994)      | (4), (5)                                             | Rossi (1994)                | Rossi (1994)               | Rossi (1994)               |

<sup>(1)</sup> PENROD et al. (1995)  
<sup>(2)</sup> ZERDA & GERBA (1984)  
<sup>(3)</sup> Buoyant density of bacteriophage usually varies from 1.4 to 1.54 g/ml.  
<sup>(4)</sup> ACKERMANN & DuBOW (1987a)  
<sup>(5)</sup> ACKERMANN & DuBOW (1987b)  
 ND Values not determined or known by authors.

In addition, the "signals" observed at each sampling point could be better quantified due to a more extended injection time. Higher concentrations and extended presence at each sampling location would be expected with longer injection periods thereby increasing the quality of the comparative data set for all tracers.

Test 1 was done using salt and Phages H40 and H4 (no uranine) to document phage efficacy in conjunction with the salt/tank environment. Duration of the phage injection time was about 1.2 h compared to 4.5 h for the salt. The difference in injection times for the two tracer types was unique to Test 1 and was necessitated by logistics. The test successfully indicated that both salt and phage migration responses could be well documented in a period of from about 24 to 36 h. Test 2, carried out back-to-back with Test 1, was done with equal injection times to develop more suitable sampling strategies and to compare a) salt versus phage responses and b) two different phage behaviours (H40 and H6). Test 3 was done under higher flow rates and salt concentrations to a) address sample data gaps, b) compare, for the first time, uranine and salt solute responses, c) compare the influence of ionic strength on recovery and d) repeat the Phage H40 and H6 comparison. Test 4 was done to a) compare two non marine Phages, T7 and Psf2, with the marine Phage H40 since all were potential phages to be used during an upcoming field test and b) provide replicate data for the Test 3 uranine and salt response. Tests 5 and 6 were done principally to compare the behaviour of MS2, a non-marine Phage with the marine Phage H40 and to use different ionic strength conditions as part of that comparison. MS2 is widely used for viral transport studies in the USA. We wanted to compare its behaviour with the marine Phage H40, developed at Univ. Neuchatel and used in Switzerland. At this time, both phages had already been used in tracer tests at field sites. However, the fact that MS2 is commonly developed from a genetically altered source has resulted in restrictions now being placed on the use of MS2 in Switzerland. This comparison was specifically targeted at evaluating the possibility that MS2 can be replaced by H40 in viral transport behaviour studies.

First, the test results can be grouped and compared related to the different biocolloids used under variable test conditions:

- Phage H40: six tests with variable flow rate and electrical conductivity (Tests 1 through 6);
- comparative tests with six different phages in salt conditions (Tests 1 through 5);
- comparison of H40 and MS2 in salt and no salt environments (Tests 5 and 6).

Second, the results that were observed portray and exemplify heterogeneity in a porous media system. The differences in response within the heterogeneity of MARCEAUS as it is observed with the different flow path conditions within the tank are highly illustrative of what can take place in aquifers in real world situations.

**Table 2-3. MARCEAUS tracer test condition details.**

| Test Parameters |                             |                            |                                     |                                                | Sampling locations <sup>(4)</sup> |    |    |    |    |    |    |    |    |    |     |    |    |      | Tracer components injected |     |  |  |                               |  |  |  |  |  |
|-----------------|-----------------------------|----------------------------|-------------------------------------|------------------------------------------------|-----------------------------------|----|----|----|----|----|----|----|----|----|-----|----|----|------|----------------------------|-----|--|--|-------------------------------|--|--|--|--|--|
|                 |                             |                            |                                     |                                                |                                   |    |    |    |    |    |    |    |    |    |     |    |    |      | Solutes <sup>(3, 5)</sup>  |     |  |  | Bacteriophages <sup>(6)</sup> |  |  |  |  |  |
| Test            | Inj'n <sup>(1)</sup><br>(h) | Time <sup>(2)</sup><br>(h) | Flow rate<br>(L min <sup>-1</sup> ) | EC <sup>(3)</sup><br>( $\mu\text{S cm}^{-1}$ ) | US                                | IW | PW | P1 | P2 | P3 | P4 | DS | EC | UR | H40 | H4 | H6 | Psf2 | T7                         | MS2 |  |  |                               |  |  |  |  |  |
| 1               | 1.2, 4.1                    | 14                         | 4.7                                 | 2,375                                          | y                                 | y  | y  | y  | y  | y  | y  | y  | y  |    |     | y  | y  |      |                            |     |  |  |                               |  |  |  |  |  |
| 2               | 4                           | 14                         | 4.3                                 | 2,350                                          | y                                 | y  | y  | y  | y  | y  | y  | y  | y  |    |     | y  |    | y    |                            |     |  |  |                               |  |  |  |  |  |
| 3               | 4                           | 25                         | 7.6                                 | 4,400                                          | y                                 | y  | y  | y  | y  | y  | y  | y  | y  | y  |     | y  |    | y    |                            |     |  |  |                               |  |  |  |  |  |
| 4               | 4                           | 28                         | 7.2                                 | 4,400                                          | y                                 |    | y  |    |    | y  |    | y  | y  | y  | y   | y  |    |      | y                          | y   |  |  |                               |  |  |  |  |  |
| 5               | 4                           | 25                         | 4.8                                 | 3,750                                          | y                                 |    | y  |    |    | y  |    | y  | y  | y  | y   | y  |    |      |                            | y   |  |  |                               |  |  |  |  |  |
| 6               | 4                           | 26                         | 4.9                                 | 700                                            | y                                 |    | y  |    |    | y  |    | y  | y  |    | y   | y  |    |      |                            | y   |  |  |                               |  |  |  |  |  |

(1)

Injection time reflects the time during which tracer was added to the upstream mixing volume compartment. For feasibility Test 1, dissolved salt solute was injected for about 4.1 h, phages were injected for a shorter period.

(2)

Time period during which samples were taken.

(3)

Electrical Conductivity (EC) measurements ( $\mu\text{S cm}^{-1}$ ) were made in concert with the dissolved salt that was used as the solute tracer. Background levels in the supply water were about 700  $\mu\text{S cm}^{-1}$ .

(4)

Sampling locations are shown on figures 2-2 and 2-3.

(5)

Uranine (UR) was the second solute used as a dissolved chemical tracer.

(6)

Bacteriophages were the biocolloid components compared to the solute tracers. Phage characteristics are in table 2-2.

### 2.2.6 Laboratory methods

#### On site analyses

Periodically during the testing, supplemental samples were taken for on-site measurement of temperature and EC (WTW models LF-315 and 318). They were commonly taken immediately after a timed sample. pH measurements were made prior to and at the end of testing from the US and DS locations (Metrohm Model 704).

#### Sample preservation and transport

Once samples were taken, they were kept at about 4° C initially in a thermo-cooler within the air-conditioned container. Periodically, they were transferred for storage to a refrigerator that was kept at about 4° C. Finally all samples were transported over a period of about 3 h in the ice-filled thermo coolers from the refrigerator in Strasbourg to the cold room storage facility (2 to 4° C) of the Microbiology Laboratory, Univ. Neuchâtel. Samples were commonly kept there less than one day until analysis began.

#### Bacteriophage analysis

Samples from the tank were analysed at the University of Neuchatel Microbiology Laboratory for each B/HB system using an adaptation of the classical double-layer technique on Petri dishes (P. Rossi & W. Käss 1998). Concentrations were calculated based on the visible point counts and the sample analysis dilution. More detailed descriptions of the development and improvements made in the analysis of these bacteriophages are presented in P. Rossi & W. Käss (1998). The following details summarize the methods used for the analysis. It was adapted as needed to the large number of samples from the tank at a single time.

All liquid handling was done using automatic, adjustable volume pipettes (Socorex). Phage sample analysis was done in two steps. The first step was a simple presence/absence test which, when positive, allowed effective estimation of the dilution required for the second step, a more precise enumeration.

The first step of the procedures was as follows:

Step 1 – Presence/absence test:

- mix 2 ml sample, 0.2 ml exp. growing bacterial culture (OD<sub>600</sub> ca.1) in sterile glass tube;
- leave tube at room temperature for 15 min to allow phages to adsorb onto bacteria;
- pour whole tube contents onto Petri dish with 20 ml of solid SWA medium;
- add 4 ml of molten soft SWA medium (0.6% agar) kept at 50° C;
- mix well and gently, leave Petri dish to cool and incubate at 22° C for 24 to 36 h;
- proceed to second step of the analysis if lysis plaques are detected (positive result).

The second step consists of a more precise approach to arriving at a phage yield in the Petri dishes.

Step 2 - Precision counting the number of bacteriophages:

- add precisely 4 ml of sample to sterile glass tube.
- If needed, dilute sample beforehand in saline buffer (0.9% NaCl). In this case, add 0.4 ml of last dilution tube to 3.6 ml of saline buffer in sterile glass tube. The optimal number in last tube counted should be from 100 to 500 phages  $\text{ml}^{-1}$ ;
- add to last tube 400  $\mu\text{l}$  of exp. growing bacterial culture ( $\text{OD}_{600}$  about 1) ( $\sim 5 \times 10^7$  bacteria  $\text{ml}^{-1}$ );
- leave tube at room temperature for 15 min to allow phages to adsorb onto bacteria;
- pour precisely 1100  $\mu\text{l}$  of last tube contents onto Petri dish with 20 ml of solid SWA medium;
- add 4 ml of soft molten SWA medium (0.6% agar) kept at  $50^\circ \text{C}$  onto Petri dish;
- mix well and gently leaving Petri dish to cool and incubate at  $22^\circ \text{C}$  for 24 to 36 h.

#### Phage enumeration

Counting of the plaques forming units was done at least twice. The first time was after from 18 to 24 h and the second time it was done was after about 48 h. Results are the mathematical average of the plaques counted on commonly two or sometimes more Petri dishes, adjusted for dilution.

Steps 1 and 2 could be optimised for Tests 3 through 6 by skipping the positive-negative test. This could be done because the timing of the arrival and peak concentrations was relatively predictable after the second test had been completed.

#### **2.2.7 Comparative response evaluation for transport and attenuation assessment**

Bacteriophage behaviour was compared to solute tracer responses in a manner similar to methods described originally by R. HARVEY *et al.* (1989). This included comparing: a) plots of concentration history (breakthrough curves of tracer concentration vs. time); b) plots of relative attenuation ( $C/C_{\text{max}}$  for phages,  $\text{Tr}/\text{Tr}_{\text{max}}$  for solutes where  $C_{\text{max}}$  and  $\text{Tr}_{\text{max}}$  represent phage and solute the observed maximum concentration at any sampling location, respectively); c) plots of tracer concentrations normalized to i) initial concentrations or ii) mass injected ( $[C/C_0$  or  $C/M_0]$  for phages,  $[\text{Tr}/\text{Tr}_0$  or  $\text{Tr}/M_0]$  for solutes - where  $C_0$  and  $\text{Tr}_0$  represent the phage and solute initial injection concentrations at measured at the US (Upstream) location, respectively); d) maximum dimensionless concentration (MDC) values ( $\text{Max.}(\text{Tr}/\text{Tr}_0)$  for solutes,  $\text{Max.}(C/C_0)$  values for phages); e) retardation factor values (RF – the ratio of time at which the particle vs. the solute had their maximum value ( $T_{\text{peak}}$ )); f) colloid relative breakthrough values (RB – particle mass recovered compared to solute mass recovered, normalized to 100 percent using non-reactive solute assumptions); g) colloid attenuation (1 minus RB); and h) values for time of first detection (arrival) ( $T_{\text{arr}}$ ) of solutes and phages.

**Table 2-4. Solute and biocolloid tracer component response characteristics.**

| Sample location<br>by test | Solute Response                               |                          |            |                                              |                          |            | Bacteriophage Response                       |                          |     |            |           |              |                                               |                          |     |            |           |              |
|----------------------------|-----------------------------------------------|--------------------------|------------|----------------------------------------------|--------------------------|------------|----------------------------------------------|--------------------------|-----|------------|-----------|--------------|-----------------------------------------------|--------------------------|-----|------------|-----------|--------------|
|                            | Salt (EC)                                     |                          |            | Uranine                                      |                          |            | Phage: H40                                   |                          |     |            |           |              | Second Phage                                  |                          |     |            |           |              |
|                            | T <sub>arr</sub><br>(h)                       | T <sub>peak</sub><br>(h) | MDC<br>(%) | T <sub>arr</sub><br>(h)                      | T <sub>peak</sub><br>(h) | MDC<br>(%) | T <sub>arr</sub><br>(h)                      | T <sub>peak</sub><br>(h) | RF  | MDC<br>(%) | RB<br>(%) | Att'n<br>(%) | T <sub>arr</sub><br>(h)                       | T <sub>peak</sub><br>(h) | RF  | MDC<br>(%) | RB<br>(%) | Att'n<br>(%) |
| T 1                        | Tr <sub>0</sub> – max dEC 1.7e <sup>+03</sup> |                          |            | Tr <sub>0</sub> – max UR none                |                          |            | C <sub>0</sub> - max H40 1.1e <sup>+05</sup> |                          |     |            |           |              | C <sub>0</sub> - max H4 2.7e <sup>+04</sup>   |                          |     |            |           |              |
| US                         |                                               |                          | 100        |                                              |                          |            |                                              |                          |     | 100        | 100       | 0            |                                               |                          |     | 100        | 100       | 0            |
| IW                         | 1.0                                           | 3.0                      | 100        |                                              |                          |            | 0.3                                          | 1.3                      | na  | 42         | 94        | 6            | 0.3                                           | 1.0                      | na  | 18         | 38        | 62           |
| PW                         | 2.0                                           | 4.5                      | 101        |                                              |                          |            | 1.7                                          | 2.0                      | na  | 41         | 73        | 27           | 1.7                                           | 2.0                      | na  | 19         | 31        | 69           |
| P1                         | 3.7                                           | 8.0                      | 78         |                                              |                          |            | 3.0                                          | 5.5                      | na  | 4          | 29        | 71           | 3.3                                           | 5.5                      | na  | 4          | 21        | 79           |
| P2                         | 4.0                                           | 10.0                     | 82         |                                              |                          |            | 4.0                                          | 7.0                      | na  | 3          | 20        | 80           | 4.0                                           | 7.0                      | na  | 1          | 8         | 92           |
| P3                         | 2.7                                           | 5.7                      | 98         |                                              |                          |            | 2.3                                          | 3.0                      | na  | 12         | 29        | 71           | 2.3                                           | 3.0                      | na  | 4          | 8         | 92           |
| P4                         | 3.0                                           | 11.5                     | 68         |                                              |                          |            | 4.0                                          | 6.0                      | na  | 2          | 12        | 88           | 4.0                                           | 6.5                      | na  | 0.4        | 4         | 96           |
| DS                         | 3.0                                           | 7.2                      | 55         |                                              |                          |            | 3.0                                          | 4.0                      | na  | 9          | 43        | 57           | 3.0                                           | 3.0                      | na  | 3          | 24        | 76           |
| Injection (h):             | 1.2, 4.1                                      |                          |            | Q (L min <sup>-1</sup> ) 4.7                 |                          |            | Phage: H40                                   |                          |     |            |           |              | Phage: H4                                     |                          |     |            |           |              |
| T 2                        | Tr <sub>0</sub> – max dEC 1.6e <sup>+03</sup> |                          |            | Tr <sub>0</sub> – max UR none                |                          |            | C <sub>0</sub> - max H40 1.3e <sup>+04</sup> |                          |     |            |           |              | C <sub>0</sub> - max H6 3.8e <sup>+04</sup>   |                          |     |            |           |              |
| US                         |                                               |                          | 100        |                                              |                          |            |                                              |                          |     | 100        | 100       | 0            |                                               |                          |     | 100        | 100       | 0            |
| IW                         | 1.0                                           | 3.5                      | 98         |                                              |                          |            | 0.5                                          | 3.8                      | 1.1 | 46         | 45        | 55           | 0.5                                           | 3.2                      | 0.9 | 6          | 8         | 92           |
| PW                         | 2.0                                           | 4.0                      | 100        |                                              |                          |            | 1.7                                          | 4.0                      | 1.0 | 40         | 46        | 54           | 1.7                                           | 3.7                      | 0.9 | 2          | 3         | 97           |
| P1                         | 3.3                                           | 8.0                      | 82         |                                              |                          |            | 3.3                                          | 9.0                      | 1.1 | 20         | 27        | 73           | 3.3                                           | 8.5                      | 1.1 | 2          | 2         | 98           |
| P2                         | 4.0                                           | 10.5                     | 80         |                                              |                          |            | 4.0                                          | 8.5                      | 0.8 | 6          | 11        | 89           | 4.3                                           | 9.0                      | 0.9 | 0.3        | 0.5       | 99.5         |
| P3                         | 2.7                                           | 5.4                      | 95         |                                              |                          |            | 2.7                                          | 5.1                      | 0.9 | 16         | 21        | 79           | 2.7                                           | 4.9                      | 0.9 | 0.3        | 1         | 99           |
| P4                         | 3.7                                           | 12.0                     | 66         |                                              |                          |            | 4.7                                          | 8.5                      | 0.7 | 3          | 5         | 95           | 4.3                                           | 8.5                      | 0.7 | 0.1        | 0.3       | 99.7         |
| DS                         | 2.7                                           | 6.5                      | 92         |                                              |                          |            | 2.7                                          | 6.0                      | 0.9 | 25         | 31        | 69           | 2.7                                           | 6.5                      | 1.0 | 2          | 2         | 98           |
| Injection (h):             | 4.0                                           |                          |            | Q (L min <sup>-1</sup> ) 4.3                 |                          |            | Phage: H40                                   |                          |     |            |           |              | Phage: H6                                     |                          |     |            |           |              |
| T 3                        | Tr <sub>0</sub> – max dEC 3.7e <sup>+03</sup> |                          |            | Tr <sub>0</sub> – max UR 9.1e <sup>+02</sup> |                          |            | C <sub>0</sub> - max H40 7.5e <sup>+03</sup> |                          |     |            |           |              | C <sub>0</sub> - max H6 3.6e <sup>+04</sup>   |                          |     |            |           |              |
| US                         |                                               |                          | 100        |                                              |                          | 100        |                                              |                          |     | 100        | 100       | 0            |                                               |                          |     | 100        | 100       | 0            |
| IW                         | 0.8                                           | 3.2                      | 99         | 0.8                                          | 3.2                      | 98         | 0.3                                          | 3.0                      | 0.9 | 79         | 85        | 15           | 0.5                                           | 3.0                      | 0.9 | 10         | 11        | 89           |
| PW                         | 1.7                                           | 4.7                      | 99         | 1.7                                          | 4.7                      | 98         | 1.7                                          | 5.0                      | 1.1 | 72         | 78        | 22           | 1.7                                           | 5.0                      | 1.1 | 6          | 6         | 94           |
| P1                         | 3.0                                           | 9.0                      | 81         | 3.7                                          | 9.0                      | 80         | 3.3                                          | 9.0                      | 1.0 | 33         | 41        | 59           | 3.3                                           | 9.0                      | 1.0 | 4          | 4         | 96           |
| P2                         | 3.9                                           | 9.5                      | 78         | 3.9                                          | 9.5                      | 71         | 3.9                                          | 8.0                      | 0.8 | 29         | 30        | 70           | 3.9                                           | 8.0                      | 0.8 | 3          | 3         | 97           |
| P3                         | 2.7                                           | 5.8                      | 96         | 2.7                                          | 5.8                      | 94         | 2.7                                          | 5.0                      | 0.9 | 39         | 41        | 59           | 2.7                                           | 5.0                      | 0.9 | 2          | 2         | 98           |
| P4                         | 3.7                                           | 11.0                     | 50         | 3.7                                          | 11.0                     | 42         | 3.7                                          | 7.5                      | 0.7 | 11         | 15        | 85           | 3.7                                           | 8.5                      | 0.8 | 1          | 2         | 98           |
| DS                         | 2.7                                           | 7.0                      | 76         | 2.7                                          | 7.0                      | 69         | 2.3                                          | 7.0                      | 1.0 | 28         | 51        | 49           | 3.0                                           | 7.0                      | 1.0 | 3          | 4         | 96           |
| Injection (h):             | 4.0                                           |                          |            | Q (L min <sup>-1</sup> ) 7.6                 |                          |            | Phage: H40                                   |                          |     |            |           |              | Phage: H6                                     |                          |     |            |           |              |
| T 4                        | Tr <sub>0</sub> – max dEC 3.7e <sup>+03</sup> |                          |            | Tr <sub>0</sub> – max UR 2.6e <sup>+02</sup> |                          |            | C <sub>0</sub> - max H40 7.6e <sup>+03</sup> |                          |     |            |           |              | C <sub>0</sub> - max Psf2 6.2e <sup>+02</sup> |                          |     |            |           |              |
| US                         |                                               |                          | 100        |                                              |                          | 100        |                                              |                          |     | 100        | 100       | 0            |                                               |                          |     | 100        | 100       | 0            |
| PW                         | 1.5                                           | 4.3                      | 99         | 1.7                                          | 4                        | 97         | 1.5                                          | 5                        | 1.1 | 84         | 76        | 24           | 2.0                                           | 4                        | 0.9 | 40         | 33        | 67           |
| P2                         | 3.3                                           | 9.0                      | 84         | 3.7                                          | 9                        | 82         | 3.0                                          | 7                        | 0.8 | 34         | 29        | 71           | 7.0                                           | 8                        | 0.8 | 0.2        | 0         | 100          |
| P4                         | 3.3                                           | 10.0                     | 57         | 3.0                                          | 10                       | 40         | 3.0                                          | 7                        | 0.7 | 13         | 19        | 81           | None                                          |                          |     | 0          | 100       |              |
| DS                         | 2.7                                           | 7.0                      | 77         | 2.7                                          | 7                        | 70         | 2.3                                          | 7                        | 1.0 | 37         | 42        | 58           | 3.0                                           | 4                        | 0.6 | 1          | 1         | 99           |
| Injection (h):             | 4.0                                           |                          |            | Q (L min <sup>-1</sup> ) 7.2                 |                          |            | Phage: H40                                   |                          |     |            |           |              | Phage: Psf2                                   |                          |     |            |           |              |
| T 5                        | Tr <sub>0</sub> – max dEC 3.0e <sup>+03</sup> |                          |            | Tr <sub>0</sub> – max UR 2.9e <sup>+02</sup> |                          |            | C <sub>0</sub> - max H40 1.5e <sup>+04</sup> |                          |     |            |           |              | C <sub>0</sub> - max MS2 4.0e <sup>+04</sup>  |                          |     |            |           |              |
| US                         |                                               |                          | 100        |                                              |                          | 100        |                                              |                          |     | 100        | 100       | 0            |                                               |                          |     | 100        | 100       | 0            |
| PW                         | 2.5                                           | 6.5                      | 102        | 2.5                                          | 6.0                      | 98         | 2.5                                          | 7.0                      | 1.1 | 71         | 66        | 34           | 2.5                                           | 5.5                      | 0.8 | 61         | 59        | 41           |
| P2                         | 4.5                                           | 11.0                     | 65         | 4.5                                          | 11.5                     | 55         | 3.9                                          | 9.5                      | 0.9 | 10         | 24        | 76           | 4.1                                           | 9.5                      | 0.9 | 6          | 11        | 89           |
| P4                         | 4.5                                           | 10.5                     | 45         | 4.5                                          | 11                       | 38         | 3.5                                          | 9.0                      | 0.9 | 13         | 28        | 72           | 3.9                                           | 7.5                      | 0.7 | 6          | 11        | 89           |
| DS                         | 4.1                                           | 8.0                      | 70         | 4.1                                          | 8                        | 66         | 3.5                                          | 9.0                      | 1.1 | 29         | 48        | 52           | 3.5                                           | 8.5                      | 1.1 | 25         | 31        | 69           |
| Injection (h):             | 4.0                                           |                          |            | Q (L min <sup>-1</sup> ) 4.8                 |                          |            | Phage: H40                                   |                          |     |            |           |              | Phage: MS2                                    |                          |     |            |           |              |
| T 6                        | Tr <sub>0</sub> – max dEC None                |                          |            | Tr <sub>0</sub> – max UR 2.7e <sup>+02</sup> |                          |            | C <sub>0</sub> - max H40 9.9e <sup>+03</sup> |                          |     |            |           |              | C <sub>0</sub> - max MS2 2.3e <sup>+04</sup>  |                          |     |            |           |              |
| US                         |                                               |                          |            |                                              |                          | 100        |                                              |                          |     | 100        | 100       | 0            |                                               |                          |     | 100        | 100       | 0            |
| PW                         |                                               |                          |            | 2.5                                          | 5.0                      | 94         | 2.5                                          | 4.5                      | 0.9 | 42         | 45        | 55           | 2.5                                           | 5.0                      | 1.0 | 43         | 44        | 56           |
| P2                         |                                               |                          |            | 5.5                                          | 12.5                     | 56         | 5                                            | 12.0                     | 1.0 | 7          | 13        | 87           | 5                                             | 12.0                     | 1.0 | 2          | 3         | 97           |
| P4                         |                                               |                          |            | 3.9                                          | 12.0                     | 27         | 3.5                                          | 8.5                      | 0.7 | 8          | 16        | 84           | 3.5                                           | 8.5                      | 0.7 | 5          | 8         | 92           |
| DS                         |                                               |                          |            | 3.5                                          | 8.0                      | 65         | 3.5                                          | 7.5                      | 0.9 | 25         | 28        | 72           | 3.5                                           | 7.0                      | 0.9 | 22         | 25        | 75           |
| Injection (h) :            | 4.0                                           |                          |            | Q (L min <sup>-1</sup> ) 4.9                 |                          |            | Phage: H40                                   |                          |     |            |           |              | Phage: MS2                                    |                          |     |            |           |              |

## 2.3 Results and Discussion

The purpose of this section is to present the results of the work done with the six different phages and the two conservative tracer solutes in the tank. The results are presented as follows: 1) summary by test; 2) H40 behaviour during testing; 3) six phages compared among tests; 4) MS2 and H40 behaviour compared; and 5) heterogeneity within the tank.

### 2.3.1 Individual test summaries

The transport assessment related characteristics associated with each of the tests are presented for each test, by location, in tables 2-4 and 2-5. The following descriptions summarize the major results compared to the objectives of each of the six tests.

#### Test 1

This 14 h test represented a feasibility assessment related to the tank and measurement and sampling protocols to define phage migration through it. Measured outflow rate and EC values were about  $5 \text{ L min}^{-1}$  and  $2400 \mu\text{S cm}^{-1}$ , respectively. Two Phages (H4, H40) were injected over about 1 h and salt over about 4 h. Figures 2-4-1 through 2-4-3 illustrate the input signal, concentration history by well and by component and relative recovery of salt and Phages H4 and H40 values at the eight sampling locations. Only infrequent US sampling was done during this test and therefore, neither  $C/C_0$  nor  $C/M_0$  plots are included. Principal test results indicated, respectively:

- solute, phage arrival velocity were 0.8 to 2.0 and 1.1 to 2.4  $\text{m h}^{-1}$ ;
- solute, phage peak velocity were 0.3 to 0.9 and 0.6 to 2.0  $\text{m h}^{-1}$ ;
- H4, H40 recovery were 24 and 43% at the outlet (DS);
- H40 relative breakthrough was about 1.4 to 3.7 fold higher than H4.

#### Test 2

This 14 h test had a 4 h injection duration for two Phages (H40, H6) and salt used as the conservative solute tracer. This test was done back-to-back with Test 1 to compare Phage H6 and H4 with the consistent use of Phage H40 for migration evaluation. Measured outflow rate and EC values were about  $4 \text{ L min}^{-1}$  and  $2300 \mu\text{S cm}^{-1}$ , respectively. Figures 2-5-1 through 2-5-3 illustrate the input signal, concentration history,  $C/M_0$  values and relative breakthrough at the eight sampling locations. Principal test results indicated, respectively:

- solute, phage arrival velocity were 0.8 to 2.1 and 1.0 to 2.4  $\text{m h}^{-1}$ , similar to Test 1;
- solute, phage peak velocity were 0.2 to 1.0 and 0.2 to 1.1  $\text{m h}^{-1}$ , similar to Test 1 for the solutes but decreased for the phages;
- similar arrival, peak times for both phages;
- H6, H40 recovery of 2 and 31% at the outflow (DS);
- H40 relative breakthrough was about 6 to 35 fold higher than H6.

#### Test 3

This 25 h test had a 4 h injection duration for two Phages (H40, H6) and salt and uranine which were used as conservative solute tracer. This test was done to again compare Phage H6 and H40 migration and in this case, incorporate uranine since this solute was to be used as the principal field solute tracer. The test was done with higher flow rates and salt concentrations. Measured outflow rate and EC values were about  $7.6 \text{ L min}^{-1}$  and  $4400 \mu\text{S cm}^{-1}$ , respectively. Figures 2-6-1 through 2-6-3 illustrate the input signal, concentration history,  $C/M_0$  values and relative recovery at the eight sampling locations. Principal test results indicated, respectively:

- solute, phage arrival velocity both ranging from 1.1 to 2.4  $\text{m h}^{-1}$ ;
- solute, phage peak velocity from 0.2 to 0.9 and 0.3 to 0.9  $\text{m h}^{-1}$ ;
- similar solute and except at IW and DS, phage arrival (detection) times;
- earlier phage peak times than solutes except at PW, P1 and DS where they were similar;
- both solutes had a dual peak at P4 that the phages did not;
- H6, H40 recovery were 4 and 51% at the outflow (DS), greater than in Test 2;
- H40 relative breakthrough was about 8 to 23 fold higher than H6.

The H40 and H6 DS recovery were higher than in Test 2. There was a higher salt concentration (ionic strength) during Test 3. What were surprising were the similar arrival velocities for phages and solutes despite the increase in flow rate. Normally, we would have expected that the higher flow rate would have resulted in a corresponding increase in arrival velocities. The timing of the samples was possibly too far apart to effectively discriminate among arrival times. For subsequent testing, sampling density was increased.

**Table 2-5. Velocity ( $\text{m h}^{-1}$ ) calculated from times to initial detection ( $T_{\text{arr}}$ ) and peak ( $T_{\text{peak}}$ ).**

| # | Arrival  |              |         |     |        |      | Peak    |     |        |      |
|---|----------|--------------|---------|-----|--------|------|---------|-----|--------|------|
|   |          |              | Solutes |     | Phages |      | Solutes |     | Phages |      |
|   | Location | Distance (m) | dEC     | UR  | H40    | H4   | dEC     | UR  | H40    | H4   |
| 1 | IW       | 0.8          | 0.8     | -   | 2.4    | 2.4  | 0.3     | -   | 0.6    | 0.8  |
|   | PW       | 4.0          | 2.0     | -   | 2.4    | 2.4  | 0.9     | -   | 2.0    | 2.0  |
|   | P1       | 4.2          | 1.1     | -   | 1.4    | 1.3  | 0.5     | -   | 0.8    | 0.8  |
|   | P2       | 4.3          | 1.1     | -   | 1.1    | 1.1  | 0.4     | -   | 0.6    | 0.6  |
|   | P3       | 4.6          | 1.7     | -   | 2.0    | 2.0  | 0.8     | -   | 1.5    | 1.5  |
|   | P4       | 4.6          | 1.5     | -   | 1.2    | 1.2  | 0.4     | -   | 0.8    | 0.7  |
|   | DS       | 5.6          | 1.9     | -   | 1.9    | 1.9  | 0.8     | -   | 1.4    | 1.9  |
|   | Location | Distance (m) | dEC     | UR  | H40    | H6   | dEC     | UR  | H40    | H6   |
| 2 | IW       | 0.8          | 0.8     | -   | 1.6    | 1.6  | 0.2     | -   | 0.2    | 0.2  |
|   | PW       | 4.0          | 2.0     | -   | 2.4    | 2.4  | 1.0     | -   | 1.0    | 1.1  |
|   | P1       | 4.2          | 1.3     | -   | 1.3    | 1.3  | 0.5     | -   | 0.5    | 0.5  |
|   | P2       | 4.3          | 1.1     | -   | 1.1    | 1.0  | 0.4     | -   | 0.5    | 0.5  |
|   | P3       | 4.6          | 1.7     | -   | 1.7    | 1.7  | 0.8     | -   | 0.9    | 0.9  |
|   | P4       | 4.6          | 1.3     | -   | 1.0    | 1.1  | 0.4     | -   | 0.5    | 0.5  |
|   | DS       | 5.6          | 2.1     | -   | 2.1    | 2.1  | 0.9     | -   | 0.9    | 0.9  |
|   | Location | Distance (m) | dEC     | UR  | H40    | H6   | dEC     | UR  | H40    | H6   |
| 3 | IW       | 0.8          | 1.1     | 1.1 | 2.4    | 1.6  | 0.2     | 0.2 | 0.3    | 0.3  |
|   | PW       | 4.0          | 2.4     | 2.4 | 2.4    | 2.4  | 0.9     | 0.9 | 0.8    | 0.8  |
|   | P1       | 4.2          | 1.4     | 1.1 | 1.3    | 1.3  | 0.5     | 0.5 | 0.5    | 0.5  |
|   | P2       | 4.3          | 1.1     | 1.1 | 1.1    | 1.1  | 0.4     | 0.4 | 0.5    | 0.5  |
|   | P3       | 4.6          | 1.7     | 1.7 | 1.7    | 1.7  | 0.8     | 0.8 | 0.9    | 0.9  |
|   | P4       | 4.6          | 1.3     | 1.3 | 1.3    | 1.3  | 0.4     | 0.4 | 0.6    | 0.5  |
|   | DS       | 5.6          | 2.1     | 2.1 | 2.4    | 1.9  | 0.8     | 0.8 | 0.8    | 0.8  |
|   | Location | Distance (m) | dEC     | UR  | H40    | Psf2 | dEC     | UR  | H40    | Psf2 |
| 4 | PW       | 4.0          | 2.7     | 2.3 | 2.7    | 2.0  | 0.9     | 1.0 | 0.9    | 1.0  |
|   | P2       | 4.3          | 1.3     | 1.2 | 1.4    | 0.6  | 0.5     | 0.5 | 0.6    | 0.6  |
|   | P4       | 4.6          | 1.4     | 1.5 | 1.5    | -    | 0.5     | 0.5 | 0.7    | -    |
|   | DS       | 5.6          | 2.1     | 2.1 | 2.4    | 1.9  | 0.8     | 0.8 | 0.8    | 1.3  |
|   | Location | Distance (m) | dEC     | UR  | H40    | MS2  | dEC     | UR  | H40    | MS2  |
| 5 | PW       | 4.0          | 1.6     | 1.6 | 1.6    | 1.6  | 0.6     | 0.7 | 0.6    | 0.7  |
|   | P2       | 4.3          | 0.9     | 0.9 | 1.1    | 1.0  | 0.4     | 0.4 | 0.4    | 0.4  |
|   | P4       | 4.6          | 1.0     | 1.0 | 1.3    | 1.2  | 0.4     | 0.4 | 0.5    | 0.6  |
|   | DS       | 5.6          | 1.4     | 1.4 | 1.6    | 1.6  | 0.7     | 0.7 | 0.6    | 0.7  |
|   | Location | Distance (m) | dEC     | UR  | H40    | MS2  | dEC     | UR  | H40    | MS2  |
| 6 | PW       | 4.0          | -       | 1.6 | 1.6    | 1.6  | -       | 0.8 | 0.9    | 0.8  |
|   | P2       | 4.3          | -       | 0.8 | 0.9    | 0.9  | -       | 0.3 | 0.4    | 0.4  |
|   | P4       | 4.6          | -       | 1.2 | 1.3    | 1.3  | -       | 0.4 | 0.5    | 0.5  |
|   | DS       | 5.6          | -       | 1.6 | 1.6    | 1.6  | -       | 0.7 | 0.7    | 0.8  |

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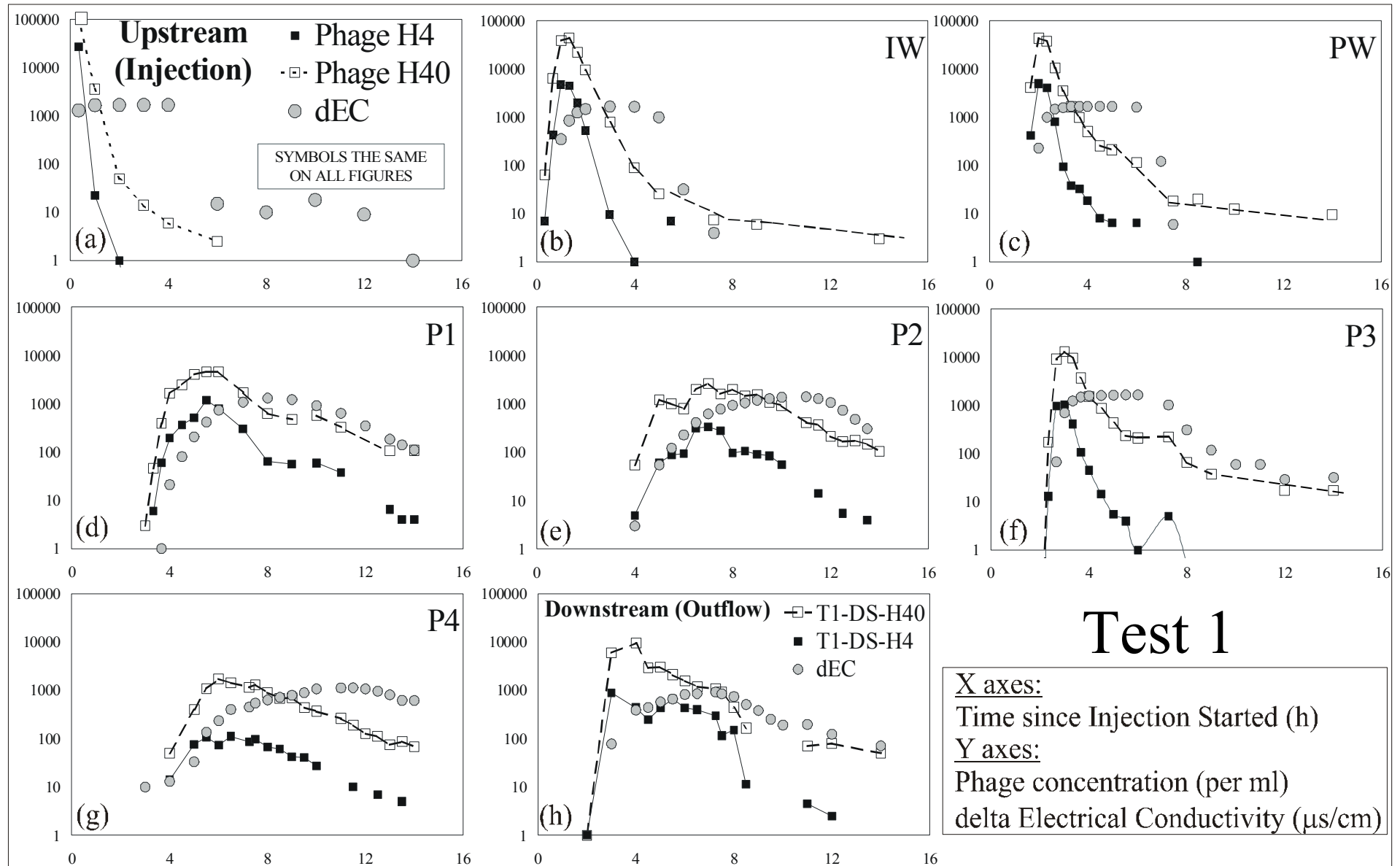


Figure 2-4-1. Test 1 concentration history by well location.

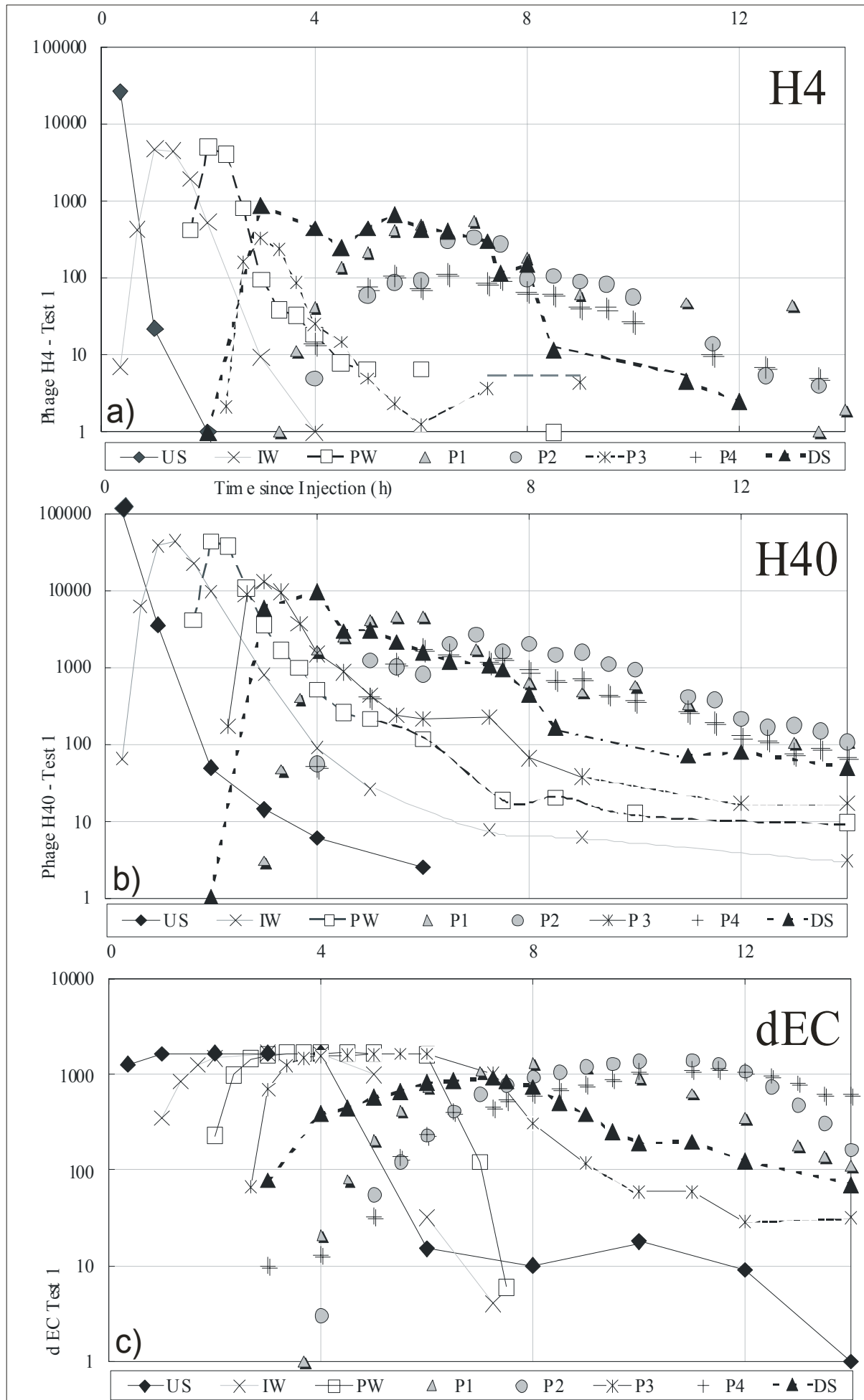


Figure 2-4-2. Test 1 concentration history by tracer component (all locations).

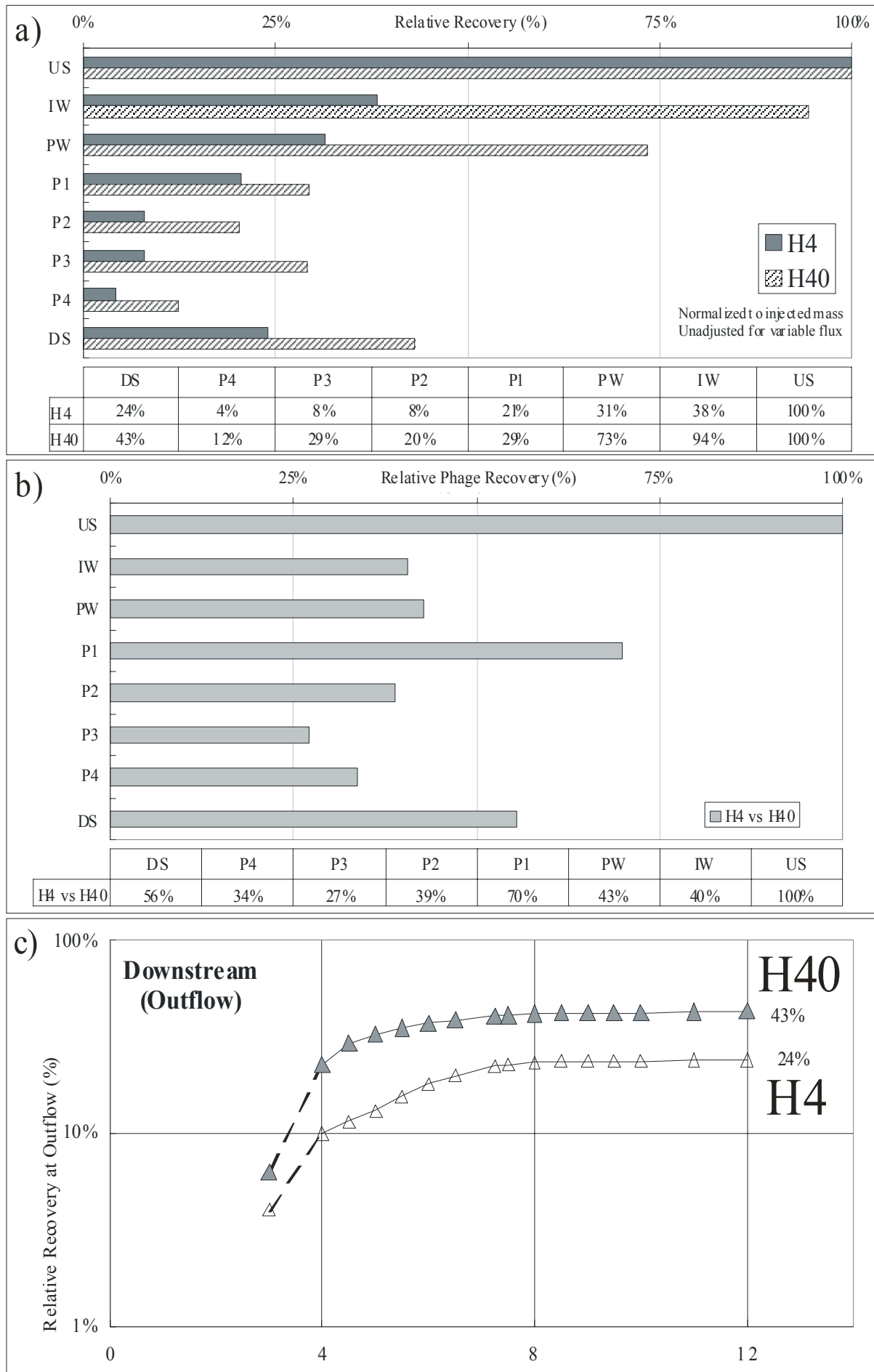


Figure 2-4-3. Test 1 relative breakthrough (RB) of Phages H4 and H40 with outflow total recovery.

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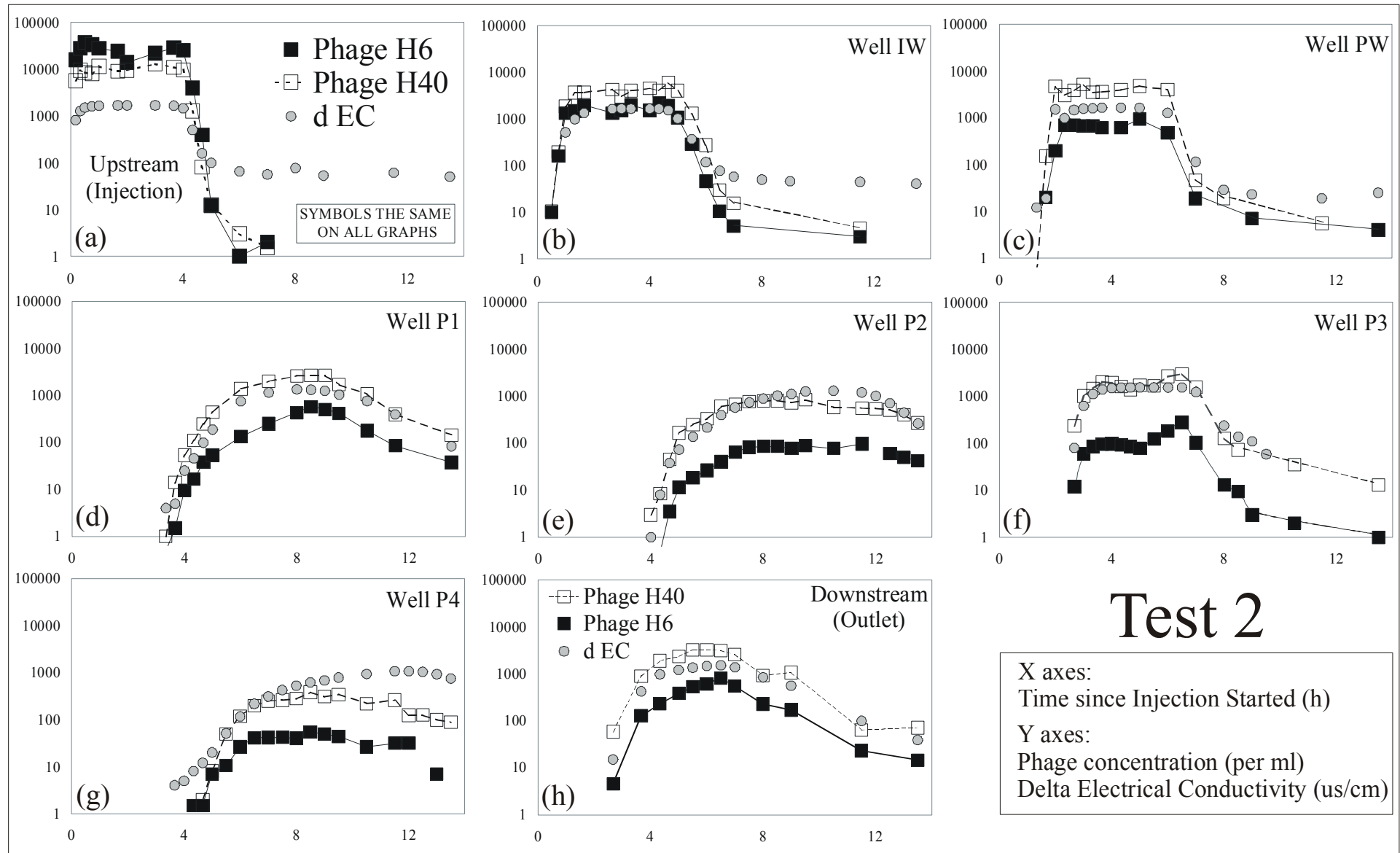


Figure 2-5-1. Test 2 concentration history by well location.

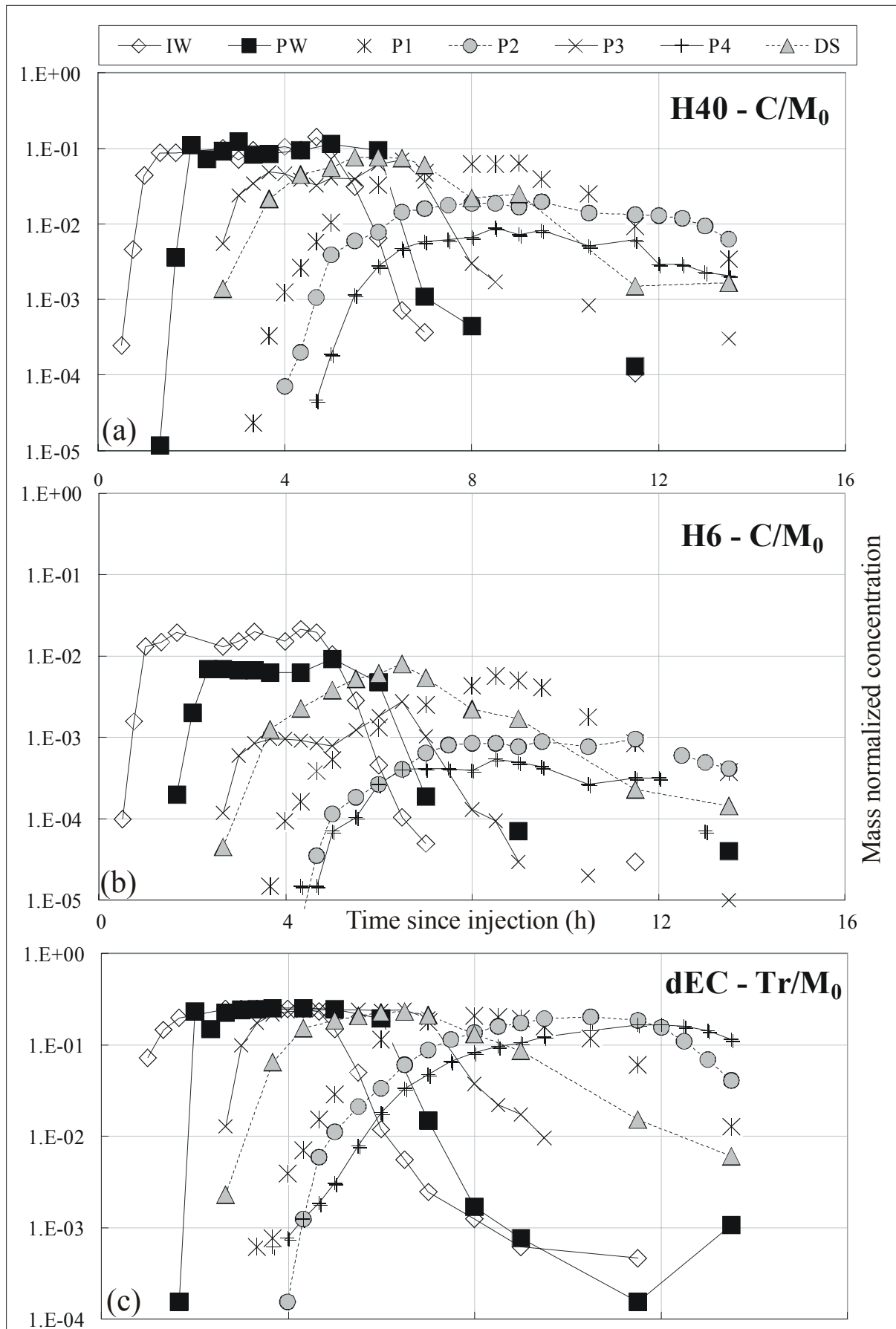


Figure 2-5-2. Test 2 tracer component response normalized to injected mass ( $C/M_0$ ).

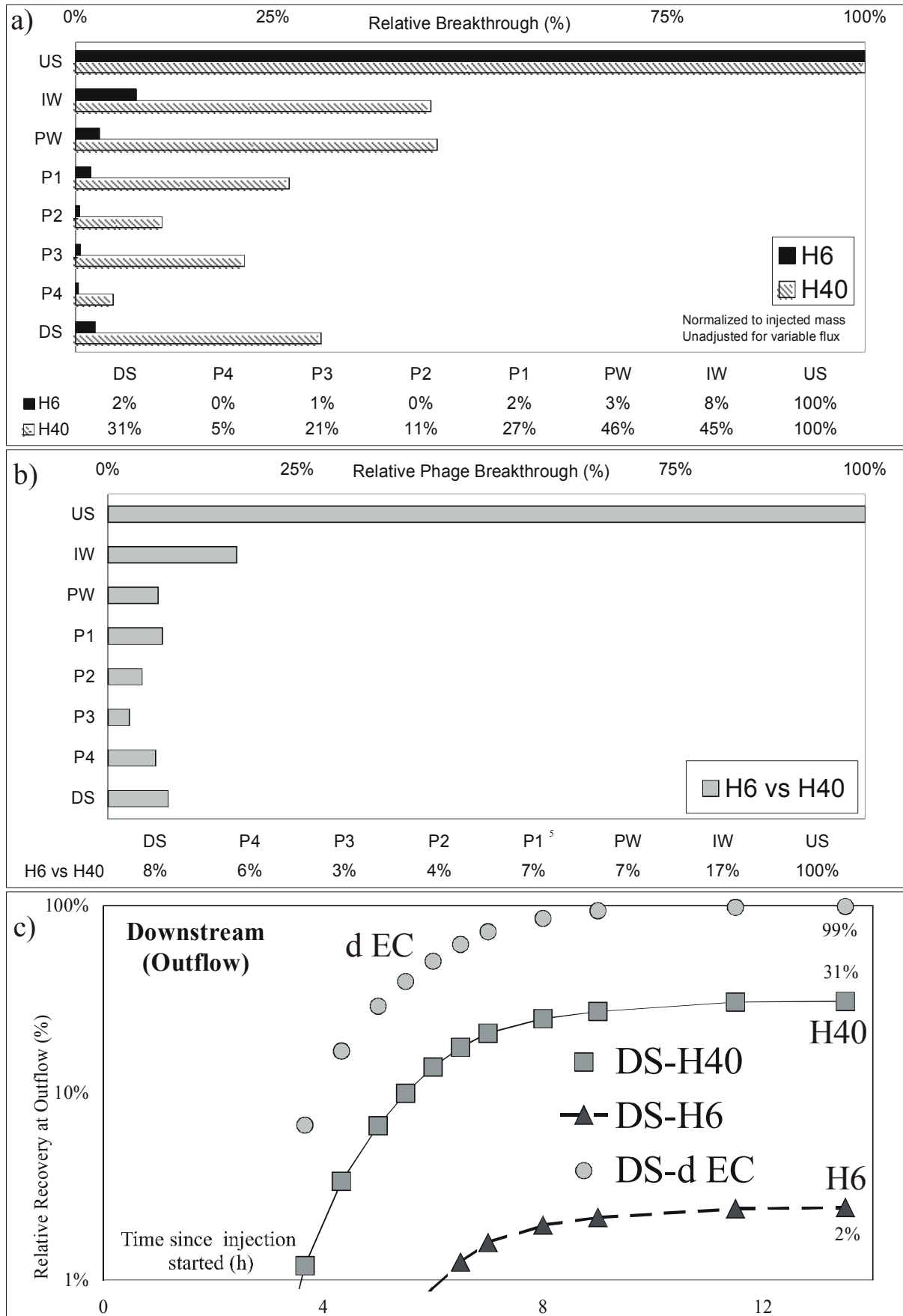


Figure 2-5-3 . Test 2 relative breakthrough (RB) of Phages H6 and H40 with outflow total recovery.

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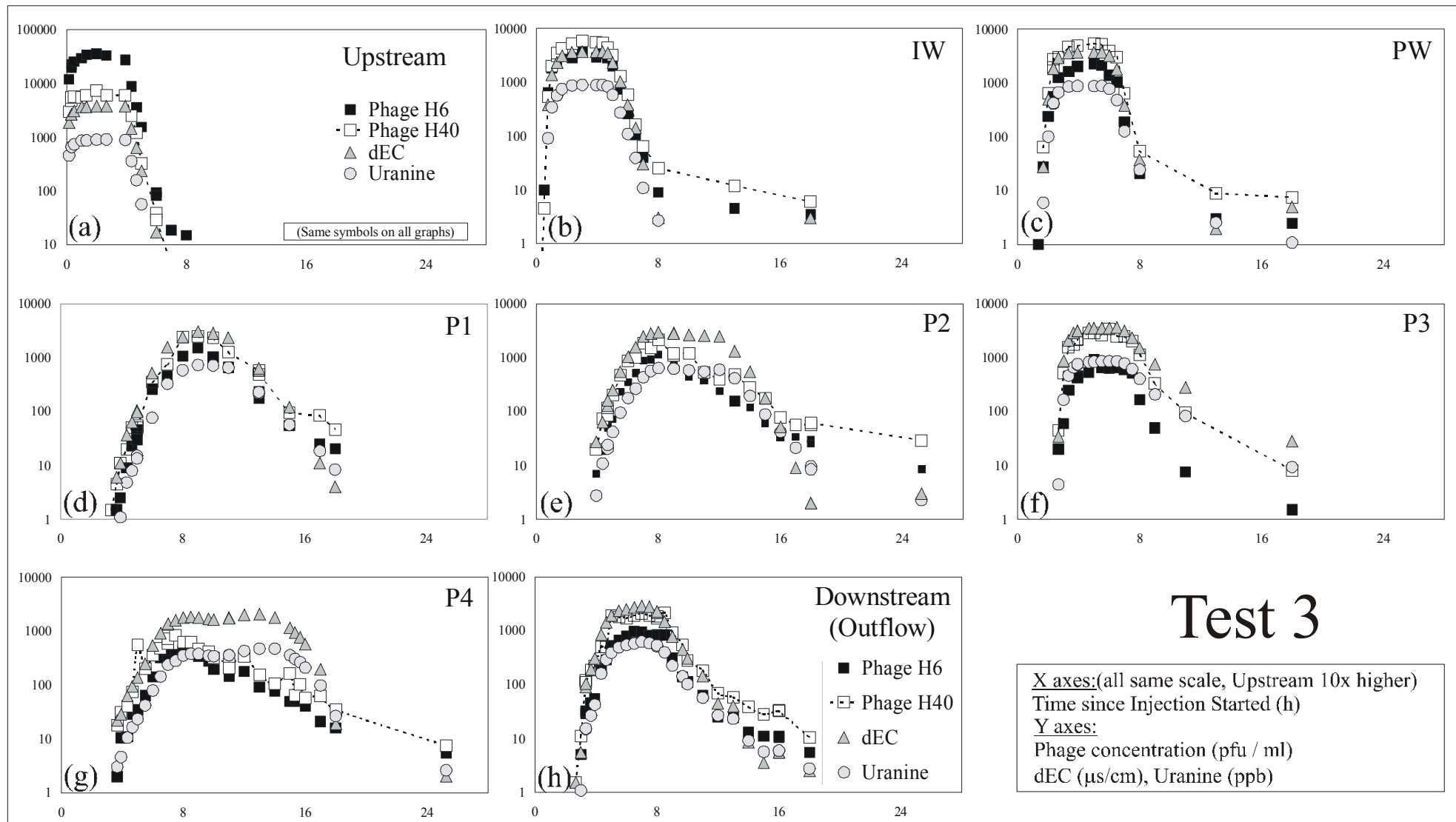


Figure 2-6-1 Test 3 concentration history by location.

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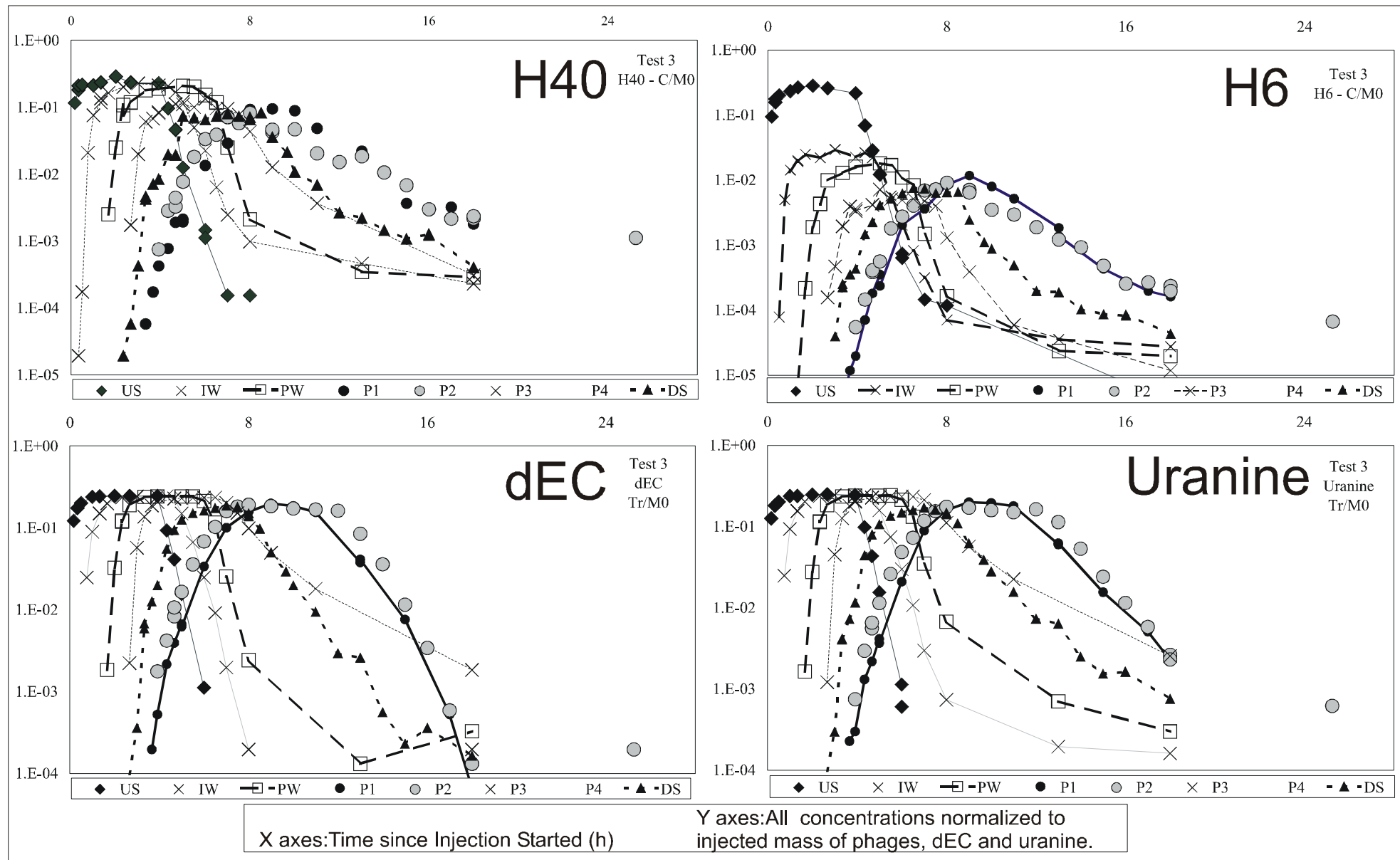


Figure 2-6-2. Test 3 tracer component response normalized to injected mass ( $C/M_0$ ).

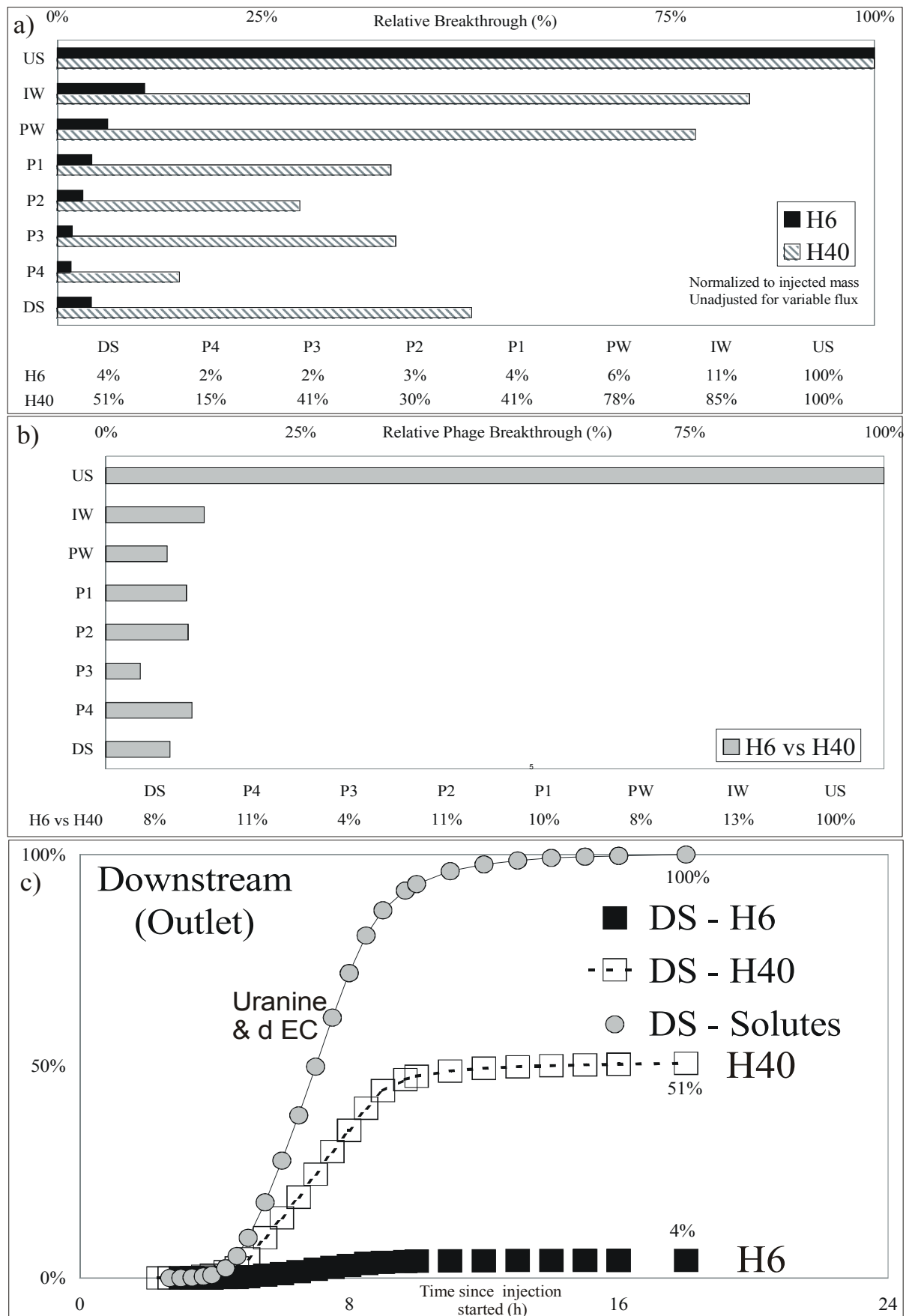


Figure 2-6-3 . Test 3 relative breakthrough (RB) of Phages H6 and H40 with outflow total recovery.

### Test 4

This 28 h test had a 4 h injection duration for three Phages (H40, T7, Psf2) with salt and uranine used as conservative solute tracers. This test was done to compare Phage H40 with Phages T7 and Psf2 migration and to compare their responses with the two solutes. The test was done with the same flow rates and salt concentration as in Test 3. Measured outflow rate and EC values were about  $7.2 \text{ L min}^{-1}$  and  $4400 \text{ } \mu\text{S cm}^{-1}$ , respectively. In-tank sampling locations were reduced from six to three in this test with samples no longer being taken at IW, P1 or P3. Figures 2-7-1 through 2-7-3 illustrate the input signal, concentration history and  $C/M_0$  values and relative recovery at the five sampling locations. The principal results of the test indicated, respectively:

- increase (cf. Test 3) solute, Phage H40 arrival velocity from 1.2 to 2.7 and 1.4 to 2.7  $\text{m h}^{-1}$ ;
- solute, phage peak velocity from 0.5 to 1.0  $\text{m h}^{-1}$  increasing to about 20% compared to Test 3;
- earlier time to phage maximum concentrations at the two non-channel locations (P2, P4);
- no detection of T7 at in-tank or outflow sampling locations, no Psf2 at P4;
- similar solute and phage arrival at PW, but earlier phage detection at P2, P4 and DS;
- both solutes had a dual peak at P4 which phages did not;
- Psf2, H40 recovery were 1 and 42% at the outflow (DS);
- H40 relative breakthrough was about 2 to 2900 fold higher than Psf2;
- Psf2 initial injection concentration likely too low for valid comparison at all sampling locations.

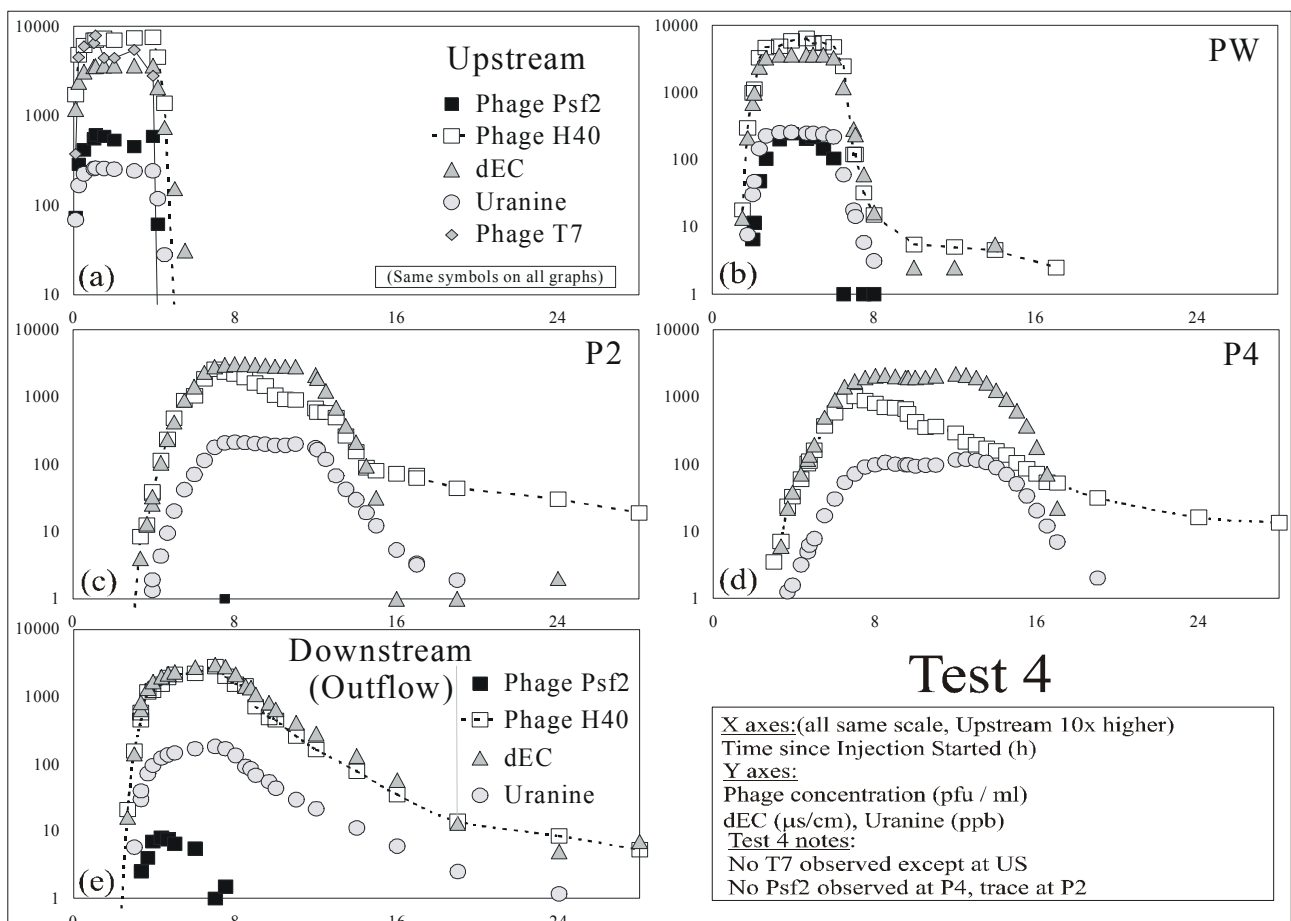


Figure 2-7-1 Test 4 concentration history by location

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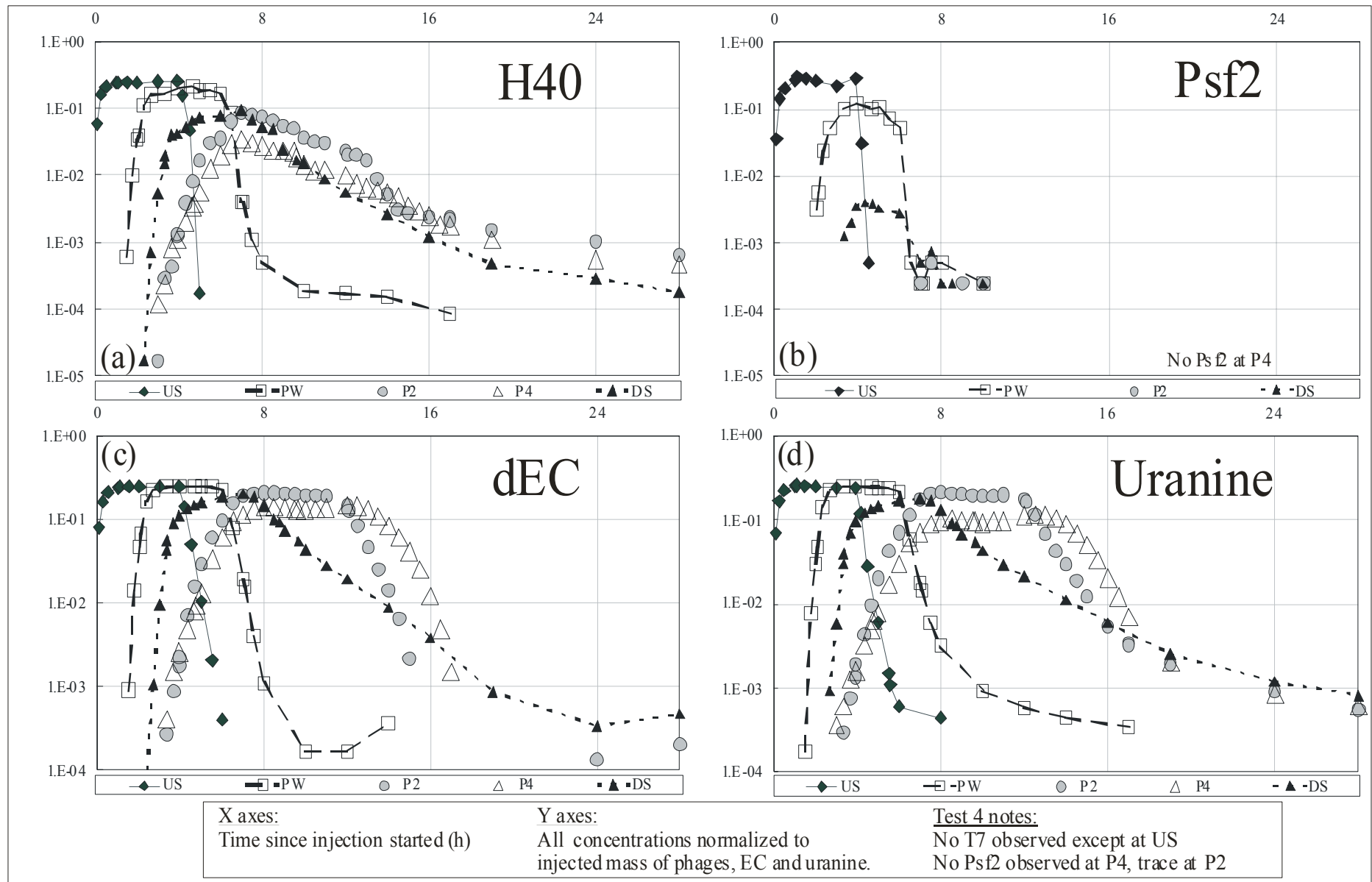


Figure 2-7-2. Test 4 tracer component response normalized to injected mass ( $C/M_0$ ).

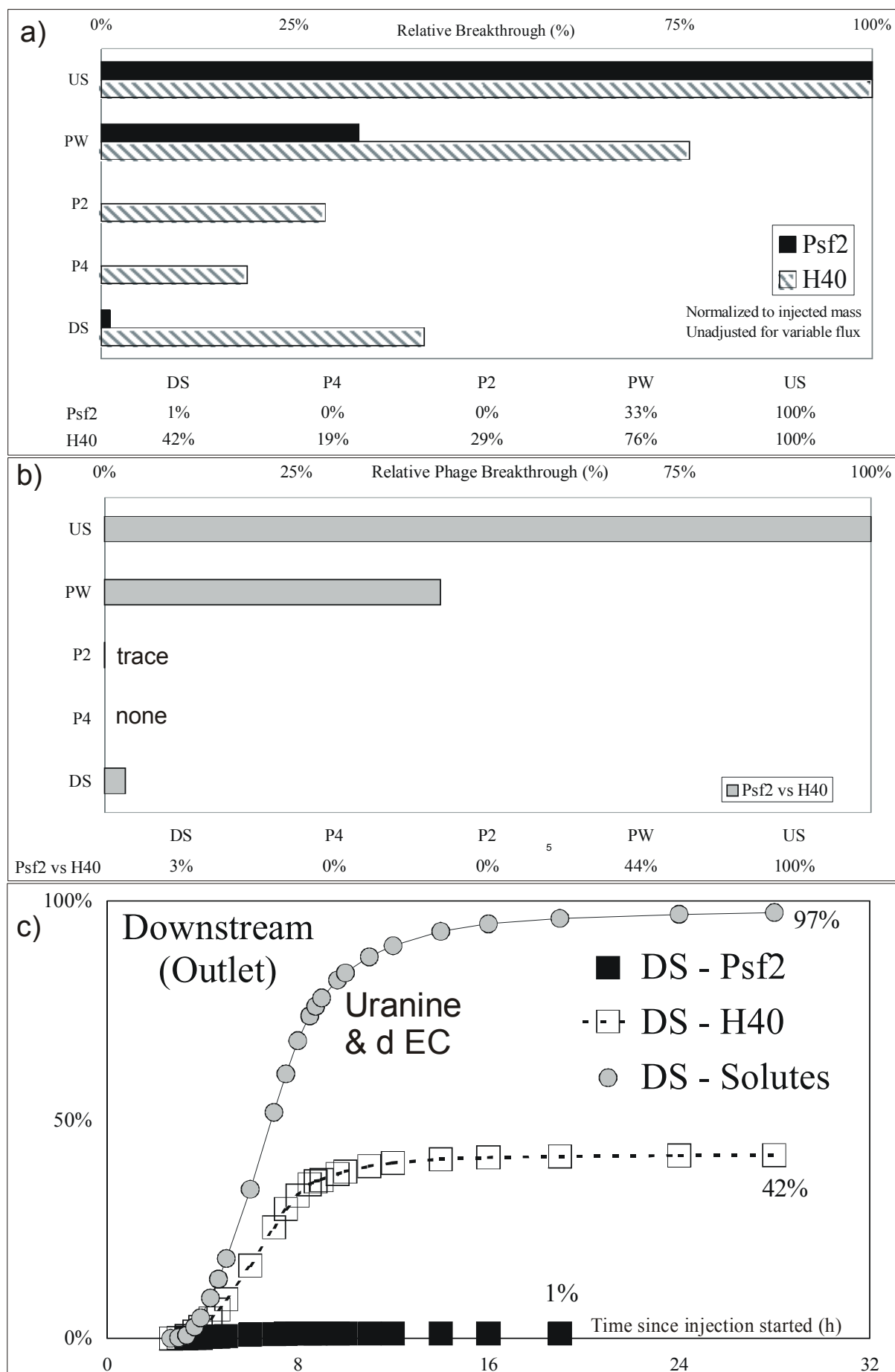


Figure 2-7-3. Test 4 relative breakthrough (RB) of Phages Psf2 and H40 with outflow total recovery.

### Test 5

This 25 h test had a 4 h injection duration for both the two Phages (H40, MS2) and salt and uranine used as conservative solute tracers. This test was done to compare Phage H40 and MS2 migration and to compare their responses with the two solutes. The flow rates were similar to tests 1 and 2 and salt concentrations were between those in Tests 1 and 2 and Tests 3 and 4. Measured outflow rate and EC values were about  $4.8 \text{ L min}^{-1}$  and  $3700 \text{ } \mu\text{S cm}^{-1}$ , respectively. Figures 2-8-1 through 2-8-3 illustrate the input signal, concentration history and  $C/M_0$  values and relative recovery at the five sampling locations. The principal results of the test indicated, respectively:

- solute, phage arrival velocity from 0.9 to 1.6 and 1.0 to 1.6  $\text{m h}^{-1}$ ;
- solute, phage peak velocity were both from 0.4 to 0.7  $\text{m h}^{-1}$ ;
- similar solute, phage detection at PW, earlier for phages at DS, P2 and P4;
- both solutes with a dual peak at P4, and a similar, but less distinct pattern for Phage H40;
- MS2, H40 recovery were 31 and 48% at the outlet (DS);
- H40 relative breakthrough was about 1.1 to 2.7 fold higher than MS2.

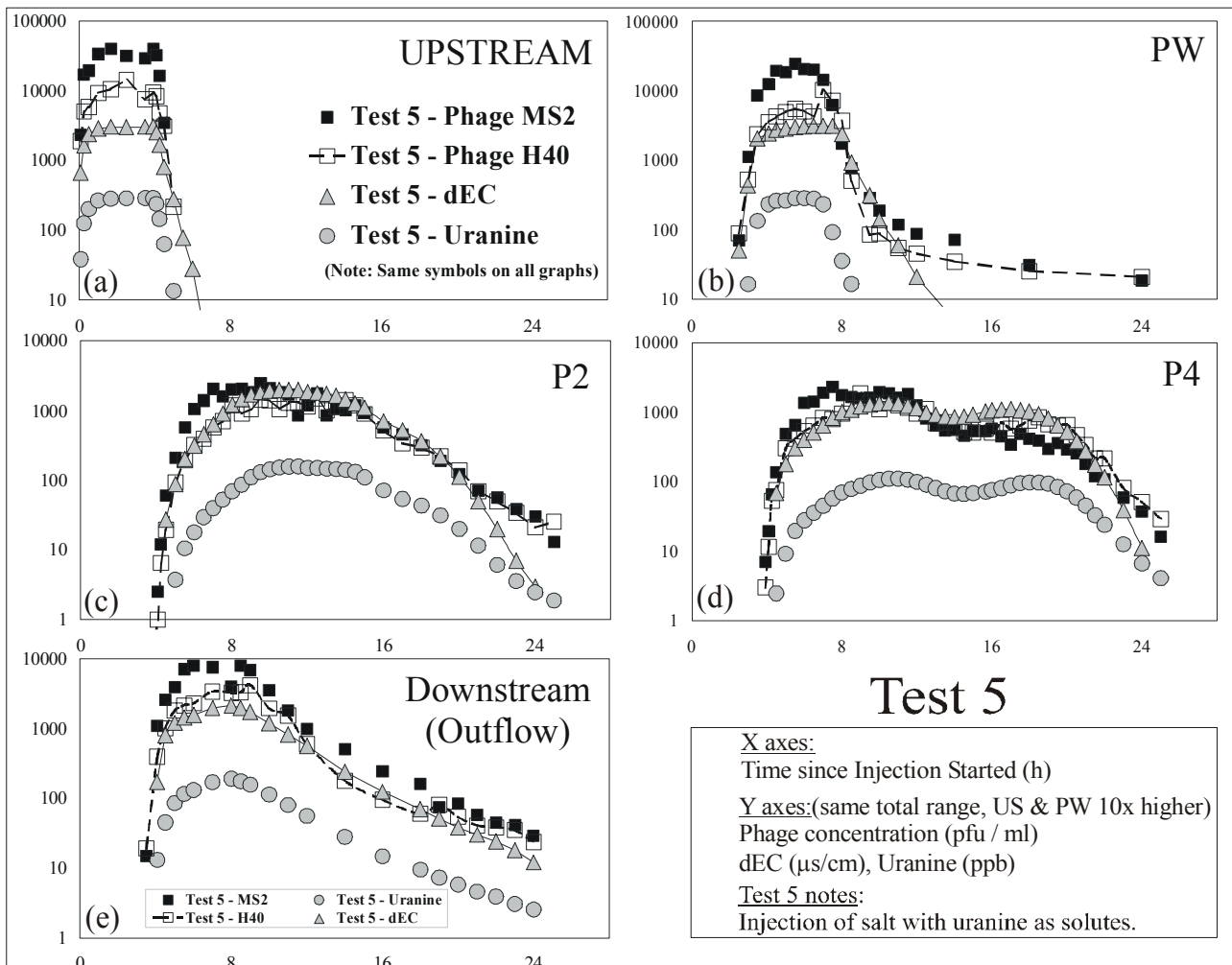


Figure 2-8-1. Test 5 concentration history by location

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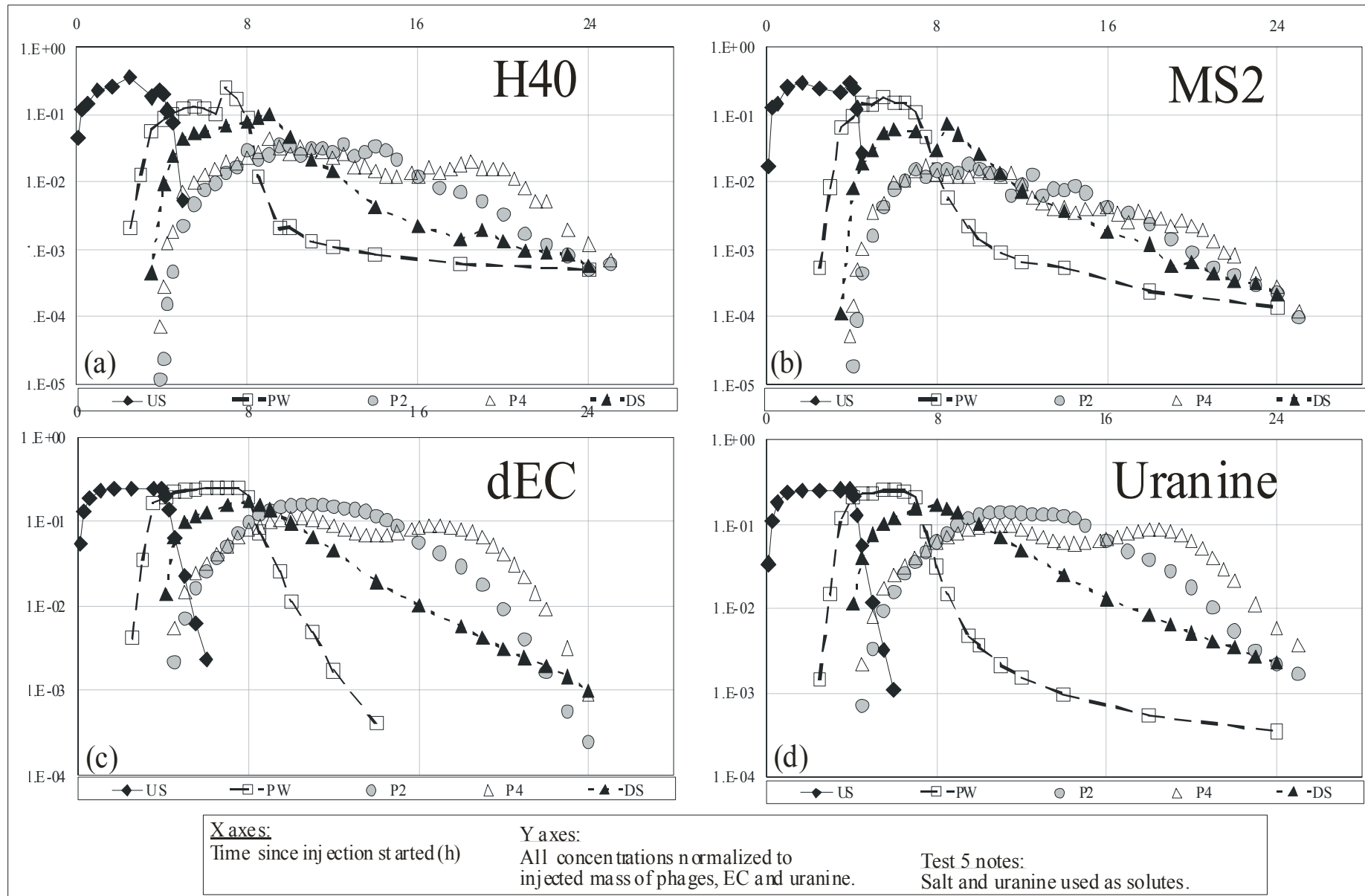


Figure 2-8-2. Test 5 tracer component response normalized to injected mass ( $C/M_0$ ).

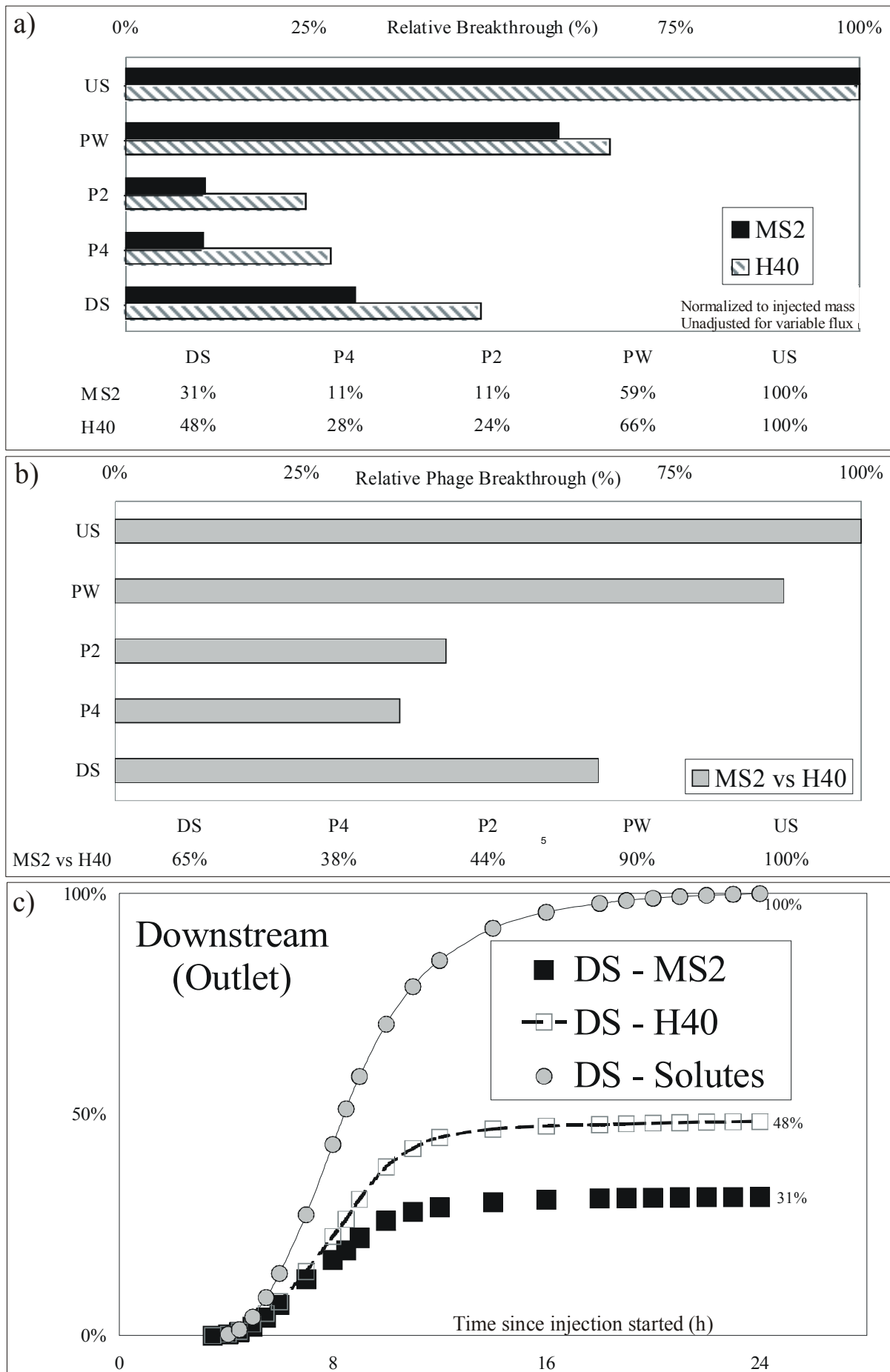


Figure 2-8-3. Test 5 relative breakthrough (RB) of Phages MS2 and H40 with outflow total recovery.

### Test 6

This 26 h test had a 4 h injection duration for two Phages (H40, MS2) and uranine used as the conservative solute tracer. This test was done to compare Phage H40 and MS2 migration under lower ionic strength (no salt added) conditions and to compare their responses with the solute. The flow rates were similar to Tests 1, 2 and 5. Measured outflow rate and EC values were about  $4.9 \text{ L min}^{-1}$  and about  $700 \text{ } \mu\text{S cm}^{-1}$ , respectively. Figures 2-9-1 through 2-9-3 illustrate the input signal, concentration history and  $C/M_0$  values and relative recovery at the five sampling locations. The principal results of the test indicated, respectively:

- uranine, Phage H40 arrival velocity from about  $0.8$  to  $1.6 \text{ m h}^{-1}$ , similar to Test 5;
- uranine, Phage H40 peak velocity from  $0.3$  to  $0.8$  and  $0.4$  to  $0.9 \text{ m h}^{-1}$ ;
- similar solute, phage detection times at PW and DS, earlier for phages at P2 and P4;
- both solutes had a dual peak at P4 that the phages did not;
- MS2, H40 recovery were 25 and 28% at the outlet (DS);
- H40 relative breakthrough about equal to 4 fold higher than MS2.

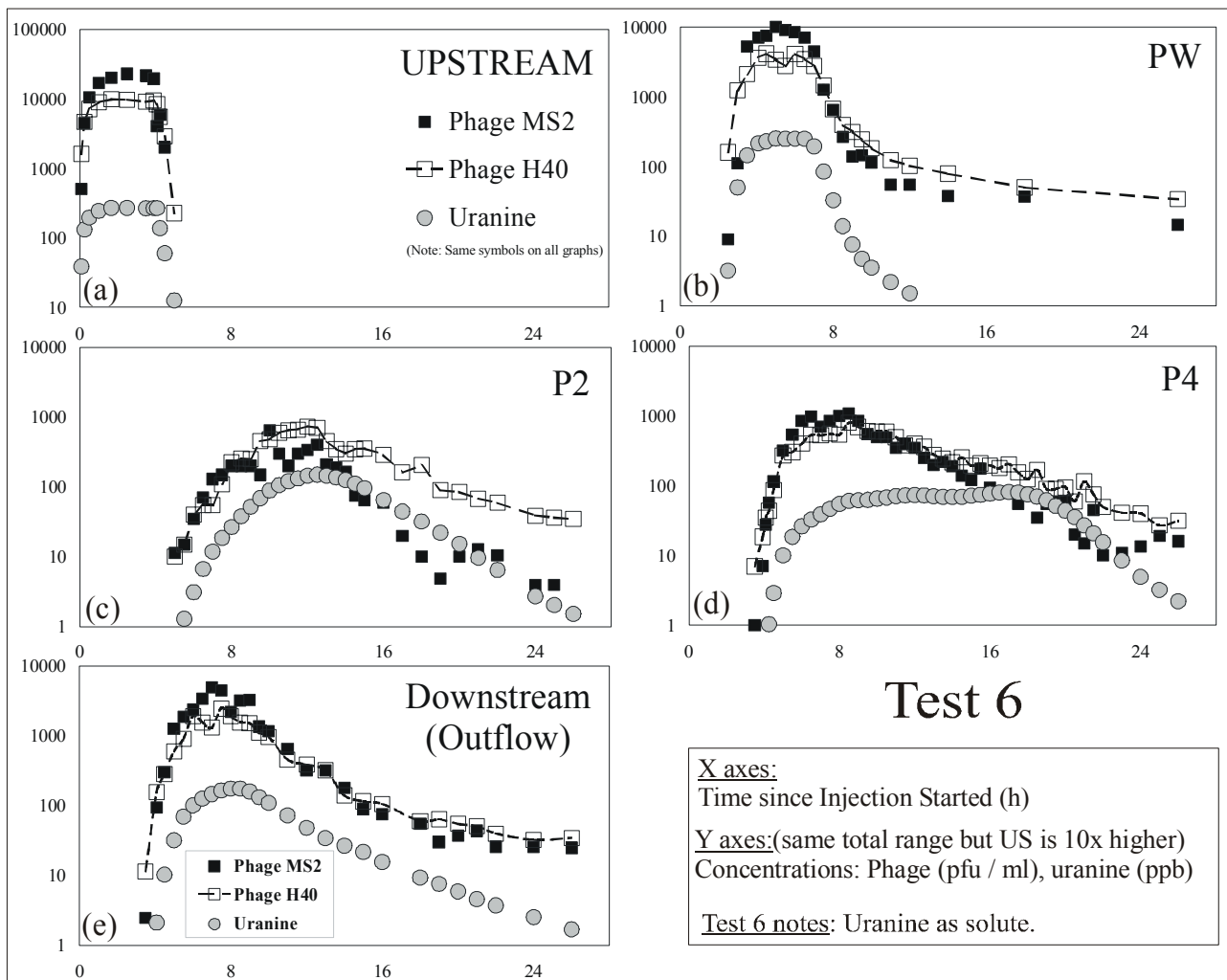


Figure 2-9-1. Test 6 concentration history by location.

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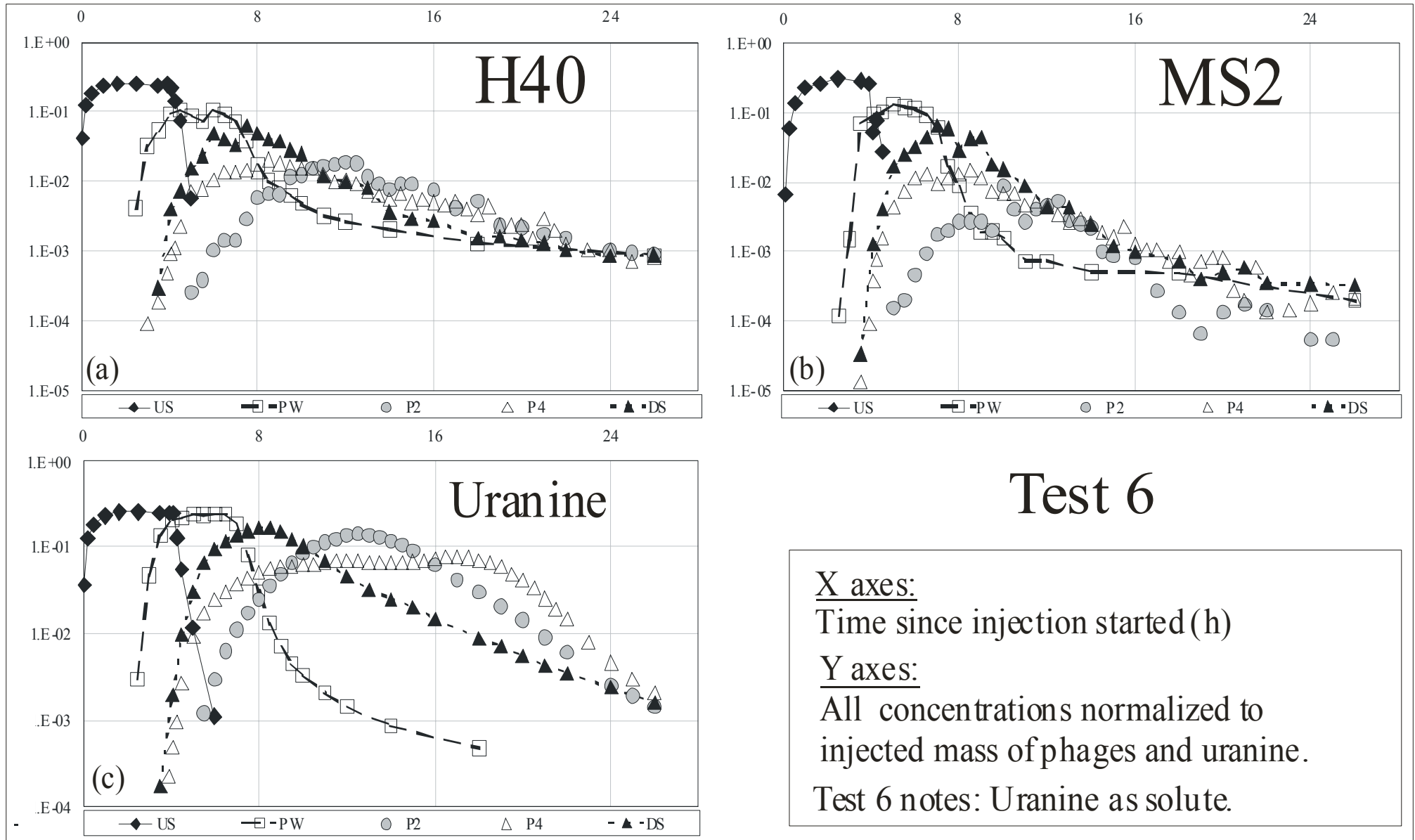


Figure 2-9-2. Test 6 tracer component response normalized to injected mass ( $C/M_0$ ).

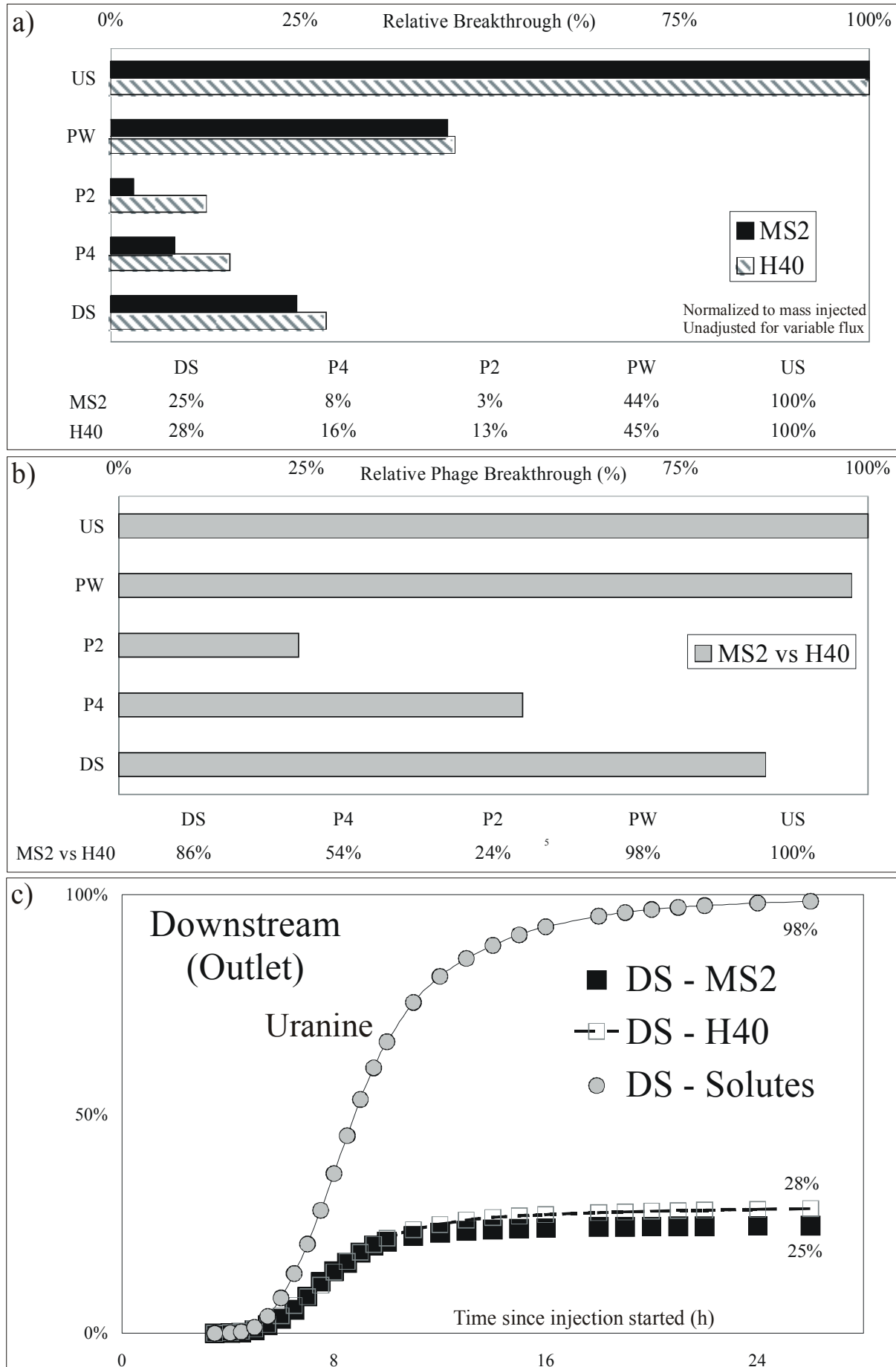


Figure 2-9-3. Test 6 relative breakthrough (RB) of Phages MS2 and H40 with outflow total recovery under no additional salt loading.

### 2.3.2 Bacteriophage H40 under varying ionic strength conditions

Tests 2 through 6 involved the use of Phage H40 each time, under similar injection conditions with respect to the solute injection time and phage concentrations but with differences in the electrical conductivity reflecting ionic strength. Test 1 had an unequal time for solute and phage injection so it is not included in this comparison. The change in EC injection concentrations varied as a function of the salt mixture and varied from 0 (Test 6) to about 1600 (Test 2) to 3000 to 3700 (Tests 3, 4, 5)  $\mu\text{S cm}^{-1}$ . Flow rates varied during these five tests to a factor of about two. Flow rate variation would not be expected to influence the migration processes or recovery results given the environment in which the testing was carried out. Increased ionic strength would have theoretically reduced recovery. What we compare in this section are the results of the calculated Maximum Dimensionless Concentrations (MDC) and the Relative Breakthrough (RB) values – at each location sampled during testing.

Table 2-6 and figure 2-10 show the variation in the MDC (a) and RB (b) compared to the EC for the five tests at all sampling locations. Both plots show that except for at the P4 location in Test 5, the migration or transport indicator values generally increased after a value of about 2500  $\mu\text{S cm}^{-1}$  as EC increases. The increase is not expected compared to current theoretical suggestion that ionic strength increases would result in an increase adsorption of the colloids onto the matrix materials thereby leading to a decrease in both parameter values. At P4, both the MDC and the RB values were trend-consistent in so far as Test 5 had higher values than Tests 6 and 2 with lower ECs. Also, Tests 3 and 4 which had the highest EC had higher MDC and RBs than Tests 6 and 2. However, Test 5 MDC and RB values were two to three times above the trend based on the other four tests. P4 during Test 5 also showed a double peak in the H40 concentration history pattern, the only time that such a peak occurred for the biocolloids at P4. A possible explanation relates to the flow rates and relative pathways followed by the colloids through the system with changes in mobility and flow regimes.

Test 5 had the same flow rate as in Tests 6 and 2 but a higher EC. Thus, the results are consistent with increased mobility and penetration throughout the system as the conductivity increased. Tests 3 and 4 had increased flow rates with increased mobility. However, assuming that for H40 there is an increased mobility and reduced removal with increasing EC, the 75% higher flow rates in Tests 3 and 4 could have also resulted in a change in the timing of the distribution of the pathways used for the colloidal particles to follow. Reduced relative removal combined with the heterogeneity of the channel could account for a continuing change in the pathways as the colloid mass flows through the various channels and laterally migrates into the slower flow fields.

For DS during Test 5, the RB value was intermediate between the two values of Tests 3 and 4 although the MDC was not. The recovery increased from 28% at the background EC level of 700  $\mu\text{S cm}^{-1}$  (Test 6) to 31% at 2400 (Test 2) to 51% and 42% at 4400  $\mu\text{S cm}^{-1}$  (Tests 3 and 4), respectively. The Test 5 total recovery with an EC value of 3700  $\mu\text{S cm}^{-1}$  was intermediate between those of Tests 3 and 4 at 48%.

This differences in Test 5 may suggest that over a certain level of conductivity there is less impact on the change in mobility or that there were other factors that had affected the tank environment between the periods of Test 5 and the previous series of Tests 3 and 4. Regardless, the results between the two series of tests conducted one after another (2, 3, 4 and 5, 6), all show the same consistent trend of increasing H40 recovery and MDC levels with increasing EC values at all sampling locations within the tank.

**Table 2-6. Phage H40 relative recovery by location for all tests <sup>(1)</sup>.**

| Test | Flow<br>(L min <sup>-1</sup> ) | EC <sup>(2)</sup> | H40 MDC (%) |    |    |    |    |    |    | H40 Relative Breakthrough (%) |    |    |    |    |    |    |
|------|--------------------------------|-------------------|-------------|----|----|----|----|----|----|-------------------------------|----|----|----|----|----|----|
|      |                                |                   | IW          | PW | P1 | P2 | P3 | P4 | DS | IW                            | PW | P1 | P2 | P3 | P4 | DS |
| 6    | 4.9                            | 700               |             | 42 |    | 7  |    | 8  | 25 |                               | 45 |    | 13 |    | 16 | 28 |
| 1    | 4.7                            | 2350              |             |    |    |    |    |    |    | 94                            | 73 | 29 | 20 | 29 | 12 | 43 |
| 2    | 4.3                            | 2300              | 46          | 40 | 20 | 6  | 16 | 3  | 25 | 45                            | 46 | 27 | 11 | 21 | 5  | 31 |
| 5    | 4.8                            | 3700              |             | 71 |    | 10 |    | 13 | 29 |                               | 66 |    | 24 |    | 28 | 48 |
| 3    | 7.6                            | 4400              | 79          | 72 | 33 | 29 | 39 | 11 | 28 | 85                            | 78 | 41 | 30 | 41 | 15 | 51 |
| 4    | 7.2                            | 4400              |             | 84 |    | 34 |    | 13 | 37 |                               | 76 |    | 29 |    | 19 | 42 |

<sup>(1)</sup> Maximum Dimensionless Concentration (MDC) values are relative to the injected concentrations measured at the US (Upstream) sampling location. No values reported for test 1 as salt and phage input signals' duration were different.

<sup>(2)</sup> Salt was measured by electrical conductivity and uranine (UR) by fluorometer measurements. The background EC value for the City of Strasbourg municipal public water supply varied by test from about 670 to 710  $\mu\text{S cm}^{-1}$ .

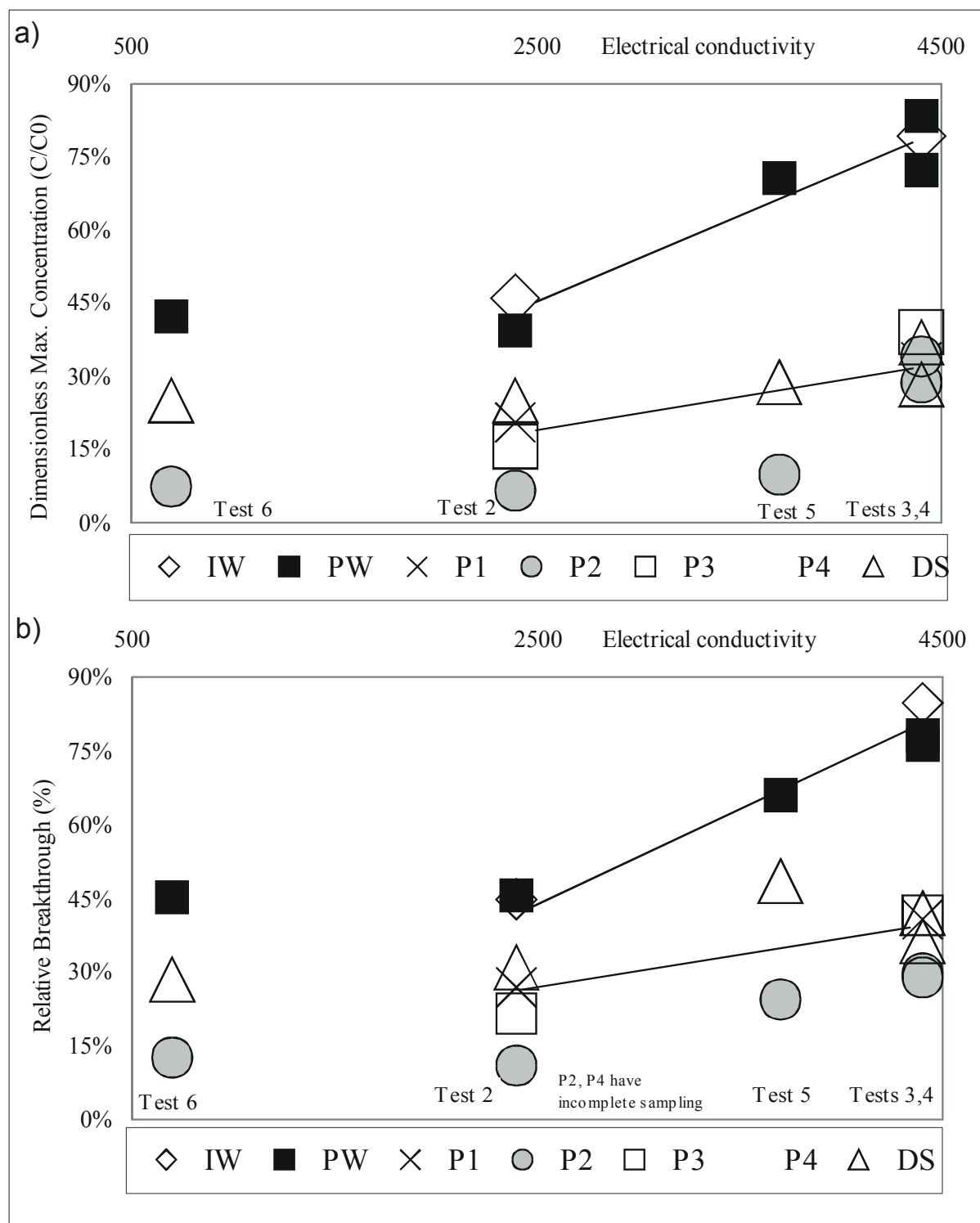


Figure 2-10. Phage H40 maximum dimensionless concentration and RB variation with EC ( $\mu\text{S cm}^{-1}$ ).

### 2.3.3 Phage responses – all tests

H40 was used in all tests and as discussed in Section 3.2, there was relatively consistent recovery at the sampling locations throughout the tank. Of interest is how recovery of the other phages, and in particular, MS2, a phage that is widely used as a surrogate for studies of viral transport outside Switzerland, compared to that of Phage H40. H40 MDC and RB values were compared to the 2<sup>nd</sup> phage used in each test and their ratio, compared to H40 was calculated (table 2-7 and figure 2-11). We have used these two parameters as global indicators of the overall response of the phages although there are undoubtedly individual differences at each sampling location that, when considered in detail, could also indicate further insight into such a comparison. The relative ranking reflecting higher persistence/recovery (lower attenuation/removal) within the tank-testing environment as based on the MDC and RB results from all sampling locations is:

$$H40 = \text{or} > MS2 > H4 >> H6 >>> Psf2 >>>> T7$$

MS2 most closely matched H40. The detailed comparison of the MS2 and H40 responses in Tests 5 and 6 is in Section 3.4. The H4 Phage used in Test 1 was similar to MS2, but less present than MS2 except at the outflow where its recovery was the similar to MS2 in Test 6 at 24%. H4 had MDC and RB values of from 1.4 to 4 fold less than H40. H4 was only used in Test 1 and that test was done with different injection conditions than the other tests. Therefore, comparing the H4 response in detail with other phages than H40 may not be as justified as for other tests' phage indicator values. It is clear, however, that the H4 results from the initial feasibility trial in Test 1 show that while more attenuated than the H40, it responded with an efficacy less than, but on the same order of magnitude as, MS2 at the locations where both were sampled. This was not the case with the other three phages used (H6, Psf2 and T7).

**Table 2-7. H40 comparison with other phage MDC and RB responses <sup>(1)</sup>.**

|        |                       | a) MDC of H40 vs. 2 <sup>nd</sup> Phage at sampling locations |           |           |           |           |                          |           |
|--------|-----------------------|---------------------------------------------------------------|-----------|-----------|-----------|-----------|--------------------------|-----------|
|        | 2 <sup>nd</sup> Phage | <u>IW</u>                                                     | <u>PW</u> | <u>P1</u> | <u>P2</u> | <u>P3</u> | <u>P4</u> <sup>(3)</sup> | <u>DS</u> |
| Test 1 | H4                    | 2.3                                                           | 2.5       | 1.0       | 2.0       | 3.2       | 3.9                      | 2.7       |
| Test 2 | H6                    | 8                                                             | 21        | 13        | 25        | 60        | 20                       | 12        |
| Test 3 | H6                    | 8                                                             | 11        | 8         | 9         | 22        | 9                        | 11        |
| Test 4 | Psf2 <sup>(2)</sup>   |                                                               | 2         |           | 210       |           | nd                       | 33        |
| Test 5 | MS2                   |                                                               | 1.2       |           | 1.6       |           | 2.2                      | 1.1       |
| Test 6 | MS2                   |                                                               | 1.0       |           | 4.3       |           | 1.7                      | 1.2       |

|        |                       | b) RB of H40 vs. 2 <sup>nd</sup> phage at sampling locations |           |           |           |           |                          |           |
|--------|-----------------------|--------------------------------------------------------------|-----------|-----------|-----------|-----------|--------------------------|-----------|
|        | 2 <sup>nd</sup> Phage | <u>IW</u>                                                    | <u>PW</u> | <u>P1</u> | <u>P2</u> | <u>P3</u> | <u>P4</u> <sup>(3)</sup> | <u>DS</u> |
| Test 1 | H4                    | 2.5                                                          | 2.3       | 1.4       | 2.6       | 3.7       | 3.0                      | 1.8       |
| Test 2 | H6                    | 6                                                            | 19        | 14        | 22        | 35        | 16                       | 13        |
| Test 3 | H6                    | 8                                                            | 13        | 10        | 9         | 23        | 9                        | 16        |
| Test 4 | Psf2 <sup>(2)</sup>   |                                                              | 2.3       |           | 2900      |           | nd                       | 38        |
| Test 5 | MS2                   |                                                              | 1.1       |           | 2.3       |           | 2.7                      | 1.4       |
| Test 6 | MS2                   |                                                              | 1.0       |           | 4.2       |           | 1.9                      | 1.2       |

(1) The number shown indicated the multiplier for which H40 was greater than the other phage response during that test at each sampling location for the parameters compared.

(2) No T7 was detected at any sampling location during Test 4.

(3) Psf2 was not detected (nd) at P4 during Test 4. Numbers of 999 and 9999 were used on the graph to indicate a H40 value for its high relative presence (figure 2-11).

Phage H6 was removed significantly more than the H4 and MS2. Depending on location, RB and MDC levels were from 8 to 60 and 6 to 35 fold, respectively, less than H40 values. H6, like H40, also

demonstrated a consistent and significant increase in both MDC and RB values at all locations in Tests 2 and 3 where the EC value was increased from about 2300 to 4400  $\mu\text{S cm}^{-1}$ .

Phage Psf2 MDC and RB values were 2 to 10 fold lower than H6 except at PW where they were 5 to 10 times higher. Phage T7 was not detected in any of the sampling locations and therefore it was the most attenuated and least mobile within the tank. This may, however, have been due to its inability to migrate when there were high ionic strength levels due to the presence of salt in the transporting fluid.

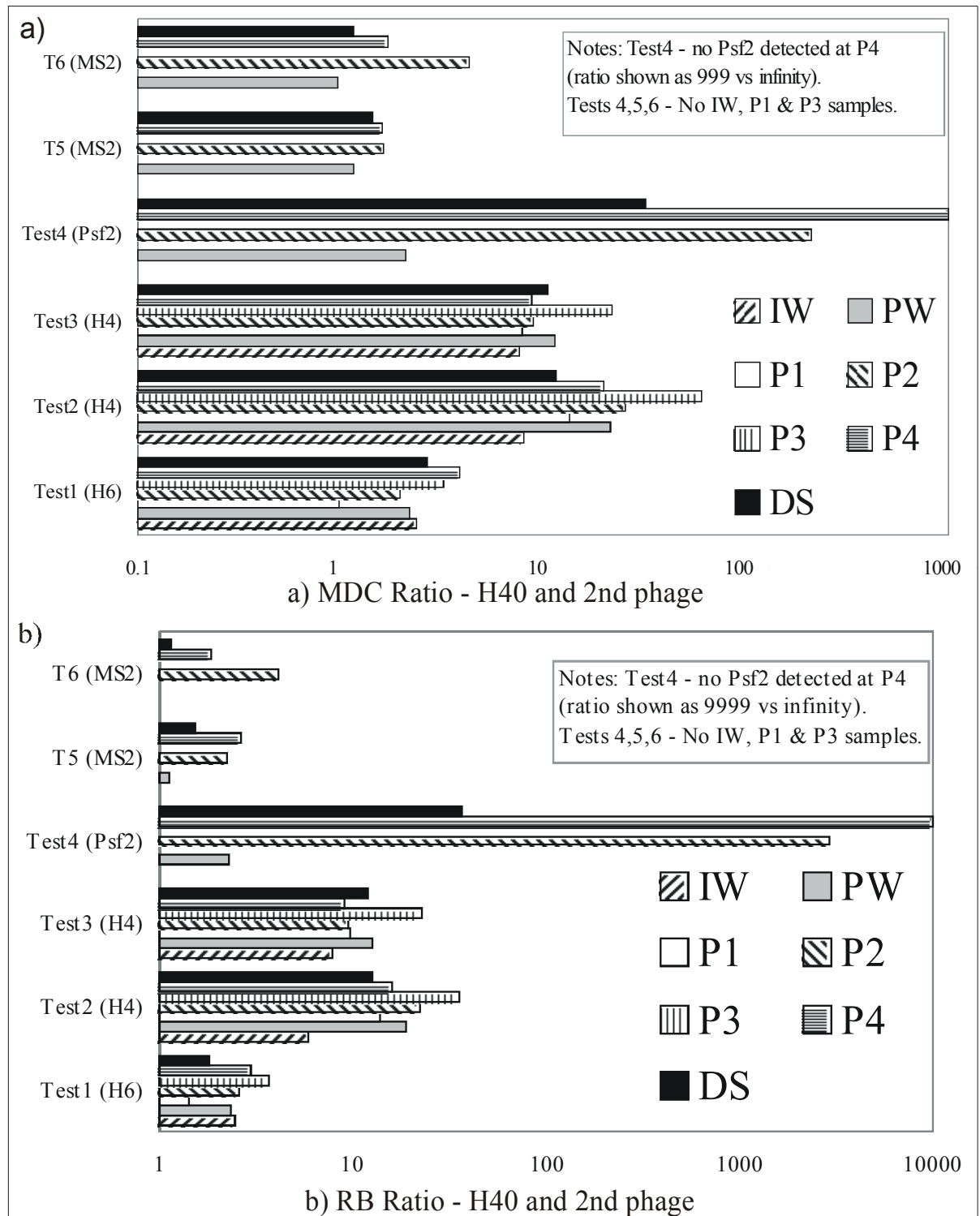


Figure 2-11. Ratio of H40 to other phage MDC and RB responses. (a) maximum dimensionless concentration (MDC) (b) relative breakthrough (RB).

### 2.3.4 H40 and MS2 comparison

Tests 5 and 6 were carried out specifically to evaluate the response of MS2 and H40 as both had been or were used at three different test sites as part of the field-oriented bacteriophage response characterization research programs (K. KENNEDY 2000, K. KENNEDY at al. 2001a, K. KENNEDY at al. 2001b, K. KENNEDY at al. 2001c). Concentration histories of the two test results along with the solute responses are in figure 2-12. Comparisons of their relative responses with respect to MDC and RB values are in table 2-8. Comparative C/C<sub>0</sub> and RB plots are on figure 2-13.

Phage detection times (table 2-4) in Test 5 were generally the same for each phage at PW and DS. H40 arrived earlier at P2 by 0.2 h and at P4 by 0.4 h being present in the sample prior to the one in which the MS2 was detected. Detecting the absolute arrival times in Test 6 was more difficult since there were background levels on the order of 1 to 4 pfu ml<sup>-1</sup> for the MS2 and H40 Phages, respectively, at most sampling locations. In Test 6, the detection of the two phages appeared to be the same at all locations. Comparing the detection/arrival times between the two tests showed that they were identical in both tests at PW and DS. H40 arrived earlier at P4 in Test 6 by 0.4 h and MS2 arrived at the same time. The consistent difference was at P2 where both phages were detected above apparent background levels about 1 h later in Test 6 arriving at 5 h. The lower ionic strength during Test 6 seems to have caused retardation in detection/arrival at this P2 location.

Peak concentrations in Test 5 with the higher ionic strength occurred earlier for MS2 than H40 at PW and P4 by 1.5 h and DS by 0.5 h and were the same at P2. In Test 6, MS2 peak values were more similar to H40 being the same at P2 and P4, and 0.5 h later and earlier at PW and DS, respectively. The 0.5 h difference in these instances represented the difference in time for one sampling event. Comparing the peak times showed that in Test 6 compared to Test 5, the maximum concentrations were, at:

- PW, earlier for MS2 and H40 by 0.5 to 2.5 h, respectively;
- P2, 2.5 h later;
- P4, 0.5 h earlier for H40 and 1 h later for MS2;
- DS, earlier for both phages by 1.5 h.

Comparing the H40 response at PW and P4 during the two tests is difficult to clearly interpret. Test 5 value for the H40 maximum was unusually high at PW and considerable later than MS2. At P4, there were continuing fluctuations in the H40 concentrations in Test 5 and an apparent dual peak. However, the overall comparative pattern for timing of the maximum concentrations of the phages was that Test 6, under lower ionic strength, had later peaks at P2, and earlier peaks at the other three locations.

MDC and RB history at each sampling location are shown on figure 2-13 and summarized in table 2-8. In all cases, a) H40 RB was greater – from 7 to 18 percent in Test 5 and from 1 to 10% in Test 6, and b) the RB and MDC of both phages decreased with the lower ionic strength in Test 6 compared to Test 5. In addition, the difference between the RB of the two phages also decreased from Test 5 to Test 6 indicating more similar responses under the lower ionic strength conditions. The differences between the RB of H40 and MS2 decreased from Tests 5 to 6 from 7% to 1% at PW, 13% to 10% at P2, 18% to 8% at P4 and 16 to 3% at DS, respectively (table 2-8). MDC values were also greater for H40 than MS2 except in Test 6 at PW where they were about the same (42, 43%).

**Table 2-8. Comparison of Test 5 and Test 6 MS2 and H40 maximum dimensionless concentration (MDC) and relative breakthrough (RB) values.**

| Test (Phage)                  | a) MDC (%) |    |    |    | b) RB (%) |    |    |    |
|-------------------------------|------------|----|----|----|-----------|----|----|----|
|                               | PW         | P2 | P4 | DS | PW        | P2 | P4 | DS |
| Test 5 (H40)                  | 71         | 10 | 13 | 29 | 66        | 24 | 28 | 48 |
| Test 5 (MS2)                  | 61         | 6  | 6  | 25 | 59        | 11 | 11 | 31 |
| Test 5 H40 vs. MS2 Difference | 10         | 4  | 7  | 4  | 7         | 13 | 17 | 17 |
| Test 6 (H40)                  | 42         | 7  | 8  | 25 | 45        | 13 | 16 | 28 |
| Test 6 (MS2)                  | 43         | 2  | 5  | 22 | 44        | 3  | 8  | 25 |
| Test 6 H40 vs. MS2 Difference | -1         | 5  | 3  | 3  | 1         | 10 | 8  | 3  |

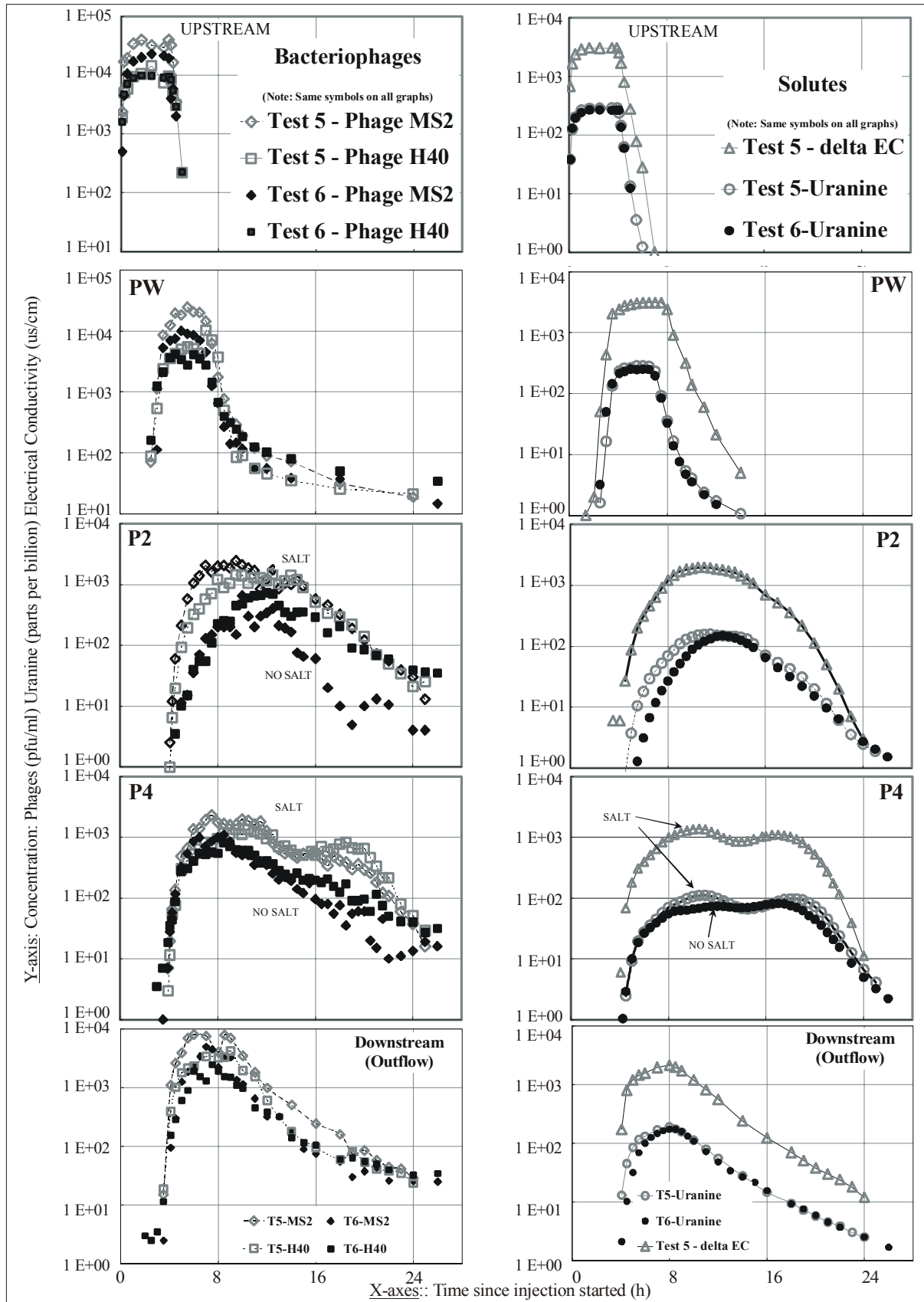


Figure 2-12. Tests 5 and 6 tracer component concentration histories.

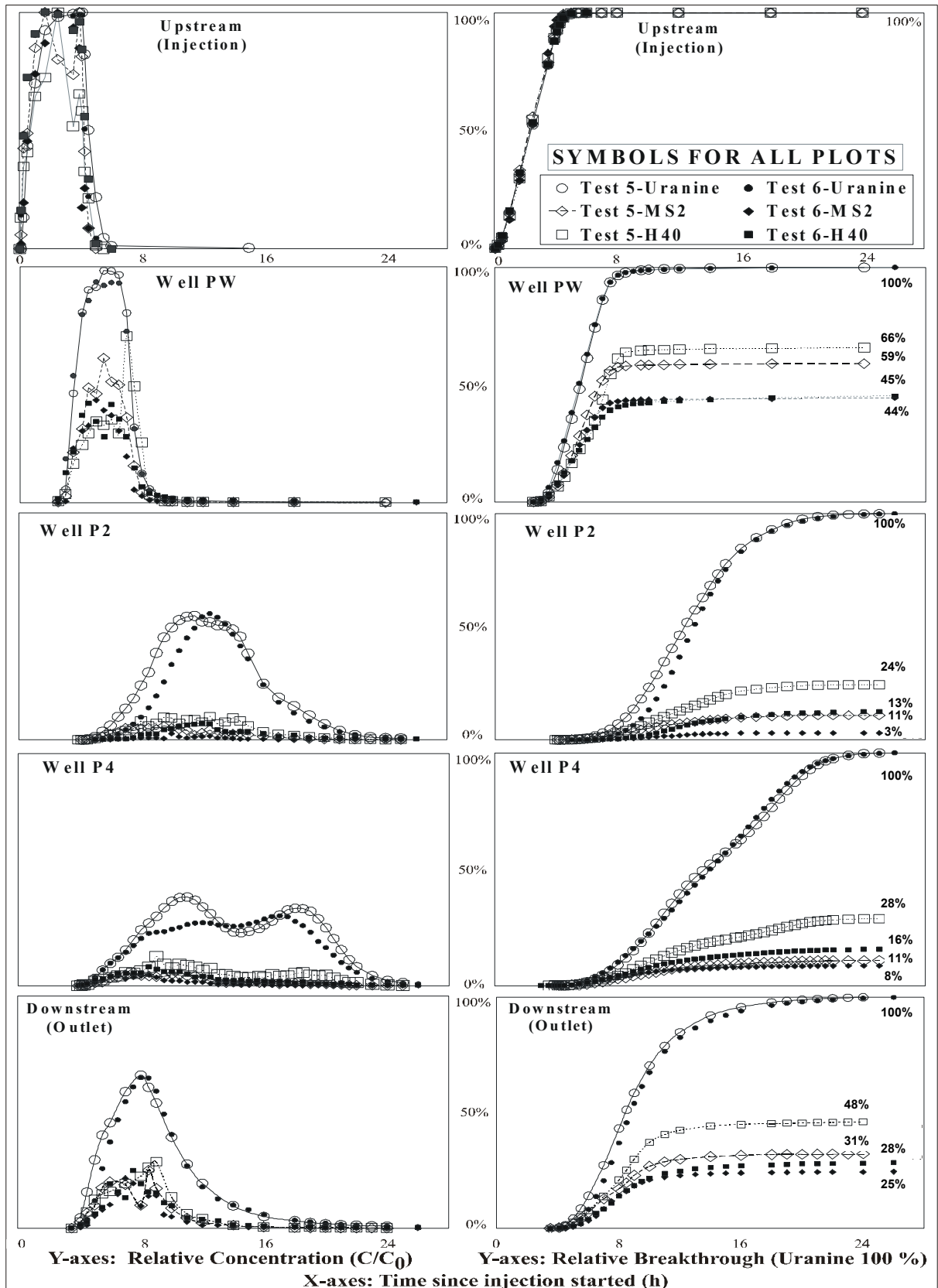


Figure 2-13. Phages H40 and MS2 concentrations normalized to initial injection values ( $C/C_0$ ) and relative breakthrough for Tests 5 and 6.

### 2.3.5 Comparative response by location

As a final comparison, we consider how differences in the response patterns of the solutes and phages throughout the tank reflect heterogeneity within the system. It is clear from the data that have been already presented that the response of the solutes and phages differ in terms of relative and total recovery and in terms of the timing of the breakthrough curve characteristic responses at the sampling locations. The difference in both solute and particle transport behaviour is also considerable throughout the tank despite the fact that the tank is filled with relatively clean sands that have less than a 1½ order of magnitude change in hydraulic conductivity.

The DS outlet location reflected the total response of the system over time. There were evident, more permeable pathways in the tank through which the transport of the injected materials took place preferentially. This included the locations where there were samples taken at IW, PW and to a lesser degree P1 and P3. P2 and P4 were located in areas where there was retarded arrival relative to absolute distance from the inflow compared to P1 and P3, respectively. A way to illustrate the response within the heterogeneity from the standpoint of particle migration was to consider variability in the arrival velocities for the H40 Phage at each location (table 2-9a) – normalized against the measured average flow rate through the tank (table 2-9b). Tests 4 through 6 (results with larger emphasis in bold in table 2-9) had the highest arrival time sampling density. Therefore, the values have the most accurate response in terms of documented arrival times. At PW and DS, the flow rate normalized values range from 0.32 to 0.37 and 0.33 to 0.34, respectively. At P2 and P4, the values range from 0.17 to 0.23 and 0.21 to 0.27, respectively. Thus, the flow rate-normalized arrival velocity at the wells located in channelled environments was about 50 percent higher than for less permeable pathways.

Ranges in the Phage H40 MDC and RB also differed according to location (table 2-10). RB values (excluding Test 1) were from: 45 to 85% at IW and PW; 21 to 41% at P1 and P3; 11 to 30% at P2; 5 to 28% at P4, and 28 to 51% at DS. Looking at the differences in detail by test shows that there was a relatively consistent ranking in all tests for H40 RB (figure 2-14) by location as follows:

$$IW = \text{or} > PW > DS > P1 = \text{or} > P3 > \text{either } P2 > P4 \text{ or } P4 > P2$$

In general, the H4, H6, Psf2 (where detected) and MS2 Phages followed the same patterns as the H40 in terms of relative abundance at these locations. However, as described in the earlier sections, the recovery of the other phages was consistently lower than the H40.

**Table 2-9. First detection velocity ( $\text{m h}^{-1}$ ) and flow-rate normalized value for H40 during testing.**

| a) Velocity of first H40 phage detection (m h <sup>-1</sup> )                            |              |      |      |      |             |             |             |      |      |      |
|------------------------------------------------------------------------------------------|--------------|------|------|------|-------------|-------------|-------------|------|------|------|
| Loc'n                                                                                    | Distance (m) | Test |      |      |             |             |             | min  | max  | sd   |
|                                                                                          |              | 1    | 2    | 3    | 4           | 5           | 6           |      |      |      |
| IW                                                                                       | 0.8          |      | 1.6  | 2.4  |             |             |             | 1.58 | 2.37 | 0.56 |
| PW                                                                                       | 4.0          |      | 2.4  | 2.4  | <b>2.7</b>  | <b>1.6</b>  | <b>1.6</b>  | 1.60 | 2.66 | 0.50 |
| P1                                                                                       | 4.2          |      | 1.3  | 1.3  |             |             |             | 1.26 | 1.26 | -    |
| P2                                                                                       | 4.3          |      | 1.1  | 1.1  | <b>1.4</b>  | <b>1.1</b>  | <b>0.9</b>  | 0.85 | 1.42 | 0.20 |
| P3                                                                                       | 4.6          |      | 1.7  | 1.7  |             |             |             | 1.73 | 1.73 | -    |
| P4                                                                                       | 4.6          |      | 1.0  | 1.3  | <b>1.5</b>  | <b>1.3</b>  | <b>1.3</b>  | 0.99 | 1.53 | 0.20 |
| DS                                                                                       | 5.6          |      | 2.1  | 2.4  | <b>2.4</b>  | <b>1.6</b>  | <b>1.6</b>  | 1.60 | 2.40 | 0.40 |
| ~ Flow rate (L min <sup>-1</sup> )                                                       |              | 4.7  | 4.3  | 7.6  | 7.1         | 4.8         | 4.9         |      |      |      |
| b) Flow-rate normalized first H40 detection (velocity divided by tank average flow rate) |              |      |      |      |             |             |             |      |      |      |
|                                                                                          |              | Test |      |      |             |             |             | min  | max  | sd   |
|                                                                                          |              | 1    | 2    | 3    | 4           | 5           | 6           |      |      |      |
| IW                                                                                       | 0.8          |      | 0.36 | 0.31 |             |             |             | 0.31 | 0.36 | 0.04 |
| PW                                                                                       | 4.0          |      | 0.55 | 0.31 | <b>0.37</b> | <b>0.33</b> | <b>0.32</b> | 0.31 | 0.55 | 0.10 |
| P1                                                                                       | 4.2          |      | 0.29 | 0.17 |             |             |             | 0.17 | 0.29 | 0.09 |
| P2                                                                                       | 4.3          |      | 0.25 | 0.14 | <b>0.20</b> | <b>0.23</b> | <b>0.17</b> | 0.14 | 0.25 | 0.04 |
| P3                                                                                       | 4.6          |      | 0.40 | 0.23 |             |             |             | 0.23 | 0.40 | 0.12 |
| P4                                                                                       | 4.6          |      | 0.23 | 0.17 | <b>0.21</b> | <b>0.27</b> | <b>0.27</b> | 0.17 | 0.27 | 0.04 |
| DS                                                                                       | 5.6          |      | 0.48 | 0.32 | <b>0.34</b> | <b>0.33</b> | <b>0.33</b> | 0.32 | 0.48 | 0.07 |

# CHAPTER 2 - COMPARATIVE BACTERIOPHAGE TRANSPORT IN A SAND TANK MODEL

**Table 2-10. Tracer component response characterization by location and by test.**

| SOLUTES           |                       |      |                                  |                      |                       |         |                      |                       |         | BACTERIOPHAGES       |                       |     |         |        |           |                      |                                 |     |         |        |           |             |                          |           |  |  |
|-------------------|-----------------------|------|----------------------------------|----------------------|-----------------------|---------|----------------------|-----------------------|---------|----------------------|-----------------------|-----|---------|--------|-----------|----------------------|---------------------------------|-----|---------|--------|-----------|-------------|--------------------------|-----------|--|--|
| Sampling Location | Distance from US (cm) | Test | Flow rate (L min <sup>-1</sup> ) | SALT (EC)            |                       |         | URANINE              |                       |         | Phage H40            |                       |     |         |        |           |                      | 2nd phage (according to Test #) |     |         |        |           |             |                          | Detection |  |  |
|                   |                       |      |                                  | T <sub>arr</sub> (h) | T <sub>peak</sub> (h) | MDC (%) | T <sub>arr</sub> (h) | T <sub>peak</sub> (h) | MDC (%) | T <sub>arr</sub> (h) | T <sub>peak</sub> (h) | RF  | MDC (%) | RB (%) | Att'n (%) | T <sub>arr</sub> (h) | T <sub>peak</sub> (h)           | RF  | MDC (%) | RB (%) | Att'n (%) | 2nd Phage   | H40 (m h <sup>-1</sup> ) | V/Q       |  |  |
| IW                | 79                    | 1    | 4.7                              | 1.0                  | 3.0                   | 100     |                      |                       |         | 0.3                  | 1.3                   |     | 42      | 94     | 6         | 0.3                  | 1.0                             | na  | 18      | 38     | 62        | H4          | 2.4                      | 0.51      |  |  |
|                   |                       | 2    | 4.3                              | 1.0                  | 3.5                   | 98      |                      |                       | none    | 0.5                  | 3.8                   | 1.1 | 46      | 45     | 55        | 0.5                  | 3.2                             | 0.9 | 6       | 8      | 92        | H6          | 1.6                      | 0.36      |  |  |
|                   |                       | 3    | 7.6                              | 0.8                  | 3.2                   | 99      | 0.8                  | 3.2                   | 98      | 0.3                  | 3.0                   | 0.9 | 79      | 85     | 15        | 0.5                  | 3.0                             | 0.9 | 10      | 11     | 89        | H6          | 2.4                      | 0.31      |  |  |
| PW                | 399                   | 1    | 4.7                              | 2.0                  | 4.5                   | 101     |                      |                       |         | 1.7                  | 2.0                   |     | 41      | 73     | 27        | 1.7                  | 2.0                             | na  | 19      | 31     | 69        | H4          | 2.4                      | 0.51      |  |  |
|                   |                       | 2    | 4.3                              | 2.0                  | 4.0                   | 100     |                      |                       |         | 1.7                  | 4.0                   | 1.0 | 40      | 46     | 54        | 1.7                  | 3.7                             | 0.9 | 2       | 3      | 97        | H6          | 2.4                      | 0.55      |  |  |
|                   |                       | 3    | 7.6                              | 1.7                  | 4.7                   | 99      | 1.7                  | 4.7                   | 98      | 1.7                  | 5.0                   | 1.1 | 72      | 78     | 22        | 1.7                  | 5.0                             | 1.1 | 6       | 6      | 94        | H6          | 2.4                      | 0.31      |  |  |
|                   |                       | 4    | 7.2                              | 1.5                  | 4.3                   | 99      | 1.7                  | 3.9                   | 97      | 1.5                  | 4.7                   | 1.1 | 84      | 76     | 24        | 2.0                  | 3.9                             | 0.9 | 40      | 33     | 67        | Psf2        | 2.7                      | 0.37      |  |  |
|                   |                       | 5    | 4.8                              | 2.5                  | 6.5                   | 102     | 2.5                  | 6.0                   | 98      | 2.5                  | 7.0                   | 1.1 | 71      | 66     | 34        | 2.5                  | 5.5                             | 0.8 | 61      | 59     | 41        | MS2-salt    | 1.6                      | 0.33      |  |  |
|                   |                       | 6    | 4.9                              |                      | none                  |         | 2.5                  | 5.0                   | 94      | 2.5                  | 4.5                   | 0.9 | 42      | 45     | 55        | 2.5                  | 5.0                             | 1.0 | 43      | 44     | 56        | MS2-no salt | 1.6                      | 0.32      |  |  |
| P1                | 420                   | 1    | 4.7                              | 3.7                  | 8.0                   | 78      |                      |                       |         | 3.0                  | 5.5                   |     | 4       | 29     | 71        | 3.3                  | 5.5                             | na  | 4       | 21     | 79        | H4          | 1.4                      | 0.30      |  |  |
|                   |                       | 2    | 4.3                              | 3.3                  | 8.0                   | 82      |                      |                       | none    | 3.3                  | 9.0                   | 1.1 | 20      | 27     | 73        | 3.3                  | 8.5                             | 1.1 | 2       | 2      | 98        | H6          | 1.3                      | 0.29      |  |  |
|                   |                       | 3    | 7.6                              | 3.0                  | 9.0                   | 81      | 3.7                  | 9.0                   | 80      | 3.3                  | 9.0                   | 1.0 | 33      | 41     | 59        | 3.3                  | 9.0                             | 1.0 | 4       | 4      | 96        | H6          | 1.3                      | 0.17      |  |  |
| P2                | 427                   | 1    | 4.7                              | 4.0                  | 10.0                  | 82      |                      |                       |         | 4.0                  | 7.0                   |     | 3       | 20     | 80        | 4.0                  | 7.0                             | na  | 1       | 8      | 92        | H4          | 1.1                      | 0.23      |  |  |
|                   |                       | 2    | 4.3                              | 4.0                  | 10.5                  | 80      |                      |                       | none    | 4.0                  | 8.5                   | 0.8 | 6       | 11     | 89        | 4.3                  | 9.0                             | 0.9 | 0.3     | 0.5    | 99.5      | H6          | 1.1                      | 0.25      |  |  |
|                   |                       | 3    | 7.6                              | 3.9                  | 9.5                   | 78      | 3.9                  | 9.5                   | 71      | 3.9                  | 8.0                   | 0.8 | 29      | 30     | 70        | 3.9                  | 8.0                             | 0.8 | 3       | 3      | 97        | H6          | 1.1                      | 0.14      |  |  |
|                   |                       | 4    | 7.2                              | 3.3                  | 9.0                   | 84      | 3.7                  | 9.0                   | 82      | 3.0                  | 7.0                   | 0.8 | 34      | 29     | 71        | 7.0                  | 7.5                             | 0.8 | 0.2     | 0      | 100       | Psf2        | 1.4                      | 0.20      |  |  |
|                   |                       | 5    | 4.8                              | 4.5                  | 11.0                  | 65      | 4.5                  | 11.5                  | 55      | 3.9                  | 9.5                   | 0.9 | 10      | 24     | 76        | 4.1                  | 9.5                             | 0.9 | 6.1     | 11     | 89        | MS2-salt    | 1.1                      | 0.23      |  |  |
|                   |                       | 6    | 4.9                              |                      | none                  |         | 5.5                  | 12.5                  | 56      | 5.0                  | 12.0                  | 1.0 | 7       | 13     | 87        | 5.0                  | 12.0                            | 1.0 | 2       | 3      | 97        | MS2-no salt | 0.9                      | 0.17      |  |  |
| P3                | 460                   | 1    | 4.7                              | 2.7                  | 5.7                   | 98      |                      |                       |         | 2.3                  | 3.0                   |     | 12      | 29     | 71        | 2.3                  | 3.0                             | na  | 4       | 8      | 92        | H4          | 2.0                      | 0.42      |  |  |
|                   |                       | 2    | 4.3                              | 2.7                  | 5.4                   | 95      |                      |                       | none    | 2.7                  | 5.1                   | 0.9 | 16      | 21     | 79        | 2.7                  | 4.9                             | 0.9 | 0.3     | 0      | 99        | H6          | 1.7                      | 0.40      |  |  |
|                   |                       | 3    | 7.6                              | 2.7                  | 5.8                   | 96      | 2.7                  | 5.8                   | 94      | 2.7                  | 5.0                   | 0.9 | 39      | 41     | 59        | 2.7                  | 5.0                             | 0.9 | 2       | 2      | 98        | H6          | 1.7                      | 0.23      |  |  |
| P4                | 460                   | 1    | 4.7                              | 3.0                  | 11.5                  | 68      |                      |                       |         | 4.0                  | 6.0                   |     | 2       | 12     | 88        | 4.0                  | 6.5                             | na  | 0.4     | 4      | 96        | H4          | 1.2                      | 0.25      |  |  |
|                   |                       | 2    | 4.3                              | 3.7                  | 12.0                  | 66      |                      |                       | none    | 4.7                  | 8.5                   | 0.7 | 3       | 5      | 95        | 4.3                  | 8.5                             | 0.7 | 0.1     | 0.3    | 99.7      | H6          | 1.0                      | 0.23      |  |  |
|                   |                       | 3    | 7.6                              | 3.7                  | 11.0                  | 50      | 3.7                  | 11.0                  | 42      | 3.7                  | 7.5                   | 0.7 | 11      | 15     | 85        | 3.7                  | 8.5                             | 0.8 | 1       | 2      | 98        | H6          | 1.3                      | 0.17      |  |  |
|                   |                       | 4    | 7.2                              | 3.3                  | 10.0                  | 57      | 3.0                  | 10.0                  | 40      | 3.0                  | 7.0                   | 0.7 | 13      | 19     | 81        | none                 | none                            | -   | none    | 0      | 100       | Psf2        | 1.5                      | 0.21      |  |  |
|                   |                       | 5    | 4.8                              | 4.5                  | 10.5                  | 45      | 4.5                  | 11.0                  | 38      | 3.5                  | 9.0                   | 0.9 | 13      | 28     | 72        | 3.9                  | 7.5                             | 0.7 | 6       | 11     | 89        | MS2-salt    | 1.3                      | 0.27      |  |  |
|                   |                       | 6    | 4.9                              |                      | none                  |         | 3.9                  | 12.0                  | 27      | 3.5                  | 8.5                   | 0.7 | 8       | 16     | 84        | 3.5                  | 8.5                             | 0.7 | 5       | 8      | 92        | MS2-no salt | 1.3                      | 0.27      |  |  |
| DS                | 560                   | 1    | 4.7                              | 3.0                  | 7.2                   | 55      |                      |                       |         | 3.0                  | 4.0                   |     | 9       | 43     | 57        | 3.0                  | 3.0                             | na  | 3       | 24     | 76        | H4          | 1.9                      | 0.40      |  |  |
|                   |                       | 2    | 4.3                              | 2.7                  | 6.5                   | 92      |                      |                       | none    | 2.7                  | 6.0                   | 0.9 | 25      | 31     | 69        | 2.7                  | 6.5                             | 1.0 | 2       | 2      | 98        | H6          | 2.1                      | 0.48      |  |  |
|                   |                       | 3    | 7.6                              | 2.7                  | 7.0                   | 76      | 2.7                  | 7.0                   | 69      | 2.3                  | 7.0                   | 1.0 | 28      | 51     | 49        | 3.0                  | 7.0                             | 1.0 | 3       | 4      | 96        | H6          | 2.4                      | 0.32      |  |  |
|                   |                       | 4    | 7.2                              | 2.7                  | 7.0                   | 77      | 2.7                  | 7.0                   | 70      | 2.3                  | 7.0                   | 1.0 | 37      | 42     | 58        | 3.0                  | 4.3                             | 0.6 | 1       | 1      | 99        | Psf2        | 2.4                      | 0.34      |  |  |
|                   |                       | 5    | 4.8                              | 4.1                  | 8.0                   | 70      | 4.1                  | 8.0                   | 66      | 3.5                  | 9.0                   | 1.1 | 29      | 48     | 52        | 3.5                  | 8.5                             | 1.1 | 25      | 31     | 69        | MS2-salt    | 1.6                      | 0.33      |  |  |
|                   |                       | 6    | 4.9                              |                      | none                  |         | 3.5                  | 8.0                   | 66      | 3.5                  | 7.5                   | 0.9 | 25      | 28     | 72        | 3.5                  | 7.0                             | 0.9 | 22      | 25     | 75        | MS2-no salt | 1.6                      | 0.33      |  |  |

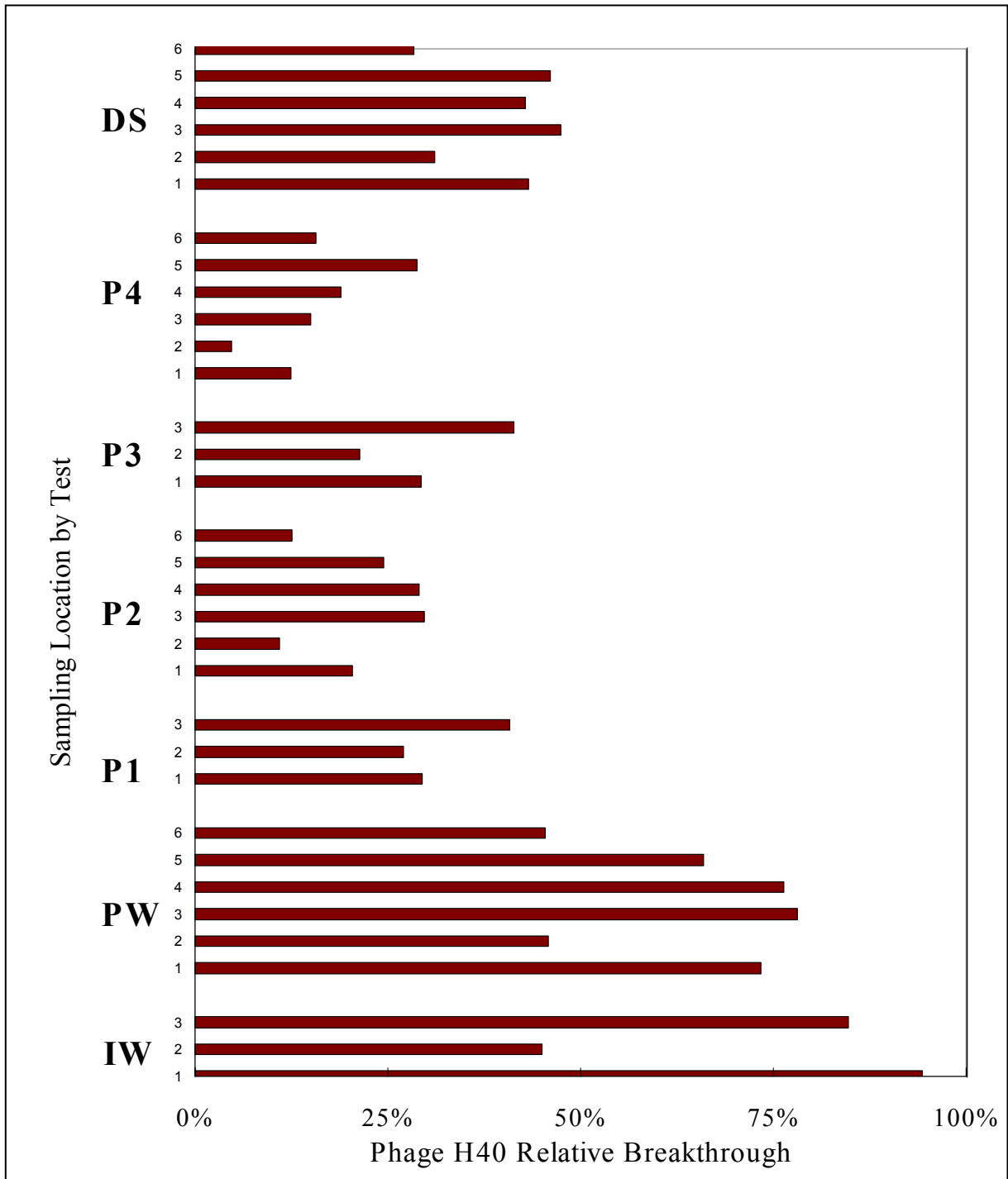


Figure 2-14. Comparative relative breakthrough for Phage H40 by location and by test.

## 2.4 Summary and Conclusions

### 2.4.1 Equipment – analyses – environment applicability

From an experimentalist's viewpoint, the success of research is highly dependent upon the adequacy and performance of the equipment selected for use, the sampling strategy and the analytical quality. It is rarely certain at the outset that the contributions to the eventual results from these specific requirements are going to be routine, correct or appropriate. Considerable time and effort went into planning the layout, sampling and analyses within the constraints of the available project resources. The flow rate manipulation within the tank was achievable although attaining a consistent inflow percentage of the concentration in the upstream source (yellow) tank was not always possible. Maintaining a constant gradient regardless of the source to inflow volume tank ratio variability and the outflow flow rate and gradient level were relatively straightforward. Electrical perturbations in the system of automated EC measurements were overcome by leaving all equipment turned on over the duration of the testing. Constant background water quality conditions were favourable for continuity and reproducibility of results.

Over 1500 samples were obtained during this research work with an average of about 250 samples taken per test. The continuous pumping of a small volume at each of the eight locations was effective to obtain sampling consistently from the porous and relatively clean sandy media. It appears to have provided a solid basis for and yielded well-reproducible and reliable results according to the analytical data. Each set of test samples was analysed for at least two phages, and uranine and/or EC. Phage analyses commonly involved two steps and duplicate aliquots at each of the two analytical procedures. Test 4 involved analyses for three different phages. Over 10,000 analytical/counting results were involved in the phage data that was generated as input to the work related to the particulate tracer component aspect of the characterization. The two most logistically-difficult parts of the work were: a) acquiring the samples at the correct time for the different locations in the tank and continuing this effort over a concentrated 24 h period (no automation possible), and b) handling the large volume of samples for phage analysis once they reached the laboratory (compared to the common 50 sample/day capacity). The solute data from the EC probes in the tank could not be relied upon for either arrival or peak concentration timing for the phages and not all sampling points were close enough to in-tank EC probes as to be representative for the samples. Performing consistent experiments reduced the uncertainty in the timing and allowed optimising of laboratory analytical steps. The uranine and EC analyses made manually were done so in order to effectively compare to sample results from the solute characterization with the phage sample data.

When the data were analysed, we believed that the tank environment had provided a sound basis for ranking the phage performance and comparing the particle and conservative solute responses. There were slight differences in the character of the two solutes' responses. However, in general they responded in a similar manner. Therefore, we were confident that the use of uranine in the field would be analogous to the salt tracer and that, within the expected relatively clean gravel settings in which the phages were to be used, combining the phage and uranine in the tracer cocktail would be appropriate and adequate.

### 2.4.2 Tracer component arrival/detection and maximum concentration characterization

The in situ EC probes were useful to scope, monitor and modify the sampling of the solute tracer components to better establish their characterization response. However, these data were not able to be relied upon for the particle sampling strategy or to estimate the particle response breakthrough curve characteristics. Initial detection of the particles was a more reliable indicator of the true arrival of the initial breakthrough than that of the solutes. The detection limits of the particles were several orders of magnitude lower (i.e. better) than for the solutes in terms of molar quantification. This detection limit difference alone could explain the consistent earlier or no-later-than-the-uranine detection of the H40 Phage at all seven downstream sampling locations in all six tests. The other possibility is that there was preferential particle advective transport that occurred during some of the testing to some of the locations. However, detection of the solute uranine and the phage particle was in the same sample in about 40% of the cases at all downstream locations except the P1 location where the phages were always detected earlier. Phages were detected earlier in 48% of the cases. In two exceptional cases, the phages were detected after the solute at the P4 location.

Another factor that could have influenced the results is the sampling frequency. With such rapid migration, it is also possible that there were not enough samples to distinguish accurately. The last three tests had sampling densities that focussed on assisting us discriminate well between the intra-

phage and solute-phage differences, in their early times and maximum values. Here, the two component types were still detected about 33% of the time in the same sample. We conclude that there is not adequate evidence to support preferential advection based solely on the initial detection of the two types of tracers. Additional analyses could be undertaken using approaches recently described in D. GROLIMUND & M. BORKOVEC (1999).

Comparing intra-phage initial detection, the H40 and second Phage (H4, H6, MS2, Psf2) occurred in the same sample in 21 of the 33 sampling situations. T7 was never detected anywhere downgradient in Test 4. Psf2 was always initially detected after H40 where it was found and it was not detected at P4. H4 was detected in the same sample at 6 of the 7 locations sampled during Test 1. H6 was initially detected in the same sample as H40 in 9 of the 14 cases, later in 4 cases, and earlier at P4 in the single instance of the second phage detected earlier than H40 in all the testing. MS2 was initially detected in the same sample as H40 in 6 of the 8 cases. H40 was detected in earlier samples in Test 5 (higher ionic strength conditions) than MS2 was at both the P2 and P4 locations.

In terms of comparing the time that solute maximum concentrations occurred, they were essentially equal or quite similar for the uranine and the EC in 11 of the 15 cases. In two instances the EC and uranine maximum values both occurred earlier than each other's but the maximum concentration occurred in just the previous sample in these four cases and these samples were only separated by 0.5 h. The solute and phage maximums could be compared for all except Test 1. In the four channel locations and at the outlet, DS, the timing of the two component type maximum concentrations were more similar with ranges in Retardation Factor (RF) consistently at each of these four locations and including DS from 0.9 to 1.1. At the P2 and P4 locations, the RF values reflected earlier times at which the phage maximums occurred with value ranges averaging about 0.84 at P2 and 0.74 at P4. The intra-phage comparison to H40 was less conclusive. Maximum concentration times were equal for H40 and the 2<sup>nd</sup> phage in 14 of 33 cases, earlier for the 2<sup>nd</sup> phage in 12 cases, later in six cases and did not occur for the Psf2 situation at P4 in Test 3. Accepting the premise that the earlier time to maximum concentration is influenced at least in part by the increased removal or attenuation, then the data are consistent to a large degree in pattern with the RB values. RB values show higher attenuation of the 2<sup>nd</sup> phages compared to Phage H40 in almost all cases. In 26 of the 33 cases there was similarity or earlier time for the 2<sup>nd</sup> phage's maximum. This suggests that comparing the times of maximum values among particles by themselves would not be a characteristic that is conclusive and it is certainly not always consistent with the relative breakthrough characterization. Thus, the RF value seems to be of less diagnostic significance in assessing the extent to which there are changes in attenuation relative to particles or even the solute. That being the case, it also suggests that the processes that are occurring to cause attenuation along the pathways may not always be reflected in the differences in the shape and occurrence of the particles and co-relative solute maximum values alone (pseudo-peak or peak). Increased contrasts in solute and particle maximum times generally reflected longer pathway and lower hydraulic conductivity settings, at least in this laboratory sandy environment. In addition, the similarity in the maximum timing in the channelled environments also did not reflect the relatively large changes in the RB values seen at these same locations.

### **2.4.3 Environmental heterogeneity**

Overall, the four in-tank piezometers plus the two sampling wells IW and PW, provided an adequate base to demonstrate the influence of heterogeneity within the system. The difference in both solute and particle transport behaviour at the six sampling locations within the tank was considerable. The tank is filled with relatively clean sands that have less than 1½ order of magnitude range in hydraulic conductivity. Despite this, there were marked changes in the patterns of not only the arrival/detection times at similar distances from the injection point, but also in the presence, concentrations and duration and form of the phage breakthrough curves. Clear distinctions are evident for the solutes reflecting channel flow areas compared to areas of slower migration. Phages illustrated similar patterns. In the case of Psf2, it was largely not detected outside the channel areas yet was readily characterized at the channel sample locations. The variability in magnitude and extent of the migration response with time in the spatial environment of the tank clearly leads to a greater awareness of the need to establish reliable and adequate sampling density in flow systems. This is shown here to be true even where there was only a minor degree of heterogeneity.

Within the heterogeneity, the particles had substantially lower recovery in the lower permeability than in the channel areas. This was to some extent surprising given the absence of silt and clay fractions within the sand environment. It suggests that the presence of these smaller grained materials are not always be required to reduce the particle concentrations along the flow path but that the combined

longer residence and path lengths can also have similar influences with flow in fine grained sand conditions.

#### **2.4.4 Tracer component response characterization**

At a final collection or total recovery points in a flow field, the comparison of the responses of particle and solute tracers is readily accomplished using the product of the flow rate and the sampling concentrations to calculate the component mass recovered which can be directly contrasted among components. At individual in-field or along pathway locations, the flow rate through the pathway point is not known and likely is variable. At these locations it is useful to combine the data graphically so as to evaluate the component responses and to depict the plot features that are evident and can characterize either the component or the location response. Using different diagnostic plots facilitates this comparison. Such diagnostic plots illustrate time versus component concentration with the concentration data depicted a) normalized to their maximum concentration, b) normalized to their original injected concentration and c) normalized to their original injected mass.

Component recovery comparison can be readily quantified at individual sampling points by comparing the time-based concentration product normalized to each component's originally injected mass or concentration. To characterize and compare the response at different locations throughout the flow field, the pattern as well as the total breakthrough at those points can be quantified when related to the response of a solute at each location assuming that the solute response is conservative throughout the flow field. This approach results in the quantity termed Relative Breakthrough (RB) which provides a consistent approach for evaluation of the relative total recovery anywhere within a flow field. Comparison of the pattern of changes in RB with time also shows where differences are occurring among particles and solutes.

#### **2.4.5 Intra-phage response characterization and ranking**

The tank activities allowed us to compare the efficacy of different phages as they migrated throughout the flow system. This approach was the basis for the planning of field experiments that were following and continuing at different sites, at each of which there was a relatively clean and highly permeable gravel-based porous media. It was important therefore to rank the phage responses and to use this ranking to select those that were the most resilient, i.e. least attenuated, for the related field studies. The results of the ranking of the six phages that were evaluated in the MARCEAUS environment indicated the relative persistence in the tank in almost all test cases and at most sampling points. The relative ranking reflecting higher persistence/recovery (lower attenuation/removal) within the tank-testing environment as based on the MDC and RB results from all sampling locations was:

$$H40 = \text{or} > MS2 > H4 >> H6 >>> Psf2 >>>> T7$$

There were similarities in the response of MS2 and H40 in Tests 5 and 6 at the PW and DS locations. MS2 MDC and RB values were equal to 1.4-fold lower than H40. At the P2 and P4 locations, MS2 values were from about 1.6- to 4.3-fold lower than H40. In general, the MS2 response more closely resembled the H40 response values in Test 6 with lower ionic strength. H4 was also similar to H40 during Test 1 at P1 with MDC and RB values equal and 1.8-fold lower than H40. At the other six locations, the H4 values were from 1.8- to 3.9-fold lower than H40. The H6 Phage was attenuated more than the H4 Phage. MDC and RB were about 6- to 60-fold lower than H40. The Phage Psf2 was attenuated more than the H6 Phage. Psf2 MDC and RB values were about 30-to 3000-fold lower than H40 except at PW where it was 2-fold lower. Phage T7 was completely attenuated in the tank.

H40 was observed under variable ionic strength conditions. Except at P4 in Test 5, the migration or transport indicator values generally increased after a value of about  $2500 \mu\text{S cm}^{-1}$  as EC increases. The increase was not expected compared to current theoretical prediction that as ionic strength increases, increased adsorption of the colloids would occur onto the matrix materials thereby leading to a decrease in both parameter values. We cannot explain this apparent contradiction at this time. Originally, we thought that it could relate directly to the marine nature of the H40 Phage. Specifically, MS2 and H40 responses were compared under salt and no salt conditions in Tests 5 and 6. In these tests the MS2 and H40 MDC and RB values were consistently and significantly higher in the higher ionic strength environment at all four downstream locations. These consistent results between the two tests suggest that both MS2 and H40 attenuation decreased with higher salt content in the water albeit that MS2 attenuation was greater than H40 attenuation as the salt content increased.

## **Chapter 3**

### ***Documented Transport Conditions and Migration Rates in Gravel Deposits – a Ground Water Protection Perspective*<sup>1</sup>**

#### ***Abstract***

Contaminant migration in a highly permeable gravel geologic setting was documented using tracer surrogates to evaluate adequacy of ground water protection rationale. Solute and biocolloid transport rates to 22 m were from 130 to 480 m day<sup>-1</sup>, up to 40 times faster than predicted using conventional flow/effective porosity approaches. In one well, a 1 m vertical heterogeneity dominated transport in 12 m of saturated thickness. Biocolloids were consistently detected earlier due to lower than solute detection limits. Maximum biocolloid concentrations commonly occurred prior to solute peaks as colloid removal processes rather than media properties control their breakthrough. Relative colloid recovery was typically 1.5 to 4 percent and in one case, 72 percent of solutes, illustrating low attenuation. Transport direction differed by up to 90 degrees from expected local scale gradients and not always along regional trends. Results demonstrate the special field testing and detailed considerations needed to develop realistic protection strategies in gravel environments.

#### **3.1 Introduction**

The effective environmental protection of the public and the ground water resources requires a profound understanding of the environmental setting of aquifers used for public water supplies. However, protection schemes can be based on out-of-date input from widespread field measurements or colloquial rules-of-thumb more appropriate to ground water yields than to contaminant transport issues. Transmissivity data from long-term pumping tests and data-based networks of regional well water levels from which hydraulic gradients can be determined are basic information that, used as a basis in developing a protection plan, are important. However, this level of basic 'quantitative' hydrodynamic data can be inadequate to document or reasonably estimate the true migration rates of potential pollutants in heterogeneous aquifers, particularly those with important preferential pathways found in high energy deposition environments such as river gravel.

Previous work understanding the nature of hydrogeologic heterogeneity in gravel aquifers has been based for the most part on geological mapping and geophysical characterization of specific water supply settings. These data can be useful 1) to correlate spatially with yields and transmissivity and 2) as part of schemes for improving well head protection that rely on aquifer characterization. However, the vertical resolution of most practical VLF, EM, Micro seismic or Radar aerial geophysical techniques is commonly inadequate to identify whether important preferential pathways exist even locally (J. McNeill and V. Labson, 1991). Furthermore, well density in these types of river gravel aquifers, even where high-resolution borehole geophysics and detailed geological sampling and logging has been carried out (E. Yearsley *et al.*, 1990) is rarely sufficient to effectively document these important types of heterogeneity. Even more detailed hydrogeological characterization commonly carried out at individual water supply wells can rarely address this issue. Due to their high permeability, it is not possible to adequately stress the aquifer over prolonged periods and distances during exploration phases to identify such contributing zones. Infrequently, detailed flow logging is conducted opposite contributing screened intervals in water supply wells. The emphasis is oriented to total yield rather than possible contaminant transport. Work by P. Rossi *et al.*, (1994) at another site in a fluvio-glacial aquifer in Switzerland suggested transport dependence on lateral variations in river deposit

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<sup>1</sup> This chapter is based on K. Kennedy *et al.* 2001a and a broader article currently under review (K. Kennedy & M. Schürch).

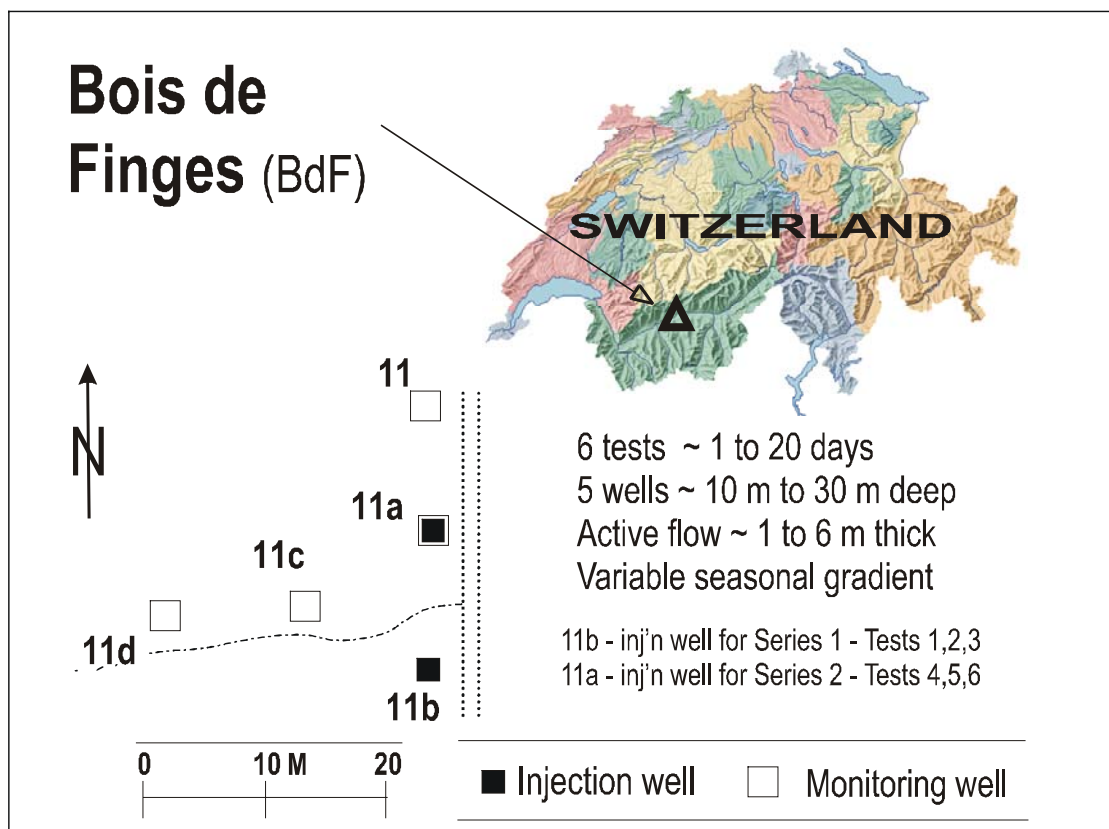


Figure 3-1 Site location map with well layout.

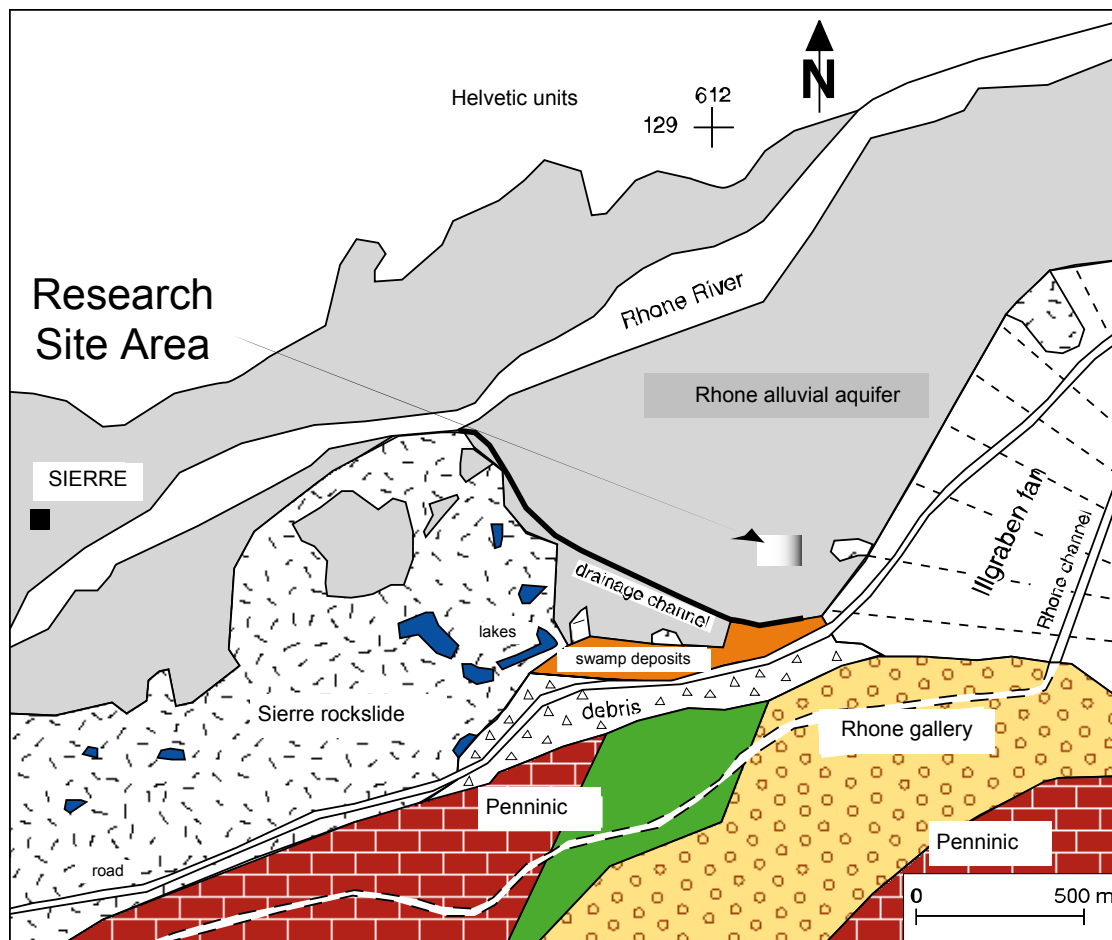


Figure 3-2 Site regional geology.

heterogeneity. However, subsequent testing at this site (K. Kennedy *et al.*, 2001a) illustrated that the effective contributing section within the previously tested 10 m of saturated thickness were likely limited to less than 2 m. While this does not change the documented migration rate results, it would materially change the predicted contaminant concentration magnitude at a receptor well.

Recent equipment and analytical advances have increased the feasibility of aquifer tracer studies previously considered logistically difficult and too expensive to justify for ground water protection investigations. We believe that an approach utilizing these newer methods can better serve protection strategies since they can provide real rates of migration to define protection zones and validate transport models.

The objective of this research work was to document the magnitude and direction of ground water flow and transport rates and concentrations in a highly conductive gravel environment believed to have a highly variable gradient direction. We evaluated the heterogeneity and determined the variability in magnitude and direction of contaminant migration at a local scale. We used both an artificial solute and biocolloid particles as tracers to emulate non-attenuating chemical and viral-sized ground water contaminants, respectively. The results reported herein were derived from work undertaken jointly by the two authors. They were interested in how modern tracer techniques could be applied in high permeability environments (K. Kennedy) as part of the input to characterizing the hydrogeology and geochemistry of an important research site where river gravel makes up a significant part of the subsurface flow setting (M. Schürch, 2000).

### 3.2 Study Area

The Bois de Finges (BdF) study area is located in the upper Rhone River valley of Switzerland (Canton Valais, Figure 3-1). It has been investigated for over the past decade as part of a long term environmental and hydrogeologic characterization of the valley in a location subject to stress from aggregate mining, water deviation, lateral recharge from higher salinity waters and land development. In 1997, four new wells supplementing an existing well were installed over a 25 by 25 m area referred to as the P11 site. It was done here as this location was believed representative of the BdF alluvial aquifer where its flow gradient magnitude and direction varied seasonally. Details of this and other pertinent BdF studies are in M. Schürch, 2000.

#### 3.2.1 Geologic setting

The regional geologic setting is depicted in Figures 3-2 and 3-3. Figure 3-2 illustrates the aerial plan of the geology with the Rhone River alluvial deposits in the center of the valley and pinching out against the valley walls to the north and south. Figure 3-3 shows that the gravel extends to depths of over 50 m. Southward, the river alluvial deposits directly overlie lacustrine, landslide and sandy fan deposits. Beneath the investigation site, the Rhone River gravel is about 15 m thick and directly overlie the upper Illgraben alluvium and its underlying silt- and clay-dominated deposits. Detailed geology in the immediate site area was investigated with core logging and geophysical methods. Figure 3-4 shows the results from the 30 m deep well (P11a) installed in 1997 illustrating the dominance of sand and gravel in the upper 12 m section. Cobbles with diameters of from 10 to 20 cm were reported in over 50 percent of this depth. Fluid logging conducted in Well 11A in 1999 indicated significant flow zones from between 5 and 7 m, 11 and 12 m, and 25 and 26 m (M. Schürch *et al.*, 2001).

#### 3.2.2 Hydrogeologic setting

The site is located in a region where there are about 60 widely spaced monitoring wells in the river plain. The data collected over the past four years suggest that the flow gradients on a regional basis can change seasonally (Figure 3-5 a, b). During low water level conditions in winter, the flow in the Rhone River is low and contributions to ground water flow increase from the walls of the valley suggesting the gradient towards the walls would be at this time perpendicular to the river. During the high water period when there is increased flow in the Rhone River, the gradient appears to be more parallel to the river channel. Vertical gradients in the area are not well understood due to the paucity of 1) flow measurements in wells intersecting both formations and 2) locations where the upper and deeper sections are isolated in adjacent wells. However, at well P11A, a vertical flow of from 2 to 3 L min<sup>-1</sup> was documented during the low water period (Feb. 1999) suggesting that the heads in the lower formations are higher than in the overlying alluvium, at least during the low ground water level water conditions.

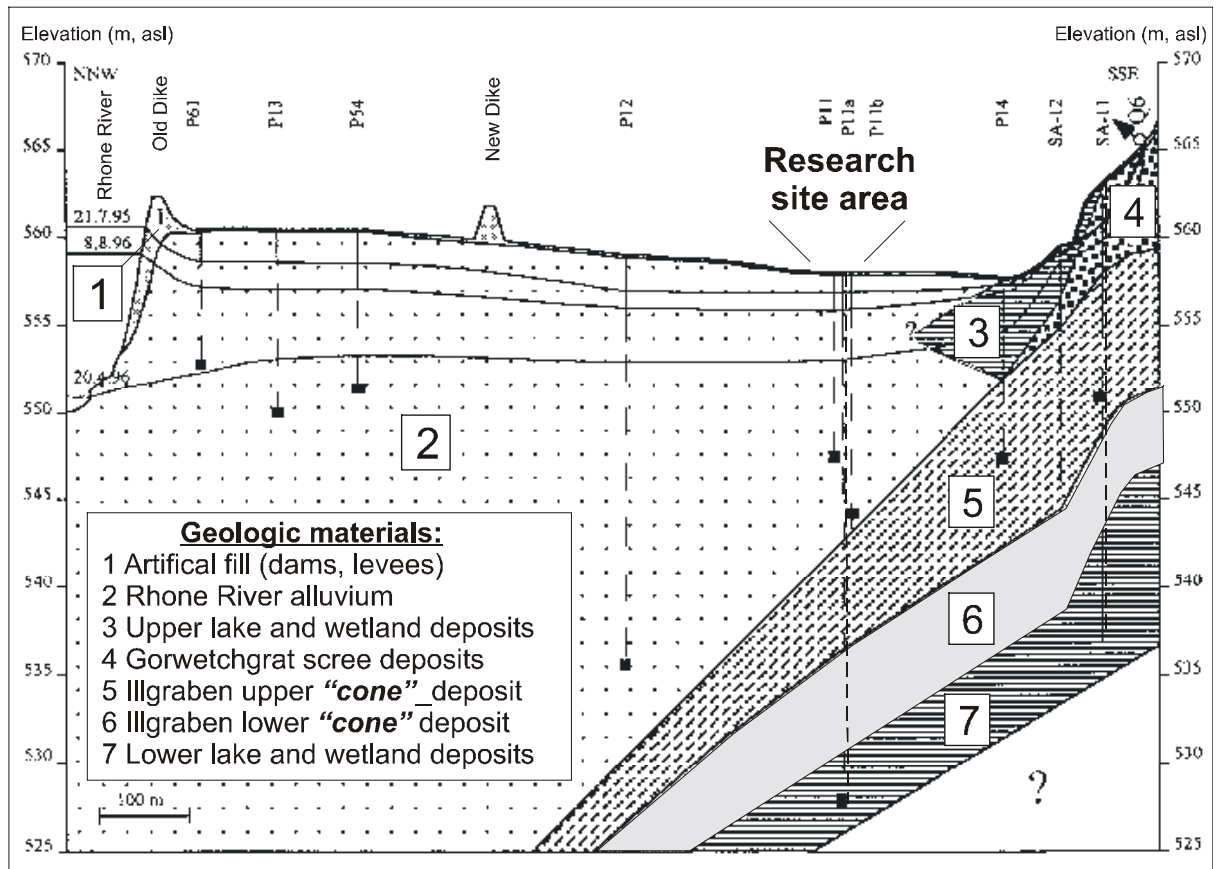


Figure 3-3 Bois de Finges (BdF) geologic setting cross section.

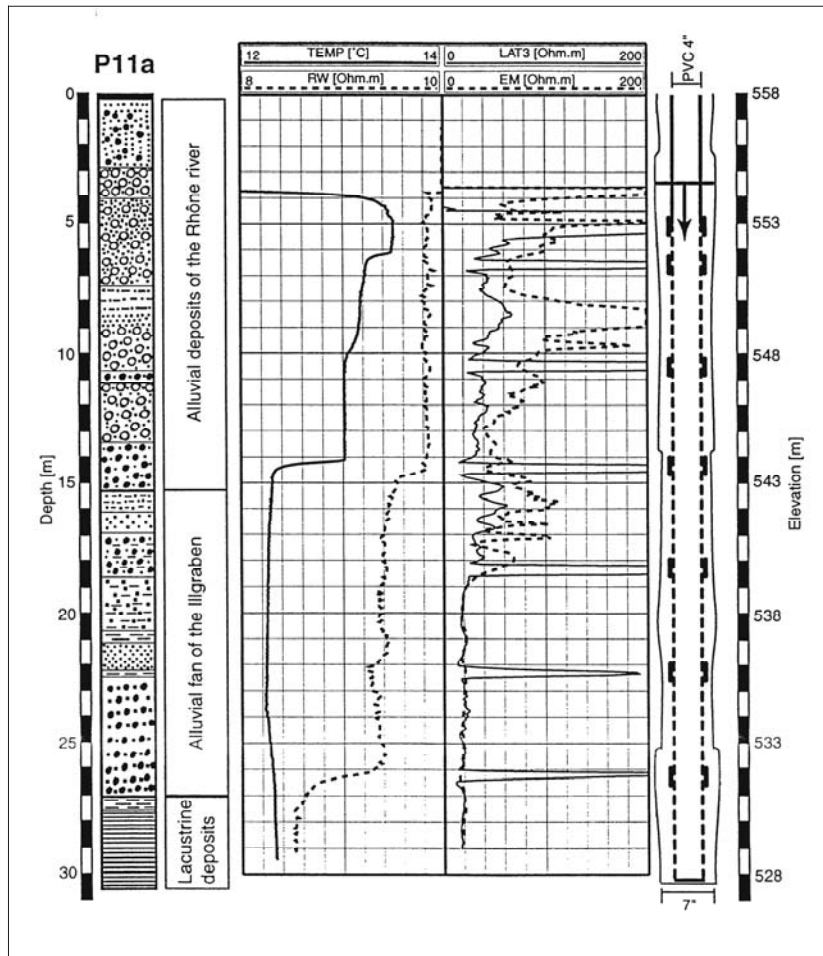


Figure 3-4 Representative geologic and geophysical logs (Well 11A).

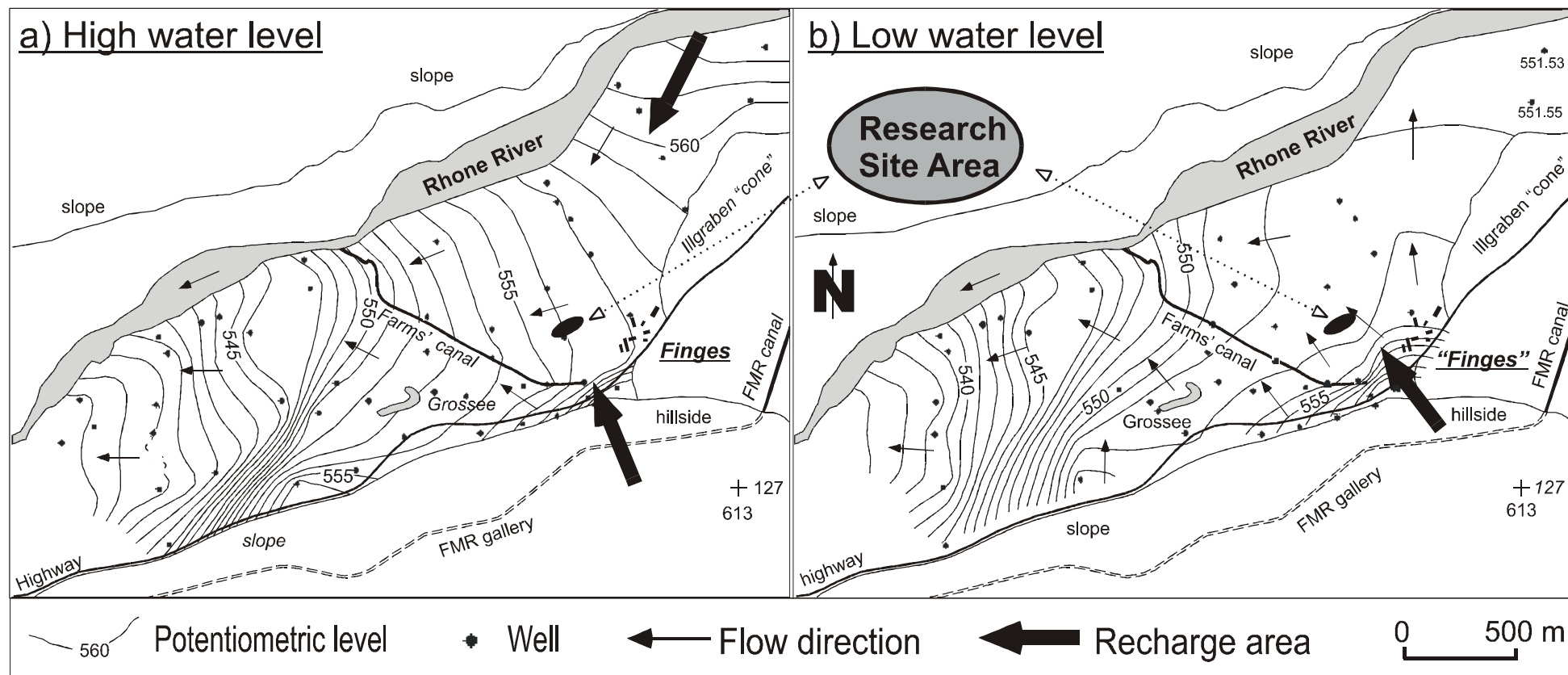


Figure 3-5 Site regional hydraulic gradients

- a) high water level
- b) low water level.

Hydraulic conductivity

Results of drilling and borehole logging geophysics done at the site indicated that there was a sequence of about 15 m of sand, gravel and silt layers throughout the area, consistent with the Rhone River environment. Interpretation of detailed SP and resistivity logs done immediately after drilling in the new boreholes suggested that a 7 m zone from about 5 to 12 m had the highest likelihood of transport. It was in this zone that the migration testing was carried out during the Series 1 and attempted at the beginning of the Series 2 tests. Borehole lateral migration fluid logging carried out in the 11A borehole 6 months after completion of the tracer work showed potential for more discrete widths between 5 and 7 and 11 and 12 m. Sand and gravel total porosity values range in the literature from about 25 to 50 percent and are believed to be in this range for the saturated thickness in the site and surrounding vicinity.

Local variability in hydrodynamic properties of the alluvial deposits have been investigated by pump testing in the 4 new wells at the site (M. Schürch, 2000). The hydraulic conductivity ranged between  $3e^{-3}$  and  $8e^{-3} \text{ m s}^{-1}$  (Dupuit, Thiem-Dupuit methods). These values are based on the assumption that the entire saturated thickness of about 12 m contributed equally to the observed water level drawdown and recovery responses. Our assessment of the alluvial wells monitored in the entire BdF suggests that they generally respond to pumping in a similar manner as at the P11 site.

Local hydraulic gradient during testing

Table 3-1 summarizes the hydraulic gradient conditions that were present during the two test series at three different water level stages. Ranges in hydraulic gradient from the injection to the monitoring wells varied from 1 to 2‰ and 2 to 5‰ during the low and high water level conditions, respectively.

| Test Series | Test  | Water level condition | Injection |                            |                    |                       | Monitor |                            |                    |                       |                        | Gradient |
|-------------|-------|-----------------------|-----------|----------------------------|--------------------|-----------------------|---------|----------------------------|--------------------|-----------------------|------------------------|----------|
|             |       |                       | Well      | Ref. Point Elev'n (m, asl) | Depth to water (m) | Water Elev'n (m, asl) | Well    | Ref. Point Elev'n (m, asl) | Depth to water (m) | Water Elev'n (m, asl) | Injection distance (m) |          |
| 1           | 1     | Medium Nov '97        | P11b      | 558.61                     | 4.59               | 554.02                | P11     | 559.17                     | 5.21               | 553.96                | 20                     | 0.0030   |
|             |       |                       |           |                            |                    |                       | P11a    | 557.94                     | 3.96               | 553.98                | 10                     | 0.0040   |
|             |       |                       |           |                            |                    |                       | P11c    | 558.93                     | 4.94               | 553.99                | 11                     | 0.0027   |
|             |       |                       |           |                            |                    |                       | P11d    | 558.57                     | 4.59               | 553.98                | 22                     | 0.0018   |
|             | 2     | Low Mar '98           | P11b      | 558.61                     | 5.89               | 552.72                | P11     | 559.17                     | 6.49               | 552.69                | 20                     | 0.0018   |
|             |       |                       |           |                            |                    |                       | P11a    | 557.94                     | 5.24               | 552.70                | 10                     | 0.0020   |
|             |       |                       |           |                            |                    |                       | P11c    | 558.93                     | 6.22               | 552.71                | 11                     | 0.0009   |
|             |       |                       |           |                            |                    |                       | P11d    | 558.57                     | 5.87               | 552.70                | 22                     | 0.0009   |
|             | 3     | High Aug '98          | P11b      | 558.61                     | 3.35               | 555.27                | P11     | 559.17                     | 4.00               | 555.17                | 20                     | 0.0048   |
|             |       |                       |           |                            |                    |                       | P11a    | 557.94                     | 2.72               | 555.22                | 10                     | 0.0045   |
|             |       |                       |           |                            |                    |                       | P11c    | 558.93                     | 3.72               | 555.21                | 11                     | 0.0050   |
|             |       |                       |           |                            |                    |                       | P11d    | 558.57                     | 3.37               | 555.20                | 22                     | 0.0030   |
| 2           | 4,5,6 | High Aug '98          | P11a      | 557.94                     | 2.72               | 555.22                | P11     | 559.17                     | 4.00               | 555.17                | 10                     | 0.0050   |
|             |       |                       |           |                            |                    |                       | P11b    | 558.61                     | 3.35               | 555.27                | 10                     | (0.004)  |
|             |       |                       |           |                            |                    |                       | P11c    | 558.93                     | 3.72               | 555.21                | 10                     | 0.0010   |
|             |       |                       |           |                            |                    |                       | P11d    | 558.57                     | 3.37               | 555.20                | 20                     | 0.0010   |

**Table 3-1 Water level conditions and hydraulic gradients during testing.**

**3.2.3 Geochemical setting**

Groundwater of the river gravel deposits is characterized by the Ca, Mg-SO<sub>4</sub>, and HCO<sub>3</sub> geochemical type with average total dissolved ions (TDI) of about 1850 mg L<sup>-1</sup>. An increase in ground water level of about 2.5 m at the end of April decreases the mineralization, as well as the concentration of the dominant ions, such as calcium representing 65% of the cations and sulfate representing 85% of the anions. Furthermore, ground water measurements show a seasonal temperature cycle with temperature varying from a maximum in September (about 15 °C) to a minimum in April (about 12 °C). Table 3-2 contains selected site geochemical data.

All wells of the P11 site are situated completely in the Rhone River gravel except well P11A, which crosses three different stratigraphic horizons: Rhone River gravel (0 to 15 m), sandy fan (15 to 27 m) and lacustrine (27 to 30 m) deposits. The evolution with depth of the chemical composition follows the

varied geological setting. The redox conditions are highly variable: 450 mV and pH 7.4 in the Rhone River gravel, - 50 mV and pH 7.6 in the sandy fan deposits and -100 mV in the lacustrine sediments, where the maximum mineralization was observed. The 3-D hydrogeochemical conditions of the site are described in detail in M. Schürch *et al.* (2000).

| Well & Depth (m) | Date     | T °C | EC µS/cm | pH   | Eh mV | O <sub>2</sub> mg/l | Ca mg/l | Mg mg/l | Na mg/l | K mg/l | Sr mg/l | SO <sub>4</sub> mg/l | HCO <sub>3</sub> mg/l | Cl mg/l | NO <sub>3</sub> mg/l | Bal % |
|------------------|----------|------|----------|------|-------|---------------------|---------|---------|---------|--------|---------|----------------------|-----------------------|---------|----------------------|-------|
| P11              | 12.11.97 | 14.0 | 2120     | 7.54 | 387   | 5.5                 | 346     | 103     | 27      | 4.4    | 5.4     | 1204                 | 165.0                 | 9.8     | 3.7                  | -2.0  |
| P11              | 17.03.98 | 11.8 | 2180     | 7.45 | 428   | 8.0                 | 360     | 104     | 25      | 4.0    | 6.1     | 1216                 | 165.0                 | 11.6    | 6.9                  | -1.3  |
| P11a/8           | 20.11.97 | 12.2 | 2130     | 7.48 | 458   | 6.3                 | 346     | 109     | 27      | 4.5    | 5.3     | 1215                 | 182.2                 | 10.4    | 4.4                  | -2.1  |
| P11a/12          | 20.11.97 | 12.9 | 2140     | 7.42 | 468   | 5.4                 | 348     | 106     | 27      | 4.4    | 5.4     | 1223                 | 181.5                 | 10.4    | 4.7                  | -2.5  |
| P11a/16          | 20.11.97 | 12.7 | 2300     | 7.44 | -41   | 2.6                 | 376     | 106     | 27      | 4.3    | 5.1     | 1287                 | 202.1                 | 8.7     | 2.2                  | -2.8  |
| P11a/19          | 20.11.97 | 13.1 | 2290     | 7.46 | -67   | 3.7                 | 373     | 104     | 26      | 4.3    | 5.5     | 1269                 | 199.8                 | 9.0     | 2.6                  | -2.7  |
| P11a/24          | 20.11.97 | 12.9 | 2300     | 7.49 | -102  | 1.0                 | 376     | 104     | 25      | 4.2    | 5.2     | 1266                 | 201.3                 | 8.8     | 2.5                  | -2.6  |
| P11a/28          | 20.11.97 | 12.9 | 2440     | 7.48 | -102  | 1.3                 | 433     | 106     | 25      | 4.2    | 5.5     | 1428                 | 211.2                 | 7.0     | 1.0                  | -2.8  |
| P11b             | 12.08.98 | 15.0 | 2120     | 7.29 | 460   | 5.6                 | 352     | 105     | 28      | 4.4    | 6.6     | 1181                 | 187.6                 | 9.0     | 3.1                  | -0.9  |

**Table 3-2 Site ground water chemical data.**

P11: sample taken over the whole screen length;

P11a/8: sample taken in a depth of 8 m;

EC: electrical conductivity at 25°C;

Bal: Ionic balance =  $[(\sum \text{cat.} + \sum \text{an.})/(\sum \text{cat.} - \sum \text{an.})] * 100 (\%)$

### 3.3 Methods and Materials

Documenting contaminant transport behavior consisted of determining the rates and recovery of both solute and biocolloid particle tracers based on an injection and various monitoring wells during two series of test conditions. The first series (Tests 1,2,3) was done at the site during each of intermediate, low and high water level periods over transport distances to 22 m with circulation between about 6 and 12 m. Well P11b was used for injection and wells P11, P11a, P11c and P11d were monitored for tracer arrival. The second series (Tests 4,5,6) was carried out during high water level conditions over transport distances to 20 m with reduced circulation depths as described above. Well P11a was used for injection and wells P11, P11c and P11d were monitored for tracer arrival. A summary of the methods and equipment used for this type of tracer testing are in K. Kennedy (2000). Data for all pertinent sample analyses are in Appendix A3.

#### 3.3.1 Injection and sampling - tracers used

The solute and biocolloid particles were mixed together as a single tracer cocktail in a dedicated container at the surface. Injection lasted over a period of 10 to 75 min. The tracer was released into the well under a Venturi effect into an already established internal closed circuit flow circulation regime. Monitoring wells were sampled using the same well-internal circulation. Water was pumped at about 4 L/min using a battery-driven downhole pump (Reich, 12v 18L min<sup>-1</sup>) and tubing from downhole just below, to up hole and back down to just above, the well's expected contributing interval (Figure 3-6).

The injection method had to be modified for the second configuration of tests during high water level because of a preferential path found in well P11a which dominated the horizontal flow from the well. The original plan for circulation at injection well P11a was with the pump at 12 m and the return line at 5 m. This separation was proposed as opposite the active contributing interval based on the geophysical and geological logging. This separation, however, did not result in any tracer return with circulation. Tracer was observed at the surface only when the pump was raised to 5.9 m with the return line left at 5 m. This configuration was used for Tests 4, 5 and 6.

The solute tracer used in all tests was the fluorescent dye, uranine, also commonly referred to as fluoresceine. Field fluorimeters (P.-A. Schnegg and K. Kennedy, 1998) were modified to provide results onsite from a) downhole, b) inline flow during well pumping/circulation, and c) discrete site aliquots/samples. The resolution was from 0.1 to 0.5 ppb with either rapid (each 10 s for 15 h) or longer-term (each 4 min for 15 days) acquisition capacity.

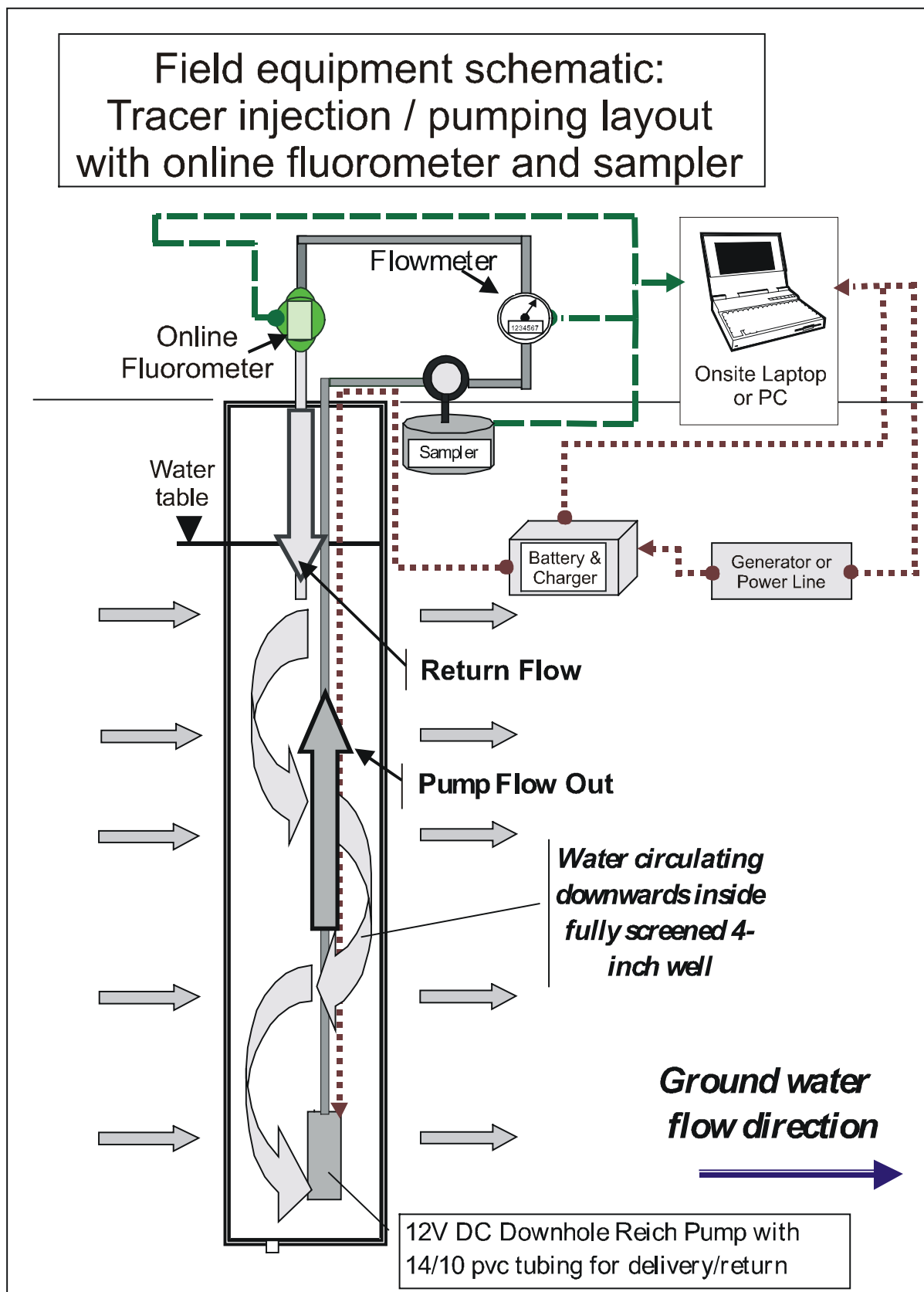


Figure 3-6 Well circulation system for injection and sampling activities.

The biocolloid particle tracers used were three different bacteriophages (H40, MS2, H4). Bacteriophages (B) are non-pathogenic, non-toxic biological tracers. They are virus of a specific host bacteria (HB) ranging in size from about 20 to 350 nm. They are commonly injected with high concentrations ( $>10^{10}$  plate forming units (pfu/ml), can have a large percentage removal in the subsurface, yet are useful due to their low detection level (0.5 pfu/ml) and uniqueness. Samples from the field were analyzed at the Univ. Neuchatel Microbiology Laboratory for each B/HB system using an adaptation of the classical double-layer technique on Petri dishes (P. Rossi and W. Käss, 1998). Concentrations were calculated based on the visible point counts and the sample analysis dilution.

### 3.3.2 Preliminary travel time estimates

The expected travel times to 10 and 22 m was estimated using the given ranges in hydraulic conductivity and an effective porosity ranging from 25 to 75 percent of the observed gravel and sand deposit total porosity value. This was done using the standard formula:

$$\bar{v} = \frac{K}{n_e} \frac{\partial h}{\partial l}$$

where:  $\bar{v}$  is average linear velocity, K is hydraulic conductivity (m/s),  $n_e$  is effective porosity (dimensionless) and  $\partial h / \partial l$  is the site hydraulic gradient (dimensionless).

|                              |           | $n_e$ (25% n)           | $n_e$ (75% n) | $n_e$ (25% n) | $n_e$ (75% n) |
|------------------------------|-----------|-------------------------|---------------|---------------|---------------|
|                              |           | 6.25 %                  | 18.75 %       | 6.25 %        | 18.75 %       |
| $\delta h / \delta l$        | K (m/s)   | $\bar{v}$ (m/h)         |               |               |               |
|                              |           | Arrival<br>10, 22 m (h) |               |               |               |
| <b>0.001</b><br>(low water)  | $3e^{-3}$ | 0.17                    | 0.06          | 58, 127       | 174, 382      |
|                              | $8e^{-3}$ | 0.46                    | 0.15          | 22, 48        | 65, 143       |
| <b>0.005</b><br>(high water) | $3e^{-3}$ | 0.86                    | 0.29          | 12, 25        | 35, 76        |
|                              | $8e^{-3}$ | 2.3                     | 0.77          | 4, 10         | 13, 29        |

**Table 3-3 Preliminary estimates of tracer migration rates.**

Table 3-3 summarizes planning calculations with the predicted average linear velocity from 0.06 to 0.46 and 0.29 to 2.3 m/h under low and high water conditions, respectively. Estimated travel time to the monitoring wells at distances of 10 and 22 m also varied over a considerable range from about 22 to 174 and 48 to 382 h under low water level conditions. Travel times to the 10 and 22 m during high water level conditions with larger gradient ranged from 4 to 35 and 10 to 76 h, respectively.

### 3.3.3 Sampling strategy

A sampling plan was developed for the initial test conducted in the late fall, a period of the year when typically moderate water level and gradient conditions exist. A pertinent sampling strategy is important. Since field studies are event driven and time consuming, biocolloid analyses need to be optimized. They are costly to repeat. In particular, initial tests at a new site, such as this one, typically have a high degree of uncertainty. The sampling strategy would commonly be based on existing site hydraulic parameters. Subsequent tests had the benefit of the early documented responses and lowered uncertainty.

Considering the travel estimates in Table 3-3, tracer detection would occur at 10 m, the nearest downgradient location, at between 4 to 22 h after injection. To compensate for the high uncertainty, a biocolloid sampling frequency on the order of every  $\frac{1}{4}$  hour would be adequate for this set of conditions and would reasonably begin about 2 h after injection. The in-situ, real-time solute tracer response at the monitoring locations facilitated changes in the sample plan after documenting the solute tracer presence. However, once detected, it would be difficult to determine if the biocolloids arrived beforehand. Changes to the sampling start time for biocolloid samples would be in order if the observed injection dilution was rapid. Other similar gravel-dominated deposits have shown such effects when preferential pathways were influencing migration (K. Kennedy, 2000). Therefore, the rate of falloff in concentration at the injection well would be the first indication of a more rapid than expected system response.

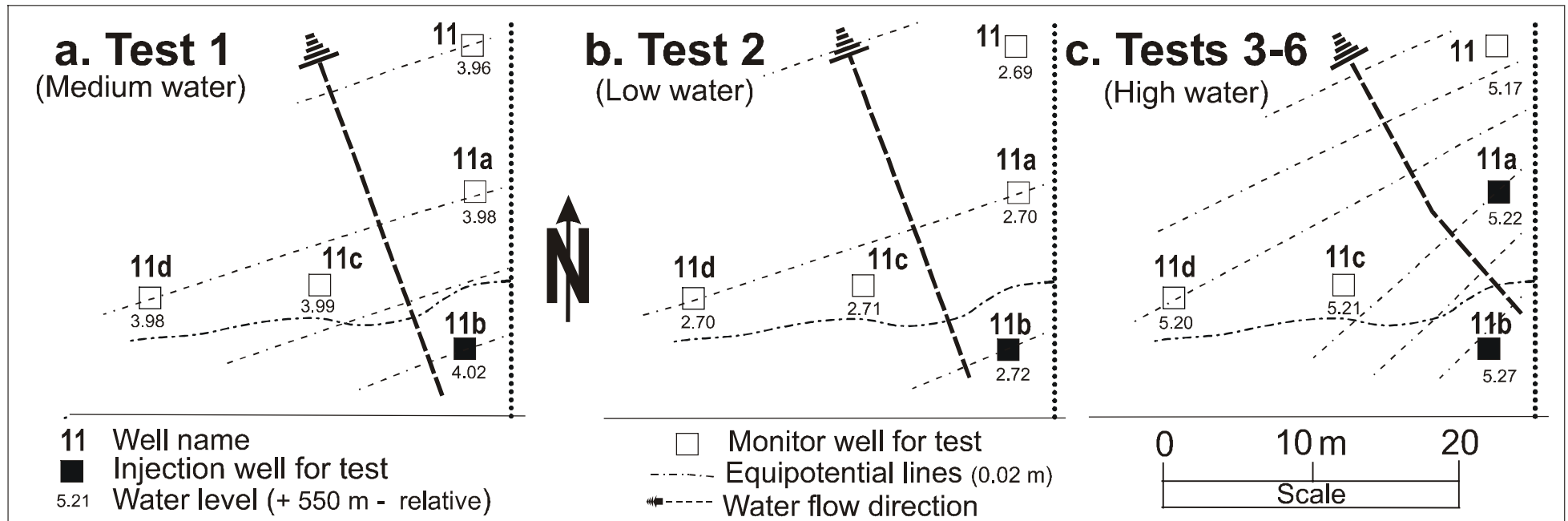


Figure 3-7

Local hydraulic gradient conditions during testing

a) Medium water level conditions (Test 1)

b) Low water level conditions (Test 2)

c) High water level conditions (Tests 3,4,5,6)

Sample planning was left flexible with 1) the start of sampling on the observed injection well falloff dilution and 2) the frequency during the peak and tailing breakthrough range based on the in-situ tracer solute responses available from the site instrumentation.

### 3.3.4 Sampling locations

In which monitoring wells to sample was also in question due to the implied seasonal variability in the hydraulic gradient direction at the site. The more classic approach to evaluating ground water flow focuses on both direction and magnitude of hydraulic gradient. Figure 3-7 shows that the aerial hydraulic gradient varied by a factor of about 3 over the site during the seasonal variations measured. The highest gradient occurred during the high water level period and was about 5%. However, there was little difference in flow direction based on interpretation of traditional potentiometric data from the five well locations. The small change in direction observed on Figure 3-7c suggests an irregularity in the site gradient conditions, but this by itself is not adequate to identify heterogeneity or the differences in expected vs. actual results at the site. Sampling was done in all nearest wells at the outset of each of the tests and appropriately modified after the results of initial detection and experience at the site

### 3.3.5 Comparative analysis

Biocolloid behavior was compared to solute tracer responses in a manner similar to methods described originally in R. Harvey *et al.*, 1989. This included comparing relative accumulation ( $C/C_{\max}$ ), maximum dimensionless concentrations ( $[C/C_0]_{\max}$  or  $[Tr/Tr_0]_{\max}$ ), retardation factor (RF) – the ratio of particle vs. solute maximum times, relative breakthrough (RB) – particle mass recovered compared to solute recovery (normalized to 100 percent using non-reactive solute assumptions), equivalent to mass recovery under dipole conditions, and attenuation (1 minus RB). The 1-D analytical program 'TRACI95' (A. Werner, 1998), was used to best fit the uranine results based on the method of moments, averages and maximum concentration. Mean transit time, mean flow velocity and longitudinal dispersivity and dispersion were obtained based solely on the distance from the injection well to each location and that location's response.

## 3.4 Results and Discussion

Three significant results related to our research at BdF are the direction and rate of migration and the biocolloid particle relative recovery. Each of these results was different than expected based on conventional hydrogeologic approach and published results of biocolloid particle behavior compared to solute responses. Before describing and discussing the specific solute and particle behavior during the two test series, it is useful to understand the tracer injection results and in particular, how they differed according to injection well location.

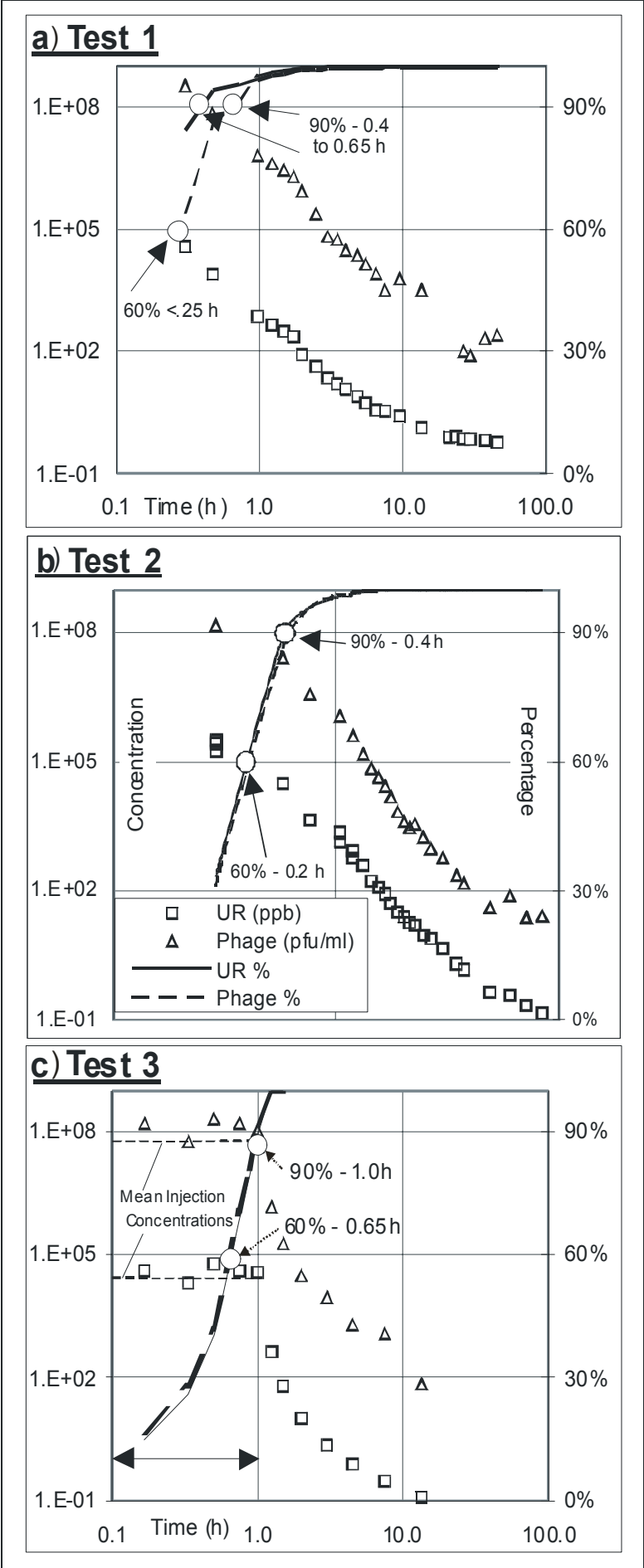
| Test | Injection Time (h) | Uranine Injection Concentration (ppb - $Tr_0$ ) | Particle Injection Concentration (pfu/ml - $C_0$ ) | Injection Falloff          |                       |
|------|--------------------|-------------------------------------------------|----------------------------------------------------|----------------------------|-----------------------|
|      |                    |                                                 |                                                    | Time to 60% $M_0$ (h)      | Time to 90% $M_0$ (h) |
| 1    | 0.2                | $7.e^{+05}$                                     | $7.e^{+09}$                                        | < 0.25                     | 0.4                   |
| 2    | 0.2                | $3.e^{+05}$                                     | $2.e^{+08}$                                        | 0.2                        | 0.4                   |
| 3    | 1.1                | $4e^{+04}$                                      | $1.e^{+08}$                                        | 0.65                       | 1.0                   |
| 4    | 1.1                | $8.e^{+05}$                                     | $2.e^{+09}$                                        | No return – no calculation |                       |
| 5    | 1.1                | $1.e^{+05}$                                     | none used                                          | No effective return        |                       |
| 6    | 1.2                | $2.e^{+05}$                                     |                                                    |                            |                       |

**Table 3-4 Injection conditions and concentration falloff results.**

### 3.4.1 Injection conditions

The injection conditions, record and concentration profile at Well P11b for the first series of three tests is shown in Figure 3-8 and summarized in Table 3-4. Samples taken from the circulation injection system show that from the start of injection, 60 and 90 percent of the total tracer mass was injected within from about 15 to 40 and from about 24 to 60 min, respectively. These contrast

Figure 3-8 Injection record and falloff – Series 1: a) Test 1, b) Test 2, c) Test 3.



with the results from Series 2 tests where there was essentially an injection of the total amount of the tracer into the preferential pathway directly as it was introduced (Figure 3-9). For Series 2, the flow rate laterally into the preferential pathway at well 11A from a depth between about 5 to 6 m exceeded the internal pump circulation rate of 4 L/min.

There was no return due to the high conductivity pathway into which the injection occurred. Therefore, the falloff in injection concentration could not be documented. We assume that there would have been a relatively constant rate of dilution over this period with a step-type function during the longer injection periods of about one hour during the three tests.

### **3.4.2 Detected tracer response locations – Migration direction**

A standard method of predicting where a contaminant will migrate is routinely based the assumption that it will follow the direction of flow determined from a potentiometric surface map of an aquifer. Certainly, we would expect there to be minor differences in the interpretation according to scale being considered and the resolution of both the measuring point elevation and water level measurements. However, in most situations where there is no controlling heterogeneity within the flow domain, this approach is considered acceptable. However, it is important to recognize that the strong heterogeneity within a river alluvium environment can result in uncertainty in predicting the direction of migration at scales smaller than the aquifer's total area. Results from the historic method may not be correct and if so, the reliability of a well head protection program based on this approach is questionable.

Figure 3-10 illustrates where tracers were detected for the two sets of test series events. Tracers injected at well 11b were found only at wells 11c and 11d. Tracers were detected at only well 11c for injection at well 11a. During both test series the classic hydraulic gradient direction does not correlate well with the direction between the injection and detection locations. Based on local well elevations over the 25 m distances, the hydraulic gradient at this local scale was about 20 to 25 degrees west of north during tests 1 and 2. Tracers were detected in locations about 45 to 60 degrees further west, which corresponds to the regional flow direction of the aquifer during high water level conditions. During tests 3 through 6, the high water level conditions the gradient depicts the flow about 40 degrees west of north. The tracers were detected however about 45 degrees further to the west during test 3, and almost perpendicular to the local flow gradient during tests 4 to 6 with the injection done at well 11A.

The gradient flattening shown at the site scale in Figure 3-7a and the possible change in direction shown in Figure 3-7c may be evidence of an irregularity or heterogeneity in the subsurface conditions. Typically such changes or differences would not manifest themselves over such short distances when highly permeable conditions such as exist at this site are present.

### **3.4.3 Tracer response timing – Migration rate**

Characterizing migration velocity is conventionally done using a rudimentary analysis of solute tracer migration responses commonly dividing the distance to the observation well divided by the time to the tracer arrived. However, approaches vary in defining what to use as the 'arrival time'. Frequently referred to in the classical approach to tracer studies are comparison of the arrival time functions at the point of either 50 percent or maximum (peak) concentration of the tracer breakthrough. Studies in closely-spaced fractured settings under low-flow and readily controlled dipole test conditions have adopted a convention of considering and comparing arrival results based on a '5 percent approach'. This method is applied in a closed dipole testing configuration and compares the time at which 5 percent of the total material injected arrives at the extraction point. Comparing tracer percentage recovery or peaks determined from their breakthrough curves is of interest particularly when comparing relative sorption and reactivity within the testing environment.

Some workers have also argued, as we do, that related to contaminant migration, particularly as it relates to travel times used for defining well head protection zones, a more pertinent consideration of arrival time is the first arrival (referred to hereafter as first detection). This is especially true for biocolloids that are typically subject to extremely high percentage removal from the flow system. However, despite this large removal, their presence at a low concentration is important. Their occurrence at exceptionally low levels can represent a significant health risk for public drinking water supplies. We also recognize that using a first arrival based on the contaminants' or surrogates' first

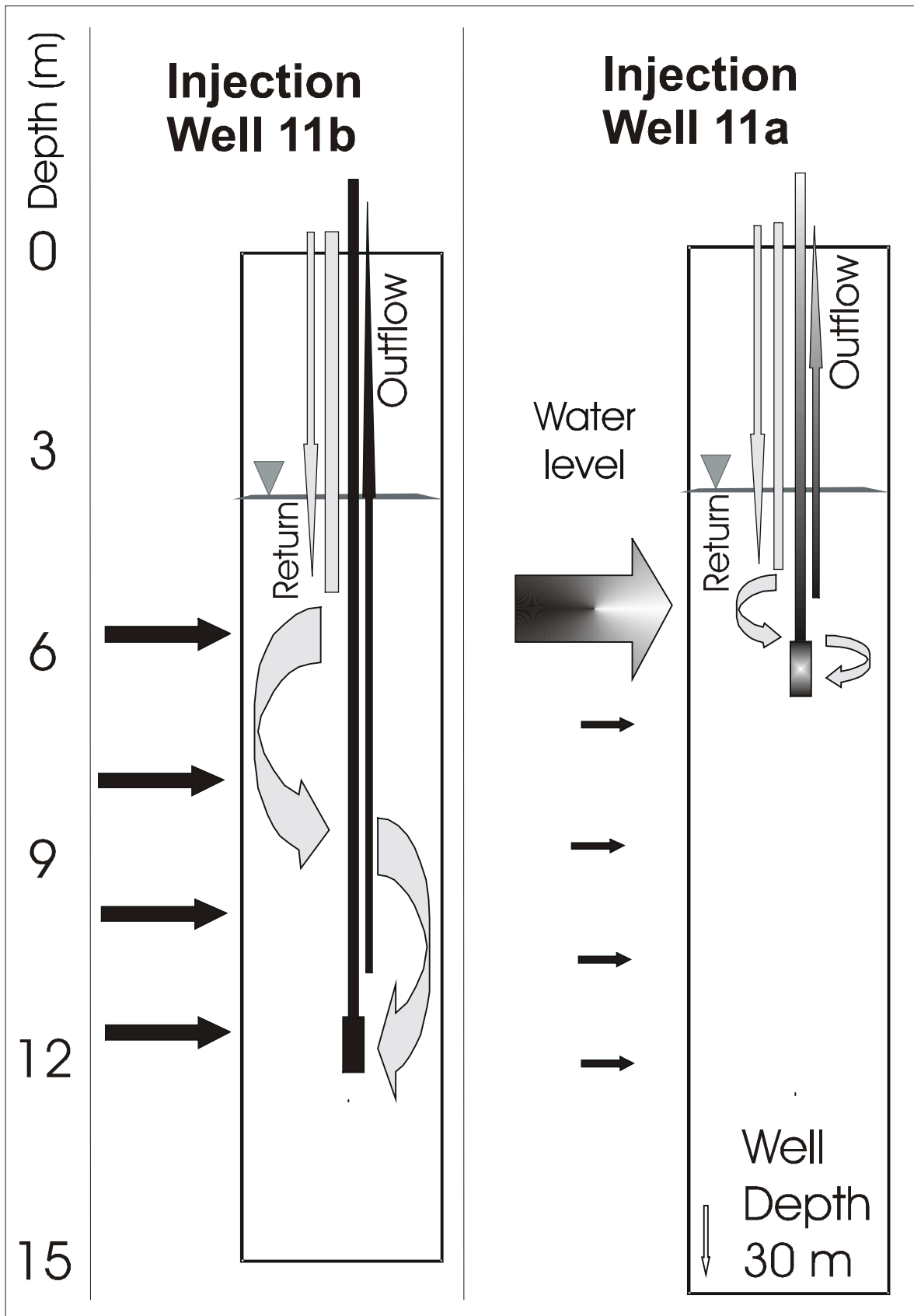


Figure 3-9 Injection well lateral flow dynamics.

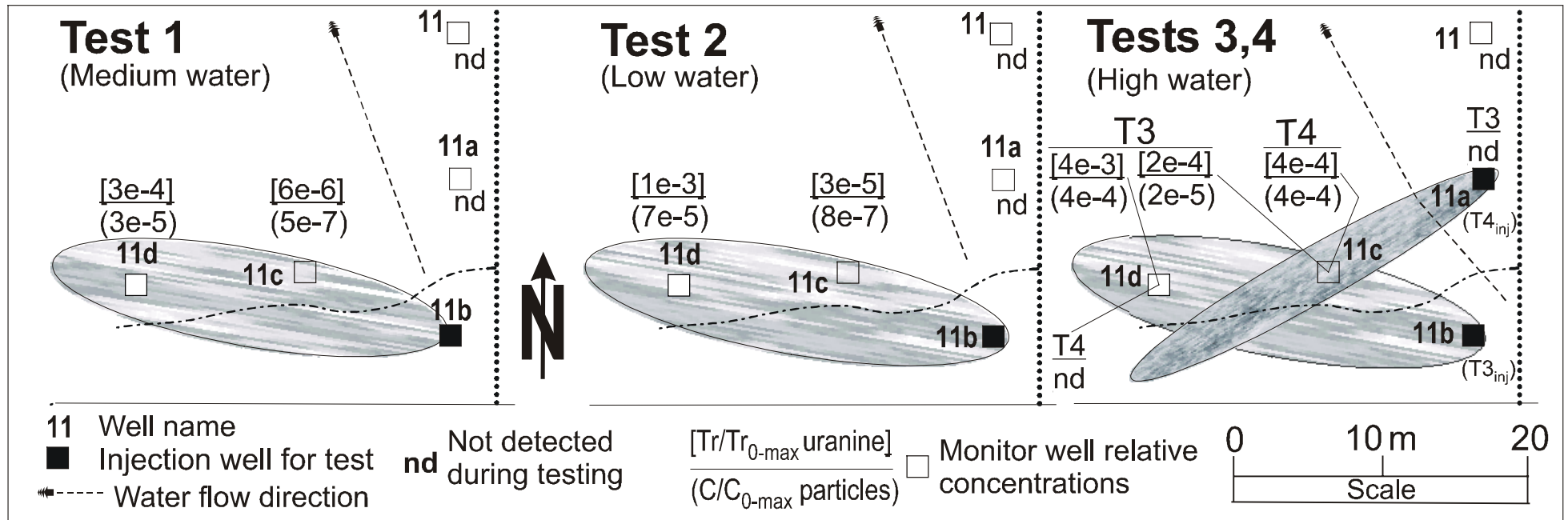


Figure 3-10 Tracer response locations.

detection can be biased depending on the precision of the analysis technique including both the method and instrumentation being used. So the question of whether one particular type of tracer, the solute or the particle, 'arrived' first can rarely be answered. The analysis detection limits are commonly not equal.

For the results, we present the migration rates expressed in terms of both arrival based on first detection and peak concentration. The peak concentrations can also arguably be compared with the preliminary estimates (also in Table 3-5) made using traditional calculations from values of hydraulic conductivity, effective porosity and hydraulic gradient. We suggest that the appropriate migration rate for contaminant studies within the context of water supply protection is first detection related to biocolloids and conservatively, also for solutes. The responses of the tracers during the two series of tests are shown on Figures 3-11 and 3-12, respectively. Concentrations have been normalized to their maximum observed concentrations on these plots to more easily compare the times to the peak abundance among different tracers during each test. Migration rates are graphically represented on Figure 3-13 and summarized in Table 3-5.

| Test                                    | Estimate <sup>(1)</sup> |          |               | Solute         |               |                                        |                |               |                                        | Biocolloids        |               |                                        |                |               |                                        |                   |
|-----------------------------------------|-------------------------|----------|---------------|----------------|---------------|----------------------------------------|----------------|---------------|----------------------------------------|--------------------|---------------|----------------------------------------|----------------|---------------|----------------------------------------|-------------------|
|                                         |                         |          |               | Detection      |               |                                        | Peak           |               |                                        | Detection          |               |                                        | Pseudo Peak    |               |                                        |                   |
|                                         | Arrival<br>(h)          | Gradient | Rate<br>(m/d) | Arrival<br>(h) | Rate<br>(m/d) | Rate/ <i>i</i> <sup>(2)</sup><br>(m/d) | Arrival<br>(h) | Rate<br>(m/d) | Rate/ <i>i</i> <sup>(2)</sup><br>(m/d) | Arrival<br>(h)     | Rate<br>(m/d) | Rate/ <i>i</i> <sup>(2)</sup><br>(m/d) | Arrival<br>(h) | Rate<br>(m/d) | Rate/ <i>i</i> <sup>(2)</sup><br>(m/d) | RF <sup>(3)</sup> |
| <b><u>To 11m (Wells 11b to 11c)</u></b> |                         |          |               |                |               |                                        |                |               |                                        |                    |               |                                        |                |               |                                        |                   |
| 1                                       | 9                       | 0.0027   | 30            | 3.7            | 71            | 3.e <sup>+04</sup>                     | 13.5           | 20            | 7.e <sup>+03</sup>                     | 2.0                | 132           | 5.e <sup>+04</sup>                     | 4.0            | 66            | 2.e <sup>+04</sup>                     | 0.3               |
| 2                                       | 26                      | 0.0009   | 10            | 4.3            | 61            | 7.e <sup>+04</sup>                     | 24.1           | 11            | 1.e <sup>+04</sup>                     | 3.5                | 77            | 8.e <sup>+04</sup>                     | 6.3            | 42            | 5.e <sup>+04</sup>                     | 0.26              |
| 3                                       | 5                       | 0.0050   | 55            | 2.1            | 126           | 3.e <sup>+04</sup>                     | 6.8            | 39            | 8.e <sup>+03</sup>                     | 1.0                | 264           | 5.e <sup>+04</sup>                     | 3.8            | 69            | 1.e <sup>+04</sup>                     | 0.56              |
| <b><u>To 22m (Wells 11b to 11d)</u></b> |                         |          |               |                |               |                                        |                |               |                                        |                    |               |                                        |                |               |                                        |                   |
| 1                                       | 26                      | 0.0018   | 20            | 2.5            | 211           | 1.e <sup>+05</sup>                     | 6.5            | 81            | 4.e <sup>+04</sup>                     | 2.0                | 264           | 1.e <sup>+05</sup>                     | 4.2            | 126           | 7.e <sup>+04</sup>                     | 0.65              |
| 2                                       | 53                      | 0.0009   | 10            | 4.1            | 129           | 1.e <sup>+05</sup>                     | 11.6           | 46            | 5.e <sup>+04</sup>                     | 3.0                | 176           | 2.e <sup>+05</sup>                     | 8.7            | 61            | 7.e <sup>+04</sup>                     | 0.75              |
| 3                                       | 16                      | 0.0030   | 33            | 1.7            | 311           | 1.e <sup>+05</sup>                     | 4.7            | 112           | 4.e <sup>+04</sup>                     | 1.5                | 352           | 1.e <sup>+05</sup>                     | 3.5            | 151           | 5.e <sup>+04</sup>                     | 0.74              |
| <b><u>To 10m (Wells 11a to 11c)</u></b> |                         |          |               |                |               |                                        |                |               |                                        |                    |               |                                        |                |               |                                        |                   |
| 4                                       | 22                      | 0.001    | 11            | 0.5            | 480           | 5.e <sup>+05</sup>                     | 1.4            | 171           | 2.e <sup>+05</sup>                     | < 0.75             | >320          | 3.e <sup>+05</sup>                     | 1.7            | 141           | 1.e <sup>+05</sup>                     | 1.21              |
| 5                                       | 22                      | 0.001    | 11            | 0.6            | 400           | 4.e <sup>+05</sup>                     | 1.7            | 141           | 1.e <sup>+05</sup>                     | no biocolloid used |               |                                        |                |               |                                        |                   |
| 6                                       | 22                      | 0.001    | 11            | 0.6            | 400           | 4.e <sup>+05</sup>                     | 2              | 120           | 1.e <sup>+05</sup>                     |                    |               |                                        |                |               |                                        |                   |

**Table 3-5 Predicted vs. documented migration rates at Bois de Finges.**

**Footnotes:**

1 Estimates based on effective porosity (~ 6 %), K (8e<sup>-3</sup> m s<sup>-1</sup>) and gradient in place during test dates.

2 Gradient-normalized rate: calculated velocity divided by gradient between the injection and each monitoring well.

3 Recovery Factor (RF) is a measure of the difference between the peak time to the maximum concentration of the biocolloid compared to the reference peak time for the solute tracer assumed to be conservative in the testing environment as described by R. Harvey *et al.* (1989).

### 3.4.4 Migration rates - Test series 1 (Tests 1,2,3)

Solute detection during the three tests suggest transport rates of from 60 to 125 and 130 to 310 m/d at wells 11c and 11d, respectively (Table 5). Gradient change was generally consistent with increased rates. At well 11c, the lowest gradient during Test 2 had a gradient-normalized transport rate about 2.5 times higher than the two other tests. Gradient-normalized transport rates to well 11d were within about 35 percent.

Transport rates based on solute peaks at Well 11c (11 m) (~11 to 40 m/d) were similar in all tests to those predicted using the traditional transport rate estimates (~10 to 55 m/d). However, rates to Well 11d at 22 m were about 4 to 5 times greater (~50 to 110 m/d) than predicted (10 to 30 m/d). Solutes, based on first detection and peak times migrated about 2 to 3 and about 3 to 4 times, respectively, faster to 22m than to 11m. This strongly displays the dominance of a channeled environment with a preferential pathway from Well 11b to Well 11d lateral to the plotted hydraulic gradient shown in Figure 3-7.

Biocolloid detection resulted in calculated transport rates of from 80 to 260 and from 175 to 350 m/d at wells 11c and 11d, respectfully. Gradient change was generally consistent with increased rates. At well 11c, the lowest gradient during Test 2 had a gradient-normalized transport rate about 1.7 time higher than the two other tests. Gradient-normalized transport rates to well 11d ranged over about 65 percent showing a wider variability than for the solute.

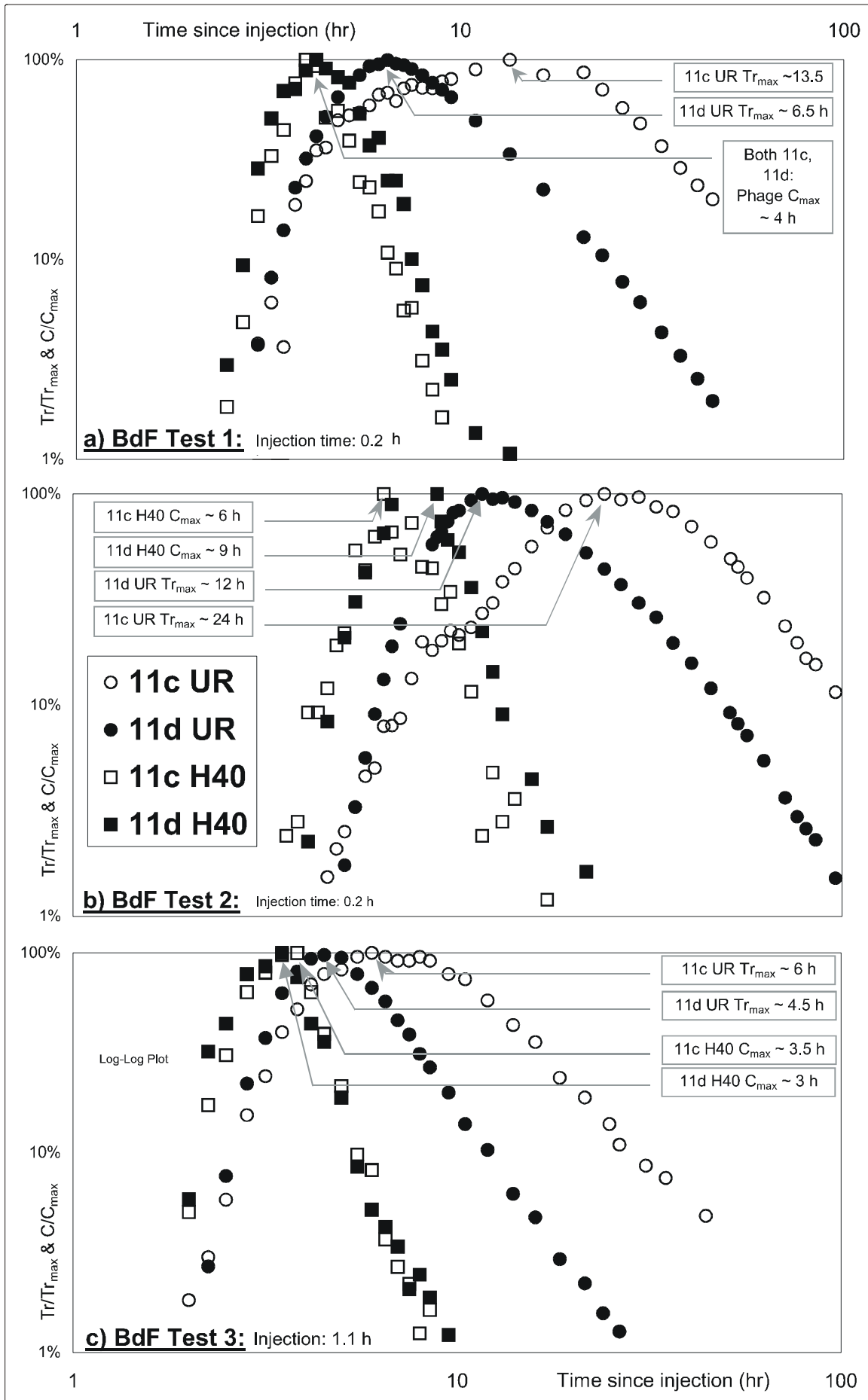


Figure 3-11 Tracer magnitude response - Series 1: a) Test 1, b) Test 2, c) Test 3.

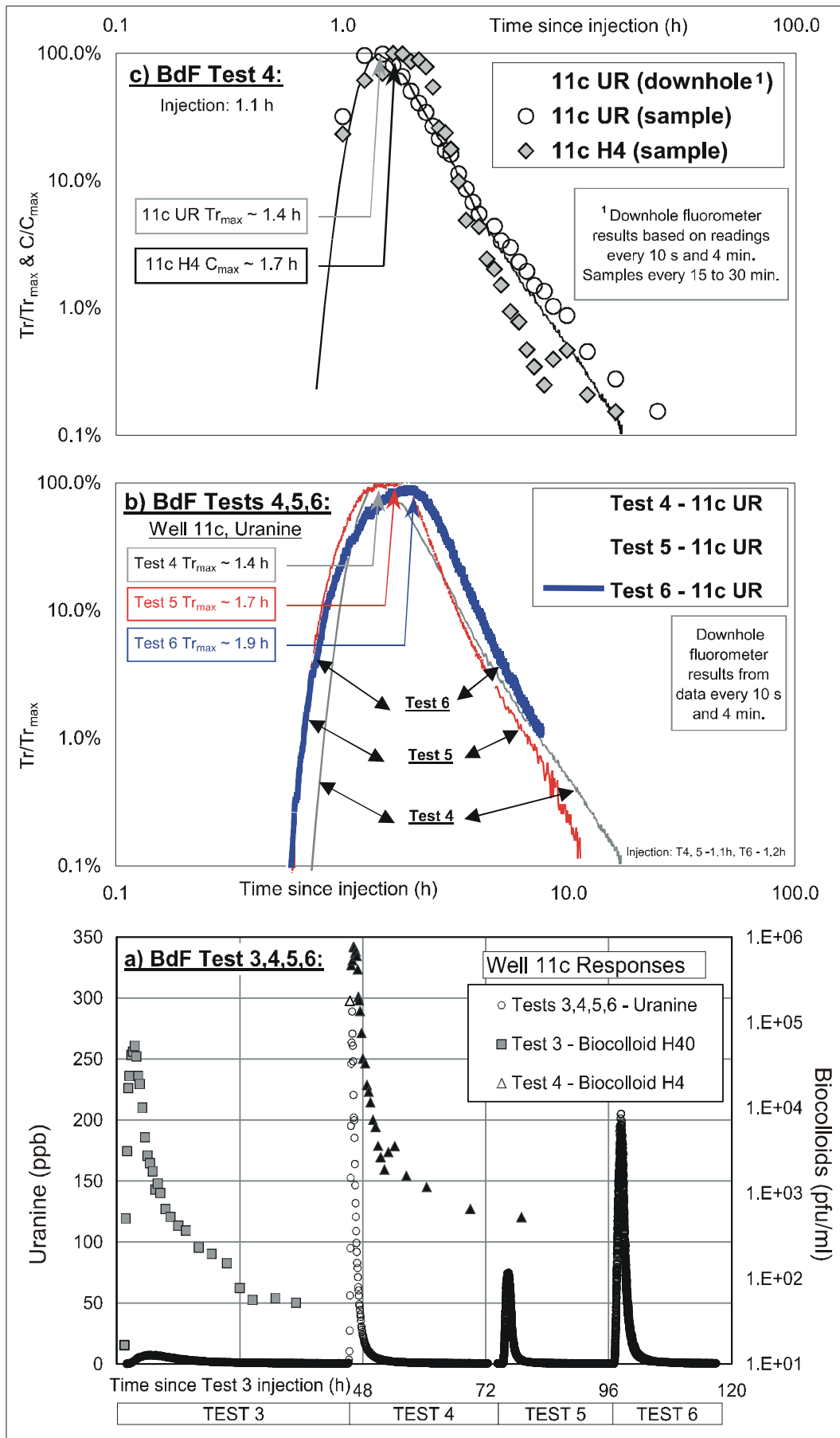


Figure 3-12 Tracer magnitude response at well 11C under high water level conditions:

- a) Uranine and biocolloids in Test 4
- b) Comparative uranine for Tests 4,5,6
- c) Sequential uranine in Tests 3, 4, 5, 6.

Biocolloid maximum concentrations occurred consistently earlier than those of the solutes. They were from 80 to 400 and 30 to 60 percent earlier at wells 11c and 11d, respectively. However, this response is not surprising. There is a large percentage removal of biocolloids from the flow system in comparison to a conservative solute. The removal response reduces the number of particles available for transport and with distance there is an effective source reduction just as if there were retardation. The removal mechanisms are complex resulting from different particle, geochemistry and subsurface environment interactions. The biocolloid removal action is believed to be more responsible for the timing of the breakthrough curve peak than the response of the biocolloid migration rate within the flow system. As a result, we refer to the biocolloid maximum as a 'pseudo-peak' whose character is more a function of particle removal than on biocolloid transport rate in the subsurface. Therefore, comparison of the time to maximum concentrations of the biocolloid particle (particle pseudo-peaks) with that of the solute tracers was considered by R. Harvey *et al.*, (1989) as a retardation factor (R.F.) whose values are also shown on Table 3-5. RF values in these tests were from 0.25 to 0.56 and 0.65 to 0.75 at wells 11c and 11d, respectively.

Biocolloids were consistently detected earlier than the solutes. Detection occurred from 25 to 110 and 15 to 40 percent earlier at Wells 11c and 11d, respectively. At well 11c, the higher the gradient, the greater the difference in detection between the biocolloids and the solutes. At well 11d, the opposite occurred with lower gradients resulting in higher differentiation between the two tracer types. Some of the earlier detection difference is related to the lower detection limit for the biocolloid analysis compared to the fluorescent tracer. In fact, the colloid detection limit is at a minimum 1 particle in 2 ml, compared to 0.5 ppb for the fluorometers. Therefore it is not surprising to be able to detect the colloid earlier than the solute since the fluorometric analysis method can be 8 to 11 orders of magnitude less sensitive at a molecular level.

#### **3.4.5 Migration rate - Test series 2 (Tests 4,5,6)**

Solute detection during the three tests occurred only at well 11c from injection at well 11a. Calculated transport rates were from 400 to 480 m/d over the distance of 10 m. We had expected to see a response at well 11d, but it did not occur. There was no notable gradient change over the week that the three tests were performed. Gradient-normalized transport rates were 3 to 4 times higher than the highest observed during the Series 1 tests. Transport rates based on solute peaks at Well 11c (~120 to 170 m/d) were 10 to 15 times greater than those predicted using traditional transport rate estimates (~10 m/d). However, site geophysical characterization activities had not included testing under high water level conditions. The preferential pathways suggested as playing a dominant role during this series of tests are included in the calculation of the hydraulic conductivity during pumping tests under high water level conditions. The hydraulic conductivity was calculated by dividing the transmissivity by 12 m, representing the total saturated aquifer thickness. Indeed, the gravel between 5 and 6 m depth at well 11a has a much higher influence on the transport than the rest of the saturated 11 m. This masking effect which hides the presence of the preferential pathway influence during pump testing accounts for the large order of magnitude difference in the predicted compared to our documented transport rates.

The first biocolloid detection at well 11C in Test 4 was not documented well enough to compare with that of the solute. The first biocolloid detection in the initial sample taken was at 60 minutes, about 25 minutes after the solute had appeared and it had a concentration of  $1.8 \times 10^8$  pfu. This first sample value shows that the sample was taken later than the actual biocolloid arrival. A first detection time of no later than 45 minutes was extrapolated from the concentration increase in the samples. Therefore, a minimum transport rate of 320 m/d is calculated, lower than that for the solute. The biocolloid pseudo-peak time occurred after the solute peak as shown by an RF value of 1.21 for this test (Table 3-5). This is the first time in our testing of gravel aquifer environments at three different sites in Switzerland that this has occurred (K. Kennedy, 2000). This supports, as discussed in the next section, the suggestion that, at least in this pathway that was tested, there was only limited removal of the biocolloid from the transport zone.

#### **3.4.6 Tracer concentration response – magnitude and relative recovery**

The previous section dealt with rates of transport based on the time of the tracers' first detection and maximum concentrations. This section deals with both absolute and relative amounts or mass of tracer observed and compares these amounts with respect to tracer type at locations observed. Magnitude and relative recovery assists evaluation of transport mechanisms, degree of heterogeneity, nature and extent of biocolloid reduction and relative efficacy of transport. When natural gradient

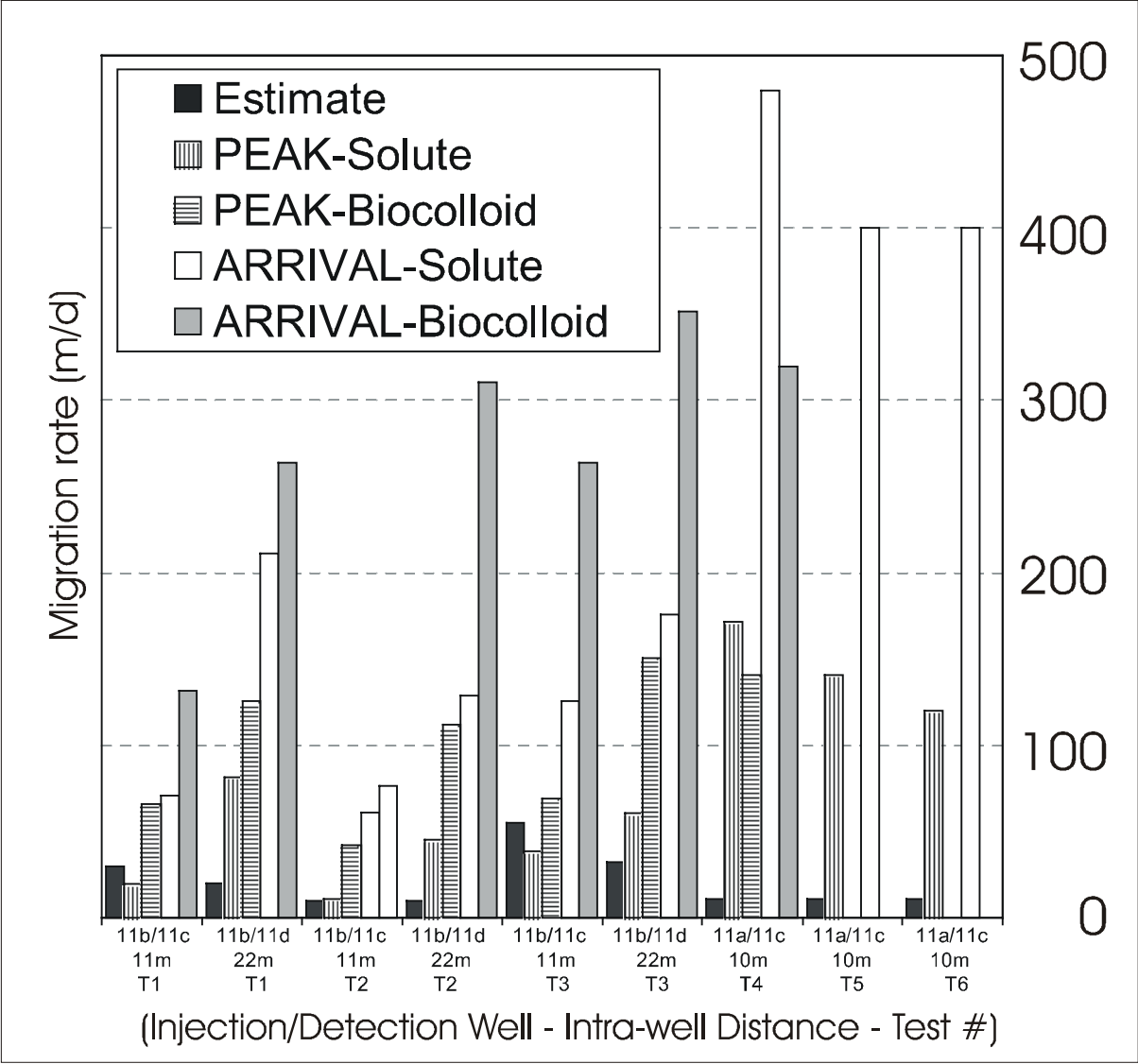


Figure 3-13 Migration Rates from different calculation methods.

conditions are used for testing, there is no artificial means used to capture all of the injected tracer mass. In these cases, one analysis process compares first relative magnitude and relative recovery by location in the flow field and second, relative recovery of biocolloid vs. solute. The solute response is assumed conservative, *i.e.* no retardation or removal in the testing pathway. Further, the flow system transport mechanism is evaluated by calculating values of dispersivity for the conservative solute, even when determined from a basic 1 D model.

Results for the six tests are summarized in Tables 3-6 and 3-7. Figures 3-11 and 3-12 illustrate the relative time responses of the two tracers. Absolute concentration histories and the relative recovery of both tracer types are shown on Figures 3-14 and 3-15 for Series 1 and 2 tests, respectively.

| WELLS |            |              |           | URANINE RESPONSE        |                      |                                      | BIOCOLLOID RESPONSE                         |                           |                      |                                    | RELATIVE RESPONSES |        |           |
|-------|------------|--------------|-----------|-------------------------|----------------------|--------------------------------------|---------------------------------------------|---------------------------|----------------------|------------------------------------|--------------------|--------|-----------|
| Test  | Inj'n Well | Monitor Well | Apart (m) | Tr <sub>max</sub> (ppb) | T <sub>max</sub> (h) | (Tr/Tr <sub>0</sub> ) <sub>max</sub> | Phage                                       | C <sub>max</sub> (pfu/ml) | T <sub>max</sub> (h) | (C/C <sub>0</sub> ) <sub>max</sub> | RF                 | RB (%) | Att'n (%) |
| 1     | 11B        | 11C          | 11        | 4                       | 13.5                 | 6e <sup>-06</sup>                    | H40                                         | 3600                      | 4                    | 5e <sup>-07</sup>                  | 0.3                | 0.6    | 99.4      |
|       |            | 11D          | 22        | 196                     | 6.5                  | 3e <sup>-04</sup>                    |                                             | 229000                    | 4                    | 3e <sup>-05</sup>                  | 0.7                | 3.9    | 96.1      |
| 2     | 11B        | 11C          | 11        | 9                       | 24.1                 | 3e <sup>-05</sup>                    | H40                                         | 125                       | 6                    | 8e <sup>-07</sup>                  | 0.3                | 0.2    | 99.8      |
|       |            |              |           |                         |                      |                                      | MS2                                         | 8                         | 6                    | 1e <sup>-07</sup>                  | 0.3                | 0.03   | 99.97     |
|       |            |              |           |                         |                      |                                      | H40                                         | 7750                      | 9                    | 5e <sup>-05</sup>                  | 0.7                | 1.3    | 98.7      |
|       |            | 11D          | 22        | 274                     | 11.6                 | 1e <sup>-03</sup>                    | MS2                                         | 420                       | 9                    | 6e <sup>-06</sup>                  | 0.8                | 0.13   | 99.87     |
| 3     | 11B        | 11C          | 11        | 8                       | 6.8                  | 2e <sup>-04</sup>                    | H40                                         | 2200                      | 4                    | 2e <sup>-05</sup>                  | 0.6                | 1.4    | 98.6      |
|       |            | 11D          | 22        | 150                     | 4.7                  | 4e <sup>-03</sup>                    |                                             | 53000                     | 3.5                  | 4e <sup>-04</sup>                  | 0.7                | 4.4    | 95.6      |
| 4     | 11A        | 11C          | 10        | 289                     | 1.4                  | 4e <sup>-04</sup>                    | H4                                          | 765000                    | 1.7                  | 4e <sup>-04</sup>                  | 1.2                | >72    | <28       |
| 5     | 11A        | 11C          | 10        | 75                      | 1.7                  | 6e <sup>-04</sup>                    | No biocolloid injected during these 2 tests |                           |                      |                                    |                    |        |           |
| 6     | 11A        | 11C          | 10        | 205                     | 2.0                  | 1e <sup>-03</sup>                    |                                             |                           |                      |                                    |                    |        |           |

**Table 3-6 Tracer concentration response characteristics at Bois de Finges.**

|          |                | Well 11c           |                        | Well 11d                                    |                        | Ratio 11c/11d |
|----------|----------------|--------------------|------------------------|---------------------------------------------|------------------------|---------------|
| Test     | Injected grams | RR <sub>N-UR</sub> | RR <sub>N-UR</sub> /gm | RR <sub>N-UR</sub>                          | RR <sub>N-UR</sub> /gm | %             |
| Series 1 |                |                    |                        |                                             |                        |               |
| 1        | 63             | 110                | 2                      | 1866                                        | 30                     | 6%            |
| 2        | 71             | 362                | 5                      | 5173                                        | 73                     | 7%            |
| 3        | 65             | 85                 | 1                      | 645                                         | 10                     | 13%           |
| Series 2 |                |                    |                        |                                             |                        |               |
| 4        | 62             | 358                | 6                      | No recovery of uranine detected at Well 11d |                        |               |
| 5        | 25             | 114                | 5                      |                                             |                        |               |
| 6        | 25             | 345                | 14                     |                                             |                        |               |

**Table 3-7 Uranine comparative relative recovery.**

### 3.4.7 Tracer magnitude

Magnitude can be compared using the maximum dimensionless concentrations of the injected solution for uranine  $(Tr/Tr_0)_{max}$  and the biocolloids,  $(C/C_0)_{max}$  (Table 3-6). For Series 1 Tests 1,2,3, at wells 11c and 11d,  $(Tr/Tr_0)_{max}$  values ranged from  $6e^{-6}$  to  $2e^{-4}$  and  $3e^{-4}$  to  $4e^{-3}$  and biocolloid  $(C/C_0)_{max}$  values ranged from  $1e^{-7}$  to  $2e^{-5}$  and  $6e^{-6}$  to  $4e^{-4}$ , respectively. Peak dimensionless concentrations for each tracer type were consistently greater at well 11d than well 11c, ~ 30 to 60 fold in tests 1 and 2 and ~ 20 fold in test 3. The uranine peak abundance  $(Tr/Tr_0)_{max}$  occurred from 1.5 to 2 times faster at the more distant well 11d than at well 11c suggesting longitudinal flow along a strong preferential pathway from the injection well to the further of the two wells and lateral dispersion to the closer one. The differences in response in the two wells illustrate the strong heterogeneity in the part of the system tested. For biocolloids, the pseudo-peak abundance  $(C/C_0)_{max}$  was from 0.9 to 1.5 faster at the more distant well compared to well 11c. The pseudo-peaks however, cannot be similarly compared due to variability in the removal effects associated with the biocolloid itself rather than the characteristics of the flow system.

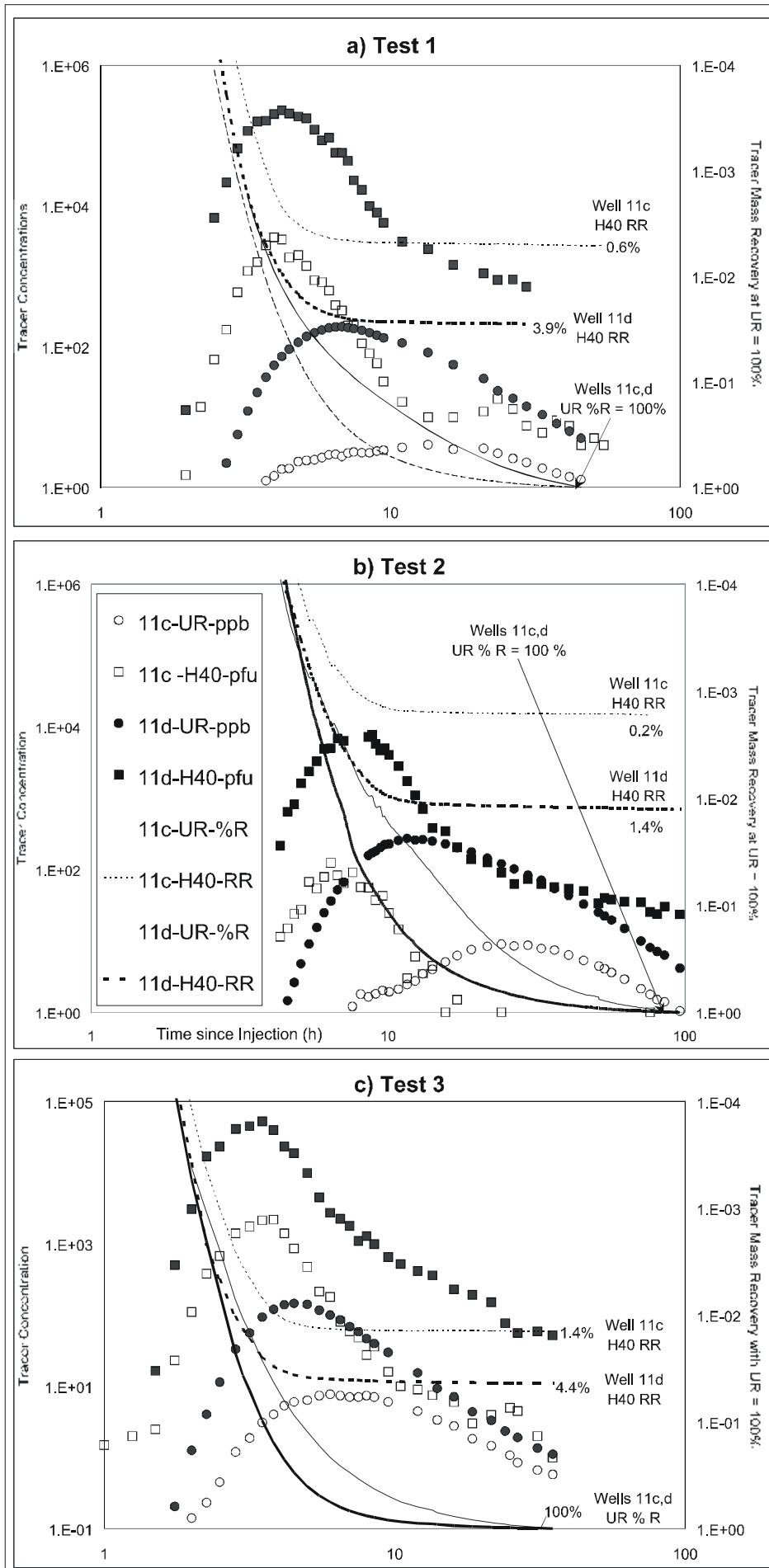


Figure 3-14 Tracer history and recovery - Series 1: a) Test 1, b) Test 2, c) Test 3.

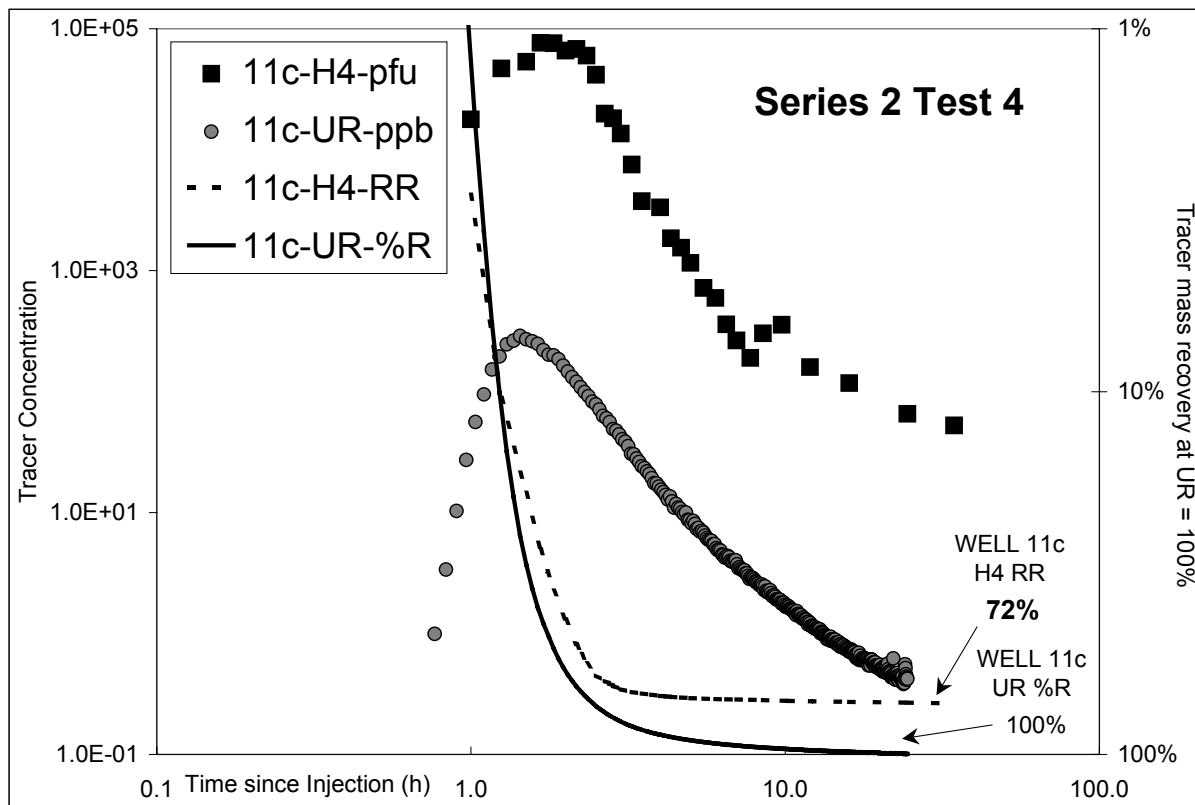


Figure 3-15 Tracer history and recovery - Series 2: Test 4.

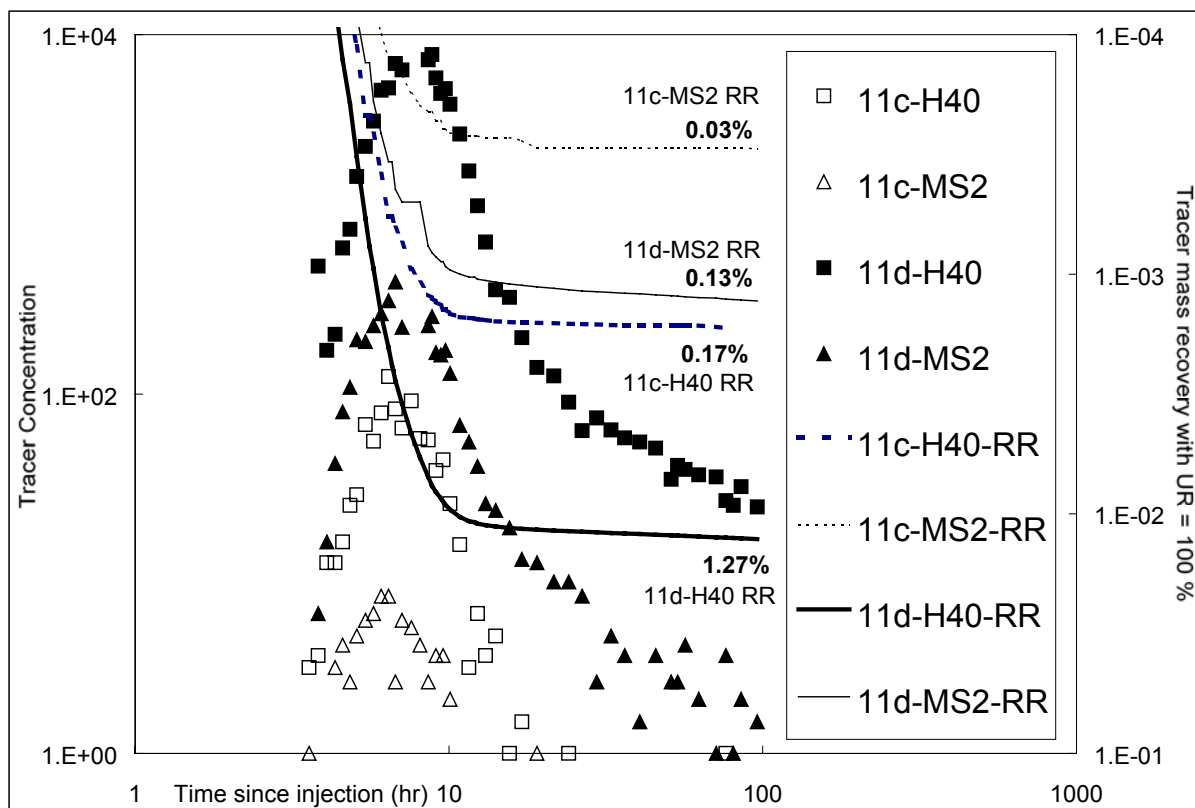


Figure 3-16 Biocolloid response and recovery comparison.

For Series 2 Tests 4,5,6, at well 11c, uranine ( $(Tr/Tr_0)_{max}$ ) and the biocolloid  $(C/C_0)_{max}$  values were similar to the highest ones observed at well 11d and were from  $4e^{-4}$  to  $1e^{-3}$  and  $4e^{-4}$ , respectively. These high values appear consistent with restricted and limiting channel flow in high velocity environments.

#### 3.4.8 Tracer recovery

Total recovery of the conservative solute uranine can be contrasted to the original sum injected for each site where it was found (Table 3-7). Observed concentrations, multiplied by the period of time over which they occurred, and summed for the entire experiment reflect the point recovery at the well. The sum, divided by injected solute weight results in a relative recovery normalized ( $RR_N$ ) to mass that was injected. Totals observed at each site are comparable assuming that the total flux through the contributing thickness in each well is equal over time. Uranine relative recovery ( $RR_{N-UR}$ ) was from 1 to 5 at well 11c and from 10 to 73 at well 11d during Series 1. During Tests 1 and 2 there were ~ 10 to 12 min injection periods and recovery lasted over several weeks at both wells. In these cases, ~ 7 percent of the mass observed at well 11d was detected at well 11c. It doubled to about 13 percent during test 3 that had a ~70 min injection period.

During Series 2 Tests 4, 5 and 6, the  $RR_{N-UR}$  at well 11c was 6, 4 and 13, respectively, with these values being reached in 24 h periods. The first two of three similar tests had similar  $RR_{N-UR}$  values but the third was about 2.5 fold greater. We believe this increase is attributable to the sampled interval in monitor well 11c being decreased by a similar factor from 6 to 2 m for Test 6. The concentration increased with less dilution in the sampling system volume than occurred during Tests 4 and 5.

The relative recovery of particles compared to that of uranine at each well has been referred to as relative breakthrough (RB) in formative work done by Harvey *et al.*, (1989) describing responses of different sized microspheres and bacteria at the Cape Cod site in the mid-1980s. Colloid attenuation is referred to as 100 percent minus the RB percent. In much of the porous media work to date, this standard comparison has commonly shown attenuation over 99 percent. Only a small percentage of colloids are detected compared to the conservative non-sorbing solute over the same period. Values of RB and Attenuation in Table 3-6 show that biocolloid H40 had RB values more than an order of magnitude higher than documented at Cape Cod in finer grained sediments. At well 11d, RB varied from about 1.4 to 4.4 percent for phage H40. Well 11c had attenuation more typical of finer sediments ranging from 99.9 to 99.99 percent. The results from Test 4 were surprising. In this case, with an RB of 72 percent, only 28 percent of the colloid was removed from the system relative to what occurred with the conservative solute. Clearly the amount of attenuation over this 10 m flow path was small and this limited degree of removal of the colloids is not expected in the majority of porous media pathways over such distances.

#### 3.4.9 Biocolloid response differences

The particle migration patterns of different colloids are expected to be dissimilar. Their surface charge, size and shape vary. These factors and others cause differences in their behavior and their interaction within the aquifer as relates to both the water and granular material. Therefore migration responses of unlike colloids in the subsurface environment are therefore not easily predicted. Of interest, however, for ground water protection studies, is how colloids behave compared to norms or standards assumed or presented as representative viral surrogates. In Test 2, we used two different bacteriophage biocolloids, H40 and MS2. MS2 was chosen since it is one of the most widely studied phage associated with viral surrogates experiments at field sites in North America and occasionally in Europe. H40, developed and widely used at the Univ. of Neuchatel for particle tracer studies was chosen because it is a strong and resistant phage that seems to survive well in a variety of different subsurface settings. Both phages were injected at the same time so that their magnitude and recovery could be compared directly. Results are in Table 3-6 and comparative responses are plotted in Figure 3-16. The dimensionless maximum concentrations were ~ 8 to 9 fold higher for H40 than MS2 at wells 11c and 11d, respectively. Their peak abundance and therefore the RF values were similar, 0.3 at well 11c and 0.7 to 0.8 at well 11d. Their RBs reflecting total recovery differed. H40 had a recovery rate 6 to 10 fold higher at wells 11c and 11d, respectively, compared to MS2.

The greater H40 migration efficacy is demonstrated clearly by almost an order of magnitude higher RB and  $C/C_0$  values. The similar pseudo-peak times to each well suggests that they are effectively transported in a similar manner through the sediments along the pathways that were followed to reach each well despite the fact they have dissimilar rates of removal during migration along the flow system.

### 3.4.10 Dispersivity

This property of the medium is best evaluated assuming conservative transport conditions with no adsorption or removal of the tracer. Therefore, the values of longitudinal dispersivity and dispersion coefficient were determined based only on the solute transport alone (Table 3-8). A typical curve-fitting result based on the 1D analysis method in the model Traci 95 (A. Werner, 1998) is in Figure 3-17. For well 11c, 11 m from the source in the Series 1 tests, longitudinal dispersivity values were 1.9 m during the low and high water level condition tests and 3.7 m during the intermediate water level test. The dispersion coefficient values, which incorporates the velocity calculated for the peak corresponded to  $1.9 \text{ m}^2 \text{ h}^{-1}$  during high water,  $0.5 \text{ m}^2 \text{ h}^{-1}$  during low water and  $1.4 \text{ m}^2 \text{ h}^{-1}$  during intermediate water level conditions. For well 11d, 22 m from the source in the Series 1 tests, longitudinal dispersivity values were similar from 1.2 to 1.3 m during the low and high water level condition tests and 1.6 m during the intermediate water level test. Corresponding, dispersion coefficient values were  $1.9 \text{ m}^2 \text{ h}^{-1}$  during the high water level condition,  $4.2 \text{ m}^2 \text{ h}^{-1}$  during low water and  $2.7 \text{ m}^2 \text{ h}^{-1}$  during intermediate water level conditions.

| Well | Tracer <sup>2</sup> | Test 1<br>(Nov. '97)   |                          |                   |                                       | Test 2<br>(Mar. '98)   |                          |                   |                                       | Test 3 & 4,5,6<br>(Aug. '98) |                          |                   |                                       |
|------|---------------------|------------------------|--------------------------|-------------------|---------------------------------------|------------------------|--------------------------|-------------------|---------------------------------------|------------------------------|--------------------------|-------------------|---------------------------------------|
|      |                     | T <sub>50</sub><br>(h) | V <sub>50</sub><br>(m/h) | $\alpha_L$<br>(m) | D <sub>L</sub><br>(m <sup>2</sup> /h) | T <sub>50</sub><br>(h) | V <sub>50</sub><br>(m/h) | $\alpha_L$<br>(m) | D <sub>L</sub><br>(m <sup>2</sup> /h) | T <sub>50</sub><br>(h)       | V <sub>50</sub><br>(m/h) | $\alpha_L$<br>(m) | D <sub>L</sub><br>(m <sup>2</sup> /h) |
| 11c  | UR-samples          | 29                     | 0.4                      | 3.7               | 1.4                                   | 43                     | 0.3                      | 1.9               | 0.5                                   |                              |                          |                   |                                       |
|      | UR-continuous       |                        |                          |                   |                                       | 43                     | 0.3                      | 1.9               | 0.5                                   | 11.2                         | 1.0                      | 1.9               | 1.9                                   |
| 11d  | UR-samples          | 8.4                    | 2.6                      | 1.6               | 4.2                                   | 17.4                   | 1.3                      | 2.0               | 2.6                                   |                              |                          |                   |                                       |
|      | UR-continuous       |                        |                          |                   |                                       | 17.7                   | 1.2                      | 2.2               | 2.7                                   | 5.4                          | 4.1                      | 1.0               | 4.0                                   |
|      | UR-T4               |                        |                          |                   |                                       |                        |                          |                   |                                       | 1.7                          | 5.9                      | 0.3               | 2.0                                   |
| 11c  | UR-T5               |                        |                          |                   |                                       |                        |                          |                   |                                       | 1.8                          | 5.5                      | 0.5               | 2.8                                   |
|      | UR-T6               |                        |                          |                   |                                       |                        |                          |                   |                                       | 2.2                          | 4.5                      | 0.6               | 2.6                                   |

**Table 3-8 Dispersivity, dispersion coefficient results using data matching.**(TRACI 95<sup>1</sup>).

**Footnotes:**

1 TRACI 95 – Authored by Andreas Werner, Koln, DE. (Formerly at Uni Karlsruhe)

2 Tracer – results of Uranine according to analysis method: (a) average, (m) moments, (c) concentration maximum based on results from samples. Continuous differentiates between data records from fluorimeters in continuous mode versus discrete samples taken during the testing.

During Series 2 tests which occurred during the high water level condition, the instantaneous injection condition associated with the analysis method was not true. Therefore, the values calculated do not have the same degree of reliability as those for the Series 1 test. For well 11c, 10 m from injection, longitudinal dispersivity values were from 0.3 to 0.6 m with dispersion coefficient values of 2.0 to 2.8  $\text{m}^2/\text{h}$ . These results are also within the range reported by L. Gelhar *et al.*, (1985) (Figure 3-18).

The dispersivity results judged most reliable from our testing in this gravel environment are those at well 11d. Well 11c, compared to well 11d, had 20 to 50 times lower solute maximums and reached a maximum 50 to 100 percent later. Therefore, well 11d appears to have behaved more longitudinally down gradient from the injection well and has the more reliable longitudinal dispersivity values. They varied by a factor of 3 from 1.2 to 3.7 m over the 22 m distance. These longitudinal dispersivity results also fit within the range of values presented by L. Gelhar *et al.* (1985) as shown on Figure 3-18. Since they are consistent with those reported by others, this suggests that the large range in heterogeneity in the gravel aquifer system tested is not able to be diagnosed in calculations of dispersivity, at least within the limits of the analysis undertaken in this exercise.

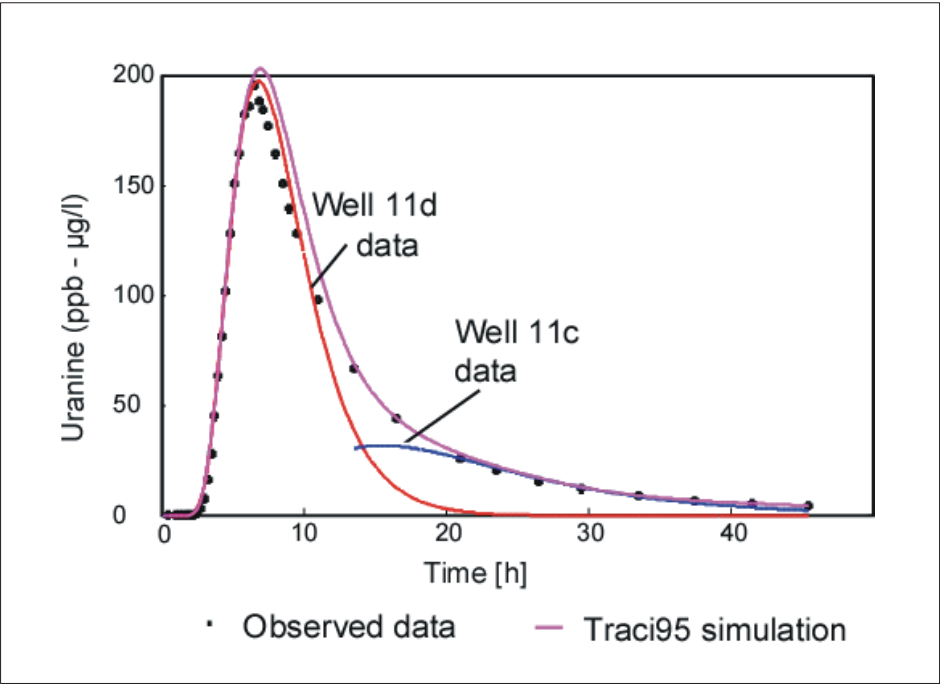


Figure 3-17      Dispersivity by data matching using TRACI 95.

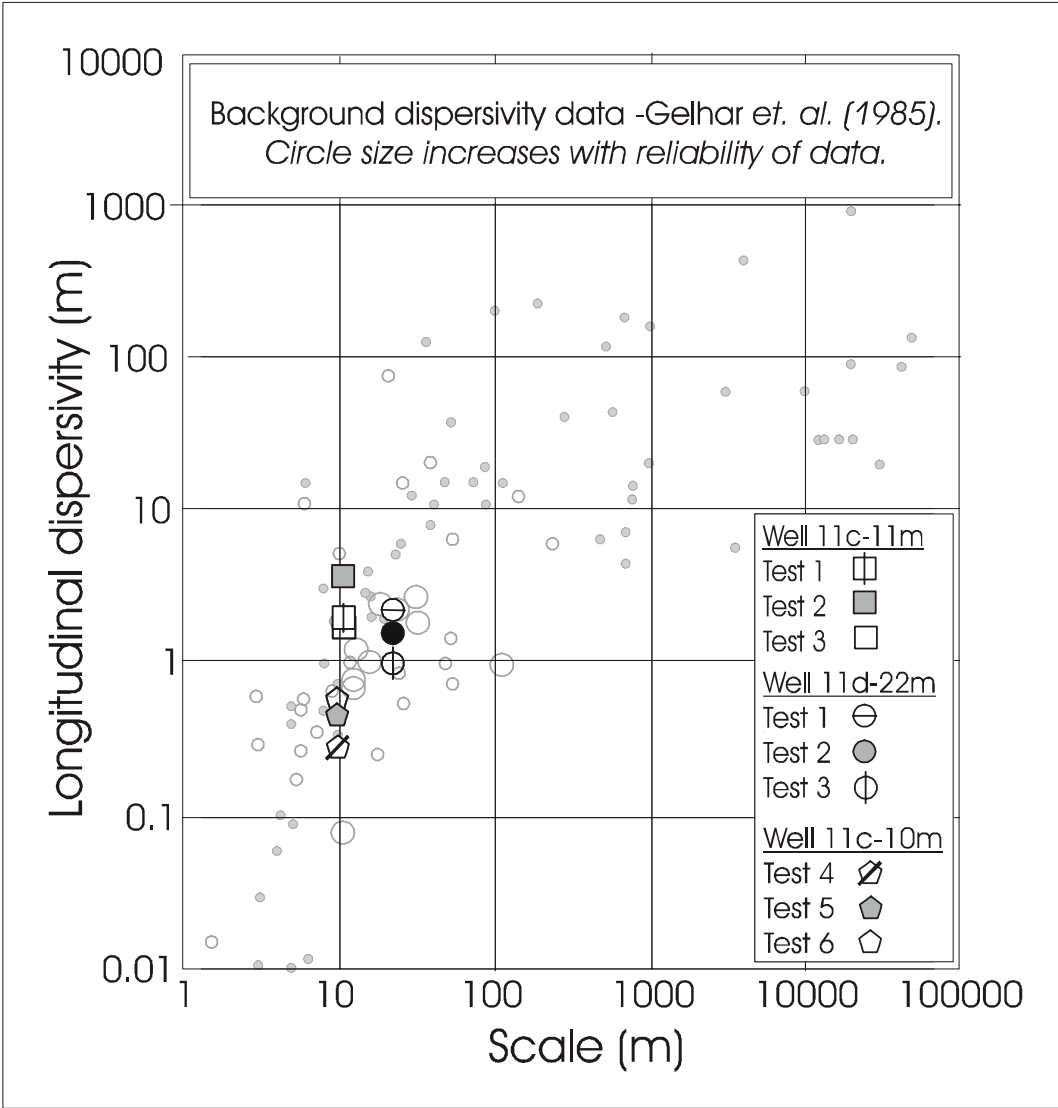


Figure 3-18      Comparative dispersivity values.

### 3.5 Conclusions

In considering how to best protect wells and other sources of water supply coming from aquifers situated in highly permeable gravel settings, the heterogeneity of preferential pathways, transverse directional gradients, rapid and low attenuation transport phenomena should be well understood and defined on a site specific basis. Failing this, no effective ground water protection strategy can be assured in this type of hydrogeologic setting. It is inadequate to rely solely upon applying regional models that do not include the possibility to depict potential selective transport paths.

Direction of transport does not always occur along regional or even local scale flow lines. The characteristic gravel aquifer responses seen at BdF illustrate anomalies compared to traditionally expected hydrodynamic results. Dominant responses occurred in a direction, likely along preferential pathway channels that were as much as 90 degrees different from what was anticipated flow based on local water level gradient data. Despite the local 10 to 25 m scale investigated in this work, conventional gradient data would have been relied upon in most situations to reflect general and site specific transport direction. Our work shows that in that case, the direction suggested for investigation would have been wrong. It further points out the need to have a well spacing that is adequate to define migration paths. For contaminant and protection studies in heterogeneous gravel aquifers, well spacing cannot be effective on the typical hundreds of m scale. Rather it needs to be targeted to site conditions, include a close understanding of the depositional setting and can likely be likely no more than 10 m near important release point scenarios.

Vertical segregation of pathways in individual wells and the aquifer setting markedly influenced the results in the series 2 tests. The expected 6 m contributing interval was defined as about 1 m with tracer methods. The expected detection at 24 m did not occur. This type and degree of heterogeneity is hard to define with precision except within the well. Detailed flow logging and related tracer work is valuable and should always be considered in this type of setting.

Travel times in the gravel preferential pathways were documented at several hundreds of m day<sup>-1</sup>. Measured arrival times at BdF were from two to six times faster to well 11c and about 10 times faster to well 11d than estimated from conventional flow-related and aquifer pump testing data. For our series 2 tests, arrival was from 35 to 45 times faster than predicted. The presence of extremely low concentrations of harmful material, particularly biocolloidal materials such as virus or bacteria can represent a health threat. Therefore, estimates of when a contaminant will arrive is not adequately based on average pore water velocity or a time to maximum concentration. It needs to be documented based on actual field testing at a site, particularly if the site is providing a public water supply and ground water protection zones need to be established.

Injection lasted over a period of from 10 to 75 min. No significant influence of the injection length was observed on transport rates or migration conditions. The recovery rate, however, seemed to increase with increasing injection time length.

The solute dimensionless concentration magnitudes in preferential pathways from  $3e^{-04}$  to  $4e^{-03}$  are relatively high given the flow distances of 10 to 22m. The RB for biocolloid particles was also high from ~ 1.5 to 4 percent, and in test series 2 extreme at ~ 72 percent. Therefore, there is a strong potential for significantly less attenuation in flow systems in these types of gravel environments than typically is considered in most particle transport evaluations.

We believe that the better proof upon which ground water protection studies should be based to achieve less uncertainty is detailed field investigations and tracer testing with tracer components involving multiple types of materials. The applicability of real input to such strategies is considerable compared to theoretical models that rely on for the most part only conventional flow data. Geologic information derived from understanding of sediment deposition patterns and settings should be relied upon to define the scale and types of experiments that need to be conducted to best characterize the subsurface environments. Using the results from a correctly developed and fully applied investigative approach will then allow a logical extrapolation from the field-derived conceptual transport model to a more predictive larger scale mathematical model whose range in transport estimates can be better validated to be consistent with field observations.

## **Chapter 4**

### ***Characterization of the Kappelen Ground Water Research Site (BE), Switzerland, and Preliminary Bacteriophage and Solute Tracer Component Responses<sup>1</sup>.***

#### **Abstract**

Characterization of a ground water research site near the Swiss village of Kappelen (BE) began in 1996. The site, located about 15 km northwest of Bern, covers about 15000 m<sup>2</sup> in a forested area with relatively flat terrain. Its near-surface ground water flow system is part of the prolific Seeland aquifer. Surface geophysical studies, first at a regional scale and then at two targeted sites, evaluated and defined the limits, nature, depth and extent of the fluvio-glacial materials. A preferred site was selected at which the aquifer depth extended to between 12 and 20 m. It also had a relatively consistent and smooth undulating contact between the high-resistivity permeable gravel layers and the underlying lower permeability silt aquitard. Hydrogeologic work consisted of defining the regional and site gradient and subsequently installing, developing and testing of two shallow piezometers and 16 deeper wells. Regional well measurements confirmed the historical gradient as applicable to the Kappelen site. Onsite data defined a relatively low gradient of about 0.0005 to 0.001. The subsurface instrumentation was completed at 11 locations in both a lower (10 to 15 m) and upper (4 to 9 m) zone in the aquifer. Drilling results corresponded well with geophysical depth predictions. Hydraulic conductivity was estimated for the aquifer materials at  $1\text{e}^{-04}$  to  $1\text{e}^{-02}$  m/s and the underlying silt aquitard at  $3\text{e}^{-09}$  to  $6\text{e}^{-08}$  m/s. Partially-confined conditions in the lower zone implied potential segregation of flow and transport regimes between the two zones. Reconnaissance biogeochemical characterization was extended due to the impact of detected REDOX levels, principally in the lower zone, which has resulted from an upgradient sugar refinery's historical discharge practices. Bacterial accumulations (*Siderocapsa* sp., *Gallionella* sp. and *Leptothrix* sp) created such complete biofouling of the lower zone well screens that the two last tracer tests in this zone could not be completed successfully. Seven tracer tests were undertaken in about 15 months. Bacteriophage and uranine responses were able to be well characterized to distances of about 20 m (row 1) with the responses in the two zones exhibiting different transport direction and velocity characteristics. To 60 m, there was less detection of either phages or uranine. Only one well in each zone to date has had a response adequate to characterize tracer component behavior. Phage and solute initial detection times were not sufficiently different to conclusively document that phage preferential advective transport processes were manifest. Solute velocities based on the time of maximum concentration ranged from about 14 to 21 m/d and 3 to 7 m/d in the lower and upper zones, respectively. Transport velocities based on arrival times were 2- to 4- fold higher. Phage maximum concentrations consistently occurred earlier than those of uranine consistent with diminishing mass along the pathway due to attenuation. Retardation values were from 0.2 to 0.9. Phage H40 recovery, based on relative breakthrough normalized to uranine recovery was about 0.02 % and 0.005% in the lower zone at 20 and 60 m distances, respectively. In the upper zone, it was 0.01 % at two locations at about 20 m. Results presented herein are based principally on field experimental work sponsored by the Swiss National Science Foundation (FNS 20-46726.96) and conducted at the site for the contract funding period between Oct. 1996 and Sept. 1998.

<sup>1</sup> This chapter forms the basis of a paper (K. Kennedy, I. Müller, P.-A. Schnegg, P. Rossi and R. Kozel, 2001) published in *Beiträge zur Hydrogeologie*, Jargang 2001, Band 52, pp. 158-180 (Tracer studies in the Unsaturated Zone and Groundwater - Investigations 1996-2001, Eds. M. Probst & H. Zojer, Joanneum Research, Graz 231p.).

## **4.1 Introduction**

Ground water research activities at the Kappelen test site (Figure 4-1) were initiated in 1996 following its sponsorship by the Swiss National Science Foundation (FNS) as part of contract FNS 20-46726.96. The purpose of this chapter is to outline the work that has been undertaken at the site and present results to date in terms of site and tracer component (bacteriophages and uranine) transport characterization in this setting. The principal work activities were undertaken at the site over a period of two years ending in October 1998. Further work is ongoing at the site under continued sponsorship of the FNS related to aquifer heterogeneity.

### **4.1.1 Objectives**

The objectives of the field research work at Kappelen were part of a continuing scientific program to characterize bacteriophage (phage) migration and to develop and carry out comparative particle and solute transport investigations in porous media aquifers. The three principal goals of the field research program were to: 1) define the patterns of phage behavior as they interact with porous media environments in terms of physical, chemical and biological processes in the subsurface, 2) evaluate phages as tracers by conducting repetitive multi-phage tracer experiments in field environments and 3) compare phage responses to classical artificial fluorescent tracers. Meeting these goals would ultimately develop a better understanding of how to document and define particle transport conditions and assess their related migration processes. This could provide critical input needed to improve ground water protection strategies for aquifers particularly with respect to viral and other biological particles.

### **4.1.2 Scope of work**

The activities undertaken at the site have followed four programs. The first program consisted of surface geophysical investigations, first at a regional and subsequently at a site scale. Follow-up detailed work addressed technique improvement. The second program addressed hydrogeological characterization. Regional wells within 2 km were measured and gradients were confirmed. Installing two shallow piezometers filled a water level data gap in the immediate site vicinity allowed confirmation that it aligned with regional hydrodynamic gradients. Sixteen, 102-mm diameter wells were installed to depths of about 15 m, developed and hydraulically tested over a 90 by 40 m area. The third program was a reconnaissance biogeochemical characterization that was intensified due to the impact of REDOX condition in the lower part of the site aquifer. Finally, a tracer testing program was initiated. Seven tests were undertaken, with two in the upper and five in the lower part of the aquifer. The results presented herein are for the most part based on work conducted during the contract period between Oct. 1996 and Sept. 1998. Two recent graduate student diploma projects at CHYN (M. Ousseni, 1998; N. Nosedá, 1999) were also conducted at Kappelen and involved work related to site characterization and hydrogeology.

### **4.1.3 Site setting**

The site is located in an area of the Seeland aquifer, which consists principally of post-glacially deposited sand and gravel overlying Tertiary marl and sandstone. This important aquifer has been the subject of studies for several decades. P. Kellerhals & B. Tröhler (1976) provide a comprehensive description of the regional geological setting and ground water conditions. The aquifer is an important public water as well as agricultural supply. It extends over an area of about 70 km<sup>2</sup>, has a depth up to 25 m, and a water table about 2 to 5 m below ground surface. Work to date has included: regional aquifer modeling (D. Biaggi et al., 1994), impact definition from sugar refining and other waste site releases (C. Halmes, 1993; P. Hoffmeyer, 1995), artificial recharge studies (P. Kellerhals & C. Haefeli, 1988) as well as ground water protection and monitoring of the quality of subsurface conditions (R. Kozel, 1992). While many wells and piezometers exist over the entire area, they have been installed in a variety of manners over the past 25 years, are widely spaced, are of uncertain construction and many do not have well-documented lithology. Rarely have samples been retained. The past use of an area north of the nearby town of Aarberg included discharge of liquid wastes from a sugar refining operation to leaking lagoons. These practices extended over tens of decades and have resulted in a plume of organic and sulfate contaminants extending several kilometers down gradient. This was investigated over widely spread areas but no detailed sampling of the aquifer near the lagoons had been undertaken. The Kappelen site is less than one km from the northern extent of these historical lagoons.

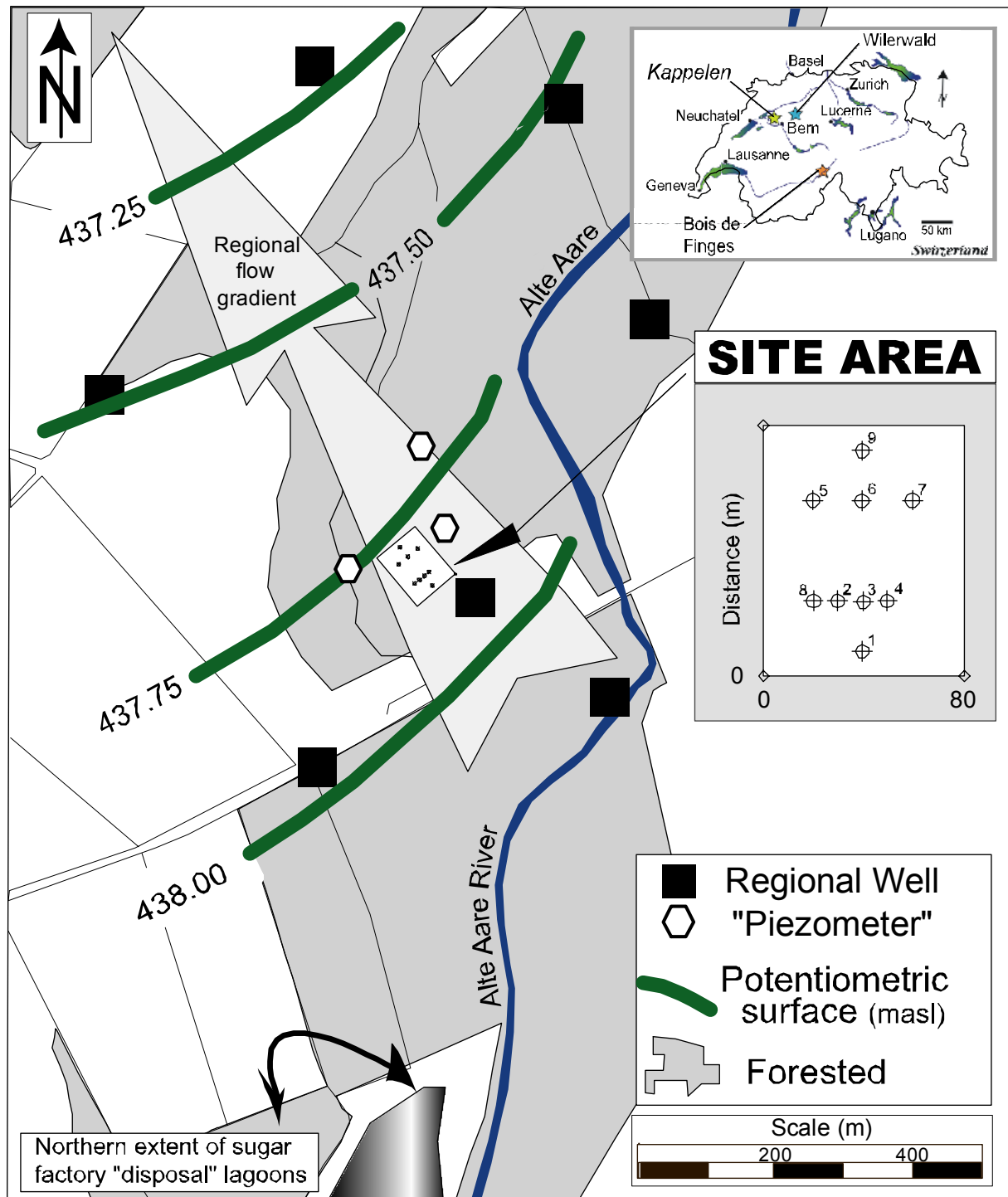


Figure 4-1 Site location map with regional wells and part of the Seeland aquifer potentiometric surface as measured in October, 1996.

The site is located in a forested area of relatively flat terrain situated at an elevation of about 440 to 445 m. The Alte Aare River lies about 250 to 300 m to the east. Incised paleochannels of the Aare account for most of this relief. Agricultural areas that surround the forest have associated consumptive ground water use in the summer months that directly influence the water table. This effect plus natural seasonal variation may result in fluctuations of up to 2 m locally. The regional hydraulic gradient is to the northwest and is relatively low at about 0.001 (0.1 %) with localized influences along the course of the Alte Aare River.

## 4.2 Geophysical Exploration

Surface geophysical exploration was done to find and develop an appropriate site took place at both a regional and then a more localized scale. Additional geophysical investigations were later done to compare the results of variable spacing and alternate methods of evaluating the subsurface conditions at the site.

RMT (Radio magneto telluric) and VLF-EM (very low frequency electro magnetic) surveys similar to those conducted at the Wilerwald (A. Carvalho Dill, 1993) and other porous media sites (P. Turberg et al., 1994) were conducted during July and August, 1996. Six sites over an area of about 6 km<sup>2</sup> were evaluated near Lyss and Aarberg, (BE). Two of the six sites had relatively homogeneous stratigraphic materials and consistent contact depths with the underlying silts. Follow-up detailed geophysics was done at both sites. One site that belonged to 14 landowners had the depth to the underlying aquitard measured at about 25 m. The other site belonged to the village of Kappelen landowners and the depth to the underlying aquitard was estimated at from 13 to 18 m. The latter site was selected and is now referred to as the Kappelen Research Site (KAP).

Detailed geophysics using both RMT and VLF-EM was done at the site on 10 m by 20 m spacing in 1996 as described in E. Oyono (1996) and on 5m by 10 m spacing by some of the current authors in 1997. Figures 4-2a and 4-2c illustrate VLF-EM data from the site. Figure 4-2a is a 3-D depiction of the interpreted VLF-EM surface based on interpolation of 10 adjacent lateral transects. The results illustrate the relatively consistent depth and type of materials found in the upper materials overlying a lower conductivity material. Figure 4-2c shows the results of one of the VLF-EM transects over a distance of about 80 m across the site showing the lateral continuity to the east. The higher percent outphase values seen to the west on this and also reflected to the northwest in the 3-D image are believed to be an artifact from a buried line or metal tubing along the access road through the forested area. Interpreted depth to the underlying aquitard and resistivity of the materials in the aquifer itself are shown on Figures 4-2b and 4-2d. The 3-D visualization of the interpreted thickness of the sand and gravel aquifer overlying the silt aquitard (Figure 4-2b) shows a variable but relatively smooth contact. The depth varied in total from about 13 to 19 m but over much of the mapped area appears to have a 3 m depth range from about 15 and 18 m. Figure 4-2d illustrates a profile along one of the RMT transects illustrating the relatively high true resistivity values (300 to 500 ohm-m) in these layered and heterogeneous gravel aquifer materials. The depth interpreted along the profile is from 13 to 18 m without the presence of sharp and incised channel features that were observed at the other locations investigated regionally.

The reliability of the estimated aquitard depth measurements was evaluated after the monitoring wells had been drilled at the site in 1996 and again in 1997. These estimated depths are shown on Figure 4-3a for the RMT transects done with a north-south orientation in 1996 and an east-west orientation in 1997. Compared to the drilled well depths (Figure 4-3a) at the locations shown on Figure 4-4, the geophysically interpreted average depth results for both surveys had differences at two locations of 1.5 and 1.7 m and at the remaining seven locations of less than 1 m.

The correlation between true resistivity defined from the RMT geophysical work in 1996 and the hydraulic conductivity (K) estimates for the underlying aquitard and overlying gravel aquifer material was evaluated (Figure 4-3b). Results showed a grouping of the underlying silt materials with true resistivity results from 70 to 100 ohm-m and 350 to 500 ohm-m corresponding to K-range estimates of about  $1e^{-05}$  m/s and  $7e^{-04}$  to  $3e^{-03}$  m/s, respectively. K-values were taken from grain size analyses as well as preliminary site hydrogeologic characterization activities at individual wells. There is a reasonable agreement in the relationship and consistency of the two values given the range in properties of the two types of materials.

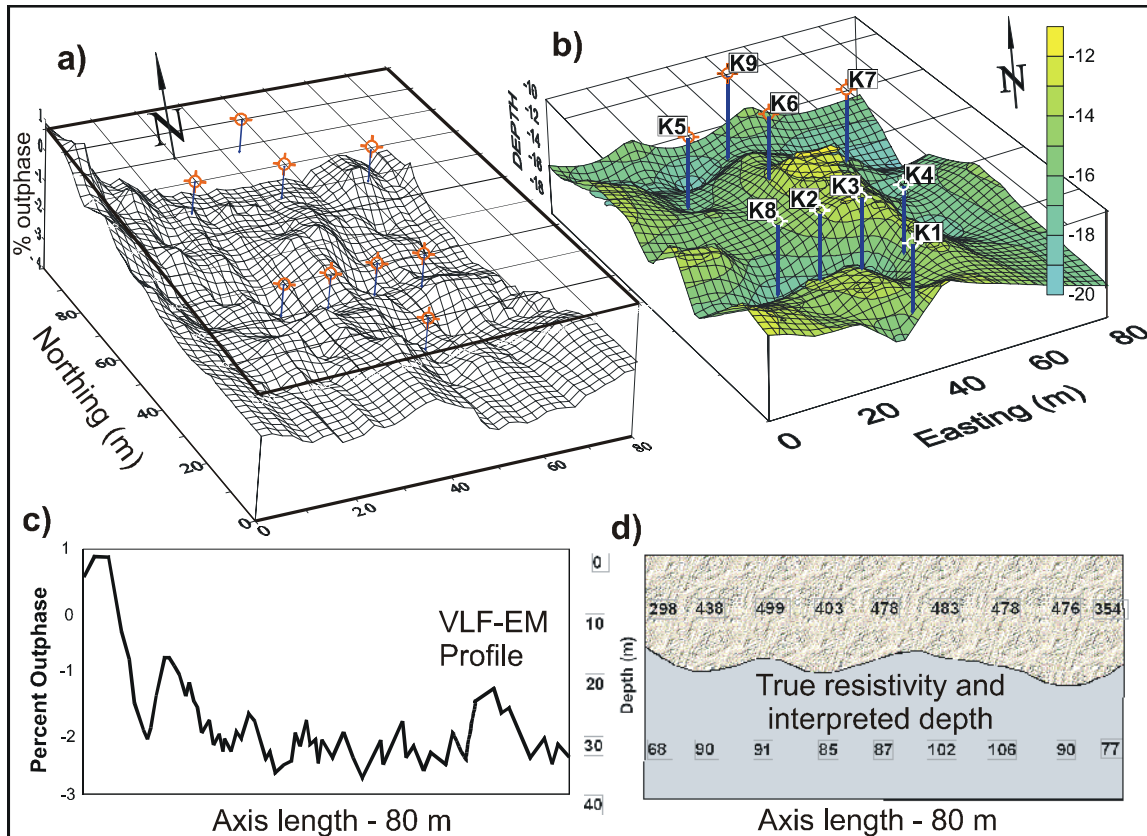


Figure 4-2 Geophysical results from the site investigations.

- a) VLF-EM 3D contour map,
- b) Interpreted resistivity data providing 3D depth projection to aquitard,
- c) VLF-EM profile along an E-W transect,
- d) True resistivity values and the interpreted depth to aquitard profile along an E-W transect.

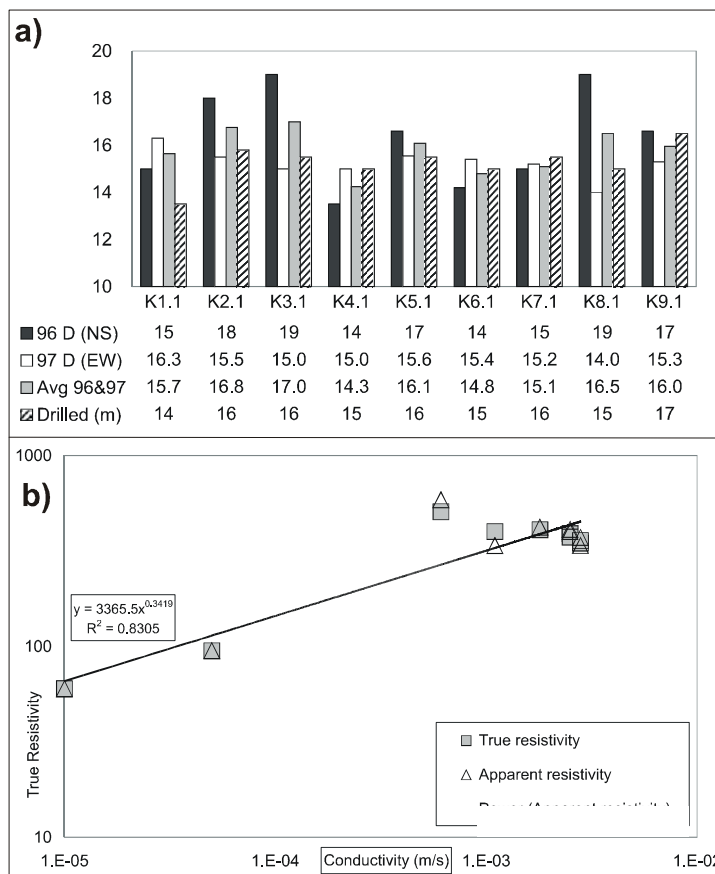


Figure 4-3 Geophysical correlation.

- a) Measured vs. geophysics-based predictions of aquitard depth,
- b) True, apparent resistivity and hydraulic conductivity.

### 4.3 Hydrogeological Characterization

Background water level information was obtained from the well-established regional ground water monitoring network. About 12 existing wells, six of which are shown on Figure 4-1, were used to update the regional picture of the ground water flow system and quantify the potentiometric surface. Water level measurements were made over a period of about two months and the ground water flow direction pattern from Sep. and Oct. 1996 was compared to the historical data going back over 10 years. The 1996 data fit well with previous records indicating a general northwesterly flow direction at the site. A visual reconnaissance revealed the existence of three nearby and previously undocumented extraction points that could be utilized for ground water monitoring locations. Two of these could be readily accessed and water levels measurements and surveying were done to combine these site-specific data with the regional information. Data gaps, however, existed in the coverage at the site and the influence of the Alte Aare River to the east of the site was not well understood. Accordingly, a shallow (rammsondierung) probe and piezometer installation program was carried out prior to proceeding with actual site drilling. Five locations were evaluated by driving a metal rod to refusal, recording the blow count and checking for the presence of water. Detection of water at three could not be confirmed. At the other two locations, slotted pipes were installed to allow continued water level measurements. Details of the rammsondierung program are in Appendix A4-1 along with the well logging details for the site.

The ground water flow direction estimated from the nearby regional well measurements and the results of the surface RMT geophysics were used to lay out the proposed initial well locations. The proposed field was designed to have an injection and pumping location separated by a distance of about 90 m. In between, additional wells at six locations were proposed at distances of about 20 m (row 1) and 40 m (row 2) from the injection well.

Well locations 1 through 7 (14 wells) were completed in Oct. and Nov. 1996 (Figure 4-4). Wells 8 and 9 were installed in Jul. 1997. Except for wells 8 and 9, each location has both a shallow well screened from about 4 or 5 m to 8 to 9 m and a deeper well screened over 4 or 5 m from about 10 to 15 m (Table 4-1). Typical geology from the wells is illustrated in Figure 4-5a. Typical well construction is shown in Figure 4-5b. All wells were completed with 102 mm ID poly-type casing, screens with a slot size of 1.5 mm and a gravel pack of 2.0 to 3.2 mm or 4 to 6 mm. Wells were surveyed and a permanent benchmark was established at the site.

|      | Borehole         |                 |       | Well  |         | Screen |        |      | Gravel Pack |      | Well seal |          |
|------|------------------|-----------------|-------|-------|---------|--------|--------|------|-------------|------|-----------|----------|
|      | Ground Elevation | Drill & Install | Depth | Depth | Stickup | Base   | Length | Top  | Base        | Top  | Plug base | Plug top |
| WELL | m asl            | Date            | m     | m     | m       | m      | m      | m    | m           | m    | m         | m        |
| K1.1 | 442.54           | 10/14-Oct-96    | 15.5  | 13.2  | -0.10   | 13.0   | 3      | 10.0 | 13.0        | 9.2  | 9.2       | 8.0      |
| K1.2 | 442.46           | 23/24 -Oct-96   | 9.3   | 7.8   | 0.25    | 7.8    | 3      | 4.8  | 8.9         | 3.8  | n/a       | n/a      |
| K2.1 | 442.24           | 28-Oct-96       | 16.0  | 15.0  | 0.00    | 15.0   | 5      | 10.0 | 14.4        | 9.2  | 9.2       | 7.8      |
| K2.2 | 442.23           | 29-Oct-96       | 8.9   | 8.0   | -0.05   | 8.0    | 3      | 5.0  | 8.5         | 4.0  | n/a       | n/a      |
| K3.1 | 442.45           | 14/16-Oct-96    | 15.0  | 14.9  | 0.15    | 14.9   | 5      | 9.9  | 15.0        | 9.0  | 9.0       | 7.1      |
| K3.2 | 442.53           | 24/25 -Oct-96   | 8.6   | 8.2   | -0.20   | 8.2    | 3      | 5.2  | 8.5         | 3.9  | n/a       | n/a      |
| K4.1 | 442.62           | 29/30 -Oct-96   | 15.7  | 15.2  | -0.15   | 15.2   | 5      | 10.2 | 15.7        | 9.1  | 9.1       | 7.6      |
| K4.2 | 442.56           | 30/31 -Oct-96   | 8.9   | 7.9   | 0.05    | 7.9    | 4      | 3.9  | 8.9         | 4.2  | n/a       | n/a      |
| K5.1 | 441.78           | 21/23 -Oct-96   | 16.2  | 15.0  | 0.00    | 15.0   | 5      | 10.0 | 15.0        | 8.9  | 8.1       | 6.7      |
| K5.2 | 442.09           | 23/25 -Oct-96   | 8.9   | 8.1   | -0.10   | 8.0    | 3      | 5.0  | 8.1         | 4.6  | n/a       | n/a      |
| K6.1 | 442.35           | 25/29 -Oct-96   | 16.5  | 15.1  | -0.10   | 15.1   | 5      | 10.1 | 15          | 9.8  | 9.8       | 8.5      |
| K6.2 | 442.33           | 29/30 -Oct-96   | 8.5   | 7.9   | 0.15    | 7.9    | 3      | 4.9  | 8.0         | 3.4  | n/a       | n/a      |
| K7.1 | 442.37           | 16/21-Oct-96    | 15.0  | 15.0  | 0.00    | 15.0   | 5      | 10.0 | 15.0        | 9.1  | 9.1       | 7.6      |
| K7.2 | 442.35           | 30-Oct-96       | 8.5   | 8.1   | -0.10   | 8.1    | 3.2    | 4.9  | 8.5         | 3.5  | n/a       | n/a      |
| K8.1 | ~442.44          | 21/22-Jul-97    | 15.7  | 15.5  | ~ 0.50  | 14.5   | 4      | 10.5 | 15.7        | 9.7  | 9.7       | 8.6      |
| K9.1 | ~442.42          | 22/25-Jul-97    | 16.3  | 16.0  | ~ 0.70  | 15.0   | 4      | 11.0 | 16.3        | 10.5 | 10.5      | 8.8      |

**Table 4-1 Kappelen well completion data.** (based on E. Oyono, 1996 with wells K8 and K9 added)

n/a-not applicable;

all wells have surface seal at <1m with 0.5 m compactonite.

After drilling, wells were initially developed by pumping continuously with an submersible pump for 30 min opposite the well screens at rates of about 180 to 200 L/min. Later, the wells were pumped at similar rates over several hours per well with a suction pump. Specific capacity of the wells was

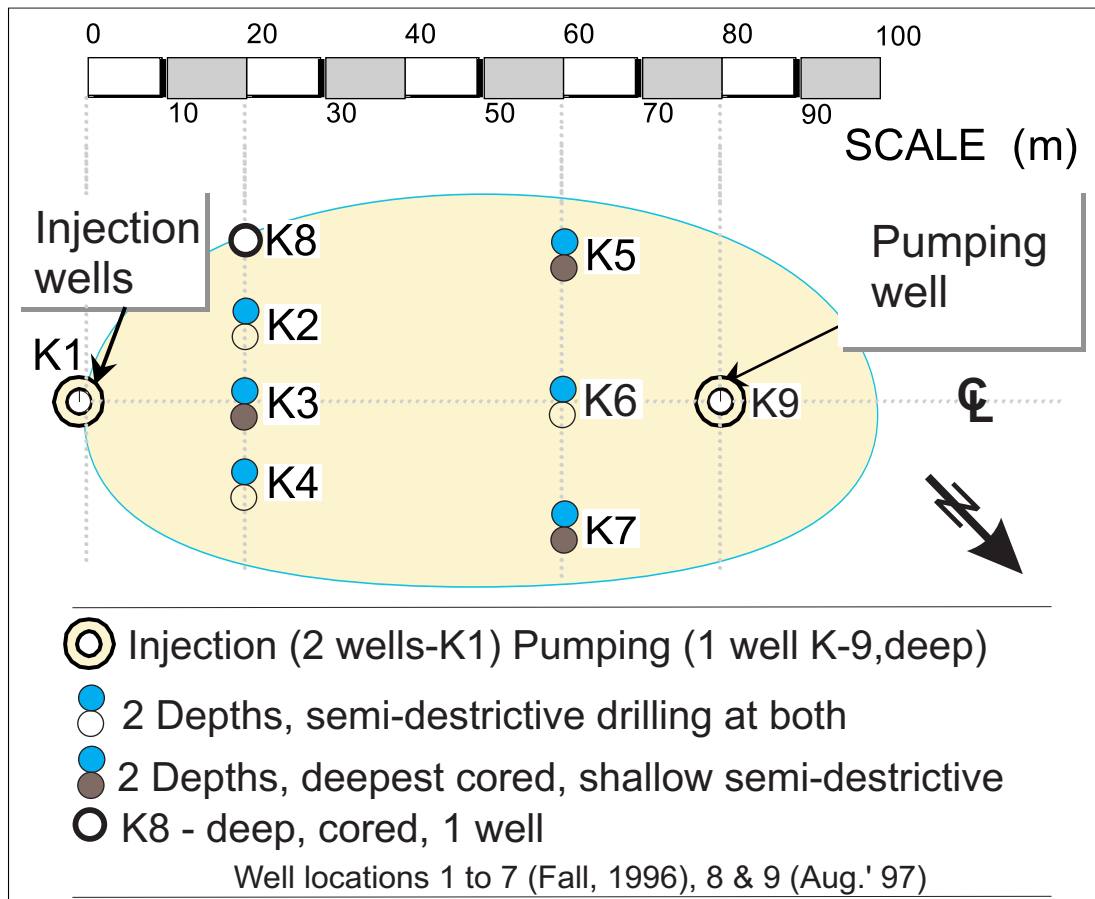


Figure 4-4 Kappelen well field layout.

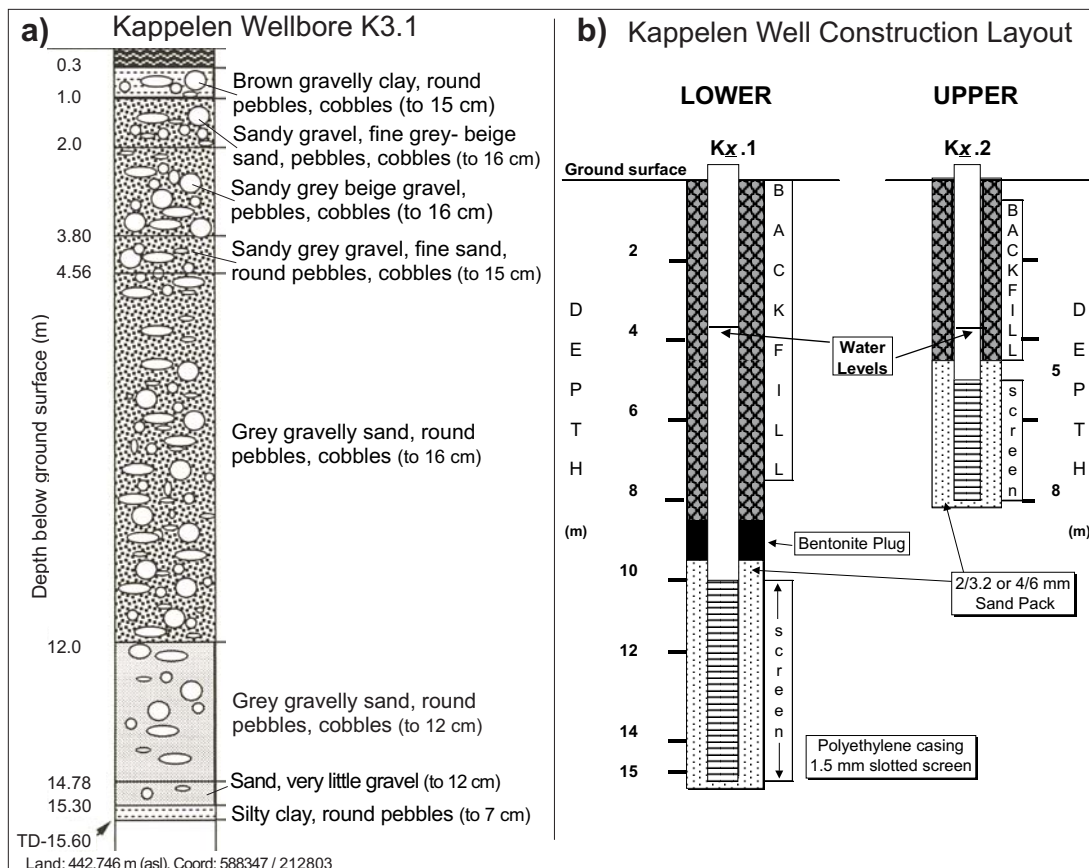


Figure 4-5 Geological interpretation and typical well completion  
a) Well log for K3.1.  
b) Schematic of typical well completions.

determined during development and in separate site testing activities. Short term pump testing was done in the deeper well locations in Nov. 1996 and in the shallow wells in 1997. In 1996, pumping was done at both wells K1.1 and K3.1 using a large capacity 600 L/min pump. Measurements were made at other wells over a period of about 2 h. Water levels at the surrounding well locations dropped initially within about two minutes and appeared to stabilize in less than 30 min. No measurements were possible in the pumping well due to the size of the suction pump intake. In May 1997, additional short-term tests were done with pumping at about 300 L/min from wells K3.1 and K7.1. Similar rapid drawdown and stability patterns were observed with the monitoring wells as had been seen earlier. In this case, water levels in the pumping wells were observed. In the two wells pumped, the water levels stabilized within a few minutes during the two tests. All indications during well testing showed that the recovery from pumping occurred within a matter of seconds to minutes and accordingly, there was little opportunity to manually document this return to stabilization. Similar effects were also observed in the observation wells. The prolific aspect of the aquifer combined with the low volume capacity of the available pumping equipment precluded inducing a significant and widespread aerial drawdown thus limiting our characterization to date as those that could be determined from individual wells.

Analysis of the hydrogeological characterization data indicated that the tested lower zone had a K-range from about  $5e^{-04}$  to  $1e^{-02}$  m/s with implied semi- to confined conditions in lower zone and water table conditions in the upper zone. Similar results were obtained from short term pumping tests in the upper zone except at K5.2 where the drawdown was rapid and reflected a lower hydraulic conductivity of about  $1e^{-04}$  m/s. Most calculations of hydraulic conductivity carried out at the site assumed the thickness of the contributing zone was equivalent to the screen for the lower zone and to the saturated thickness in the well for the upper zone.

Water level measurements repeated over about one year at the site indicated a stable hydraulic gradient in both zones that was similar and ranged from 0.0005 to 0.001. Seasonally, depth to water table changed, but the gradient remained essentially constant at the site.

Based on the determination of the hydraulic conditions, we also made scoping calculations to estimate the average transport velocity of planned tracer tests. Input was the hydraulic gradient and K-value characterizations and an estimated range in effective (transport) porosity. Preliminary average aquifer flow velocities for the site area had been estimated by Cantonal staff to be about 1 m/d in our site vicinity. We undertook scoping based on this simplistic set of assumptions using a range of hydraulic conductivity from  $5e^{-03}$  to  $1e^{-02}$  m/s, a hydraulic gradient of 0.001 and an effective (transport) porosity (n) range of 7.5 to 20 %. Well logging by P. Hoffmeyer (1995) had showed that in a well about 500 m south of the site, preferential pathways in lenses in the gravel aquifer were present in the gravel sequence. Accordingly, we used a reduced value for the lower range of the transport porosity value. Results suggested that for a conservative (non-sorptive) solute tracer, the peak concentration time to a distance of 20 m, where the first row of wells was located, would be from about 2 d to 10 d.

#### **4.4 Biogeochemical Characterization**

Diverse and widespread reduced oxygen conditions exist in the Seeland aquifer. To the southeast these are mainly due to the seepage, rich in degradable organics, from unlined discharge at the sugar refinery lagoons. Studies in the 1950s and 1970s showed an extended plume of reduced ground water deteriorating water supplies. This heterogeneity makes it difficult to determine exact horizontal/vertical plume extent, its REDOX conditions and the transport of contaminants DOC, Fe, Mn and K. Recent regional surveys showed limited rehabilitation from the infiltrating Alte Aare River. REDOX environments in ground water situations are common. However, the rates of reactive changes are typically site specific. Documenting the chemical, sedimentary and microbiologic parameters was critical to carry out further ground water research and assess the migration potential of tracers at the site.

In 1997, a program to characterize the bacteria and hydrochemistry in the wells impacted by REDOX conditions was developed. Participants included Univ. Neuchatel (CHYN) hydrogeologists and (LAMUN) microbiologists and the Canton of Bern's Analytical Laboratory and Environmental and Ground Water Protection Dept. staff. REDOX chemistry and microbial activity were evaluated in 10 wells at Kappelen. These wells are about 750 m from the northern extent of the sugar factory settling ponds (Figure 4-1). REDOX conditions varied strongly even over the limited distance of 70 m and

## CHAPTER 4 – KAPPELEN SITE AND TRACER COMPONENT RESPONSE CHARACTERIZATIONS

between the shallow and deeper aquifer layers. Characterization with results of sampling is listed in Table 4-2. Eh varied from -130 mV to +61 mV. Dissolved oxygen ranged from near zero to 25 % of saturation.

| Parameter                       | Cond  | pH  | O2   | DOC  | Dtot   | Na   | K    | Fe   | Mn   | NH4  | Cl   | SO4  | S    | NO3  | Eh   |
|---------------------------------|-------|-----|------|------|--------|------|------|------|------|------|------|------|------|------|------|
| units                           | µS/cm |     | mg/l | mg/l | mmol/l | mg/l | mg/l | mg/l | mg/l | mg/l | mg/l | mg/l | mg/l | mg/l | (mV) |
| <b>Location</b>                 |       |     |      |      |        |      |      |      |      |      |      |      |      |      |      |
| <u>Regional wells and river</u> |       |     |      |      |        |      |      |      |      |      |      |      |      |      |      |
| Alte Aare                       |       | 8.1 | 9.2  | 1.7  | 1.32   | 2.0  | 1.0  | 0.06 | 0.01 | 0.2  | 2.6  | 24.6 |      | 4.8  |      |
| 7.2                             | 705   | 7.0 | 1.3  | 1.3  | 3.91   | 4.97 | 11.5 | 0.08 | 0.02 | <    | 7.9  | 41.1 | 0.01 | 4.2  |      |
| RB1                             | 314   | 7.5 | <    | 0.6  | 1.64   | 2.23 | 1.7  | 0.05 | <    | <    | 2.4  | 27.6 | <    | 0.51 |      |
| RB1b                            | 337   | 7.5 | <    | 0.6  | 1.75   | 2.83 | 2.0  | 0.47 | 0.23 | 0.07 | 2.5  | 31.5 | 0.05 | 0.14 |      |
| A2                              | 406   | 7.3 | <    | 0.5  | 2.13   | 3.75 | 3.9  | 0.92 | 0.47 | 0.06 | 5.6  | 34.4 | 0.16 | 0.03 |      |
| A1                              | 610   | 6.9 | 7.0  | 1.4  | 3.37   | 6.32 | 5.6  | 0.38 | 0.06 | 0.02 | 7.8  | 36.7 | 0.01 | 1.17 |      |
| RB2                             | 448   | 7.2 | 1.2  | 0.9  | 2.4    | 3.29 | 1.8  | 0.05 | 0.02 | <    | 3.6  | 27.7 | <    | 0.84 |      |
| RB2b                            | 389   | 7.2 | 1.1  | 0.7  | 2.07   | 3.06 | 1.8  | 0.05 | 0.02 | <    | 3.1  | 27.4 | <    | 0.44 |      |
| <u>Site wells</u>               |       |     |      |      |        |      |      |      |      |      |      |      |      |      |      |
| <u>Upper zone</u>               |       |     |      |      |        |      |      |      |      |      |      |      |      |      |      |
| K1.2                            | 465   | 7.7 | 0.5  | 0.8  | 2.25   | 3.9  | 3.3  | 0.21 | 0.79 | 0.14 | 4.0  | 36   |      | <    | -51  |
| K3.2                            | 483   | 7.7 | 1.0  | 0.7  | 2.4    | 4.5  | 3.8  | 0.17 | 0.31 | 0.1  | 4.4  | 37   |      | <    | -91  |
| K4.2                            | 545   | 7.6 | 2.7  | 0.6  | 2.5    | 4.6  | 3.8  | 0.03 | 0.2  | 0.04 | 5.1  | 36   |      | 0.26 | 45   |
|                                 |       |     |      |      |        |      |      | 7    |      | 5    |      |      |      |      |      |
| K5.2                            | 545   | 7.6 | 0.5  | 0.7  | 2.65   | 4.0  | 5.3  | 0.76 | 0.63 | 0.02 | 5.4  | 38   |      | <    | -86  |
|                                 |       |     |      |      |        |      |      |      |      | 9    |      |      |      |      |      |
| K7.2                            | 534   | 7.6 | 1.1  | 0.6  | 2.65   | 5.0  | 3.9  | <    | 0.01 | <    | 6.0  | 36   |      | <    | 61   |
| <u>Lower zone</u>               |       |     |      |      |        |      |      |      |      |      |      |      |      |      |      |
| K1.1                            | 465   | 7.7 | 0.5  | 0.8  | 2.15   | 5.2  | 4.6  | 0.98 | 0.56 | 0.51 | 5.5  | 37   |      | <    | -85  |
| K3.1                            | 467   | 7.7 | 0.5  | 1.5  | 2.25   | 5.8  | 4.5  | 1.1  | 0.58 | 0.52 | 6.9  | 37   |      | <    | -105 |
| K4.1                            | 488   | 7.5 | 0.6  | 0.8  | 2.55   | 6.6  | 4.4  | 1.3  | 0.71 | 0.31 | 7.3  | 35   |      | <    | -90  |
| K5.1                            | 504   | 7.8 | 0.6  | 0.8  | 2.4    | 4.7  | 6.0  | 1.4  | 0.66 | 0.31 | 5.9  | 39   |      | <    | -128 |
| K7.1                            | 513   | 7.7 | 0.5  | 0.8  | 2.4    | 6.2  | 4.7  | 0.92 | 0.65 | 0.39 | 7.4  | 37   |      | <    | -38  |

**Table 4-2 Ground water chemistry in the vicinity of the Kappelen site.**

< indicates below detection limit,

no result – not measured.

Results from Canton Bern Laboratory, 1997, 1998.

Results of geochemical sampling at the site (Figure 4-6a through 4-6f) indicate that, between the upper and lower zone, there is a high degree of variability in REDOX-related indicators. Consistently, the Fe, Mn, Na, K, NH4 were significantly lower in the upper 8 to 9 m of the aquifer than in the lower 10 to 15 m zone. Some components of the regional REDOX studies have been erratic and hard to define with continuity throughout the area down gradient from the disposal lagoons. It was not always clear which depths samples actually represented. Mixing source vertical depths when depicting aerial concentrations in a heterogeneous gravel aquifer may not allow adequate characterization of a plume's nature and extent particularly when the setting is heterogeneous fluvio-glacial gravel and sand deposits. Figure 4-6g illustrates the degree of variability in Mn, Fe and DOC in regional wells, the Alte Aare River and the upper and lower zones at the site. We were interested in observing the extent to which parameters at the site varied over several 10s of meters. These data were compared to changes documented over wider areas of the Seeland aquifer. Results suggest that for parameters Mn and Fe (Figure 4-6a, 4-6b), the shallow and deep zones at Kappelen show considerable variability illustrating that at one site there is as much variability as is documented regionally at wells A1, A2 and 7.2.

There was under saturation with respect to oxygen in the lower part of the aquifer at the site. Installing the wells at the site created an artificial access source for oxygen. The well provided a vertical migration path for air to diffuse downward in its standing column of water. Oxygen reaching this under saturated environment caused changes in the bacterial activity in the near vicinity of the well, particularly in the lower zones. The oxygen appears to have caused relatively rapid indigenous iron and manganese oxidizing bacterial growth. Microbiological characterization of the strains that could have created the biofouling included *Siderocapsa* sp., *Gallionella* sp. and *Leptothrix* sp. The extent of the biofouling has not yet been well characterized. However, it has been sufficient, as described below, to effectively seal the well preventing mixing of this water with the naturally occurring recharge in the local ground water flow.

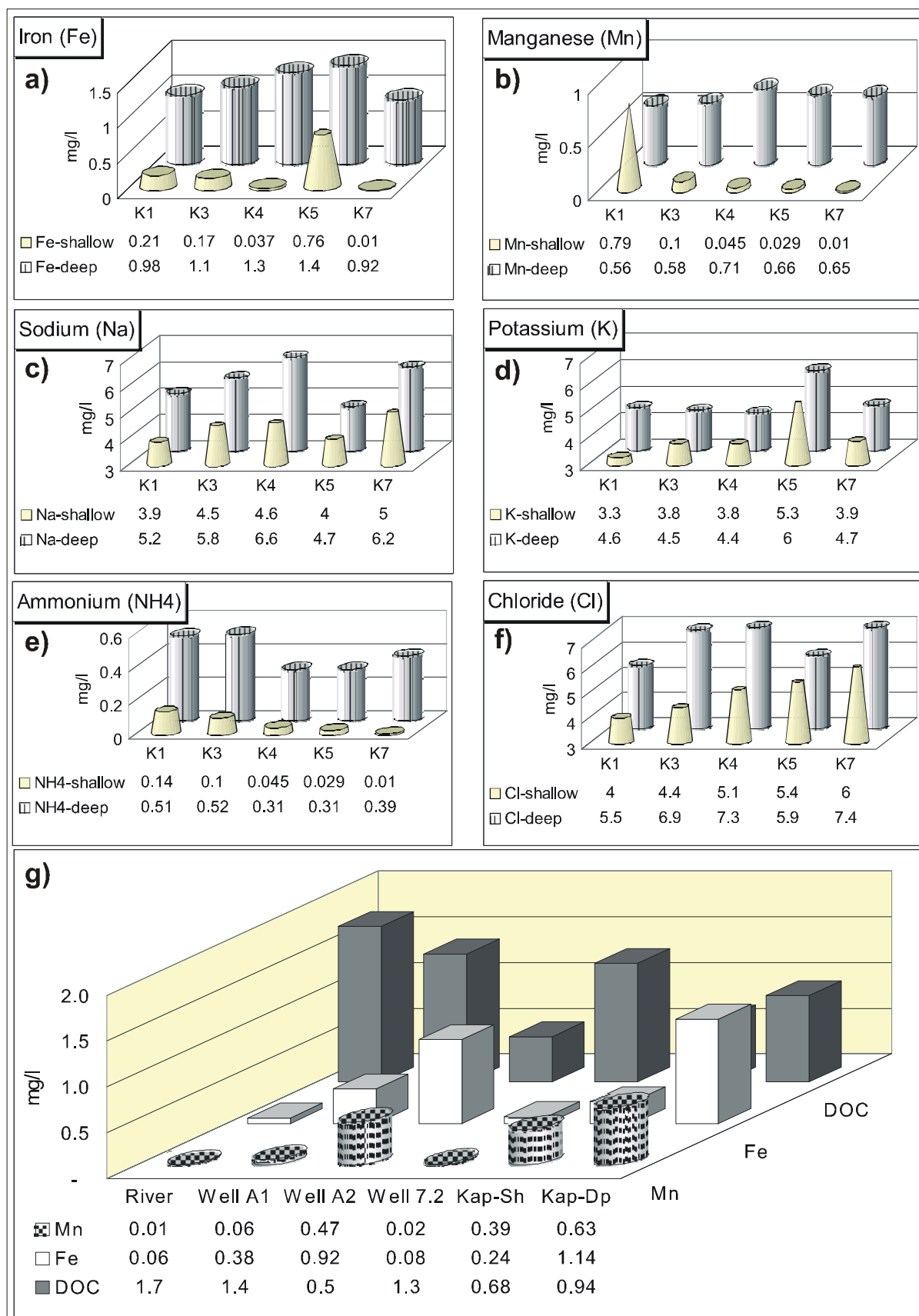


Figure 4-6 REDOX related parameter variability in the vicinity of the Kappelen site (a) Iron, b) Manganese, c) Sodium, d) Potassium, e) Ammonium, f) Chloride, g) aerial variations in Fe, Mn and DOC in the Kappelen vicinity (Data in plots a) through f) are wells at Kappelen site)).

## 4.5 Tracer Testing

The site was prepared so that tracer testing could begin in the deeper part of the aquifer in Dec. 1996. All seven deep locations were equipped with dedicated tubing sampling equipment. In addition, low volume peristaltic pumps were set up to extract from wells K2.1 and K3.1 closest to the injection well K1.1. Freezing weather precluded initiating the testing until the end of Feb. 1997. In the following 18-month period, seven tracer tests were done with five in the deep/lower and two in the shallow/upper zone. Testing focussed on the deeper zone in the first year and the shallow zone in year two. In the lower zone, the first three tests were successful in terms of defining response timing and magnitude. Tests 4 and 5 were not successful due to significant biofouling in the well screens in wells K3 and K2. Both Tests 6 and 7 in the upper zone were successful although there were only limited responses in row 2. Tables 4-3 and 4-4 summarize the tracer components, the input conditions, analytical methods used and the characterization results for the bacteriophage and uranine responses in Tests 2, 3, 6 and 7. These two tests represent the best tracer observations we have to date for the site. The test descriptions in the following section summarize the rationale and results arising from each of the seven tests undertaken. Details and analyses of tests with the significant and positive results (KAP 3, 6 and 7) are in Appendix A4-2.

| Tracer component     | Type       | Component details <sup>1</sup>                                                                                                                        | Test<br>Injected component mass |                           |                           |                                             | Analysis Method<br>(Resolution)                                                                                                                |
|----------------------|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|---------------------------|---------------------------|---------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
|                      |            |                                                                                                                                                       | KAP 2                           | KAP 3                     | KAP 6                     | KAP 7                                       |                                                                                                                                                |
| Uranine              | Solute     | Sodium fluoresceine<br>MW 376.28<br>$C_{20}H_{10}O_5Na_2$<br>44,130 nm <sup>3</sup> ,<br><i>Siphoviridae</i><br>gm (dsDNA), IP<br>(nm),<br>zp (-42.0) | 25 gm                           | 25 gm                     | 26 gm                     | 53 gm                                       | On-Line spectrofluorometry<br>CHYN/UNINE<br>Field Fluorometer <sup>2</sup><br>(~ 0.1 to 0.5 ppb resolution)                                    |
| Bacteriophage<br>H40 | Biocolloid | 24 nm <sup>4</sup> ,<br><i>Leviviridae</i> ,<br>gm (ssRNA),<br>IP (3.5, 3.9),<br>zp (nm)                                                              | 1.3e <sup>13</sup><br>pfu       | 1.5e <sup>14</sup><br>pfu | 1.7e <sup>14</sup><br>pfu | 1.6e <sup>15</sup><br>pfu                   | Bacterial host cultivation / growth<br>association,<br>plate counting - petri dish<br>(UNINE-Microbiol. Lab.)<br>(1 phage per 2 ml resolution) |
| Bacteriophage<br>H4  | Biocolloid |                                                                                                                                                       | 1.4e <sup>13</sup><br>pfu       |                           |                           | 5.7e <sup>14</sup><br>pfu<br>(Well<br>K3.2) |                                                                                                                                                |

**Table 4-3 Tracer component characteristics, analysis methods and injection conditions.** (Tests KAP 2 & KAP 3 (lower zone) and KAP 6 & KAP 7 (upper zone))

1 Colloid details are size (head, tail), morphotype (*family*), genetic material – single (ss) or double (ds) stranded, isoelectric point (pH at which the particles possess zero net charge) and zeta potential (mV – the charge balance, expressed in millivolts measured at a pH of 7.4 (at a given ionic strength) for the bacteriophages.

2 Described in P.-A. Schnegg & N. Doerfliger (1997) and similar to new equipment with the same resolution as in P.-A. Schnegg & K. Kennedy (1998).

3 Bacteriophage H40 developed for tracer studies by LAMUN, Microbiology Department, Univ. of Neuchatel (P. Rossi, 1994).

4 H.-W. Ackermann & M.S. DuBow, (1987 a, b).

### 4.5.1 Tracer Test KAP 1

KAP 1 began in late Feb. 1997 with the injection of about 80 gm of uranine and lasted about 10 days. Being the first test, its purpose was feasibility and reconnaissance, namely to document the ground water travel time to plan future tests. Injection was done by pumping about 20 L of tracer cocktail containing about 80 g of uranine into well K1.1. This was done at a rate of about 20 L/min and was followed by pumping about 40 L of clear water. Sampling concentrated on the three wells in row 1. Clear PVC tubing was used to take a vertical profile sample opposite the screen in well 4.1. We were interested in seeing if any tracer segregation would be manifest with a layer opposite discrete screened sections. Wells K2.1 and K3.1 were equipped with peristaltic pumps at the surface connected to intakes at their screen midpoint depths of about 12.5 m. The sampling flow was about 0.5 L/min. Uranine was measured every four minutes for these two wells with field fluorometers set up so that the pumped flow passed directly through the detector channel (modified from equipment described in P.-A. Schnegg and N. Doerfliger, 1997).

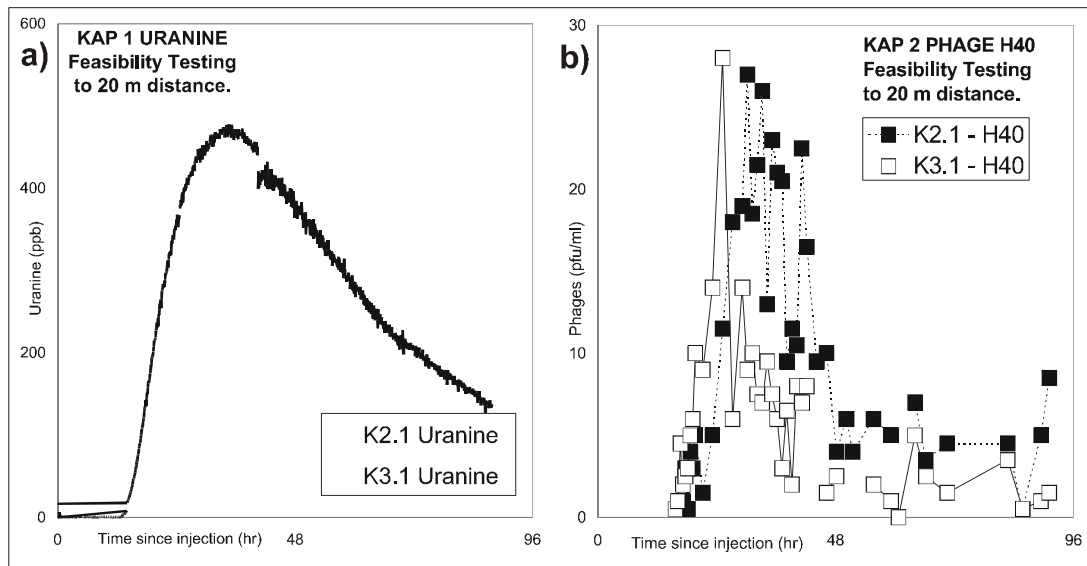


Figure 4-7 Tracer tests KAP 1 and KAP 2 concentration history at wells K2.1 and 3.1  
a) KAP 1 (Uranine) b) KAP 2 (Phage H40).

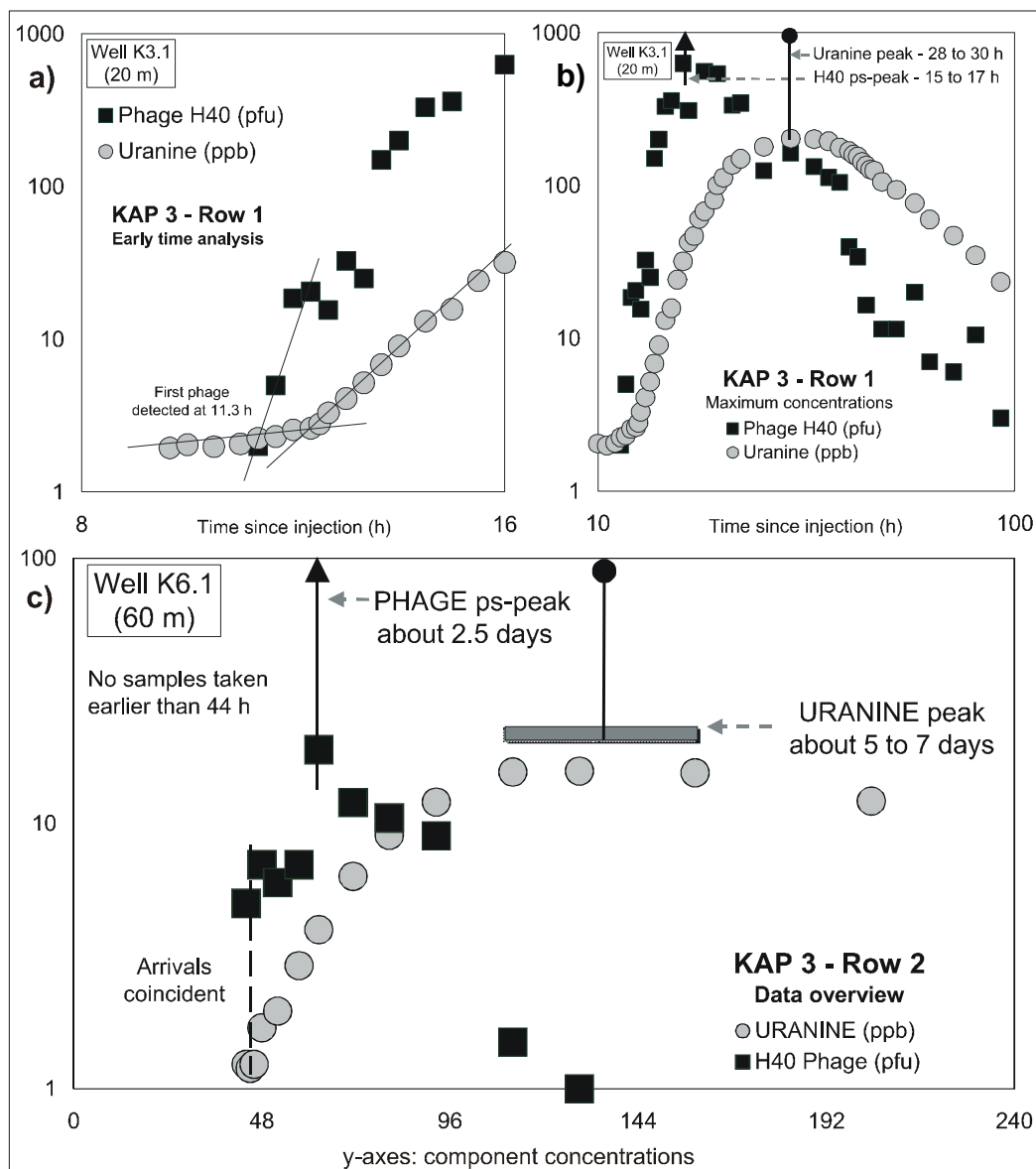


Figure 4-8 Tracer test KAP 3 concentration history  
a) Well K3.1 early time analysis  
b) Well K3.1 maximum concentrations  
c) Well K6.1 data overview.

Uranine was detected in row 1 at wells K2.1 and K3.1 (Figure 4-7a) but not at well 4.1 or any of the row 2 wells. First detection and maximum concentrations occurred at the two wells at about 14 and 40 h and 13 and 34 h, respectively. This corresponded to an arrival and transport velocity of 37 and 14 m/d, respectively. The arrival of the tracer in less than 24 hours was considered a satisfactory and encouraging response particularly considering the relatively flat hydraulic gradient (0.001).

#### 4.5.2 Tracer Test KAP 2

KAP 2 followed back-to-back KAP 1 and was carried out over about 14 days in March 1997. It was done to observe the phage efficacy and travel time to row 1 and to evaluate if the REDOX environment at the site would still allow favorable phage response. It had similar hydraulic conditions to KAP 1 but uranine levels were well above background noise. KAP 2 involved the injection of about 10 L of water containing 25 gm of uranine and two different marine phages (H4 -  $1.4 \times 10^{13}$  pfu, H40 -  $1.3 \times 10^{13}$  pfu). Injection was done by adding this solution to a volume circulating from the bottom of the well screen into a surface vessel and returning to below the water table at a rate of about 20 L/min. Sampling was done as for KAP 1 except that well K6.1 in row 2 was also equipped with a peristaltic pump and wells K5.1 and K7.1 were also sampled. Samples were taken periodically on site and transported within 12 to 24 h to the Univ. Neuchatel (LAMUN) for immediate bacteriophage analysis.

Phage H4 was not detected during the test. Phage H40 was first detected in wells K2.1 and K3.1 after about 14 h (Figure 4-7b) and continued to be present over the entire 14 d of sampling. Maximum phage concentrations reached at the two wells were about 30 pfu/ml. After 48 h, most values were at levels of less than 6 pfu/ml. Phage H40 was not detected at the other row 1 well (K4.1) nor was it detected at wells K5.1 or K7.1 in row 2. It was, however, observed to levels of about 4 pfu/ml in well K6.1 beginning about 64 h after injection and was no longer detected after about 110 h. Uranine was detected again in wells K2.1 and 3.1. The concentrations stopped decreasing in well K2.1 at about 17 h after injection reflecting the arrival of the KAP 2 injected solute at levels above the reducing background from KAP 1. The lack of testing for uranine at well K6.1 during KAP 1 precluded any comparative assessment about its behavior during KAP 2.

Results of KAP 2 were consistent with the KAP 1 results for the uranine in terms of first detection times being similar in row 1. That both phages and uranine were found at K2.1 and K3.1 and not at well K4.1 was also consistent. The first conclusion from this test was that uranine and phage H40 transport was consistent in terms of their arrival times. We did not expect similar times for the phage and solute maximum concentrations. Phage levels at both wells began to decrease after about 24 to 30 h whereas the uranine maximum values occurred at about from 36 to 52 h as during KAP 1. The second conclusion was that higher initial mass and concentrations of phages were needed to yield acceptable results. The H4 phage was not detected and the H40 phage levels did not provide sufficient resolution to well characterize their response. Finally, it was apparent that the level of attenuation of the phages to 20 and 60 m was over 99 %, as had been observed for bacteria and microspheres albeit over shorter distances in finer grained porous media at the Cape Cod site (R. Harvey et al., 1989).

#### 4.5.3 Tracer Test KAP 3

KAP 3 began May 1, 1997 and lasted 10 days. The tracer cocktail was prepared by adding 25 gm uranine and 2 L of phage H40 solution ( $1.5 \times 10^{14}$  pfu) to 8 L of water pumped from the deep zone just prior to beginning the test. Injection was done by adding this solution to a volume circulating from the bottom of the well screen into a surface vessel and returning to below the water table at a rate of about 60 L/min. The injection circulation was continued over about 30 min to ensure it was well mixed. An additional 60 L of tracer-free site ground water were pumped at a reduced rate into the well to displace the original tracer after which time it diluted under naturally conditions with flow through the well. Sampling was done with low volume peristaltic pumps at the screen mid-point at all but the injection well.

KAP 3 results are characterized by the results shown in Figure 4-8 and are quantified in Table 4-4. The early time analysis plot (Figure 4-8a) shows the row 1 well (K3.1) at which there was an apparent gradual rise in uranine levels preceding the phages first detection at about 11 h. About 1 h later, the uranine slope increased significantly and it is this slope that likely represents the uranine levels finally reaching above background variability. Figure 4-8b illustrates the breakthrough curve to 100 h

showing the maximum concentration of phages occurring at about 15 to 17 h and the uranine maximum (peak) at 28 to 30 h. In well K2.1, there was more irregularity in the phage data as had occurred during KAP 2. The phage and uranine arrival and maximum concentration times were 13 and 38 and 15 and 42 h, respectively. Maximum dimensionless concentrations (MDC) for uranine were about 2000-fold higher than for phages. Both uranine and phages were detected in well K4.1 but at concentrations 50- to 100-fold lower than in the other two row 1 wells. In row 2, uranine was detected in all three wells but only at well K6.1 were concentrations adequate for its response to be characterized. Phages were detected at K6.1 and K7.1 but were of sufficient magnitude only at K6.1 to characterize the response. The H40 and uranine response at K6.1 (Figure 4-8c), while not as well defined as in row 1, showed both components with similar early time or near-arrival patterns after about 44 h. Phage and uranine maximums were at about 2.5 d and over a broader range of 5 to 7 d, respectively. The MDC difference increased compared to row 1 with uranine about 7000-fold greater than H40. The uranine breakthrough data correspond to arrival and transport velocities of about 29 to 53 and 14 to 21 m/d in both rows, respectively.

| Down gradient well | Distance from injection (m) | Uranine response        |                  |                   | Bacteriophage H40 response |                           |                 |                   |                     |                        |
|--------------------|-----------------------------|-------------------------|------------------|-------------------|----------------------------|---------------------------|-----------------|-------------------|---------------------|------------------------|
|                    |                             | $t_{tr-arr}$ (h)        | $t_{tr-max}$ (h) | $(Tr/Tr_0)_{max}$ | $t_{c-arr}$ (h)            | $t_{c-max}$ (ps-peak) (h) | RF <sup>1</sup> | $(C/C_0)_{max}$   | RB <sup>2</sup> (%) | Att'n <sup>3</sup> (%) |
| Test 3             |                             |                         |                  |                   |                            |                           |                 |                   |                     |                        |
| K2.1               | 21                          | 15                      | 42               | 2e <sup>-03</sup> | 13                         | 38                        | 0.9             | 9e <sup>-07</sup> | 0.02                | 99.98                  |
| K3.1               | 20                          | 11                      | 29               | 2e <sup>-03</sup> | 11                         | 16                        | 0.6             | 1e <sup>-06</sup> | 0.02                | 99.98                  |
| K4.1               | 21                          | 22                      | 36               | 2e <sup>-05</sup> | Not adequately sampled     |                           |                 |                   |                     |                        |
| K5.1               | 63                          | Present, not quantified |                  |                   | None detected              |                           |                 |                   |                     |                        |
| K6.1               | 60                          | < 44                    | 129              | 2e <sup>-04</sup> | < 44                       | 62                        | 0.5             | 3e <sup>-08</sup> | 0.005               | 99.995                 |
| K7.1               | 63                          | Present, not quantified |                  |                   | Present, not quantified    |                           |                 |                   |                     |                        |
| Test 7             |                             |                         |                  |                   |                            |                           |                 |                   |                     |                        |
| K2.2               | 21                          | None detected           |                  |                   | 18                         | 30                        | na              | 3e <sup>-10</sup> | Na                  | na                     |
| K3.2               | 20                          | 13                      | 83               | 1e <sup>-03</sup> | 12                         | 27                        | 0.3             | 2e <sup>-06</sup> | 0.01                | 99.99                  |
| K4.2               | 21                          | 14                      | 89               | 1e <sup>-05</sup> | 11                         | 17                        | 0.2             | 6e <sup>-08</sup> | 0.01                | 99.99                  |
| K7.2               | 63                          | ~144                    | ~600             | 5e <sup>-06</sup> | None detected              |                           |                 |                   |                     |                        |

**TABLE 4-4 Tracer component breakthrough characteristics for phages and uranine.**

(Tracer tests KAP 3 (lower zone) and KAP 7 (upper zone)).

1 RF: Retardation Factor is the ratio of the time of maximum particle or colloid (in our case phage) concentration compared to that of the solute.

2 RB: Relative breakthrough as defined in R. Harvey et al. (1989).

3 Att'n: Attenuation % is 100 % – RB %.

Recovery calculations for phages based on relative breakthrough compared to uranine (Table 4-4) were 0.02 % for both wells in row 1 and about four times lower at 0.005 % for well K6.1. Relative breakthrough integrates the particle component responses over time and compares this total relative recovery to the integrated solute response assumed to represent 100 % recovery. In KAP 3, the MDC and RB factors decreased consistently by a factor of 3.5 to 4 over the 40 m from row 1 to row 2 wells. Retardation factor compares the time at which the particles reach their maximum with the time of maximum solute concentration. The phage maximum concentrations occurred consistently earlier than the solute maximums in this test and the RF values were 0.9, 0.6 and 0.5 at wells K2.1, K3.1 and K6.1, respectively.

#### 4.5.4 Tracer Tests KAP 4 & KAP 5

Test 4 and 5 were done in Aug. 1997 about 3 months after completion of KAP 3. The specific objective of the testing was to compare the response of 1-micron diameter fluorescent microsphere colloids with phages and uranine. Neither test was successful in that the injected component responses were either non-detectable or too low to characterize the breakthrough. The test using the microspheres was rescheduled and performed at another nearby test site, Wilerwald in Aug. and Sept. 1997.

In KAP 4, no uranine and no microspheres were detected over about one week. Phage detection was intermittent in the first two days of sampling at row 1 wells. This was in clear contrast to the patterns well established with the KAP 3 results. No response was observed in row 2 despite more frequent sampling and increased injection concentrations. The test was terminated after about 10 days and restarted as KAP 5 about a week later. In KAP 5, similar negligible responses from phages were observed and no uranine and microspheres were detected over 5 days. At this time we acknowledged that there was clearly a problem and something was taking place we did not understand. We terminated the test realizing that the lack of detected colloids was potentially reasonable, but the failure to detect uranine was not. The water levels were lower than during KAP 3, but the gradient had not changed. Our first action was to begin pumping at about 100 L/min from the K2.1 and K3.1 wells in Row 1 and the K6.1 well in row 2. K3.1 yielded water that was clear for the initial two min, became dark brownish-red with a defined residue for the next two min and was followed by water with a fluorescent green color indicating uranine in substantive concentrations. Well K2.1 yielded a similar red color and residue but no uranine was observed. Red coloration from well K6.1 lasted only a few seconds and no uranine was observed. We concluded that the KAP 4 and KAP 5 limited response had likely been caused by biofouling of the well screens. The extent of the biofouling was surprising in that it had been complete enough that the injected tracer components were prevented from entering the well. They were discovered in close proximity when active pumping took place, but had been blocked from entry by the bacterial growth.

#### **4.5.5 Tracer Test KAP 6**

KAP 6 and KAP 7 undertaken at Kappelen assessed the response characteristics of phages and uranine in the upper zone to depths from about 4 to 8 m. Pumping in the seven shallower wells had not historically shown any noticeable red discoloration. We undertook the upper zone tests hoping that to an 8 m depth, there would be a less reactive response to the introduction of oxygen than what we had occurred in the 10 to 15 m lower zone.

Tracer Test KAP 6 was similar to KAP 1 and KAP 2 in that it was a preliminary feasibility test to scope the response patterns of the upper zone. Phage H40 ( $1.7 \times 10^{14}$  pfu) and uranine (26 gm) were injected in well K1.2 on Nov. 4, 1997 and sampling carried on through Nov. 13, 1997. Sampling was done using a closed circuit circulation system. In this situation, rather than pumping from a particular depth at a low rate, the well water was circulated opposite the entire length of the screened zone thereby creating a fully mixed column of water. Flow was about 4 L/min suggesting a cycle of return for the well screen volume was from 10 to 12 min. Sampling was done for the bacteriophages by removing the required 80 ml volume from an in-line 3-way valve. The circulation flow could also be connected to the field fluorometer as in previous testing to obtain a real-time readout of the uranine concentrations. Injection was done using a similar basic system. The tracer cocktail volume flowed under Venturi conditions into the circulating line equipped with a 3-way valve. Injection circulation continued over about 4 days at which time the phage and uranine concentrations were approaching 10 pfu/ml and 1 ppb, respectively.

Both phages and uranine were detected in row 1 at the three wells but neither component was detected in row 2. The components were transported in a more northeasterly direction than had occurred in the lower zone. Phage and uranine initial detection was at about 12 and 15, 15 and 20 and 20 and 25 h at wells K4.2, K3.2 and K2.2, respectively. The concentration of phages reached a maximum at about 17, 21 and 26 h. Uranine peaks at these wells were less regular at 90, 65 and 45 h. The uranine response at well K2.2 was not well characterized. Phage concentrations were about 10-to 50-fold too low for optimal counting in row 1 and neither component was detected at row 2.

Conclusions from KAP 6 were that: no biofouling had impacted the testing, phage and uranine responses were able to be characterized at K4.2 and 3.2; phage response but not uranine could be characterized at K2.2; the direction of flow was different than in the lower zone; and, the phage injection concentrations should be increased with the mass injected over longer periods of time to establish a better breakthrough response pattern to characterize.

#### 4.5.6 Tracer Test KAP 7

KAP 7 began on Jul. 12, 1998 and lasted over 3 months. The tracer cocktail was prepared by mixing uranine (53 gm) and phage H40 ( $1.6 \times 10^{15}$  pfu) in about 20 L of solution which was injected under Venturi effect with circulation conditions over a period of 1.5 h at well K1.2. Circulation continued in the injection well over a period of about 2 days at which time uranine concentrations approached 1 ppb. H40 phage levels had decreased to about 1200 pfu/ml and remained in the range from about 350 to 2350 pfu/ml over the duration of testing. After 24 h, 38.5 L of solution with phage H4 ( $5.7 \times 10^{14}$  pfu) was injected in the first row well K3.1 using a similar method. The objective of adding the second phage was to enhance the possibility of its detection in the second row at a reduced distance of 40 rather than 60 m. Continuous monitoring of the uranine response was done at different periods at the three upper zone row 1 wells and at two row 2 wells. Sampling was done as described for the KAP 6 test. Phage sampling was done over about 10 days at all 6 wells. Uranine was measured over the full 10 days at all wells and then continued for about one and two months in wells K7.2 and K3.2, respectively.

Figure 4-9 shows five plots with different aspects of the KAP 7 test component response characterization. Table 4 summarizes the quantified aspects of these responses. Figure 4-9a shows the test record for all components detected in wells K3.2 and K4.2 in row 1. Phages were also detected in well K2.2 but only with a minor response several orders of magnitude less than in well K3.2 (Figure 4-9b). The first 6 days of the test response in row 1 illustrate that the breakthrough responses for both phages and uranine were distinct at wells K3.2 and K4.2 (Figures 4-9b, 4-9c). Phages and uranine were first detected in these two wells after 12 and 13 and 11 and 14 h, respectively. The maximum phage and uranine concentrations occurred at about 27 and 83 h and 17 and 89 h, respectively. The uranine response at well K3.2 (Figure 4-9d) illustrates that the technique of long term response characterization was effective using the closed circuit circulation combined with continuous online recording of uranine with either a surface or downhole (P.-A. Schnegg & K. Kennedy, 1998) field fluorometer unit. Only short term pump-related interruptions in the data occurred. MDCs for uranine were in the range of  $1 \times 10^{-3}$  and  $1 \times 10^{-5}$  at K3.2 and K4.2, about 640- and 260-fold higher than for phages. We observed that uranine detection was better using the newly designed downhole fluorometer located in the well directly and that it attained a resolution of about 0.1 ppb within the continuous circulation closed-circuit system.

At well K2.2, the phage maximum occurred at 18 h although its record is not considered sufficient for component response characterization at this location (Figure 4-9b). The H40 MDC at K2.2 was about 5000-fold lower than at K3.2. In row 2, neither the H40 phages injected at well K1.2 nor the H4 phage injected in well K3.2 were detected. Uranine was detected only at well K7.2 (Figure 4-9e) after about 5 days. It continued a slow and consistent increase to levels of about 2.6 ppb and stayed in this relative range over about 6 days at which time it began to decrease. MDC for the uranine at well K7.2 was about  $5 \times 10^{-6}$ , about 3-fold lower than at K4.2 and 200-fold lower than at K3.2. Monitoring ended earlier than desired at K7.2 due to other equipment commitments. The K3.2 and K4.2 uranine breakthrough data correspond to arrival and transport velocities of about 46 and 7 m/d in both rows, respectively. They would be a factor of 2 to 4 lower in row 2 based on the K7.2 response characterization.

Phage recovery at wells K3.2 and K4.2 in row 1, based on relative breakthrough compared to uranine (Table 4-4), was about 0.01 %. The site monitoring ended before there was a complete uranine recovery at either well. Therefore, these recovery numbers represent upper limits and could likely decrease by as much as a factor of 2. There was no phage recovery in row 2. Phage maximum concentrations consistently occurred earlier than the solute maximums in this test. RF values were 0.3 and 0.2 at wells K3.2 and 1 and K6.1, respectively.

Conclusions from both the KAP 6 and KAP 7 tests were that phage and uranine responses were well characterized at the row 1 distance of about 20 m but that neither phage nor uranine response was adequate at 60 m, even with a dual and prolonged injection of phages. Compared to the lower zone, the transport direction was more easterly and the solute mean transport velocity was 2- to 4-fold lower. First detection time of both tracers was similar and that the phages being detected earlier may be more dependent on the detection limit differences than on preferential colloid transport occurring in this setting.

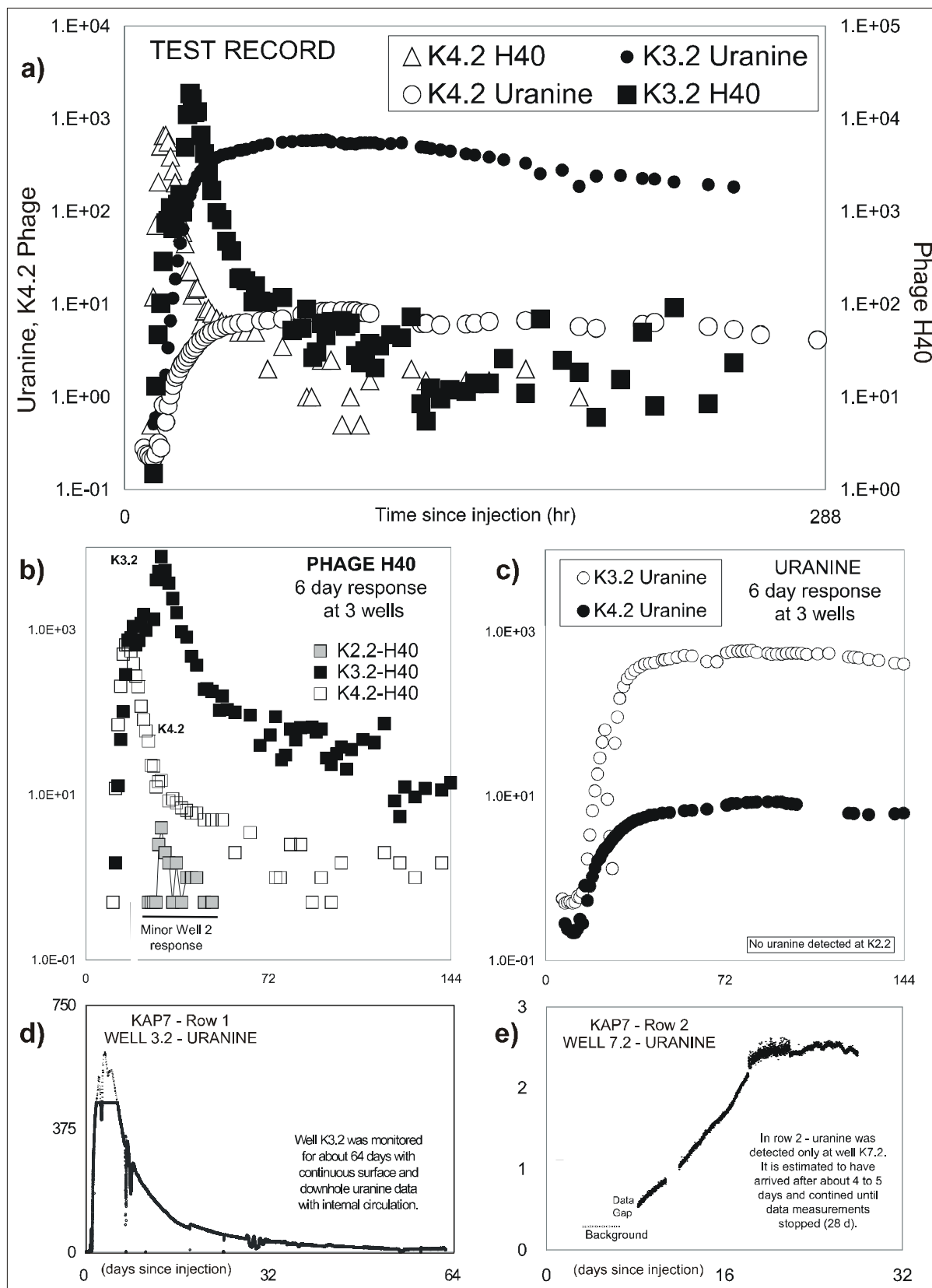


Figure 4-9 Test KAP 7 concentration history (Y-axis UNITS: Phages – pfu ml<sup>-1</sup>, uranine – ppb)

- Test record for K3.2 and K4.2 - uranine and H40,
- Phage H40 - 6 day response at K2.2, K3.2 and K4.2,
- Uranine - 6 day response at K2.2 (none), K3.2 and K4.2,
- Uranine - K3.2 (row 1) two month continuous record,
- Uranine - K7.2 (row 2) one month continuous record.

#### 4.6 **KAPPELEN - Summary and Conclusions**

Characterization of the ground water environment at the Kappelen site began by using surface geophysical exploration methods. Results from VLF-EM and RMT techniques allowed development of a conceptualized geologic model depicting the nature and extent of the relatively prolific gravel-dominated aquifer found beneath the site. This model suggested a highly permeable aquifer extended over the investigated 100 by 100 m area, that it had a high degree of continuity and that its thickness varied principally from about 15 to 18 m. Underlying this was a lower permeability zone - an aquitard, that at this site did not have the dramatic changes in relief that had been observed at other fluvial-glacial settings nearby in the region. Drilling for well installation confirmed that the actual depths to the aquitard were generally within one meter of the geophysical interpretation. The good correlation between the geophysics and the geologic data illustrates that these methods can be relied upon in this setting to distinguish between the two contrasting lithologic types and arrive at a reasonable estimate of the aquifer thickness.

Hydrogeologic characterization started with updating the regional and local water levels to document the aquifer's potentiometric surface. A relatively stable and flat gradient of about 0.0005 to 0.001 has existed during the site research. Confirming the ground water flow direction and magnitude was followed by the installation of two piezometers and 16 wells in the upper and lower parts of the aquifer. The wells were aligned along the expected northwesterly gradient over a length of 90 m and a width of 40 m. Localized pumping in each well was principally relied upon to arrive at estimates of hydraulic conductivity ranging from  $1\text{e}^{-04}$  to  $1\text{e}^{-02}$  m/s. Travel times observed in the tracer testing imply that there may be preferential pathway lenses in the gravel structure that are more transmissive than the higher value in this range.

Biogeochemical characterization of the ground water began by defining the REDOX conditions as they existed particularly in the lower part of the aquifer at the site. Site wells may have inadvertently provided a path for oxygen to migrate by diffusion to the base of the aquifer. This oxygen could have led to the bacterial biofouling that has been documented. These conditions may continue to have an impact and if so could affect the ability to successfully undertake tracer tests in the lower zone. High rate pumping and possible treatment or specialized well and cleaning of the well screen and surrounding gravel pack will likely be required for future testing in this lower zone at the site to be successful.

Bacteriophage H4 response has not been able to be well defined and it may not be suitable for use at this site. However, bacteriophage H40 could be defined and its response, along with uranine have been well characterized to distances of about 20 m (row 1). Both the particle and solute tracer component responses in the two zones exhibited different transport direction and velocity characteristics. To 60 m, there was less detection of either phages or uranine and precise arrival times have not been well documented. Only one well in each zone to date has had a response adequate to characterize tracer component behavior. The lack of a greater detection of at least the solute uranine in row 2 is surprising unless there are preferential pathways between the wells and the dispersion expected is not occurring effectively outside these channels to create a wider plume at that distance. Regardless, longer injection times on the order of days should be anticipated along with increased phage mass in order to be able to observe and better characterize the phage responses to distances greater than 20 m.

Phage and solute initial detection times were not sufficiently different to conclude whether or not there was clear evidence of phages undergoing preferential advection compared to the solute. Solute velocities based on the time of maximum concentration ranged from about 14 to 21 m/d and 3 to 7 m/d in the lower and upper zones, respectively. Transport velocities based on arrival times were 2- to 4-fold higher. MDCs for the phages were from 2- to 4- orders of magnitude lower than for the solute. Phage maximum concentrations consistently occurred substantially earlier than those of uranine consistent with diminishing mass source along the transport pathway due to particle adsorption/attenuation. Retardation Factor values were from 0.2 to 0.9. Phage H40 recovery, based on relative breakthrough normalized to uranine recovery, was about 0.02 % and 0.005% in the lower zone at 20 and 60 m distances, respectively. In the upper zone, it was 0.01 % at two locations at about 20 m. Accordingly, the phages have been attenuated to in excess of 99.9 %. Despite large attenuation, the H40 phage responses in KAP 3 and 7 have illustrated that bacteriophages do have consistent and continuous breakthrough patterns and that with adequate planning and resources even in difficult field settings, these can be well defined. This suggests that the Kappelen site's use, given that the REDOX conditions remain understood and controlled, could likely be expanded to conduct further more detailed biocolloid/particulate migration and adsorption process research in its special subsurface environment.

## **Chapter 5**

### ***Results of Bacteriophage, Microsphere and Solute Tracer Migration Comparison at Wilerwald Test Field (BE), Switzerland<sup>1</sup>***

#### **Abstract**

Migration behavior of three viral-sized bacteriophages (H40, MS2, Psf2) and a 1 micron diameter fluorescent latex bead (all with negative surface charges) were compared to the solute, uranine. Testing was done in a heterogeneous, well-channeled fluvial-glacial gravel aquifer at the Wilerwald site in western Switzerland. Injection was into a preferential flow layer between 4 and 6 m below ground and down gradient measurements were made in three wells to 64m. On site real time results allowed optimizing the sampling strategy, minimized the test duration and reduce analytical needs. Prototype instruments were successfully used under routine field conditions to provide direct inline measurement of colloids (microspheres with laser particle detection) and uranine (spectrofluorometry). Component resolutions were 0.1 microspheres/ml (latex), 0.5 to 1 ppb (uranine) and 0.5 pfu/ml (phages). The four colloids were consistently detected at similar times at each of two 'channel' (C6-14 m, D7-64 m) and an 'off-channel' (CD-34 m) wells. Average arrival velocities were 265 and 180 m/d, respectively. There was no conclusive evidence of preferential colloid advection although it may have occurred at 64 m. Average ground water velocity in the channel, based on the observed solute maximum values, decreased from about 135 m/d at 14 m to about 80 m/d at 64 m. Off channel, the average velocity was 40 m/d. Colloid maximum concentrations (pseudo-peaks) occurred at similar times at each well and were always earlier than the solute peaks. Phage relative breakthroughs (RB) were high reflecting the relatively open and clay-free pathways and large grain size in the gravel aquifer channels. They varied considerably from 16 to 41 % and 0.2 to 3 % for the phages at 14m and at both 34 and 64 m, respectively. Microsphere RBs were about 9 percent at both the C6 and D7 wells. The H40 phage had 2- to 10-fold higher recovery than the MS2 and Psf2 phages that had similar RB values at each well. Colloid concentrations normalized to the injected mass were typically 2.5 to 3 orders of magnitude lower than uranine. All phages exhibited change in their recovery behavior with relatively consistent tailing patterns influencing the late time responses. Numerical interpretation of the response at D7 was done using a 1-D finite difference code (CoTrans) based on solute migration in a dual porosity (mobile/immobile) model and a reversible sorption mechanism for particle transport. Colloid breakthroughs were used to calculate dispersivity (2.5 to 3.5 m) thereby reducing its possible range if determined only by matching solute data.

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<sup>1</sup> This chapter forms the basis of a paper (K. Kennedy, S. Niehren, P. Rossi, P.-A. Schnegg, I. Müller and W. Kinzelbach, 2001) published in *Beiträge zur Hydrogeologie*, Jargang 2001, Band 52, pp. 180-210 (Tracer studies in the Unsaturated Zone and Groundwater - Investigations 1996-2001, Eds. M. Probst & H. Zojer, Joanneum Research, Graz 231p).

## 5.1 Introduction

Comparing the behavior of colloids and solutes as tracers in heterogeneous porous media aquifer environments is complicated by a variety of factors related to the aquifer materials, water chemistry, and tracer interactions with both the solid and liquid phases with which they come into contact. Laboratory experiments tend to show idealized responses from isolated variables being evaluated rather than the composite effects that are inherent in any real world set of conditions. Field tests are the basis for documenting actual transport conditions and providing a base to better simulate and calibrate predictive transport models. The site layout and geophysically interpreted hydrogeological heterogeneity of the Wilerwald site where this work was carried out in the fall of 1997 is in Figure 5-1.

### 5.1.1 Objectives & rationale

The primary purpose of our work at this site was to document and evaluate the nature and extent of transport and attenuation of different types of phages and compare their behavior with a conventional solute tracer. We coordinated with P-A. Schnegg from the Hydrogeology Center at the University of Neuchatel and S. Niehren and W. Kinzelbach from ETH's Water Resources Management Department to evaluate field instrumentation measuring uranine and synthetic colloidal particles, respectively, during actual testing

The rationale associated with this work was three-fold. First, by using both particles and a solute tracer at the same time, we would better understand and characterize the transport-related phenomena of the subsurface media. Second, bacteriophages can mimic the manner and extent to which viral particles are removed from the environment during transport. One of the phages used was MS2 that has seen relatively widespread use internationally as a viral surrogate but has been restricted in its use here in Switzerland due to its genetically altered source. We wanted to compare that phage's performance with a marine bacteriophage, H40 that had been developed and for the most part used for particle migration studies in karst and river settings in Switzerland. Third, the sampling strategy in such testing activities is commonly difficult to determine beforehand. The use of online instrumentation to measure in real time both the particle and solute tracer concentrations at the wells allowed a focussed and more efficient approach in addition to dramatically reducing the time required to complete the work.

The four specific objectives of the tracer testing work done during this specific activity at Wilerwald were:

- to document and compare the behavior of three different bacteriophages,
- to evaluate the use of continuous flow through a field fluorometer and a prototype fluorescent microsphere counter under field/environmental conditions,
- to document and compare the response of four different particle types with a conservative solute tracer, uranine, and,
- to evaluate real-time on-line measurements as a technique for optimizing field tracer test procedures and sampling strategies.

### 5.1.2 Project scope

The scope of work involved three basic steps – preparation, testing and data analysis. First, the site was prepared for use. Well conditions were documented. Wells from the injection site to a distance of about 75 m were pumped to determine which were suitable for use and to clean out any residuals that had accumulated since the last testing by Rossi in 1995 (P. Rossi *et al.* 1998) and Hadi in 1995, 1996 (S. Hadi, 1997). Samples of water were submitted for bacteriophage analysis to determine if there was any background level in the water remaining since the last testing by Rossi in 1995. After evaluation of the pre-testing results, three wells were selected for the field trial and the site was equipped with power and a temporary shelter. Instrumentation in the wells was emplaced and tested. Second, the field testing activity was undertaken over a period of about two weeks during which time samples for uranine and microspheres were analyzed in the field and phages in an off-site laboratory. Third, the data were analyzed using both quantitative comparisons and a numerical modeling approach (S. Niehren, 1998).

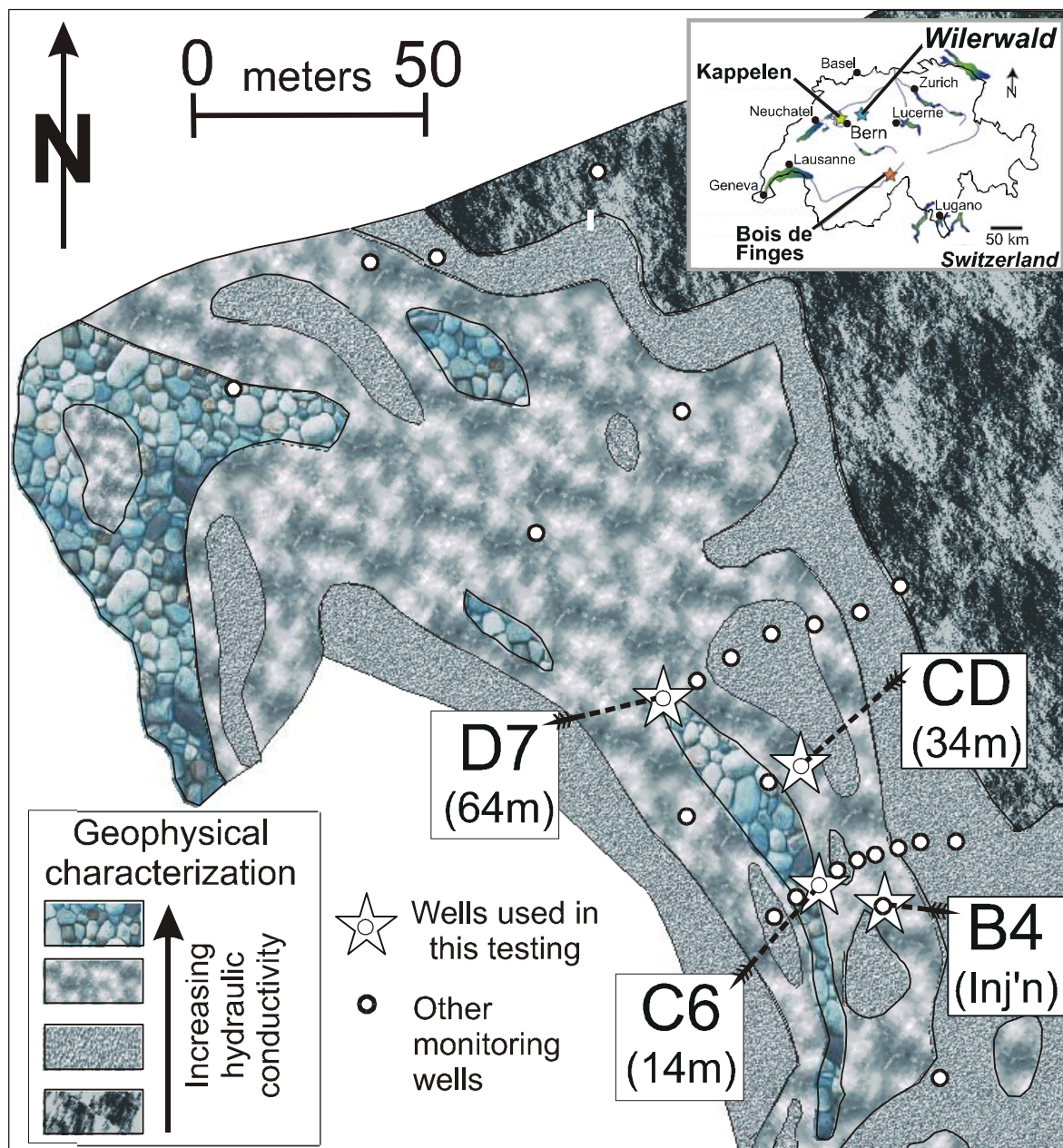


Figure 5-1 Site location map including geophysical interpretation (after A. Carvalho Dill, 1993).

## 5.2 The Wilerwald Site (Canton Bern, Switzerland)

### 5.2.1 Hydrogeologic setting

The purpose of this section is to describe the site and its characterized subsurface as it relates to a setting for migration of particles and solute tracers during the test. The site, located in the Emme valley in Canton Berne about 6 km south of Solothurn, is in a flat-lying forested area (wald) just outside the village of Wiler at an elevation of about 465m. It was instrumented originally in 1986 by driving slotted metal pipes into the ground to depths of between about 8 and 12 m. Subsequently additional metal slotted pipes were installed and a single well, CD was cored and instrumented with 2-inch OD PVC tubing (Table 5-1).

| WELL <sup>1</sup> | DEPTH<br>(m) | Internal<br>Diameter<br>(mm) | Construction <sup>2</sup> | COORDINATES    |                | MEASURED WATER DEPTH |             |             |
|-------------------|--------------|------------------------------|---------------------------|----------------|----------------|----------------------|-------------|-------------|
|                   |              |                              |                           | EAST           | NORTH          | 26-Aug               | 30-Aug      | 6-Sep       |
| <b>B4</b>         | <b>11.3</b>  | <b>32</b>                    | <b>Driven - metal</b>     | <b>609.766</b> | <b>222.05</b>  | <b>3.56</b>          | <b>3.57</b> | <b>3.57</b> |
| C0                | 4.5          | 32                           | Driven - metal            | 609.783        | 222.066        | 3.41                 | 3.41        | 3.41        |
| C1                | 4.4          | 32                           | Driven - metal            | 609.776        | 222.066        | 3.50                 | 3.50        | 3.51        |
| C2                | 11.8         | 32                           | Driven - metal            | 609.77         | 222.065        | 3.58                 | 3.58        | 3.58        |
| C3                | 10.4         | 32                           | Driven - metal            | 609.766        | 222.064        | 3.58                 | 3.58        | 3.58        |
| C4                | 12.2         | 32                           | Driven - metal            | 609.761        | 222.062        | 3.49                 | 3.49        | 3.49        |
| C5                | 12.5         | 32                           | Driven - metal            | 609.757        | 222.06         | 3.39                 | 3.40        | 3.40        |
| <b>C6</b>         | <b>11.8</b>  | <b>32</b>                    | <b>Driven - metal</b>     | <b>609.753</b> | <b>222.057</b> | <b>3.38</b>          | <b>3.39</b> | <b>3.38</b> |
| C7                | 12.1         | 32                           | Driven - metal            | 609.749        | 222.053        | 3.53                 | 3.53        | 3.53        |
| C8                | 12.2         | 32                           | Driven - metal            | 609.744        | 222.05         | 3.40                 | 3.40        | 3.41        |
| <b>CD</b>         | <b>12.3</b>  | <b>51</b>                    | <b>Borehole -PVC</b>      | <b>609.749</b> | <b>222.083</b> | <b>3.40</b>          | <b>3.39</b> | <b>3.39</b> |
| D1                | nd           | 32                           | Driven - metal            | 609.771        | 222.12         | 3.28                 | 3.28        | 3.28        |
| D2                | nd           | 32                           | Driven - metal            | 609.762        | 222.113        | 3.26                 | 3.26        | 3.26        |
| D3                | nd           | 32                           | Driven - metal            | 609.753        | 222.111        | 3.25                 | 3.25        | 3.26        |
| D4                | nd           | 32                           | Driven - metal            | 609.743        | 222.109        | 3.26                 | 3.26        | 3.26        |
| D5                | nd           | 32                           | Driven - metal            | 609.734        | 222.104        | 3.35                 | 3.35        | 3.34        |
| D6                | nd           | 32                           | Driven - metal            | 609.727        | 222.099        | 3.34                 | 3.34        | 3.35        |
| <b>D7</b>         | <b>12.3</b>  | <b>32</b>                    | <b>Driven - metal</b>     | <b>609.72</b>  | <b>222.095</b> | <b>3.27</b>          | <b>3.26</b> | <b>3.27</b> |

**Table 5-1 Site well characteristics and water level measurements.**

<sup>1</sup> Wells in bold, larger font are those used during this testing.

<sup>2</sup> Construction -driven metal slotted pipe or at CD – slotted PVC casing installed in the borehole.

The site has been used for various ground water research activities over the past 14 years (M. Sansoni *et al.*, 1987, 1988; Ch. Leibundgut *et al.*, 1992; A. Carvalho Dill, 1993 and S. Hadi, 1997). A. Carvahlo Dill (1993) describes well the setting and the rationale for the development of the site with its current ground water instrumentation. Regionally, the aquifer consists of Quaternary gravel present to variable depths from ten to about 50 m and comprised of gravel with sand and more coarse-grained fractions (pebbles, cobbles and boulders) and rarely with silt. Its composition and high degree of lithologic variability reflects its glacial provenance derived from sedimentation and erosion cycles in an alpine foreland of the Swiss Central Plateau. At a site scale, despite considerable interest and tracer-related work, there has been little lithologic documentation. The original assumption was that ground water flow and transport in the aquifer at a 100 to 200 m site scale would be relatively homogeneous and isotropic. Lithology was originally expected to be sandy gravel lying between lower permeability alluvial sediments and underlying lacustrine sediments. Accordingly, the only site borehole was at (CD on Figure 5-1) and the lithology at this location was determined only in 1992 - in part to try to understand why earlier tracer test results could not be well matched with model results.

Lacking sufficient lithologic details, variability in the aquifer subsurface materials was subsequently approached using surface and downhole geophysical investigations. The variability in hydraulic conductivity, both vertically and laterally at the site, is extensive. Significant changes in transport pathways based on interpreted lithologic variability are now believed to be on the 1 to 10 m scale.

This was determined based principally on interpretations of the limited geologic record, piezometer penetration records and the geophysics done by A. Carvalho Dill (1993).

Carvalho Dill's extensive characterization works at the site showed compelling geophysical interpretations over the instrumented and surrounding area. She interpreted aerial patterns observed from extensive and closely spaced Radio-Magnetotelluric Resistivity (RMT-R) surveys. Interpreted results indicate preferential pathways present in the form of glacial-era deposited paleo channels as well as lower permeability islands of silts and clays along and within these more highly transmissivity zones. Vertically, due to the lack of borehole data for model calibration, more detailed interpretations were not possible. Despite this work, however, the resolution of the geophysics, particularly vertically, likely does not allow a reasonable approximation of the details in transport pathways that occur in short distance at the site.

Water levels have historically been measured at from about 1.5 to 4 m below ground surface and varies seasonally. Hydraulic gradient conditions at the site are generally regarded as high and have been documented at about 0.004 m/m. Similar gradient conditions were apparent during our work.

The hydraulic conductivity at the site was determined at 21 monitoring well locations during the original work at the site as reported by Sansoni *et al.* in A. Carvalho Dill (1993). Values of transmissivity (T) were calculated for each of these wells using methods for single well pump tests. Each well was pumped at two rates over a relatively short duration and measurements were made of the stabilized drawdown. T values were calculated assuming the mean aquifer thickness and calculated values ranged over about two orders of magnitude from about  $4 \times 10^{-3}$  to  $4 \times 10^{-1} \text{ m}^2/\text{s}$ . Associated hydraulic conductivity (K) values were of from  $6 \times 10^{-4}$  to  $5 \times 10^{-2} \text{ m/s}$ . These K values, however, based on using thinner effective contributing layer thickness contributions could be much as 20 times higher.

### 5.2.2 Previous Site Tracer Testing and related activities

S. Hadi (1997) summarizes the work that was done previously at the site with works reported by M. Sansoni *et al.* (1988), J. Mägdefessel (1990) and A. Carvahlo Dill (1993). In 1995 and 1996, the Universities of Neuchatel and Freiburg both continued use of the site for focussed feasibility studies of a) biocolloid particle migration in porous media (P. Rossi *et al.*, 1998) and b) newly developed fluorescent tracers (S. Hadi, 1997), respectively.

Consistent observations and conclusions coming from the prior tracer work were that the research field was highly heterogeneous. There was no apparent or readily defined geometry for the porous media aquifer that had been well enough documented to explain the irregularity and variability in transport phenomena manifest at the site with these tests.

Carvalho Dill summarizes the early idealized and analytically derived solution attempts at modeling the site transport by M. Sansoni *et al.* (1988) and J. Schneider (1991), respectively, and their approach to match the earlier tracer test results. She concluded that attempts based on continuous layer or the necessarily idealized conditions associated with analytical solutions were problematic.

### 5.2.3 Current research perspective

Our research furthered earlier work at Wilerwald by Carvalho Dill and Rossi (P. Rossi, 1994; P. Rossi *et al.*, 1994) that established bacteriophages as feasible to use to document transport behavior of viral sized particles in porous media. We selected the Wilerwald site specifically to undertake a detailed sampling program at three wells – two believed to be located in the high permeability paleo channel (C6, D7) and one situated just off the defined channel but still in gravel with sand (CD). Our testing was clearly targeted at documenting behavior and evaluating the efficacy of real time online solute and particle detection analysis methods. It was not a further attempt to assess the existing and remaining high degree of uncertainty associated with transport conditions in the aquifer heterogeneity at the site. The earlier bacteriophage feasibility testing had been limited in that it did not have resources to establish the adequate sampling density required to fully evaluate the detailed patterns of breakthrough at all the wells that were sampled. Faced with similar resource constraints, we decided to reduce the number of wells sampled and increase our sampling density at three wells. This fit more within our objectives of multi-particle/solute comparison and determining the efficacy of continuous online uranine and microsphere detection field equipment.

### 5.3 Methods

The purpose of this section is to describe in detail: 1) well equipment, injection and sampling equipment, 2) test injection conditions, 3) tracer components, and 4) the manner in which the analyses were done for uranine and microspheres in the field and for bacteriophages in the laboratory. Site pH and electrical conductivity measurements were made prior to and during testing (Table 5-2). A single tracer cocktail mixture consisting of the solute uranine, three bacteriophages (H40, MS2 and Psf2) and a latex microsphere were injected into well B4. Water was circulated in the wells. Real time continuous online measurements for uranine and the microspheres was done at two of the three monitoring wells. Samples were taken periodically from the circulating flow for both uranine and bacteriophage analyses. Uranine concentration was determined onsite soon after sampling using a second field fluorometer. Bacteriophage enumeration was determined with analyses done at the Microbiology Laboratory of the University of Neuchatel commonly within 48 to 72 h of sampling.

| WELL      | pH <sup>1</sup> | TEMP (Deg C) | EC <sup>2</sup> (µs/cm) |
|-----------|-----------------|--------------|-------------------------|
| <b>B4</b> |                 | <b>10.3</b>  | <b>539</b>              |
| C3        |                 | 10.5         | 559                     |
| C4        |                 | 11.3         | 549                     |
| C5        |                 | 11.6         | 544                     |
| <b>C6</b> | <b>7.01</b>     | <b>11.6</b>  | <b>542</b>              |
| C7        |                 | 10.4         | 552                     |
| <b>CD</b> | <b>6.74</b>     | <b>11.0</b>  | <b>559</b>              |
| D4        |                 | 10.3         | 547                     |
| D5        |                 | 10.4         | 545                     |
| D6        |                 | 10.4         | 543                     |
| <b>D7</b> | <b>7.08</b>     | <b>10.6</b>  | <b>560</b>              |

**Table 5-2 Site pH and electrical conductivity (EC) measurements.**

<sup>1</sup> pH measured during tracer testing (Aug-Sep 1997).

<sup>2</sup> EC measured during site preparation 28 days before testing.

#### 5.3.1 Well instrumentation, injection and sampling methods

The equipment layout for the injection and sampling well components is schematically shown in Figure 5-2. Each of the wells were equipped with a battery-driven downhole pump (Comet/Reich, 12 to 18 L/min, 0.8 to 1.5 bar, 12V DC), a delivery line from the pump to the surface, and a return line to just below the water table (PVC tubing, 14mm OD, 10 mm ID). This established internal circulation opposite to that believed to be the actively transmissive layers. Circulation rates were from about 1.5 to 2 L/min – a rate which was considered adequate to create a homogeneous column of water moving downward between just below the water table depth and the pump return delivery point. The original design for the pump and return line depths was to have them each set at about 10 and 4 m, respectively. The pump depth was chosen based on the history of tracer test results reported by various authors for the site. The return line depth was set at from about 10 to 20 cm below the water table. Flow meters were put inline periodically to measure the circulation rate. The tubing at the surface could be passed through a field fluorometer to obtain real time online uranine measurements. At wells C6 and D7, a T-joint was installed directly inline allowing withdrawal of a representative aliquot of 1 ml/min to determine real time online microsphere particle detection.

Discrete samples were taken from the injection well and three down gradient monitoring wells during testing. To facilitate this, a three-way valve was installed in the circulating water line to allow flow deviation to the sample bottle without affecting the continuity of the return line volume. Samples were placed in new, 100ml PVC dark brown bottles, were capped and temporarily stored in a refrigerated container at the site, prior to shipment to the offsite microbiological laboratory for analysis.

A generator provided continuous power to battery chargers for downhole pumps, computer equipment, refrigerated sample storage container, microsphere counting equipment and the site shelter.

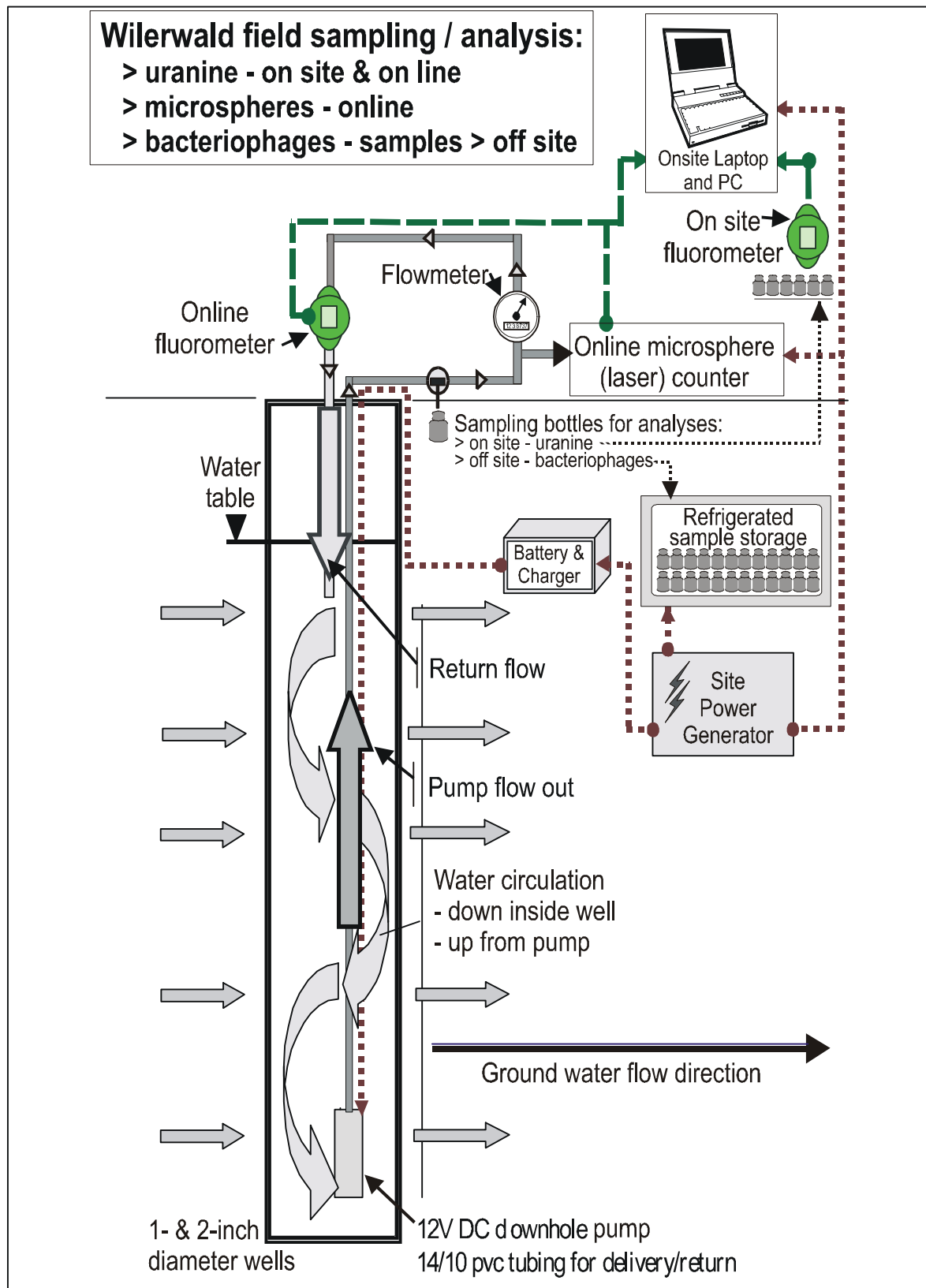


Figure 5-2 Well equipment, sampling and onsite analysis instrumentation schematic diagram.

### 5.3.2 Injection conditions

The methodology of creating the tracer test 'input signal' is important to understand and for our work this was certainly no exception. After a few minutes of injection, we received our first surprising results related to the flow conditions at the injection well. The tracer cocktail total mixed volume was injected into well B4 by connecting the container with the tracer to the flow return line and allowing the volume to flow, under Venturi effect, to be pumped at the circulation rate into the well. This process lasted 11 minutes. We realized, however, that there was no color in the return water coming from the pump depth set at 10 m. After five minutes of no return, the pump was raised to 8 and then to 6 m, but still without any visible fluorescence in the return part of the designed circulation. This condition continued with the pump set at 6 m until that remaining volume of tracer was gone. The pump was raised slowly thereafter during the rinse period up to about 5.5 m, about 1.5 m below the return line, and there was still no evidence of tracer. It was also not detected subsequently in the injection well circulation samples that had been taken during the injection period. We concluded onsite that the entire flow rate of 1.5 L/min had left the well and migrated in a high permeability layer at a depth between about 4 and 5 m. Realizing this, and wishing to have a consistent set of measurements at the down gradient wells, the down gradient well pump settings were raised from 10 m to 6 m at C6 and to 7 m at CD and D7 where they remained over the duration of testing.

To a degree, the input signal condition was not critical to our objectives – since we are focussed on comparing tracer components' response and field instrumentation efficacy. All tracer components compared were injected and sampled together. Therefore, our evaluations, given that all input and output conditions were equal and consistent were pertinent and valid regardless of the surprising situation that developed with lack of circulation return during the injection period.

### 5.3.3 Tracer components

The tracers used consisted of uranine, fluorescent microspheres and bacteriophages H40, MS2 and Psf2 biocolloids. Details related to the five tracer components are in Table 5-3. Uranine was selected as the conservative solute tracer because of its ease of use, low detection limits and the relatively low weight of material needed to undertake the test. About 154 gm of the powdered material were dissolved in 5 L of water. This amount selected was about 50% of the weight used previously by Rossi in 1995 as we were interested in lower concentrations in the observation wells than had been reported for that work (P. Rossi *et al.*, 1998).

The 1-micron diameter, carboxylate-modified, fluorescent microspheres (Molecular probes BV, Catalog No. F-8816) are artificial, commercially produced, latex beads (Figure 5-3). They have a density of 1.06 g/cm<sup>3</sup>, a highly negative surface charge and are relatively hydrophilic. They are labeled with an incorporated crimson-colored fluorescent dye with excitation and emission spectrum wavelengths of 623nm and 640nm, respectively, allowing them to be detected using a low power laser source.

Three biocolloid components – bacteriophages H40, MS2 and Psf2 were selected for this work based on prior experience at this and other karst settings and in associated migration feasibility demonstration tests in a 6 m long sand tank model (MARCEAUs) at the ULP-Strasbourg Fluid Mechanics Department (K. Kennedy, 2000). The three phages are about 8 to 40 times smaller than the microspheres ranging in size from about 25 to 130 nm. Images of MS2 and H40 phages are in Figure 5-4. Like the microspheres, they have negative surface charges. Surface charges are commonly referred to in terms of Isoelectric Point (IP) or Zeta potential (ZP). IP is the value of the pH of the solution containing the particles in suspension at which the particle exhibits a neutral surface charge and thus does not move in an electric field. Otherwise, it is simply stated as the pH at which the particles possess zero net charge. ZP is the charge balance, expressed in millivolts measured at a pH of 7.4. The higher the ZP, the more stable the colloidal suspension and the less chance of aggregation of colloidal particles.

MS2 is a member of the family *Leviviridae*, which have isometric head shapes and no tail. MS2 is a single stranded RNA phage with a size of from 24 to 26 nm and IP of from 3.5 to 3.9. Both H40 and Psf2 are members of the family *Siphoviridae* which are phages with long, non-contractile tails. Their known characteristics are limited to those defined by Rossi (1994). Psf2 family characteristics suggest that it will have a head of about 70 nm and a tail from 65 to 570 nm long and from 8 to 10 nm wide. Its ZP was measured at -36.0 mV. H40 has been characterized by Rossi (1994) indicating that its genetic material is double stranded DNA, has an icosahedral head of about 44 nm, a tail of about 85 nm, and a ZP of -42.0 mV.

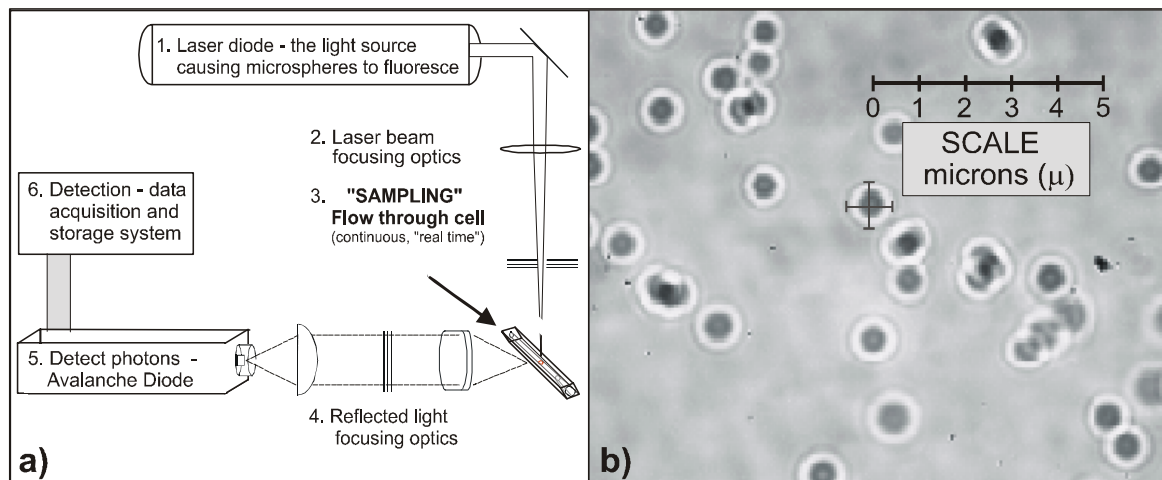


Figure 5-3 a) Microsphere detection equipment schematic  
b) microsphere photomicrograph.  
(After S. Niehren, 1998)

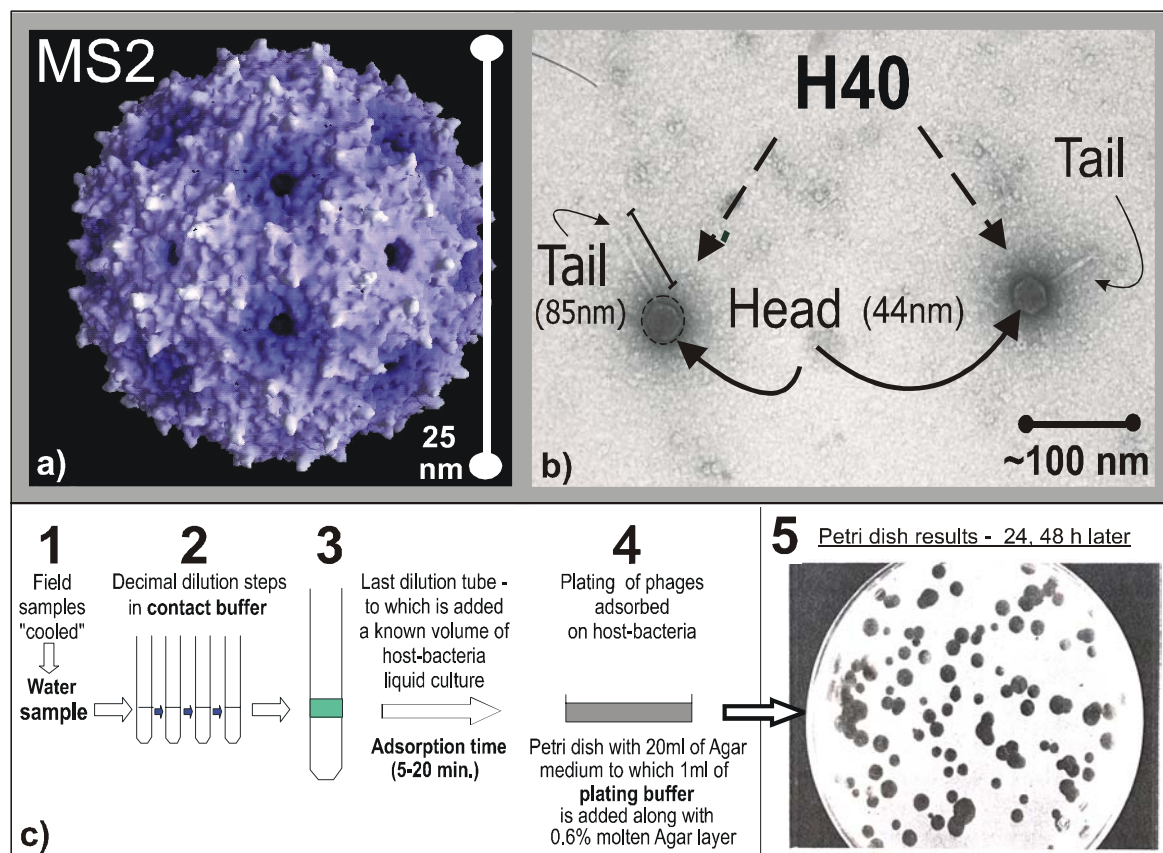


Figure 5-4 a) Bacteriophage images (after J-Y. Sgro, 1994),  
b) Phage enumeration analysis basic steps (after P. Rossi and K. Kennedy, 1999).

| Tracer component          | Type       | Component details <sup>1</sup>                                                                                  | Initial mass (M <sub>0</sub> )<br>(Volume (ml)) <sup>2</sup> | Analysis Method<br>(Resolution)                                                                                                                                              |
|---------------------------|------------|-----------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Uranine                   | Solute     | Sodium fluoresceine<br>MW 376.28<br>C <sub>20</sub> H <sub>10</sub> O <sub>5</sub> Na <sub>2</sub>              | 154 gm<br>(5000)                                             | On-Line spectrofluorometry<br>CHYN/UNINE Field<br>Fluorometer 3<br>(~ 0.1 to 1 ppb resolution)                                                                               |
| Bacteriophage<br>H40      | Biocolloid | 44,130 nm <sup>4</sup> , <i>Siphoviridae</i><br>gm (dsDNA), IP (nm),<br>zp (-42.0)                              | 1.10 e <sup>13</sup> pfu<br>(200)                            | Bacterial host cultivation /<br>growth association,<br>plate counting<br>in petri dishes<br>(UNINE - Microbiology<br>Laboratory - LAMUN)<br>(1 phage per<br>2 ml resolution) |
| Bacteriophage<br>MS2      | Biocolloid | 24 nm <sup>5</sup> , <i>Leviviridae</i> ,<br>gm (ssRNA), IP (3.5, 3.9),<br>zp (nm)                              | 1.15 e <sup>13</sup> pfu<br>(40)                             |                                                                                                                                                                              |
| Bacteriophage<br>Psf2     | Biocolloid | ~ 70, 65 to 570, <i>Siphoviridae</i><br>gm (nd), IP (nm),<br>zp (-36.0)                                         | 5.25 e <sup>12</sup> pfu<br>(750)                            |                                                                                                                                                                              |
| Microspheres <sup>6</sup> | Colloid    | 1.0 µm, Fluospheres:<br>carboxylate modified, crimson<br>fluorescent (625/645 spectra),<br>neg. surface charge. | 3.6 e <sup>10</sup> spheres<br>(250)                         | On-line laser detection <sup>7</sup><br>with flow cytometry<br>(to 1 sphere per<br>10 ml resolution)                                                                         |

**Table 5-3 Tracer test component characteristics and analysis methods.**

<sup>1</sup> Colloid details are size (head, tail), morphotype (*family*), genetic material – single (ss) or double (ds) stranded, isoelectric point (pH at which the particles possess zero net charge) and zeta potential mV (the charge balance, expressed in millivolts (mV) measured at a pH of 7.4) for the bacteriophages and the manufacturers cited characteristics for microspheres.

<sup>2</sup> The tracer cocktail contained all components and was injected in well B4 in 11 minutes at a circulation rate of 1.5 ml/min. An estimated 95 percent of the components' mass was assumed as injected (the balance input under more dilute 'rinsing' conditions).

<sup>3</sup> P.-A. Schnegg & N. Doerfliger (1997), P.-A. Schnegg & K. Kennedy (1998).

<sup>4</sup> Bacteriophage H40, P. Rossi (1994).

<sup>5</sup> H.-W. Ackermann & M.S. DuBow, 1987 (a), (b).

<sup>6</sup> Molecular Probes Europe BV, Holland, # F-8816. No IP or zeta potential values available.

<sup>7</sup> S. Niehren & W. Kinzelbach (1998).

### 5.3.4 Field analyses

Online real time measurements were made in the field for uranine, the dissolved solute and microspheres, the colloidal particle. Onsite measurements were also made for uranine analyses for the sampled water subsequently to be analyzed for bacteriophages. The data available to be observed during testing is shown on Figure 5-5.

The uranine was measured using two field fluorometers. An inline unit was placed in the immediate vicinity of the C6 wellhead through which the circulation water could flow without constriction. Readings were made every 4 minutes. The second field fluorometer measured the discrete sample values commonly within a few minutes of sampling using the faster measurement rate of 6 readings per minute. Each unit was connected over distances up to 15 m to their respective data loggers set in waterproof housings. All data collected was stored on the data logger's PCMCIA memory card as well as displayed, stored and manipulated on a site computer. The instruments' sensitivity was about 0.5 ppb with background measurements in the well waters ranging from about 0.4 to 1.0 ppb. There was good agreement between the inline and sample bottle uranine values as illustrated on Figure 5-5.

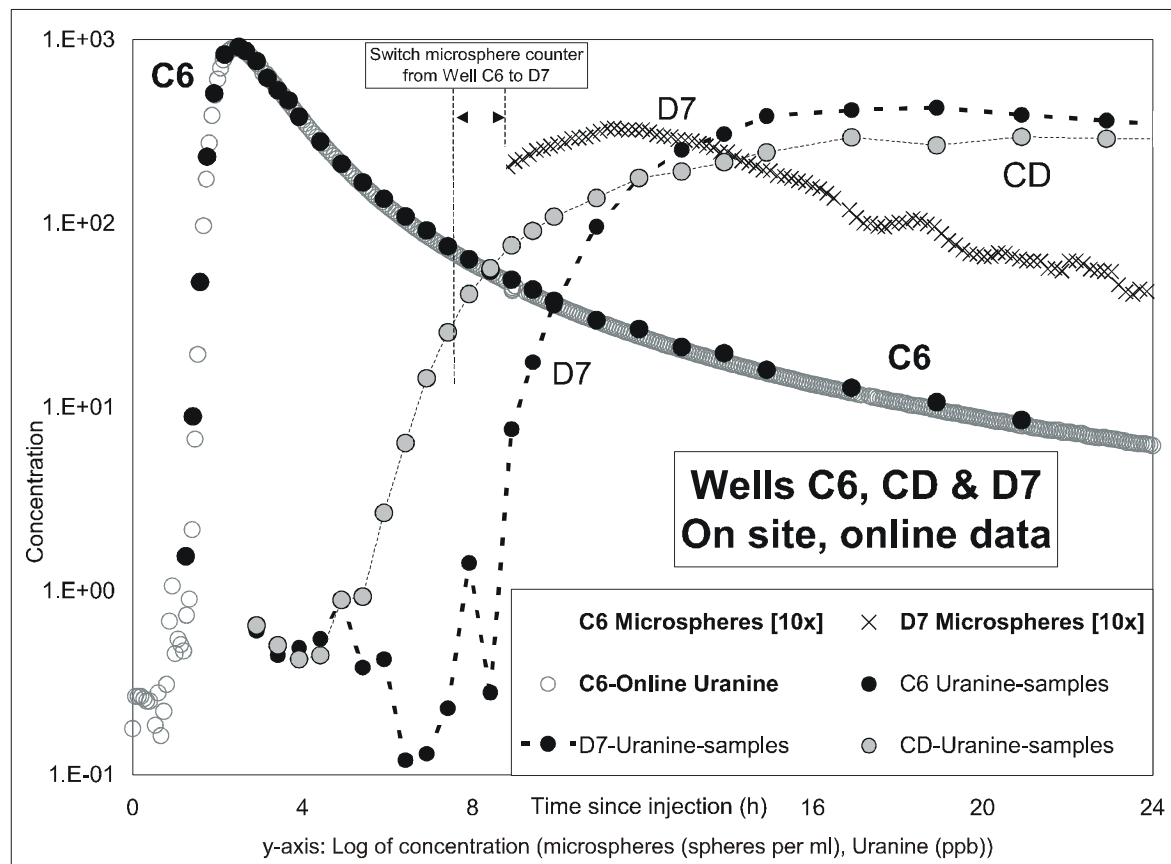


Figure 5-5 Online and onsite tracer component data available during testing (semi-log plot).

Microspheres were measured using a laser-based fluorescent particle detector, the basics of which are shown schematically on Figure 5-3 (a) along with a photomicrograph of the microspheres used in this work. This equipment was developed specifically for the purpose of measuring these particular latex beads and its use has been described related to laboratory column studies (S. Niehren and W. Kinzelbach, 1998). An aliquot of the circulating water is passed through a 250-micron diameter flow-through cell. This cell is illuminated with a precisely focussed laser beam. Fluorescent light is emitted when a microsphere passes through the beam. This microsphere-based fluorescent signal is detected in real time by a single-photon-counting avalanche diode (SPCAD). The data from the SPCAD are transmitted to a data acquisition system that stores and displays the measurements. Measurement frequency is on the order of microseconds and data are adjusted to provide a result integrated initially over 0.25 sec and subsequently as a function of the dynamic response of the system being tested. The results of the counting are displayed in Figure 5-5. There was an interruption of about 90 min in data when switching from well C6 to D7. It was unfortunate that the timing associated with end of measurements in C6 preceded the apparent arrival of the colloids at well D7. The particular and typical field-generated influences of weather and humidity, vibrations and changing operational conditions, generator power supply, line filtering of suspended sediment in the ground water were manageable. The detection of these injected colloidal particles was clearly evident and consistent at both wells that were sequentially monitored continuously.

### 5.3.5 Phage laboratory analyses

The analysis of the bacteriophages is a modified version of the method developed by M. Adams (1959), Optimization techniques were developed, used and presented by P. Rossi (1994) and P. Rossi and W. Käss (1998). Two stages of enumeration are commonly done both of which result in plaque forming units being counted in a petri dish. The first is a presence/absence test that also serves to indicate the order of magnitude for dilution of the sample so that the next step result yields a manageable number of counts on the petri dish. The basic steps associated with the analysis are summarized schematically in Figure 5-4 (b) along with the typical enumeration method illustrated. The microbiology laboratory's optimal processing capacity of about 25 samples per day was critical input to the sampling strategy. Each sample was analyzed for the concentration of each of the three phages simultaneously and in duplicate. A total of about 195 samples were taken during the testing that was concentrated heavily in the initial five days. The inactivation of the phages during the test was not considered to be a significant problem over the 12 day time period samples were taken. To evaluate this the three phages were mixed with the site water and kept in a constant temperature bath. Every one or two days, the concentrations of the phages present in the spiked site water were measured. The results confirmed our expectation that there was no significant reduction over the time period. The reduction that did occur may have been caused by the air-water interface in the laboratory vessels or other laboratory situations not occurring naturally in the field's subsurface environment.

### 5.3.6 Response assessment and quantitative analysis

Bacteriophage behavior was compared to solute tracer responses in a manner similar to methods described originally by R. Harvey *et al.* (1989). This included comparing concentration history and relative abundance ( $C/C_{max}$ ), maximum dimensionless concentrations ( $(C/C_0)_{max}$  or  $(Tr/Tr_0)_{max}$ ), retardation factor (RF) – the ratio of particle (pseudo-peak) vs. solute peak maximum times, relative breakthrough (RB) – particle mass recovered compared to solute recovery (normalized to 100 percent using non-reactive solute assumptions) and attenuation (1 minus RB). In addition, same scale plots of the early breakthrough time of colloid and uranine concentrations normalized to their original concentrations were reviewed to evaluate if there was evidence of preferential colloid transport taking place.

Transport 'arrival' velocities are calculated based on the time that a colloid is first detected at each well. Analytical detection limits affect the time at which any component is observable. Solutes commonly have practical DLs, equipment and background related, that may be orders of magnitude higher than for colloids. Colloids may have analytical-based background levels but in general have DLs several orders of magnitude lower than solutes. Differences in detection times are therefore difficult to compare. Results suggesting earlier colloid than solute detection can be in error. Recognizing this as a practical analytical limitation, an, at times, confusing practice, has developed in several disciplines wherein the time to maximum concentration ( $t_{max}$ ) or 50 % of the total recovered mass ( $t_{50}$ ), of any materials, is referred to as 'arrival time'. These arrival times were then compared and materials were referred to as arriving earlier when the mean or median mass values were different. However, this comparison is inappropriate and confusing for colloids when they are being compared to non-reactive non-sorbing solutes. Colloids undergo a high degree of attachment and

interface interaction in the subsurface environment during transport. Their mass being transported, unlike that of a conservative solute, changes, commonly decreasing, as it moves progressively down gradient during transport. When this occurs, the shape of the breakthrough curve at any further distance, including the time at which the maximum concentration occurs will be strongly influenced. Colloid attachment and removal that has taken place along the pathway to a sampling point can both lower and decrease the time at which the maximum concentration is reached. Therefore, we suggest calling a colloid's maximum concentration a "pseudo-peak" (ps-peak). It is not a parameter justified for either calculating average velocity or comparing as an arrival time relative to a conservative solute. It is a parameter that is highly variable - dependent on the media in which it is being transported as its interactions are both media and particle dependent. On the other hand, average velocities for solute migration are characteristically calculated based on  $t_{\max}$ , the time of the solute peak and reflect the effective flow velocity in the media.

### 5.3.7 Numerical modeling

A one-dimensional model was applied to the results of the solute and colloidal particle migration at well D7. The purpose of this modeling was to determine if the model parameters arrived at in matching the data were reasonable assuming a direct pathway existed over the 64 m between the injection and D7 wells. Data from the well at D7 was matched for uranine using a spherical two-region conceptualization and adsorption-desorption colloidal mechanisms. Details of adapting the combined solute advective-diffusive and colloidal attachment-detachment processes in this model (CoTrans) are in S. Niehren (1998).

## 5.4 Results and Discussion

The purpose of this section is to describe what happened experimentally at each location with respect to the different components used as tracers and discuss their implications as it relates to the components' transport characteristics under natural gradient conditions. The presentation is made by well and then by comparing the overall component behaviors and finally with the results of a 1-D numerical simulation used to compare the well D7 responses. Test data for sample results are in Appendix A5.

Figure 5-6 (semi-log) illustrates the five tracer components' concentration history as progressively observed down gradient at the three sampling wells. Figure 5-7a shows the concentration history by well for all sampled components at each well. Figure 5-7b shows the tracer component relative abundance with concentrations normalized to their maximum values detected at each well. Figures 8, 9 and 10 are log-log concentration history plots accentuating comparison of each component's time of first detection (arrival) and time to maximum concentrations by well. Figure 5-11a presents the component concentrations normalized to the original component concentrations for both solute and colloids on the same (semi-log) scale to evaluate the potential for differences between colloid and solute component advection (after P. Zhang *et al.*, in review). Figure 5-11b shows the entire data set by well (log-log) for evaluating changes in slope on the falling limb of the breakthrough curve indicating differences in later time behavior. Figure 5-12 summarizes the five tracer components' relative breakthrough both as it evolved and as absolute comparative totals at the three wells. Table 5-4 summarizes the characteristics of the breakthrough responses at the wells by tracer component. Figure 5-13 is the result of the preliminary 1-D modeling work done to compare the microsphere, bacteriophage and uranine response at well D7.

### 5.4.1 Well B4 response and injection conditions

This natural gradient test began with results at the injection well B4 that documented that the tracer cocktail had left the well in a thin lens likely on the order of 1 m at a depth of from 4 to 5.5 m below ground surface (bgs). The original design had expected circulation to occur over 6 m. No samples taken in the water pumped from the lower part of the well, either visibly or analytically, indicated that the tracer components had reached the pump depth and circulated between the pump and the surface, and therefore, the return line placed just below the water table depth. This observed result is consistent, however, with Carvalho Dill's electrical soundings at B4 (1993, Figure 34) with a specific layer of high resistivity values ( $> 500 \text{ Ohm.m}$ ) between 4 and 6 m. The 1.5 L/min circulation rate of the pump was clearly not high enough to overcome the lateral flow from the well that contained the tracer components.

## Chapter 5 – Wilerwald Bacteriophage, Microsphere and Uranine Response Characterizations

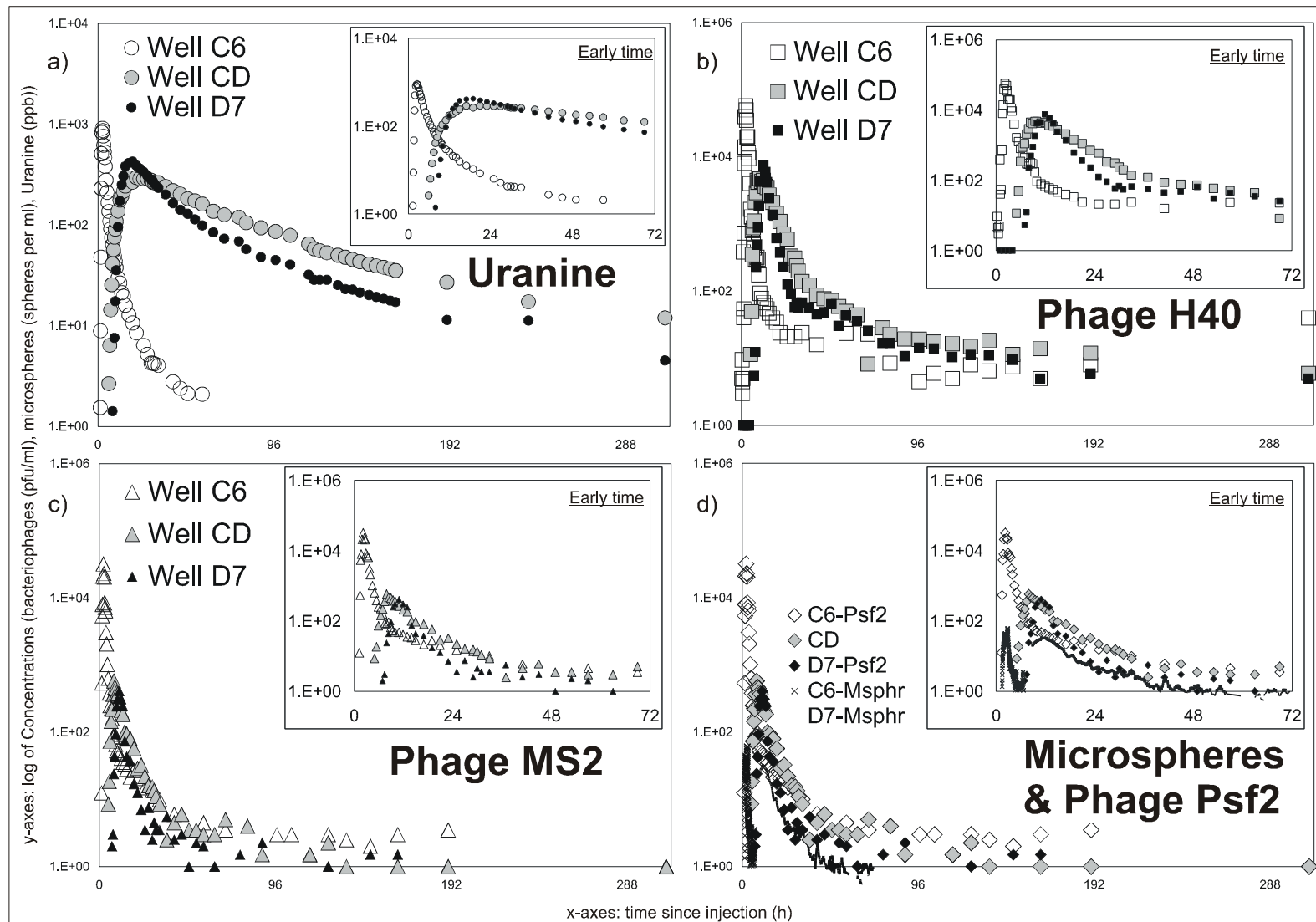


Figure 5-6 Concentration history by component at each well (semi-log plot):  
a) Uranine, b) Phage H40, c) Phage MS2, d) Microspheres & Phage Psf2.

The aquifer's vertical heterogeneity confirmed immediately in the field at B4 influenced the refined sampling design that we implemented soon thereafter at the three down gradient wells. The circulation pump depths were changed at C6, CD and D7 so that the circulating interval was reduced from 6 m to between about 3 and 4 m. Using a more narrow width impacted the maximum tracer values observed in the wells as a result of less dilution than would have occurred in the sampling wells. For example, at B4 in 1995, Rossi had injected about 300 g of uranine and would likely have followed the same narrow lateral path to leave the well. The resulting maximum uranine concentrations he obtained at C6 and D7 were about 1300 and 90 ppb, respectively. These sample results were based on removing and subsequently mixing the volume taken from the well in translucent tubing filled with a continuous column of water to a depth of about 10 m. Rossi anecdotally has described that in certain situations bands of fluorescent tracer could be observed in thin layers in the sample tubing. Using different sampling equipment and 2.6 and 3.7 m circulation intervals at the down gradient wells, the maximum uranine values during this testing were about 910 and 425 ppb. The surprising results were that the effective order of magnitude higher concentrations at D7 since we had injected only  $\frac{1}{2}$  the mass (154 g) used by Rossi. Therefore, the injection conditions and sampling protocols, possibly also along with changes in the transport pathways at the different test times clearly have significant effects on the migration response at individual wells. Hadi's 1995 and 1996 tracer work using the B4 injection well had involved injection but below the depth of a packer set at 10 m, so the results are not comparable in terms of down gradient results.

#### 5.4.2 Well C6

The concentration history to 6 h (Figure 5-7a) shows that the breakthrough curve rising and falling limbs of uranine and the phages were continuous and consistent. UR had a  $Tr_{max}$  value of 912 ppb corresponding to a  $(Tr/Tr_0)_{max}$  of  $1e^{-04}$ . H40, MS2 and Psf2 had  $C_{max}$  values of 57500, 12000 and 32000 pfu/ml corresponding to decreasing original-concentration-normalized  $((C/C_0)_{max})$  values of  $9e^{-05}$ ,  $5e^{-05}$  and  $4e^{-05}$ , respectively. The microsphere response also shows consistent data but deviates from the other colloids' response in that it depicts two separate peaks with  $C_{max}$  values of 45 and 62 microspheres/ml (ms/ml) corresponding to  $(C/C_0)_{max}$  values of 2 to  $3e^{-05}$ , similar to, but lower than the phages.

#### C6 Background levels

The C6 plot showing the time at which different tracer components were initially detected (Figure 5-8) illustrates that there was a background level associated with uranine, phage H40 and the microspheres. A background for uranine was expected based on the considerable previous use of this tracer plus the instrumentation's natural lower limit related to the instrument itself and the level of natural fluorescence in any water. We expected that these two site factors would mean that only measurements above 1 ppb would be considered as positive presence of the tracer in this test. The microsphere background level was also expected due to light scattering from particles other than the fluorescent beads injected. The counter background ranged from 0.2 to 0.6 ms/ml. The unexpected background levels were those for H40 ranging from about 4 to 10 pfu/ml. H40 was detected to concentrations of one to two hundred pfu/ml in samples taken from well B4 during the site preparation. It was also detected at levels to 10 pfu/ml in wells C6 and D7 prior to testing. The site had last been tested using the H40 phage over two years earlier. According to their typical cycle, they would not be detectable after a few months. The most likely explanation is that the phages we detected had previously become attached to adjacent matrix material but they had not lost their virulence during this attachment period. Similar results have been noted in batch tests (P. Rossi, 1994). Our pre-test pumping could have mobilized sediment particles from the aquifer to which these phages were attached and left these present in the water column prior to start of testing. The test circulation activity would have resulted in mixing of these phages within the water column and establishing a residual level at the beginning of the test. Fortunately, the background levels of H40 were not high enough to detract from the quality of the results.

#### C6 Arrival/Detection

First detection of the MS2 and Psf2 phages, for which there had been no previous site use, were both in the sample taken 75 min after injection (Figure 5-8). H40 was also present in this and the sample taken 10 min earlier. Microspheres, while being measured online, were detected above background at

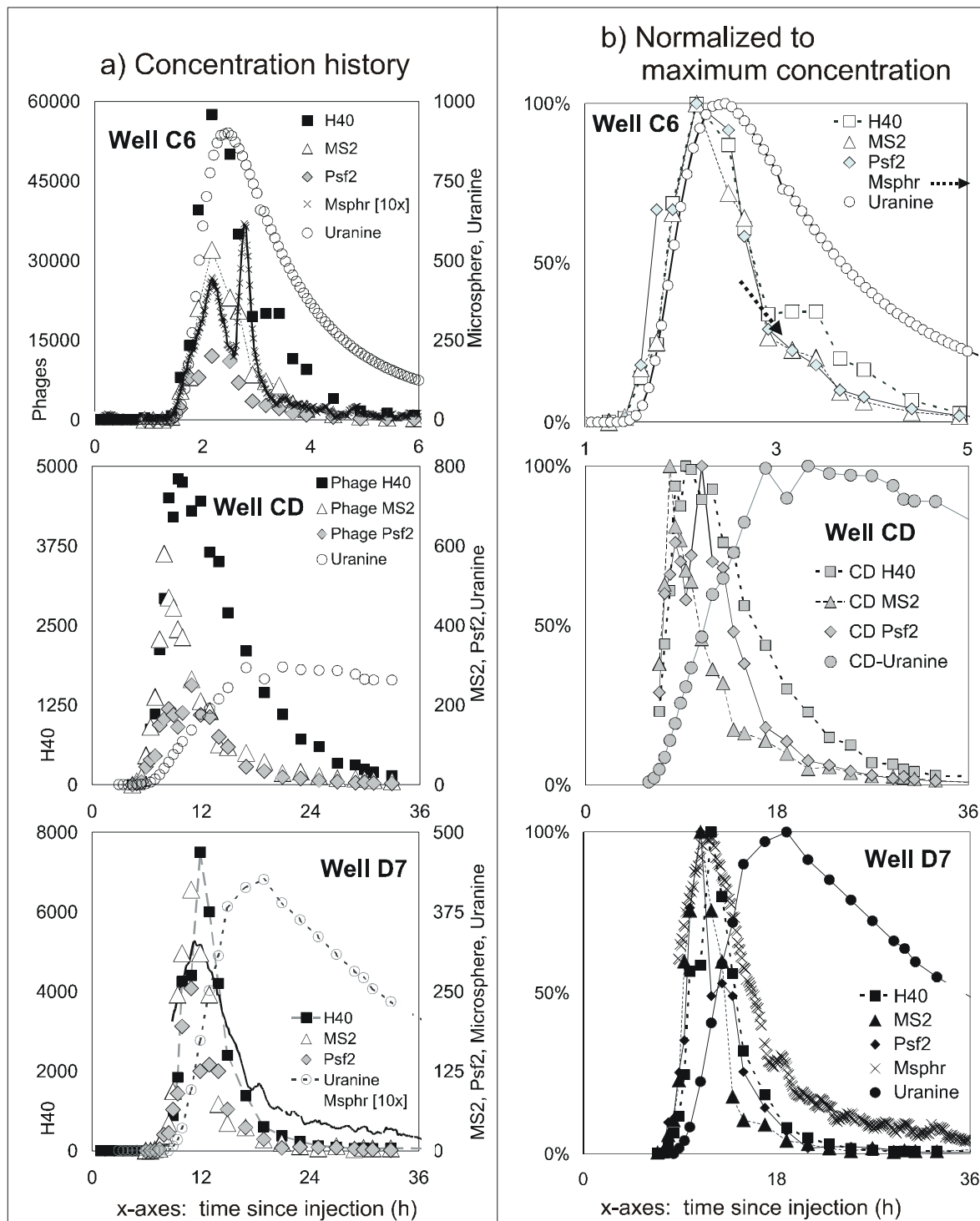


Figure 5-7 Concentration history by well for all components.  
a) arithmetic plot  
b) relative abundance

| Down<br>gradient<br>well | Distance<br>from<br>injection<br>(m) | Uranine response <sup>1</sup> |                     |                                     | Bacteriophage and microsphere responses |                    |                                              |     |                                      |           |              |
|--------------------------|--------------------------------------|-------------------------------|---------------------|-------------------------------------|-----------------------------------------|--------------------|----------------------------------------------|-----|--------------------------------------|-----------|--------------|
|                          |                                      | $t_{tr-arr}$<br>(h)           | $t_{tr-max}$<br>(h) | $Tr_{max}$<br><br>$(Tr/Tr_0)_{max}$ | Particle                                | $t_{c-arr}$<br>(h) | $t_{c-max}$ <sup>3</sup><br>(ps-peak)<br>(h) | RF  | $C_{max}$ <sup>4</sup>               | RB<br>(%) | Att'n<br>(%) |
|                          |                                      |                               |                     |                                     |                                         |                    |                                              |     | $(C/C_0)_{max}$                      |           |              |
| C6                       | 14                                   | 1.5                           | 2.5                 | 912<br><br><br><br>$1e^{-04}$       | H40                                     | 1.1                | 2.2                                          | 0.9 | $\frac{57500}{9e^{-05}}$             | 41        | 59           |
|                          |                                      |                               |                     |                                     | MS2                                     | 1.3                | 2.2                                          | 0.9 | $\frac{32000}{5e^{-05}}$             | 17        | 83           |
|                          |                                      |                               |                     |                                     | Psf2                                    | 1.3                | 2.2                                          | 0.9 | $\frac{12000}{4e^{-05}}$             | 16        | 84           |
|                          |                                      |                               |                     |                                     | Micro<br>Sphere                         | 1.4                | 2.2, 2.8 <sup>5</sup>                        | 0.9 | $\frac{45, 62}{2e^{-05}, 3e^{-05}}$  | 9         | 91           |
| CD                       | 34                                   | 5                             | 21                  | 296<br><br><br>$3e^{-05}$           | H40                                     | 4.9                | 9.4                                          | 0.5 | $\frac{4800}{8e^{-06}}$              | 3.4       | 96.6         |
|                          |                                      |                               |                     |                                     | MS2                                     | 4.4                | 7.9                                          | 0.4 | $\frac{580}{9e^{-07}}$               | 0.2       | 99.8         |
|                          |                                      |                               |                     |                                     | Psf2                                    | 4.9                | 8.4, 10.9 <sup>6</sup>                       | 0.5 | $\frac{190,250}{6e^{-07}, 8e^{-07}}$ | 0.3       | 99.7         |
| D7                       | 64                                   | 8                             | 19                  | 426<br><br><br><br>$5e^{-05}$       | H40                                     | 6.9                | 11.9                                         | 0.6 | $\frac{7500}{1e^{-05}}$              | 3.3       | 96.7         |
|                          |                                      |                               |                     |                                     | MS2                                     | 6.9                | 10.9                                         | 0.6 | $\frac{410}{6e^{-07}}$               | 0.2       | 99.8         |
|                          |                                      |                               |                     |                                     | Psf2                                    | 6.9                | 10.9                                         | 0.6 | $\frac{255}{8e^{-07}}$               | 0.2       | 99.8         |
|                          |                                      |                               |                     |                                     | Micro<br>sphere                         | nd <sup>7</sup>    | 11.3                                         | 0.6 | $\frac{33}{2e^{-05}}$                | 8.2       | 91.8         |

**Table 5-4 Tracer breakthrough characteristics for uranine, bacteriophages & microspheres.**

<sup>1</sup> Uranine is a solute that is commonly referred to as a conservative tracer – and we designate Tr as the solute component concentration label. Accordingly, we refer to  $t_{tr-arr}$  and  $t_{tr-max}$  as the times associated with arrival and maximum solute concentrations, respectively. This differentiates it from the  $t_c$  time terms associated with the colloids.

<sup>2</sup> Uranine  $Tr_0$  units are ppb.

<sup>3</sup>  $t_{c-max}$ : time of maximum colloidal particle concentration. We refer to this as a pseudo-peak (ps-peak) since it does not have the same implied or associated characteristics as conservative solute tracer peaks do for mass transport behavior.

<sup>4</sup> Bacteriophage units are pfu/ml, microsphere units are microspheres/ml (ms/ml). Microsphere values have not been corrected (increased) for the counter efficiency may be as low as 50 percent depending on environmental and water conditions during testing.

<sup>5</sup> Microspheres showed two pseudo-peaks at C6 at 2.2 and 2.8 h. The earlier pseudo-peak at 2.2 h more closely matches phage results.

<sup>6</sup> Phage Psf2 showed two pseudo-peaks at CD at both 8.4 and 10.9 h. The pseudo-peak at 8.4 h more closely matches other phage results.

<sup>7</sup> nd: not determined.

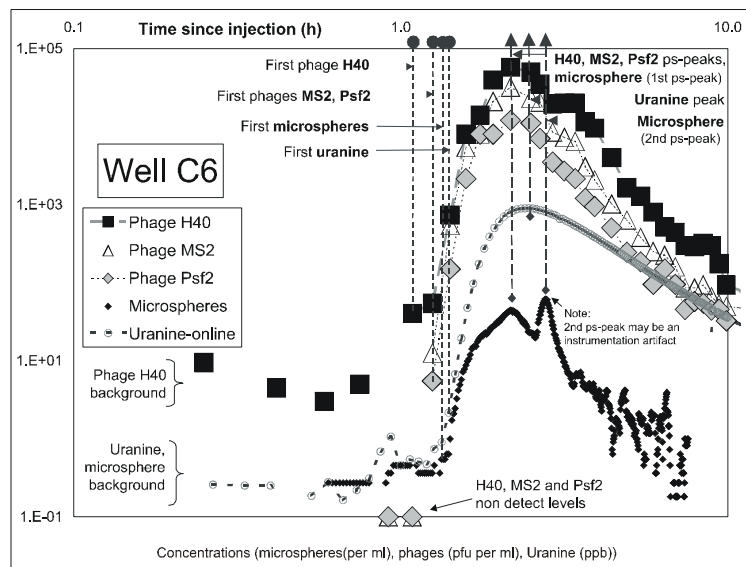


Figure 5-8 Well C6 arrival and uranine peak and colloid pseudo-peak properties (log-log plot).

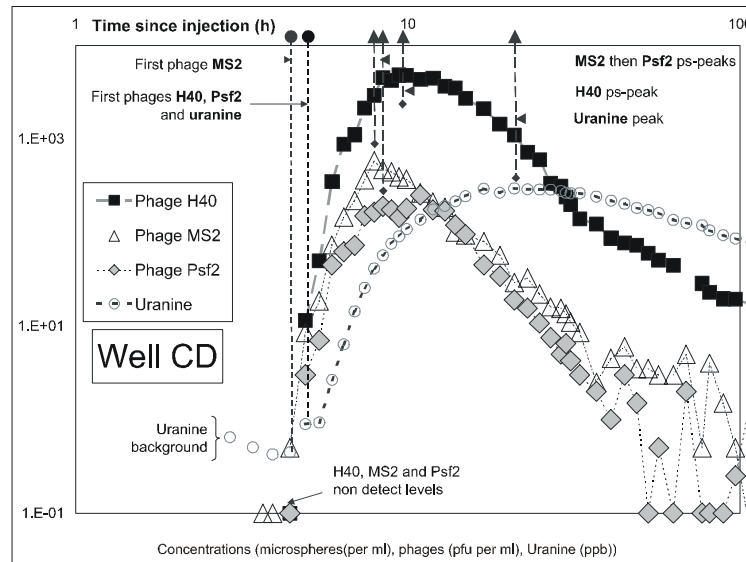


Figure 5-9 Well CD arrival and uranine peak and colloid pseudo-peak properties (log-log plot).

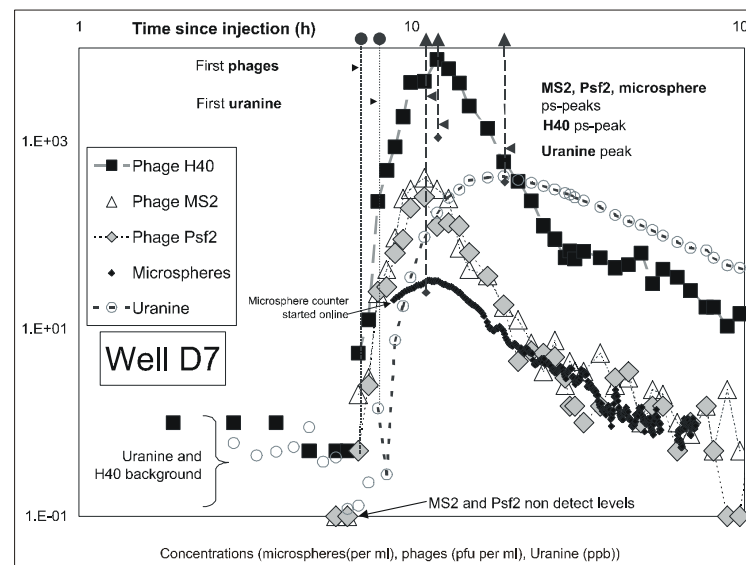


Figure 5-10 Well D7 arrival and uranine peak and colloid pseudo-peak properties (log-log plot).

about 80 min and their pattern of increase could be documented thereafter. The first inline uranine measurement occurred above 1 ppb at about 85 min after injection, demonstrating that the flow through cell measurements were systematically well documented. Phage and microsphere detection limits are about equal (0.5 particles/ml) and lower than that of the uranine so that the small differences in time of detection between the colloids and uranine are not conclusive as to preferential advection of the colloids. It was clear from the fast migration seen for both the colloids and the uranine that the advective-dispersive transport in the flow system was relatively rapid. Migration of both the colloids and the solute over the 14 m distance in from 65 to 85 min indicates an 'arrival velocity' of about 240 to 310 m/d. Figure 5-11a shows little difference in the uranine and colloids  $C/C_0$  values to about  $1e-05$  at which point the colloids begin to fall below the uranine normalized concentrations. Thus there is no clear evidence of preferential colloidal advection even with the 12- to 40-fold size difference between the bacteriophage and microsphere colloids. In this situation, then the more representative travel-time velocities are the higher ones related to the colloids with their lower detection limits.

#### C6 Maximum concentrations times and peaks

The relative abundance plot of the five components (Figure 5-7b) illustrates that the four colloids all had similar pseudo-peak times at about 2.2 h occurring about 0.3 h before the uranine peak. There was little evident error in the timing of these maximum values as the sampling density was every 10 to 15 minutes for the phages, every 4 minutes for the uranine and presented as averaged over every 10 minutes for the microspheres. The calculated mean solute velocity was about 130 m/d determined using the time to the uranine maximum concentration. The colloidal  $(C/C_0)_{\max}$  values, while predictably lower, were closer to the uranine value than expected with H40 only 13 % less and MS2, Psf2 and microspheres about 2-, 3- and 5-fold less. C6 data on Figure 5-11b show the difference in the breakthrough normalized to the total injected mass. The colloid data all appear similar with a smooth breakthrough in the rising and falling limb (slope 1) until about 6 h. At that time, the slope flattens and extends out to about 20 h for H40 and 70 h for MS2 and Psf2 (slope 2). Thereafter, there are low but relatively constant concentrations possibly associated with continuing levels of reversible detachment in the final tailing part of the curve (slope 3). The uranine data exhibits no significant changes in its pattern over the first 56 h at which time its concentration was within 2-fold of background levels and detailed monitoring ended.

#### C6 Recovery

The calculated relative breakthroughs as they evolved with time for the colloids and uranine at C6 are in Figure 5-12a. Compared to uranine assumed to reach 100 %, the colloid total relative breakthrough recovery was 41 %, 16 % and 17 % for H40, MS2 and Psf2, respectively, and about 9 % for the microspheres (Figure 5-12b). The calculated microsphere recovery was calculated to about 7 h rather than the 72 h measured for the phages. However, based on the similarity in the microsphere response to the other colloids to that point, it is unlikely that the final number rise by more than a few percent over the remaining time.

### **5.4.3 Well CD**

The concentration history to 36 h (Figure 5-7a) shows that the breakthrough curve rising and falling limbs of uranine and the H40 and MS2 phages were continuous and consistent. UR had a  $Tr_{\max}$  value of 296 ppb corresponding to a  $(Tr/Tr_0)_{\max}$  of  $3e^{-05}$ . H40 and MS2 had  $C_{\max}$  values of 4800 and 580 pfu/ml corresponding to  $(C/C_0)_{\max}$  values of  $8e^{-06}$  and  $9e^{-07}$ , respectively. Psf2 had double ps-peaks of 190 and 250 pfu/ml separated by about 2.5 h ( $(C/C_0)_{\max}$  of  $6e^{-07}$ ,  $8e^{-07}$ ). The initial ps-peak for Psf2 was similar to the other two phages. The Uranine  $C_{\max}$  decreased 3-fold compared to D6 whereas H40 decreased 12-fold and MS2 and Psf2 decreased about 50-fold. The H40, MS2 and Psf2 intra-phage  $(C/C_0)_{\max}$  difference was about 2 at C6. This increased to 9 at CD. An increased relative attenuation of the MS2 and Psf2 phage compared to H40 had occurred in transport to CD compared to C6.

#### CD Background levels

At CD, there was a background level associated only with uranine (Figure 5-9). The uranine background was expected as discussed above and was about 0.7 ppb at this location. The CD results were from discrete samples measured on site shortly after sampling rather than at C6 where they were inline. No background levels of any of the phages were detected in the samples prior to their testing-related arrival.

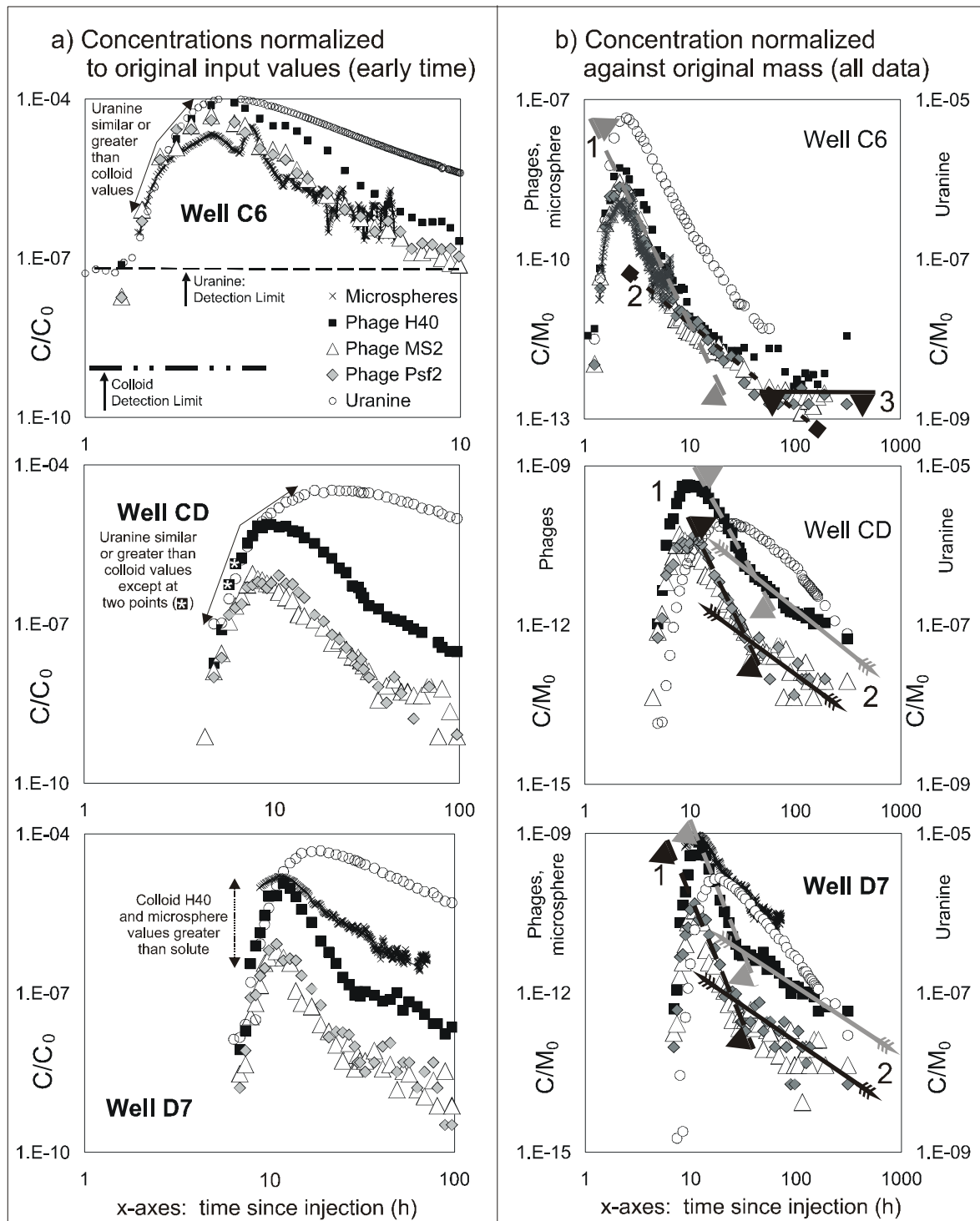


Figure 5-11 Component concentration by well normalized to a) injectate concentration ( $C/C_0$ ) and b) injectate mass ( $C/M_0$ ) (log-log plot).

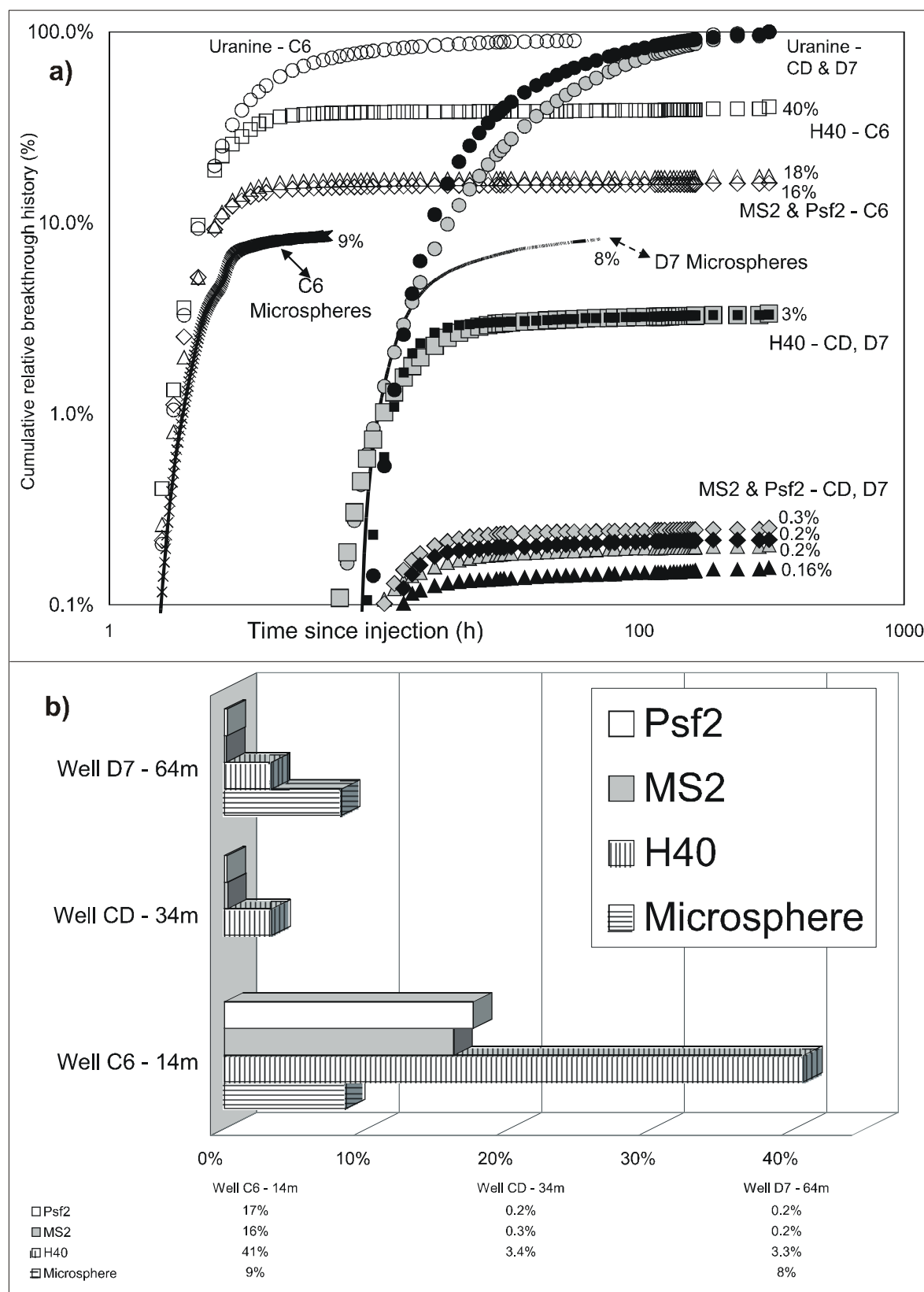


Figure 5-12 a) Cumulative relative breakthrough (log-log plot), b) Total relative breakthrough comparison by component.

### CD Arrival/Detection

First detection of the MS2 and Psf2 phages, for which there had been no previous site use, were both in the sample taken about 4.5 h after injection (Figure 5-9). H40 was first present in the sample taken 30 min later. The first uranine detected above the documented background was also at the same time as the H40 occurrence. CD is located off the main channel and exhibits a more dispersive shape for all tracer components than at C6 and D7 (Figure 5-6). Regardless, both uranine and phages exhibited relatively fast transport. Migration of both the colloids and the solute over the 34 m distance in about 4.5 to 5 h indicates an 'arrival velocity' of about 170 to 185 m/d. The 30 min difference in detection between the colloids and uranine are not conclusive as to preferential advection of the colloids. The  $C/C_0$  (Figure 5-11a) diagnostic plot does not show phage values with timing significantly in advance of the uranine suggesting there is no evidence of preferential colloidal advection. H40 values were ahead (above those) of uranine in two samples at about 6 and 6.5 h but the other phage and the remaining H40 samples show the colloids under the uranine curve.

### CD Maximum concentrations times and peaks

The relative abundance plot of the five components (Figure 5-7b) illustrates that the three phages had ps-peak times of between 8.0 and 9.4 h that occurred about 11 to 12 h before the uranine peak. There was a double Psf2 ps-peak and the second, higher one, occurred at about 11 h compared to the first one at 8.4 h. Timing of these maximum values was based on 30 min sampling. The calculated mean solute velocity was about 40 m/d determined using the  $t_{tr-max}$  value. The colloidal  $(C/C_0)_{max}$  values, were lower as expected from the off-channel setting in which this well is located according to the site geophysical studies. H40 and MS2 and Psf2 were about 4- and 40-fold less than uranine, respectively. CD data on Figure 5-11b shows the differences in the breakthrough normalized to the total injected mass. The phage responses were similar with a smooth breakthrough in the rising and falling limb (slope 1) until about 35 to 40 h. At that time, the slope flattens and extends out as the tailing features of the colloid response begin to influence the later time period of the plot (slope 2). There was no late-time constant-concentration period as had been observed in well C6. The uranine data exhibited a more dispersed form than the phages with no significant changes in its pattern over the approximate 300 h it was measured at which time its concentration was still 12-fold higher than the background levels.

### CD Recovery

The calculated relative breakthroughs as they evolved with time for the colloids and uranine at CD are in Figure 5-12a. Compared to uranine assumed to reach 100 %, the colloid total relative breakthrough recovery was 3.4 %, 0.3 % and 0.2 % for H40, MS2 and Psf2, respectively (Figure 5-12b). Compared to the recovery at C6, there was a 12-, 64- and 85-fold decrease in H40, MS2 and Psf2.

### **5.4.4 Well D7**

The concentration histories to 36 h (Figure 5-7a) shows that the breakthrough curve rising and falling limbs of uranine, phages and microspheres (once monitoring began) were continuous and consistent. UR had a  $Tr_{max}$  value of 426 ppb corresponding to a  $(Tr/Tr_0)_{max}$  of  $5e^{-05}$ . H40, MS2 and Psf2 had  $C_{max}$  values of 7500, 410 and 255 pfu/ml corresponding to  $(C/C_0)_{max}$  values of  $1e^{-05}$ ,  $8e^{-07}$  and  $9e^{-07}$ , respectively. Microspheres had a  $C_{max}$  of 33 with a  $(C/C_0)_{max}$  value of  $2e^{-05}$ , the highest of all the colloids. The Uranine  $C_{max}$  decreased 3-fold compared to the C6 value whereas H40, MS2, Psf2 and microspheres decreased 8-, 78-, 47- and 1.4-fold, respectively. The H40, MS2 and Psf2 intra-phage  $(C/C_0)_{max}$  difference of about 2 at C6 had increased to 19 and 14 for MS2 and Psf2, respectively at D7 indicating that as between C6 and CD, there had been an increase in the relative attenuation of the MS2 and Psf2 phage compared to H40.

### D7 Background levels

At D7, there were documented background levels associated with uranine and H40 (Figure 5-10). The uranine and phage backgrounds of 0.9 ppb and 1 pfu/ml, respectively, were anticipated as discussed above. No background levels of the other the phages were detected in the samples prior to their testing-related arrival. Background levels were not measured for the microspheres as the equipment was set up inline at C6 and moved to D7 only after the colloidal particles were already present.

### D7 Arrival/Detection

First detection of the MS2 and Psf2 phages, for which there had been no previous site use, were both in the sample taken about 6.9 h after injection (Figure 5-10). H40 was also first present above background in this sample. The first uranine detected above the documented background of 0.9 was at about 7.9 h. The subsequent sample 30 min later dropped below background but thereafter was again above background and continued to rise. D7 is located directly along the line of the geophysically interpreted high permeability channel and components exhibited a less dispersed shape than at CD although more dispersed than at C6 (Figure 5-6). Both uranine and phages exhibited relatively fast transport. Migration of both the colloids and the solute over the 64 m distance in about 7 to 8 h indicates an 'arrival velocity' of about 190 to 220 m/d, higher than the velocity calculated for transport to CD. This supports the preferential channel concept for the site with longitudinal migration along this pathway being dominant between B4, C6 and D7 and with lateral transport towards CD. Relative to the transport rate, the solute and colloid arrival velocities to D7 were about 72 to 82 % as fast to C6. The 1 h difference in detection time between the colloids and uranine suggests the potential for preferential colloidal advection. Indeed, the same-scale  $C/C_0$  diagnostic plot (Figure 5-11a) shows first, five H40 and second, the start of the microsphere record, with values from about 8 to 10 h above the uranine suggesting the possibility of colloids had higher advective rates than the solutes. The other phage and the remaining H40 samples show the colloids under the uranine curve.

### D7 Maximum concentrations times and peaks

The relative abundance plot of the five components (Figure 5-7b) illustrates that the three phages and microspheres had ps-peak times of between 11 and 12 h that occurred about 7 to 8 h before the uranine peak. Timing of the phage maximum values was based on 30 min samples and the uranine on 2 h samples. The calculated mean solute velocity was about 80 m/d determined using the  $t_{tr-max}$  value. Colloidal  $(C/C_0)_{max}$  values were lower than at C6 as expected and higher than at CD, the off-channel setting. H40, MS2, Psf2 and microspheres were about 4-, 78-, 57- and 3-fold less than uranine. D7 data on Figure 5-11b shows the differences in the breakthrough normalized to the total injected mass. The colloid data for the three phages are similar with a smooth breakthrough in the rising and falling limb until about 30 h (slope 1). At that time, the slope flattened and extended to late time as it did at CD (slope 2). For the microspheres, the pattern is similar but the break in slope occurs earlier than 20 h. There was no evident late-time tailing for the colloids as had been observed at C6. The microsphere data was dissimilar to the phages at the initial part of the documentation. The overall pattern however, matched well with the other colloids. The uranine data exhibited a more dispersed form than the phages with no significant changes in its pattern over the approximate 300 h it was measured at which time its concentration was about 5-fold higher than the background levels.

### D7 Recovery

The calculated relative breakthroughs as they evolved with time for the colloids and uranine at D7 are in Figure 5-12a. Compared to uranine, the colloid total relative breakthrough recovery was 3.3 %, 0.2 %, 0.2 % and 8.2 % for H40, MS2, Psf2 and microspheres, respectively (Figure 5-12b). Compared to the recovery at C6, there was a 12- and 64-fold decrease in H40 and MS2 and Psf2 values. The microspheres, however, only showed a decrease of about 10 % in their recovery. The microsphere recovery would actually be higher as the equipment was not online to monitor the entire breakthrough. This additional amount, based on the comparable phage RB evolution to those  $C/C_0$  values where monitoring began, would add about 10 percent to the total observed, raising it to ~ 9 to 10 %.

#### **5.4.5 Component response comparison**

The magnitude of the uranine and phage H40 response was greater than expected at all wells given the injection concentrations and past test experiences at the site. This was likely caused by the flow occurring in a relatively narrow layer of less than 1 m, at least in the vicinity of well B4, and this preferential lens or channel continuing without much vertical dispersion down gradient to C6. The high relative breakthrough of from 16 to 41 % for the phages at C6 supports this direct channel connection hypothesis. Both uranine and colloids thereafter had expected increases down gradient in both dispersion and attenuation, but not directly as a function of distance. There was clear influence of transverse dispersion in the component responses at CD.

The colloids were differentially attenuated as follows:

$$\begin{aligned} &\text{Phage H40} < \text{Psf2} \sim \text{MS2} < \text{Microspheres (C6)} \\ &\text{Microspheres} < \text{Phage H40} \ll \text{Psf2} \sim \text{MS2 (CD (phages) and D7)} \end{aligned}$$

Attenuation ranges (Table 5-4) at 14 m at C6 ranged from 59 to 84 % for the phages. The degree of attenuation dropped dramatically between C6 and was essentially the same for the phages at both 34 and 64 m. At CD and D7, the attenuation ranges were over 99.7 % for MS2 and Psf2. However, the attenuation values of H40 and microspheres were significantly lower in the range of 96 to 92 percent. The clean, clay-free and large pore spaces in the gravel lenses of this aquifer are likely the reasons there is such a relatively low degree of attenuation.

The four colloids arrived at each well at relatively similar times so there was no apparent transport of one phage preferentially over another. Uranine generally was detected after the colloids. Only at D7 was there enough difference to suggest that it be related to other than detection limit variability. The colloid pseudo-peaks were equal or similar at the wells in the channel, C6 and D7 with retardation factors (RF) of 0.9 and 0.6, respectively. At CD, the RF values were 0.4 for MS2 and 0.5 for H40 and Psf2. The RF value needs to be put in context based on the earlier discussion of what affects the time at which the ps-peak occurs. RF values may be useful to consider, just as plots of relative abundance to show or portray where the ps-peak and solute peaks occur in time. This is useful in a single setting assuming that the processes affecting the colloidal responses are similar throughout that setting. It is unlikely, however, that comparing the RF values among different environmental settings is appropriate.

Two dual peaks occurred that could not be readily explained based on the hydrogeological setting. The microsphere data at C6 was both continuous and consistent. Yet, it did not match any trend in the phages over this period. Despite the considerably higher sampling frequency, it is difficult to imagine a transport mechanism in this channeled rapid migration setting that would not affect all colloids in a similar manner. So the microsphere dual peak at C6 remains unexplained based on the subsurface conditions. The Psf2 dual peak was not as dramatic as the C6 microsphere pattern and could be more related to analytical variability than site conditions. Both instances had the original ps-peak patterns similar to the other colloid response at these locations. We suggest that no special significance be given to these two occurrences without further confirmation testing.

Microsphere recovery was high at both C6 and D7. Its RB at 64 m was at least 2-fold that of H40. All the colloids are negatively charged with H40 being more negative than Psf2. Unfortunately, the isoelectric point values of the microspheres and MS2 are not well documented so we cannot compare this parameter directly. The size difference is large between the microspheres and the phages. With similar negative charges, size could account for the higher microsphere recovery at the further distance. However, size differences are generally not considered as important to differentiate among colloid recovery, as are variations in colloidal surface properties. And surface properties affect the response also in accordance with the chemistry of the water in which the transport is taking place. Regardless, with H40 recovery higher than the microspheres at 14 m (C6), this suggests that colloidal differentiation may have occurred along the transport paths between the injection well and D7. There is a high degree of heterogeneity in the aquifer and there were higher  $C/C_0$  microspheres vs. uranine  $Tr/Tr_0$  values at the beginning of the D7 data record. Given these conditions, co-mingled and varying pathways for the different colloids to travel through is a possible hydrodynamic explanation for an increase in microsphere presence further down gradient.

#### 5.4.6 CoTrans model preliminary interpretation results

CoTrans calculates transport of a conservative tracer and colloids in suspension in a flow model. Details are in Niehren (1998). A double porosity (two regions, mobile/immobile zone) model is used for the ideal conservative solute tracer – in our case, uranine. The solute can occur in both the mobile and the immobile zone. CoTrans assumes that the particles follow active porosity zone 'flow paths' in which there is a physical migration (advective-dispersive conditions). The solute moves in these pores and as well, diffuses into the 'dead end' pores into which there is essentially no advective-dispersive effect, but there is diffusion. Further, this dual continuum model has the added condition that there is a diffusion 'gradient' both into and out of the adjacent immobile porosity zone as the tracer migrates along the mobile pathway pores. In this manner there is a better consistency associated with the approach to modeling not just the rising limb of the breakthrough curve but also the tailing over the long term of solute data. Colloid migration occurs only in the mobile zone and is also excluded from the low-velocity regime near matrix surfaces.

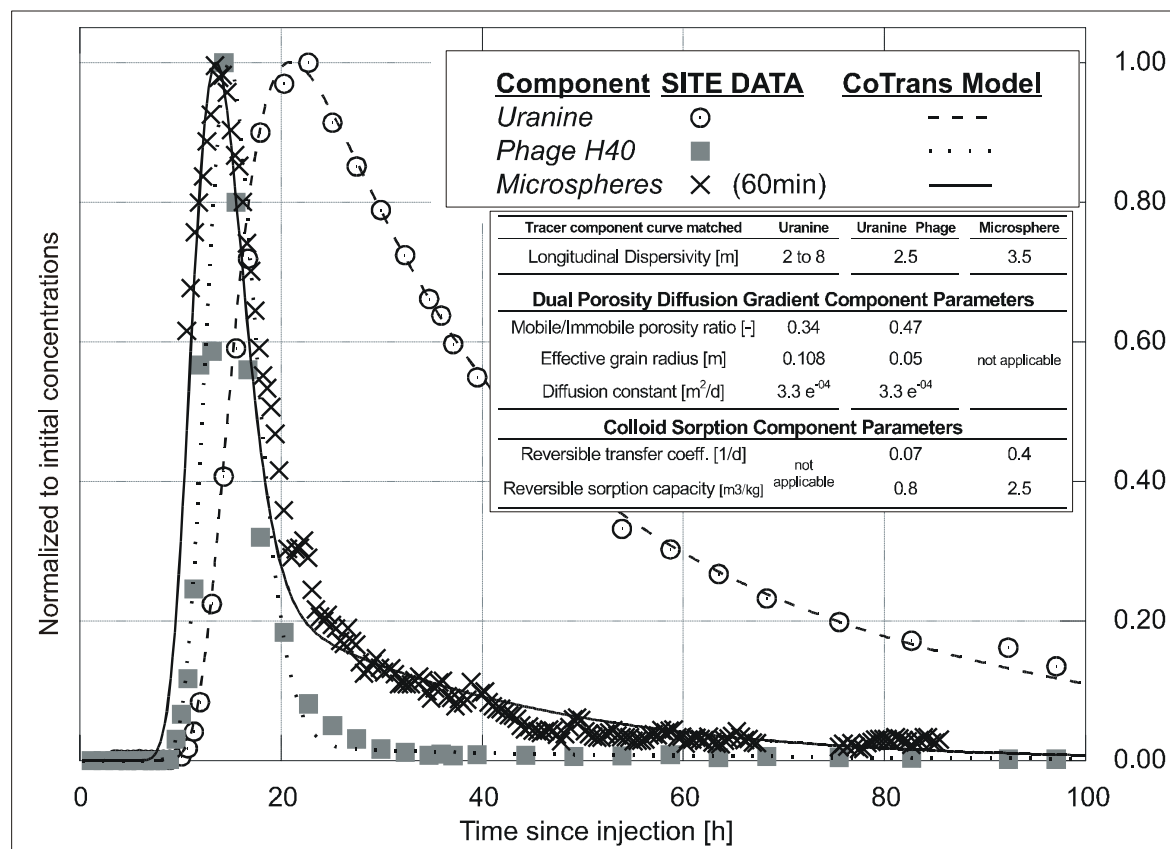


Figure 5-13 CoTrans modeling results for well D7  
(After S. Niehren, 1998 & S. Niehren and K. Kennedy, 1999)

This requires a colloid porosity parameter that is lower than the mobile zone porosity. Colloid adsorption mechanisms associated with the matrix are a) reversible adsorption of the colloid on the matrix (without maximum capacity); b) irreversible adsorption of the colloid on the matrix with a maximum capacity and c) irreversible adsorption on the already irreversibly adsorbed colloids (colony building) without a maximum capacity. Each sorption mechanism is represented in the model as a transfer coefficient and there is also a term for reversible sorption capacity.

The modeling was done to better understand and portray the behavioral characteristics of the two types of tracers in the heterogeneous porous media at Wilerwald. In choosing the 1D version of CoTrans as a preliminary model to apply at Wilerwald, we assumed that the channel well (D7) behaves as a function of distance from the injection point that is also in the active channel. CoTrans was used with uranine data that could be matched along both the rising and falling limb including tailing with dispersivity ranging from about 2 to 8m. Figure 5-13 illustrates and lists the parameter values used to fit the data. By using a dispersivity of about 2.5 to 3.5 m, we were able to match the uranine, H40 and microsphere data, respectively. By including the colloid in the matching, we narrowed the range of the possible dispersivity values selected for the system. The figure also lists the parameter values used to fit the test data. The data fit well to the model parameters selected. Despite the model simplicity and inherent assumptions, it shows that reasonable values of longitudinal dispersivity can be combined with colloid sorption and two-region solute model parameters to achieve a simulation of the observed field behavior.

## 5.5 Conclusions

We have approached this work principally from an experimentalist viewpoint with objectives targeted principally at two areas – field equipment verification and particle and solute efficacy evaluation. Injection conditions influence the test results and should not be minimized either in their importance or in their detailed presentation for any tracer work done. The higher the degree of subsurface heterogeneity, the more critical are the precise definitions of the actions taken during this phase and in the down gradient sampling locations. Comparison of test data from one series of investigators to another is typically complicated and without clear presentation of the field approach and results, it at best provides a potentially erroneous database. This is particularly true in Wilerwald with its high degree of heterogeneity. The following are our principal conclusions.

During injection at B4, we discovered flow from the well was into a discrete and relatively thin lens/layer between the depths of 4 and 6 m. A similar condition was subsequently documented at another glacio-fluvial site in the Rhone River valley. This finding suggests that hydraulic conductivity used in transport scenarios may be significantly understated. The effective zone contributing to lateral migration in this aquifer may be preferentially occurring in a limited zone on the order of 0.5 m to 1 m thick rather than the fully-screened 10 m interval. This preferential flow layer was also apparent down gradient at C6 and D7 where concentrations were higher than historical tests using the same wells and tracer components.

'Arrival' times based on first detection at each well were similar for all phages and at C6 also for the microspheres. Solute detection, principally due to lower detection limits and background levels at the site, occurred consistently later at C6 and D7 and at the same time at CD. Wells C6 and D7, based on both the tracer response and geophysical interpretation, are located along the longitudinal flow direction of the channel down gradient from the B4 injection point. Well CD is off the main channel and its results indicate more transverse dispersion than seen at C6 or D7. There was no indication of preferential colloidal advection at C6. At CD and D7, H40 exhibited a partial earlier response than uranine for periods of 30 min and 2 h, respectively, during the rising limb, but this is not considered conclusive of earlier colloid arrival.

Transport velocity values pertinent to viral surrogates, such as the colloids we used, may be more relevant when based on the first detection time rather than the time of maximum concentration. At C6 and D7, phages had migration rates of 285 (colloid average) and 223 m/d, respectively. The phage-based arrival velocity to CD off the main channel was 167 m/d. These values are from 2- to 4-fold higher than the average velocities of 135, 80 and 40 m/d calculated from the observed peak uranine concentration times at the same wells. Using colloid pseudo-peak and solute peak time to determine, which "arrived" earlier, may not be appropriate given the high colloid adsorption along the pathway.

Both H40 and microspheres were significantly less attenuated compared to MS2 and Psf2. The relative breakthrough (recovery) of each colloid at a distance of 14 m (C6) ranged from 9 to 41 %. This is a significantly higher recovery than has been reported for other similar-sized tracer components in sand and gravel porous aquifers. The high values suggest little filtration is occurring over this 14 m distance. Differences in H40 and MS2 and Psf2 recovery were only about 2.5-fold at C6, but their differences increased to about 10- to 17-fold at CD and D7. Similar results occur when comparing the  $C/C_0$  values of their pseudo-peaks. Having similar recovery results at both 34 and 64 m but in two different subsurface settings illustrates the non-uniqueness of solutions suggested for the variations in the colloid behavior. There was, however, little visual difference in the slopes and patterns of their individual responses in the falling limb and tailing section at each well suggesting the processes acting on the individual phages, prior to their arrival may be scalable and proportional, at least among these three phages. Only at CD were the shapes of the phage responses after about 50 h less alike and this may have been related to the MS2 and Psf2 concentrations approaching their detection limits. The dispersion characteristic of each phage appears similar based on the consistent breakthrough shapes at each well.

Matching the response of H40, microspheres and uranine at well D7 was done using CoTrans, a 1-D numerical code. The initial approach with attempts to match the uranine data had indicated matching the solute response was possible with a longitudinal dispersivity range of from 2 to 8 m. By matching the particle data at well D7, a longitudinal dispersivity of about 3m was determined. Using this input from the colloid data matching, the dispersivity values of all three components were able to be limited to a smaller range while matching all component data. The presence of both colloid and solute tracer helped reach consistent model results using a double porosity (two-region) model for solute migration and variable sorption conditions for the colloids.

The prototype instrumentation was able to be successfully deployed under typical field conditions. Microspheres and uranine were well documented continuously in the field and rapid analyses were possible of the discrete samples for uranine analysis as well. The inline equipment provided real time assessment of both colloid and uranine tracers allowed site sampling to be effectively modified and optimized. This was particularly important when considering the sampling for phages given that the solute arrival and maximum concentrations observed commonly followed the first colloid detection and ps-peaks. Relying on solute responses to sample for colloids was shown not to be appropriate. Phage laboratory results were also consistent and reliable. The high density sampling during this test allowed definition of precise breakthrough curve shapes and characteristics. Despite large losses in the colloid quantities, they had a regular breakthrough pattern illustrating that their persistence can be effective to evaluate and document viral migration patterns. Additional characterization of the individual colloids used for field tests, however, is needed in order to improve our detailed understanding of what particle parameters and in situ processes can cause the observed differences in their adsorption behavior.

## **CHAPTER 6**

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118. Zhang, P., W.P. Johnson, M.J. Piana, C.C. Fuller & D.L. Nafts (in review - Groundwater): Potential Artifacts in Interpretation of Differential Breakthrough of Colloids and Dissolved Tracers in the Context of Transport in a Zero-valent Iron Permeable Reactive Barrier.

# Appendix A1

## Other related presentations and publications (1997 through 2001)

1. Keith **Kennedy**, Marc Schürch, Aki Kleiner and Pierre-Andre Schnegg, 1999, **MULTIPLE TRACER TYPE RESPONSE VARIABILITY TO GRADIENT AND HETEROGENEITY IN THE UPPER RHONE RIVER AQUIFER**. European Geophysical Society – 1999 Annual Meeting. Session entitled: Intelligent Tracers. Published abstract. The Hague, 19 to 23 April 1999.

Differences in solute (uranine) and particle (bacteriophage) tracer behavior are used to better interpret aquifer responses and provide input for mass transport model verification. Six tracer tests with two configurations were used to document representative migration rates in a 15m alluvial aquifer where gradients can vary seasonally by a factor of four. Improved (< 0.5 ppb) uranine field detection limits helped address particle/solute first arrival issues. For the first configuration (6m effective contributing interval), both 'on-' and 'off-channel' wells had similar particle and uranine arrivals with  $V_{arr}$  varying seasonally from 130 to 250 m/d. On channel at 22m, particle peaks were 25 to 75 percent earlier than uranine. Off channel at 11m, uranine peaks were 70 to 270 percent later than the particles. Particle peaks were similar both on and off channel with  $V_{cmax}$  70 to 160 m/d. Uranine peaked 50 to 100 percent later off channel suggesting lateral dispersion differences or immobile zone diffusion. Tests over gradient extremes showed that the lower the gradient, the greater the peak ( $T_{cmax}$ ) difference between particles and uranine and their relative percentage recoveries. The second configuration three tests were done with a different injection well only during high water conditions. The effective injection interval was about 1 m in highly permeable 'thief zone' at about 6m depth. In this configuration, not only the arrivals, but also the peaks of particles and uranine were similar at a distance of 10m.  $V_{arr}$  was 400 m/d and  $V_{cmax}$  was 150 m/d. At the Bois de Finges site, the same down gradient sampling wells had different tracer responses as a function of tracer type, gradient, injection well and aquifer effective contributing thickness. Field results from multiple tracer types increase our understanding of, and ability to realistically characterize, mass transport of different contaminants and variable heterogeneity.

2. Keith **Kennedy**, Stefan Niehren, Pierre-Andre Schnegg, Pierre Rossi, Wolfgang Kinzelbach and Imre Müller. **MODERN COLLOID & SOLUTE DETECTION METHODS IN POROUS & FRACTURED MEDIA – LABORATORY AND FIELD SCALE EXAMPLES**. European Geophysical Society – 1999 Annual Meeting. Session entitled: Recent developments in field and laboratory methods for groundwater investigations. Published abstract. The Hague, 19 to 23 April 1999.

Representative migration rates depicting multiple contaminant types can now be measured rapidly with high resolution/accuracy. Microspheres (1 micron) are near bacteria in size, bacteriophages (0.1 micron) mimic viral and colloid contaminants and uranine can resemble solutes. Microbiology, particle physics and fluorescent tracer equipment and analysis advances were combined for this applied research. A high sensitivity on-line down hole uranine fluorometer (<0.25ppb) was developed to address solute migration. Particles and biocolloids, while having lower recovery than solutes, have better specificity and lower detection limits. Results from an on-line, in-field microsphere counter based on fluorescent laser techniques (1sphere/10ml) compares well with bacteriophage data (1 pfu/10 ml) despite particle size and surface charge differences. Tests in fractured granite and sand column (cm scale) work had high solute and 30 to 80 percent particle recovery. Tank scale tests (6m) had 25 to 50 percent particle recovery with varying ionic strength and phage type. Field trials in gravel aquifers demonstrated surprisingly consistent and reproducible phage and microsphere patterns to 70 m even with low relative recoveries. Refining these instrument/analysis methods can yield data emulating migration behavior of different contaminant types and become a practical field approach to develop realistic input to transport process models.

3. Keith **Kennedy** and Pierre Rossi, 1999, **BIOCOLLOID TRANSPORT BEHAVIOR - COMPARATIVE STUDIES IN POROUS MEDIA AQUIFERS**. Am. Soc. Microbiology -1999 Annual Mtg. Session 130: Subsurface microbiology. Poster presentation and published abstract. Chicago, 31 May to 3 June 1999.

Documenting actual colloid transport in water supply aquifers using modern 'particle' tracer methods should be a fundamental ground water protection strategy requirement. Combined microbiology and hydrogeology research was done in an experimental tank and under natural-gradient field condition. It demonstrated efficacy of various bacteriophages (Marine: H4, H6, H40; Non-marine: T7, Psf2, MS2), viral/biocolloid 'particle' migration characteristics, and differences in 'real-world' biocolloid transport behavior compared to solute tracers. Medium-scale experiments were carried out in a sandy environment in a tank model 1m by 1m by 6m with a well-defined heterogeneity. MS2 migrated well with total recovery from 23 to 31 percent, decreasing with salt concentration. Psf2 had poor transport with less than one percent recovery. T7 did not migrate in weak salt solutions. The marine phages migrated well throughout. H40 recovery was 36 to 48 percent under salt conditions and 27 percent without salt. H4 and H6 recovery were less than 5 percent. Despite lower recovery, MS2, H4, and H6 had breakthrough pattern characteristics similar to H40. Field trials done in three relatively prolific Swiss gravel-sand aquifers over distances to 70 m had reproducible and distinctive breakthrough shapes for MS2, H4, H6, H40 and Psf2. Predictably, phage recovery was small compared to the solute uranine. However, the phages had surprisingly consistent and similar overall response patterns not mimicking the differences observed in small and micro-scale laboratory column tests. Some bacteriophages, under these porous media conditions: 1) can typify expected colloid behavior to distances of hundreds of meters, 2) illustrate particle mass transport phenomena and characteristics not able to be obtained or predicted using solutes, and 3) have aquifer scale performance that does not match well with extrapolated laboratory column data. H40 was documented as 15 to 55 percent more recoverable than MS2 and may be a preferred alternative biocolloid for future field studies.

4. Keith **Kennedy**, Pierre Rossi, Robert Mosé and Imre Mueller, 1999, **BIOCOLLOID TRACER MIGRATION AS INPUT TO AQUIFER PROTECTION PROGRAMS**. AGU - 1999 Annual Spring Meeting. Session H42C-04: Boston, Poster presentation and published abstract. 01 to 04 June 1999. (AGU Student Paper Award – One of the top five student papers presented at meeting)

Actual documentation of colloid transport in water supply aquifers is feasible using modern 'particle' tracer methods and is preferable, when practical, to extrapolated column-test data. With proper colloid selection, well location and testing protocol, the practice of tracer tests with environmentally 'safe' biocolloids may be considered as an integral part of a valid ground water protection program, particularly where viral or bacterial contamination is considered. Combined hydrogeology and microbiology research was done in granular porous media at two scales: in an experimental tank over 5 m, and under natural-gradient conditions to distances of about 100m at three field sites. We tested the migration characteristics of various biocolloid, viral-sized particles ((Marine (H4, H6, H40) and non-marine (T7, Psf2, MS2) bacteriophages) and demonstrated their real-world differences compared to column tests and commonly referenced solute tracers. Medium-scale experiments were done in a tank (MARCEAUS, ULP/IMF-Strasbourg). This tank contained a sandy environment physical model 1m by 1m by 6m with 1080 cells filled with three different sized sands yielding a setting with a well-defined heterogeneity. Bacteriophage migration behavior was compared to both salt and uranine at different locations and with effectively different contaminant pathways throughout this heterogeneity. The non-marine phage MS2 migrated well with total recoveries of 23 to 31 percent, varying according to ionic strength. The phages Psf2 and T7 had poorer transport capacity with less than one percent recovery and none, respectively. The marine phages migrated well throughout with H40 recovery from 27 to 48 percent again according to ionic strength. Phages H4 and H6 recovery was less than 5 percent. Despite recovery differences, however, the three phages MS2, H4, and H6 had breakthrough pattern characteristics similar to H40. Single and double peak responses were observed and their magnitude and duration varied between the solutes and the biocolloids. Field trials were done in three prolific and highly utilized gravel-sand aquifers in Switzerland. The biocolloids had reproducible and consistent breakthrough shapes for MS2, H4, H6, H40 and Psf2 all of which were quite distinct from uranine, the solute tracer used. Predictably, the particle recovery was quite small compared to the solute. Peak and tailing responses of the two tracers types varied considerably. However, the individual phages had surprisingly consistent and similar overall response patterns in contrast to the differences commonly observed, and thus extrapolated from, small and micro-scale laboratory column tests. The utility of using column test data in viral transport models therefore becomes questionable unless its field- or aquifer- specific consistency has been demonstrated. Some biocolloids, under the tested porous media conditions: 1) can typify expected colloid behavior to distances of hundreds of meters, 2) illustrate particle mass transport phenomena and characteristics not able to be obtained or predicted using solutes, and 3) have aquifer scale performance that does not match well with extrapolated laboratory column data. Others may respond similarly but their recovery ratios are quite different. MS2 is a bacteriophage commonly used in North America as a guide to conservative viral transport rates. Our studies show the marine phage H40 from 15 to 55 percent more recoverable. MS2 is a bacteriophage commonly considered representative for viral transport models. Our research environments showed the marine phage H40 from 15 to 50 percent more recoverable than MS2. This suggests that to treat a biocolloid as conservative, it should be done in concert with field verification. Properly applied, biocolloid tracer behavior may be useful indicator input for aquifer protection programs.

5. Keith **Kennedy**, Stefan Niehren, Wolfgang Kinzelbach and Paul Marschall, 1999, **PARTICLE AND SOLUTE TRANSPORT IN A GRANITE SHEAR ZONES**. AGU - 1999 Annual Spring Mtg. Session H31B-05: Oral presentation and published abstract. Boston, 01 to 04 June 1999.

The concept of a nuclear waste repository in granite has been investigated internationally for about 25 years. Specific laboratory and field migration research continues in Switzerland at the Grimsel Test Site (GTS). The GAM project focuses on potential multiphase transport processes and pathways through shear zones. In the laboratory, the transport behavior of two colloids and a solute (uranine) were compared over 10 days as a tracer mixture migrated through a continuous shear zone planar feature along the length of a new 40 cm undisturbed core. The colloids used included 1-micron diameter fluorescent latex beads and a 0.05 by 0.15 micron diameter tailed biocolloid (bacteriophage H40). The tracer 'cocktail' was injected at a rate of 18 ml/hr for 36 hours and continued with site water for an additional 8.5 days during which time the tailing of the eluted tracers was documented. Both tracer types arrived about two hours after start of injection and the concentrations of both decreased distinctly about 2 hours after the end of injection. Solute recovery was about 90 percent whereas it was 15 and 20 percent for the biocolloid and microspheres, respectively. Particle concentrations fluctuated more than the solute. A distinctive difference between the two tracer types was that both particles tracers peaked well prior to the end of injection in contrast to the solute concentration which continued to increase in the eluted water throughout the injection phase. Solute and microsphere particle concentrations were monitored on-line in real time so sampling and test duration was optimized. Bacteriophage analyses were done offsite within 24 to 72 hours. Beginning in late 1998, a one month long pilot tracer test was carried out in the field across the GAM shear zone. Pilot phase results were needed to specify test protocols and timing for the upcoming colloid and gas migration research tests. An injection-extraction 'dipole' was set up with a stable rate of 1 ml/min at the packed off zones in the two boreholes which were 1.2m apart. A tracer solution consisting of naphthionate and dissolved Helium gas was recirculated opposite the injection packer zone for about 3.5 hours. Both tracer levels were monitored online in real time as needed. Tracers arrived after 12 hours and peaked after 32 hours yielding test condition transport rates of 4 (Vpeak) to 10 (Varr) cm per hour. Solute recovery was 30 percent after five days with a final total recovery of 40 percent. Input from the field tests and related ongoing physical core studies are directly input to the project modeling team who works closely with the test partners. Input coordinated from the three research teams will ultimately lead to better understanding and conceptualization of the processes important to multiphase flow migration in such shear zones.

6. Keith Kennedy and Imre Müller, 1999, **APPLYING DETAILED SURFACE GEOPHYSICS TO POROUS MEDIA AQUIFER CHARACTERIZATION, MODEL DEVELOPMENT AND GROUND WATER PROTECTION**, AGU – 1999 Annual Spring Meeting. Session H31C-10: H19-Effective characterization of Aquifer Heterogeneity and Incorporation into Flow and Transport Models. Oral presentation and published abstract. Boston, 01 to 04 June 1999.

Regional and detailed site specific Radio Magnetotelluric (RMT) and Very Low Frequency-Electromagnetic (VLF-EM) geophysical surveys were used to characterize porous media depositional environments and site paleogeography at a 100m scale. Extensive data and a ground water flow model existed regionally for the surrounding prolific Seeland aquifer (Switzerland, BE). Historic data were too widely spaced to locally distinguish structures, erosion surfaces and permeability resulting from the area's complex peri-glacial events. First, continuous VLF-EM profiles and RMT soundings were made to identify geologic heterogeneity in km profiles at six sites. VLF-EM data yielded a wide range in resistivity and aquifer thickness at four sites. RMT data (20m grid) confirmed high sedimentary variability and patterns of buried river channels. At one of the two sites with relatively uniform aquitard depth and topography, VLF-EM and RMT (>800 points, 5m to 20m grid, 8000m<sup>2</sup>) measurements were made and repeated. RMT and VLF-EM data were then combined to prepare a 3-D visualization of site aquifer thickness (12m to 18 m), aquitard topography with channel and ridge structures. Interpreting the geophysics data was done to characterize the degree of heterogeneity in the subsurface. Results suggested regions illustrating preferred permeability pathways (high resistivity) with limited depth variability. Doublet wells drilled at 7 locations with two depths per location has confirmed aquifer thickness, a silty underlying aquitard and we have developed a guidance protocol relating the gravel aquifer resistivity to permeability/transmissivity. Modeling site ground water flow and transport is including the structure and transmissivity interpretation, detailed core data at 6 borehole locations and both solute and colloid particles tracer test results at two levels in the aquifer. The challenge remains first to integrate the three types of local scale data into a site-scale model which is currently being completed. Once that is done, we hope to upscale from the site model with its documented (relatively high) transport rates to the regional scale characterization. This activity should fall within the standard requirements of, and provide a example of process for, the new Swiss ground water protection law.

7. Keith Kennedy and Imre Müller, 1999, **IMPROVING GROUND WATER PROTECTION STRATEGY BY USING DOCUMENTED CONTAMINANT/TRACER TRANSPORT RATES**, International Symposium on the Geology of Today for Tomorrow, Hungarian Geological Society. Session entitled: Protection of Subsurface Aquifers, Oral presentation and published abstract. Budapest, Hungary, 21 to 24 June 1999.

Documented transport rates from field data should be required input to ground water and wellhead protection programs. Such data has rarely been a part of a consistent and rigorous approach to management models that are specifically developed to address these issues. Reasons frequently cited for not undertaking the work are 1) uncertainty in applying local-scale data to larger models (up-scaling), 2) the (unjustified) perception that cost is prohibitively 'high' to conduct the tracer test work needed to document actual migration rates, and 3) developing and maintaining this input over time to the models is not necessary. Failing, however, to include such 'reality' causes misleading technical bias commonly compounded by continued misapplication. Legislated controls have also largely failed to address differences in particles and dissolved chemical behavior. Many well-intentioned protection strategies rely on overly simplistic equations and 'rule-of-thumb' regional 'flow' estimates that may be unrelated to migration potential. Multiple tracer test results over distances up to 100m in three 'managed' and significant porous media aquifers in Switzerland have illustrated effective ways to determine transport rates even in porous aquifers. Velocity, based on first detection, varied between 40m/d and 250m/d for both solute and particle tracers, orders of magnitude faster than estimated by local protection authorities. Velocity calculated from maximum concentrations was two times greater for particles than for uranine. Channels in the aquifer depicted by detailed geophysics and site tracer test results indicated the +/- 10m spacing required to document these migration rates and important aquifer geometry patterns for migration pathways. Today, we have the scientific tools available to develop more effective ground water protection strategies that rely on pertinent, real-world transport data. These data are available from either natural or injected tracers or from sites with documented contaminant migration. This approach is equally pertinent to karst, fractured and porous media environments, albeit with different field investigation and tracer methodologies.

8. Keith Kennedy, Imre Müller, Euloge Oyono & Mai Ouessini, 1999, **INTERPRETING DETAILED VLF-EM AND RMT GEOPHYSICS FOR POROUS MEDIA AQUIFER CHARACTERIZATION, MODEL DEVELOPMENT AND PROTECTION**, International Symposium on the Geology of Today for Tomorrow, Hungarian Geological Society. Session entitled: Protection of Subsurface Aquifers, Poster presentation and published abstract. Budapest, Hungary, 21 to 24 June 1999.

Regional and detailed site specific Radio Magnetotelluric-Resistivity (RMT 12 to 240 kHz) and Very Low Frequency-Electromagnetic (VLF-EM) geophysical surveys were used to characterize and discriminate between different porous media depositional environments and paleogeography. Extensive data exists on a regional scale for the prolific Seeland aquifer (Switzerland, BE). However, most historic data were too far apart to locally distinguish the differences in important sedimentary structures, erosion surfaces and permeability resulting from the area's complex peri-glacial history. Initially, continuous VLF-EM profiles and RMT sounding data were used to identify geologic heterogeneity at a regional scale over distances over 3km at six sites. A 1-D interpretation model of VLF-EM data yielded profiles with highly variable resistivity, underlying aquitard depth and topography at four of six sites. Interpreting the 2-D RMT data (20m grid) confirmed differences in sedimentary materials and yielded patterns of buried river channels and aquifer/aquitard surfaces. Two sites had more uniform aquitard depths and topography. One site was selected and VLF-EM profiles and RMT (>800 points, 5m to 20m grid, 8000m<sup>2</sup>) measurements have been made and repeated for consistency evaluation. RMT and VLF-EM data have been combined to prepare a 3-D variability model of site aquifer thickness (12m to 18 m), aquitard topography with channel and ridge structures. Interpretation of the data has yielded regions illustrating preferred permeability pathways (high resistivity). Drilling at 7 locations in the ground water research field has confirmed aquifer depth, the aquitard's silty nature and consistency and we have developed a gravel aquifer resistivity correlation. Modeling site ground water flow and transport will be able to include the structure and permeability from this geophysical characterization combined with borehole and tracer test results.

9. Keith **Kennedy**, Stefan Niehren, Wolfgang Kinzelbach and Paul Marschall, 1999, **COLLOIDAL PARTICLE AND SOLUTE MIGRATION BEHAVIOR IN GRANITE SHEAR ZONES**. International Symposium on the Geology of Today for Tomorrow, Hungarian Geological Society. Session entitled: Geological Aspects of Radioactive Waste Disposal, Poster presentation and published abstract. Budapest, Hungary, 21 to 24 June 1999

The concept of nuclear waste repository siting in granite has been actively investigated internationally by geologists and hydrogeologists including extensive work in Canada, Sweden, USA, and Switzerland. The migration of radionuclides through granite would be principally through fractures and shear zones. Characterizing these pathways has been an important interdisciplinary subject related to radioactive waste disposal. Laboratory and field studies of colloid and conventional tracer migration have been done for many shear and fracture zones including those at the Grimsel Test Site (GTS) in the central Alps of Switzerland (Canton Bern). Tests using both colloids (1-micron fluorescent latex beads, 0.2-micron biocolloid (bacteriophage H40) and uranine were done with at 18 ml/hr injection rate over 36 hours through a single planar shear feature in a 30-cm undisturbed granite core sample. Both tracer types arrived at the same time about two hours after start of injection. Solute recovery over 10 days was about 90 percent whereas particle recovery varied between 10 and 20 percent for the biocolloid and microspheres, respectively. Particle responses were similar, albeit with lower recovery than the solute. In the field at GTS, pilot phase testing was done to define the specifications for larger-scale upcoming colloid and gas migration tests to 10m distances in the same shear zone material as the core recently tested. A 16 ppm solution of naphthionate was recirculated opposite the injection packer zone over about 4 hours. An injection-extraction 'dipole' with a flow rate of 1 ml/min was set up between the two packer zones separated by 1.2m. The tracer arrived after 12 hours, peaked after about 36 hours suggesting migration rates from 3 ( $V_{peak}$ ) to 10 ( $V_{arr}$ ) cm per hour. Solute recovery of about 30 percent was obtained after five days of extraction. Applied research projects like this are targeted at better understanding and predicting gas, solute and colloid pathways. They typify modern aspects of and recent developments in geological approaches used as input to risk evaluation studies reflecting potential release scenarios from nuclear and other types of hazardous waste repository settings.

10. Keith **Kennedy** and Imre Müller, 1999, **DETAILED VLF-EM AND RMT METHODS APPLIED TO POROUS MEDIA AQUIFERS**, International Union of Geology and Geophysics (IUGG) & International Association of Hydrological Sciences (IAHS), Joint Meeting. Session entitled: Geophysical techniques applied to hydrogeology. Oral presentation and published abstract. Birmingham, UK. 18 to 31 July 1999.

Detailed Radio Magnetotelluric-Resistivity (RMT 12 to 240 kHz) and Very Low Frequency-Electromagnetic (VLF-EM) geophysical surveys were interpreted to characterize high permeability porous media depositional environments and paleogeography. Existing historic data for the prolific Seeland aquifer (Switzerland, BE) were too widely spaced to determine differences in important sedimentary structures, erosion surfaces and conductivity resulting from the area's complex periglacial history. First, continuous VLF-EM profiles and RMT soundings data identified geologic heterogeneity regionally over 3km at six sites. 1-D VLF-EM profiles showed variable resistivity, underlying aquitard depth and topography at four sites. 2-D RMT data (20m grid) confirmed sediment variability and yielded buried river channels and aquitard patterns. Of two sites with more uniform aquitard depth and topography, one was selected for VLF-EM and RMT (>800 points, 5m to 20m grid, 8000m<sup>2</sup>) measurements. These were used for a 3-D visual model of aquifer thickness (12m to 18 m), channel and ridge structures. Data interpretation showed regions with preferred permeability pathways (high resistivity). Field work took only 3 days and interpretation about two weeks. Wells drilled at 9 locations in the same area, now a ground water research field, confirmed aquifer depth, aquitard nature and consistency. A gravel aquifer resistivity correlation applied to a site flow and transport model will include the structure and permeability from this geophysical characterization combined with borehole and tracer test results. A detailed approach using these rapid, high-resolution RMT and VLF EM methods at regional and site-specific scales is an applicable technique in hydrogeology. Increasing vertical resolution can be done by incorporating recent advances in data interpretation developed jointly with our colleagues at Sopron (HU). In this way, multiple-frequency high resolution data at points can be efficiently interpreted.

11. Keith **Kennedy** and Imre Müller, 1999, **BACTERIOPHAGE MIGRATION PATTERNS IN HIGH VELOCITY AQUIFERS**, ASM International Symposium on Subsurface Microbiology (ISSM'99), Session M2A: Oral presentation and published abstract. Vail, CO, USA. 22 to 27 August 1999.

Documenting colloid transport rates in well developed water supply aquifers has been done with 'instantaneous' injections under natural gradient field conditions at three Swiss sites. Marine (H4, H40) and non-marine (Psf2, MS2) bacteriophages and at one site, microspheres, were variously compared with the solute tracer, uranine. The trials were done in three relatively prolific gravel-sand aquifers over distances from about 10m to 65m. Hydraulic gradients varied from 0.001 to 0.004. Velocity based on first arrival time varied from about 20 to 400 m/day. Arrival appeared similar for the colloidal particles and solute regardless of location in the aquifer heterogeneity. Velocity based on tracer 'peaks' was 15 to 160 m/d and, except in one extremely fast flowing setting, colloids peaked consistently two to three times earlier than uranine. Subsurface heterogeneity precludes comparing migration rates to individual wells without a more dense observation network. Colloid breakthroughs were consistent at each point. Their behavior was distinctive from, and predictably small in relative recovery compared to, uranine. In these and related clean sand tank experiments, phage H40 had higher relative breakthrough and total recovery than any other phage used, about 15 to 55 percent more than MS2. H40 may become a preferred biocolloid surrogate for viral field studies and ground water protection programs requiring tracer experiments. Bacteriophages, under these relatively fast flow conditions: 1) can likely be used to typify expected colloidal behavior to distances of many hundreds of meters given longer injection periods, 2) illustrate particle mass transport phenomena and characteristics not obtainable or predictable using solutes, 3) have patterns that do not appear to upscale well from the larger behavioral differences observed in laboratory column data.

12. Keith **Kennedy**, Stefan Niehren, Paul Marschall, Wolfgang Kinzelbach and Thomas Fierz, 1999, **TRANSPORT OF VIRAL AND BACTERIA SIZED COLLOIDS IN GRANITE FRACTURE ZONES**. ASM International Symposium on Subsurface Microbiology (ISSM'99), Session P25: Poster presentation and published abstract. Vail, CO, USA. 22 to 27 August, 1999

Subsurface colloid migration through fracture porosity can differ considerably from granular porous media yet is particularly critical to hazardous waste site-selection and wellhead protection programs. Colloids intensively attach to mineral surfaces resulting in smaller concentration along advective flow paths. Different colloids are being used to assist characterize shear-zone transport and retardation mechanisms and effective porosity quantification at a high altitude Swiss underground research site. Two colloids (bacteriophage H40, carboxylate latex microspheres) and a solute (uranine) were injected into a 40 cm long core at 18 ml/hr for 36 hours followed by formation water for 8.5 days. On-line uranine and colloid particle (microsphere) data, updated every minute, guided sampling. Both tracer types eluted together 1.8 hours after injection. The solute 'plateau' began at 20 hours and continued during tracer injection. In contrast, both colloids 'peaked' and began decreasing 14 hours prior to ending injection. Recovery and C/C<sub>0</sub> maximums were 90 and 82 percent for uranine and 15 to 20 and 25 to 45 percent for the phages and microspheres, respectively. Bacteriophage and microsphere relative breakthrough was 17 and 22 percent. In the field, a 30 day pilot transport test was completed in 1999. Naphthionate and dissolved helium migrated over a distance of 1.2m under injection/extraction 'dipole' conditions with a flow rate of 1 ml/min. Tracers arrived at 12 hours, had double peaks at 20 and 30 hours with 30 and 40 percent total recovery at 5 and 19 days, respectively. The core particle and field pilot tests were done to provide planning input to this year's bacteriophage and solute migration tests over greater fracture lengths. Biocolloid responses from 1999 field tests will be presented and compared to results from testing to date.

13. Keith **Kennedy**, Pierre Rossi and Philippe Ackerer, 1999, **BIOCOLLOID AND CHEMICAL MASS TRANSPORT IN A WELL-DEFINED HETEROGENEOUS POROUS MEDIA TANK MODEL**. ASM International Symposium on Subsurface Microbiology (ISSM'99), Session P1: Poster presentation and published abstract. Vail, CO, USA. 22 to 27 August, 1999.

Our research in this laboratory environment focussed on distinguishing flow path and mass transport rates of biocolloids and dissolved solutes in heterogeneous porous media. The clear objective is to develop better predictive tools for particulate, particulate-assisted and dissolved constituent contaminant migration/reaction processes. This began with seven tracer test campaigns repeated in a physical tank model with a well-defined physical heterogeneity (MARCEAUs). Six different biocolloids (Marine-H4, H6, H40, Non-marine-T7, Psf2, MS2 bacteriophages) were variably co-mingled with both salt and uranine to begin the experiments with a uniform input signal. The 1m x 1m x 6m tank with three different-permeability sands arranged discretely in 1080 'boxes' (10cmx10cmx40cm) has scale and pore-size conditions similar to some field sites currently used for viral migration studies. The three sand materials are washed and therefore the environment does not contain noticeable silt or clay size fractions. MARCEAU's advantage was a controllable gradient, temperature and chemistry. Salt, a 'conventional' tracer, was monitored on-line and continuously at 360 nodes and gave a fundamental solute migration setting with which to compare the bacteriophage and uranine responses. The phages and uranine were measured at six 'wells' in three of the tank's distinct transport fields. Results showed consistent salt and uranine transport throughout the tank with uranine and phage breakthrough patterns changing in response to ionic strength variations. Double peaks were clear evidence of multiple route contributions in the longer path, lower permeability areas. Biocolloid behavior differed from the solutes. Marine phages were consistent and throughout with similar breakthrough time to salt in the higher permeability regions. The subdued second response of the bacteriophages suggested more mass attachment and selectivity in pathway routing. Results have provided the starting point for currently ongoing transport modeling and biocolloid migration process evaluation.

14. Keith **Kennedy**, Wolfgang Kinzelbach and Stefan Niehren, P. Marschall, Roger Knopp and J.I. Kim, and Thomas Fierz, 1999, **SOLUTE & COLLOID TRANSPORT IN A 'GRIMSEL' GRANITE FRACTURE**. Migration'99, 7th International Conference on the Chemistry and Migration Behavior of Actinides and Fission Products in the Geosphere, Lake Tahoe NV. USA, Oral presentation and published abstract. 26 Sept to 01 Oct 1999.

International scientific research cooperation continues to evaluate solute, colloid and gas transport through fractures/shear zones. Data will help better portray aquifer structure in concert with numerical transport/sorption models. Solutes undergo matrix diffusion, colloids do not. Used together, they become 'intelligent tracers' differentiating pore and migration process characteristics. Gas tracers move with an injected air backbone and can be used to estimate water content. This research is needed to define pathway/release scenarios for radionuclides migrating from nuclear waste repositories in granite environments. Prior solute tracer work has been done in other shear zones at Grimsel (GTS), central Swiss Alps. NAGRA's current GAM project further documents hydraulic, geologic, and transport characteristics in a new shear zone which appears to have lower permeability than others investigated to date at GTS. Core and in-situ tracer tests examine the nature of near-field transport mechanisms and processes. An initial compatibility test was done through a 'planar' shear feature in a core (40-cm long, 14 cm Ø) with a ~1 percent (gross) porosity. Three colloids (1-micron fluorescent microspheres, 0.15- by 0.05-micron biocolloids (bacteriophage H40), 19 nm nanoparticles) and a solute (uranine) were used as tracers. Injection, first of tracer (1.5 days), and second of formation water (8.5 days), was done at 18 ml/hr. Pressure varied from 2.5 to 3.5 bar. Uranine concentrations and microspheres were measured online in real time. No nanoparticles were detected above background. Results showed both colloids and both tracer types eluting two hours after injection. Uranine reached an expected 'plateau/saturation' after 20 hours and increased marginally thereafter. Colloids behaved differently 'peaking' 14 hours before tracer injection ended. Recovery and C/C<sub>0</sub> were 90 and 82 percent for uranine and 15 to 20 and 25 to 45 percent for particles, respectively. Tailing behavior also differed. In-situ tracer work began with a pilot test set up with a 1.2m injection-extraction 'dipole' (1 ml/min) between two boreholes' shear zones. A solution of 16 ppm naphthionate also containing dissolved helium as a tracer was circulated opposite the interval for 3.5 hours. Both tracers responded similarly arriving 12 hours and with double peaks at from 20 to 30 hours, after injection. Naphthionate concentration peak was 60 times lower at extraction than injection. Total solute recovery reached 30 and 40 percent after 5 and 19 days, respectively. Results from future GAM multi-tracer tests will be compared to the core and pilot results and a gas tracer test protocol finalized for the Fall-1999 program.

15. Stefan Niehren and Keith **Kennedy**. 1999, **DISPERSIVE & DIFFUSIVE TRANSPORT PROCESSES IN POROUS MEDIA – COMBINED PARTICLE/SOLUTE TRACER TEST RESULTS**, European Geophysical Society – 1999 Annual Meeting. Session entitled: Intelligent Tracers, Oral presentation and published abstract. The Hague, 19 to 23 April 1999.

Aquifer transport parameters can be better evaluated when multiple tracers types are used. Migration behavior of two types of colloids (viral-sized bacteriophages, fluorescent latex beads) and one solute (uranine) were compared at three wells to distances to 64m in a heterogeneous channelled peri-glacial aquifer in western Switzerland. Recently developed portable, on-line, instrumentation was used in the field to directly measure microsphere (laser particle counting) and uranine (spectrofluorometry) concentrations. On site measurements optimized data collection and sampling strategy and minimized test duration. Analytical resolutions were 0.1 microspheres/ml for the laser detector, 0.3 ppb for the field fluorometer, and 0.5 pfu/ml for bacteriophages (off-site-24 to 72h). Both tracer types had similar arrival times at each of two 'channel' (14m, 64m) and one 'off-channel' (34m) wells.  $V_{arr}$  rates were 280, 230 and 175m/d, respectively. Particle responses were similar despite differences in their size and surface charge properties. Particle peaks were earlier than uranine by 15 to 60 and 120 percent in the channel and off-channel wells, respectively. Interpretation was done using a numerical finite difference code, CoTrans. This code is based on solute migration according to Fick's Law in a dual porosity (mobile/immobile) model and a reversible sorption mechanism for particle transport. Particle breakthrough was used to calculate dispersivity, thereby reducing its possible range if only determined by matching the solute uranine response data. Combined particle/solute tracer tests allows separation of dispersive and diffusive transport mechanisms which are not uniquely distinguishable from single tracer experiments.

16. Pierre Rossi and Keith **Kennedy**, 1999, **ENHANCED COLLOIDAL TRACER PARTICLE ENUMERATION AND IDENTIFICATION METHODOLOGIES AS APPLIED TO SUBSURFACE CONTAMINANT MIGRATION AND TRANSPORT BEHAVIOR RESEARCH**. ASM International Symposium on Subsurface Microbiology (ISSM'99), Session P1: Poster presentation and published abstract. Vail, CO, USA. 22 to 27 August, 1999.

We have used bacteriophage and latex microsphere colloidal particles parallel to conventional solute tracers for hydrogeological and contaminant transport characterization in variable porous media and fractured subsurface environments in Switzerland. These colloids can represent models emulating bacterial and viral migration and facilitated transport. For phages, we reduced the analysis time required, increased the precision and eliminated the non-discriminating nature associated with earlier methods. Our first attempts using bacteriophages as subsurface tracers showed we needed increased analytical reliability and reproducibility with reduced cost/time. We did this by adapting the classical double-layer technique in Petri dishes to each system of bacteriophage/host bacteria (B/HB). Introducing a contact period between the phage and the host bacteria before plating and carefully selecting buffers and growth media allowed both increased numbers of phages detected (>250%) and reduced analysis variability (<5%). An important step was using efficient marine B/HB systems. High salt concentration in the growth medium eliminated background and interference signal noise given by allochthonous bacteria in freshwater subsurface systems. For microspheres, we cooperate with our ETH (Zurich) colleagues who have developed a field durable, high sensitivity (1 particle/ml), on-line particle counting system. This laser-based equipment identifies fluorescent colloids with virus to bacteria size diameters. The real-time colloid identification, along side the solute data, dramatically reduces uncertainty in, and optimizes, sampling programs yielding better migration test performance without sacrificing accuracy. High-accuracy data now available for both phages and 'inactive' microspheres under field trial conditions may help us better evaluate the relevance of behavioral discrepancies frequently observed among colloid species in smaller scale laboratory column and batch tests.

17. Keith **Kennedy**, Marc Schürch, Imre Müller & Francois Vuataz, 2001, **DOCUMENTED TRANSPORT CONDITIONS AND MIGRATION RATES IN GRAVEL DEPOSITS – A GROUNDWATER PROTECTION PERSPECTIVE**. Int. Ass. Hydrogeology (IAH) – 2001 Annual Meeting. Oral presentation and published paper. Munich, Germany, 9 to 12 September, 2001.

Migration conditions in a gravel aquifer of the upper Rhone River valley were studied using particle and solutes as contaminant surrogates. Transport rates were 130 to 480 m/d over distances to 22 m, up to 40 times faster than predicted using conventional flow/effective porosity parameters. In one well, a 1-m vertical pathway heterogeneity dominated the 12-m aquifer saturated thickness. Biocolloids were consistently detected earlier than solutes due in part to their significantly lower detection limits and possibly to preferential particle advection. Biocolloid detection was from 3- to 7-fold earlier than the time to solute breakthrough peaks, those values commonly relied on when calculating reference velocity parameters. Relative colloid recovery was typically 1.5 to 4 percent and in one case was 72 % of the solute illustrating relatively low biocolloid attenuation in river gravel macropores. Transport direction was up to 90° off those determined from head-derived measurements. Results suggest that reliable groundwater protection strategy in heterogeneous gravel aquifers may improve when field-verified with migration characterization using multiple tracer types.

18. Pierre Rossi, Nathalie Dörfliger, Keith **Kennedy**, Imre Müller and Michel Aragno, 1998, **BACTERIOPHAGES AS SURFACE AND GROUND WATER TRACERS**. Published paper. Hydrol. & Earth Sys. Sci., 2 (1), 101-110.

Bacteriophages are increasingly used as tracers for quantitative analysis in both hydrology and hydrogeology. The biological particles presented here are neither toxic nor pathogenic for other living organisms as they penetrate only a specific bacterial host. They have many advantages over classical fluorescent tracers and offer the additional possibility of multi-point injection for tracer tests. Several years of research make them suitable for quantitative transport analysis and flow boundary delineation in both surface and ground waters, including karst, fractured and porous media aquifers. This article presents the effective application of bacteriophages based on their use in differing Swiss hydrological environments and compares their behavior to conventional colored dye or salt-type tracers. In surface water and karst aquifers, bacteriophages travel at about the same speed as the typically referenced fluorescent tracers (uranine, sulforhodamine G extra). In aquifers of interstitial porosity, however, they appear to migrate more rapidly than fluorescent tracers, albeit with a significant reduction in their numbers within the porous media. This faster travel time implies that a modified rationale is needed for defining some ground water protection area boundaries. Further developments of other bacteriophages and their documentation as tracer methods should result in an accurate and efficient tracer tool that will be a proven alternative to conventional fluorescent dyes.

19. Pierre-Andre Schnegg and **Keith Kennedy**, 1998, **A NEW BOREHOLE FLUOROMETER FOR DOUBLE TRACER TESTS**. Mass Transport In Fractured Aquifers And Aquitards. Published paper and oral presentation, Geoscience Cntr Copenhagen, 14 to 16 May, 1998.

This abstract describes the principle and characteristics of a field fluorometer that was designed by our institute to provide automatic monitoring of tracer concentrations. Its shape and size allows its use downhole in boreholes with diameters of 3-inches and more to depths up to 50 meters. A double-excitation, double-detection optical scheme permits simultaneous concentration measurements of two different dye tracers and water turbidity. Although the optical filter set is selected for the dye tracer pair uranine (fluorescein) - sulforhodamine, other dyes can be used. Because of a favourable separation of their characteristics in the visible spectrum, these two tracers can be used simultaneously at two different injection sites. The instrument can measure the response of both dyes even when there is an overlap of the two tracing curves. Real-time measurement of the turbidity in the range of 0.02 to 400 nephelometric units is also of great interest.

20. **Keith Kennedy**, Imre Müller and Euloge Oyono, 1998, **SURFACE GEOPHYSICS APPLIED TO PERMEABILITY DISCRIMINATION, CHARACTERIZATION AND SITE MODEL DEVELOPMENT**. European Geophysical Society – 1998 Annual Meeting. Session HSA1/01 – Characterization and modelling of the 2-D and 3-D structure of porous and fractured formations. Oral presentation and published abstract. Nice, April 1998.

Radio Magnetotelluric-Resistivity (RMT 12 to 240 kHz) and Very Low Frequency-Electromagnetic (VLF-EM) surveys were used to characterize and discriminate between different porous media depositional environments as part of a site selection process. Despite extensive data for the prolific Seeland aquifer (Switzerland, BE), most data were too far apart to locally distinguish the differences in important sedimentary structures, erosion surfaces and permeability resulting from the area's complex depositional history. Continuous VLF-EM profiles and RMT data identified geologic heterogeneity over about 3km at six sites (Canton Berne). A 1-D interpretation model of VLF-EM data yielded profiles with highly variable resistivity, underlying aquitard depth and topography at four of six sites. Interpreting the 2-D RMT data (20m spacing) confirmed these differences in the sedimentary materials and yielded patterns of buried river channels and aquifer /aquitard surfaces. The other two sites had more uniform aquitard depths and topography. Land ownership issues and aquifer depth resulted in the selection of one 'preferred site' where detailed VLF-EM profiles and RMT (>300 points, 5m to 20m, 8000m<sup>2</sup>) measurements were made. RMT and VLF-EM data were combined to prepare a 3-D variability model of site aquifer thickness (12m to 18 m), aquitard topography (channel and ridge), and preferred permeability pathways (high resistivity). Drilling at 7 points confirmed aquifer depth, silty aquitard and gravelly aquifer resistivity interpretation. Modeling site ground water flow and transport will include structure and permeability from this geophysical characterization.

21. **Keith Kennedy**, Imre Müller and Marc Schürch, 1998, **DOCUMENTED CONTAMINANT TRANSPORT RATES – THEIR USE TO REDUCE UNCERTAINTY AND IMPROVE MODEL PREDICTION**. European Geophysical Society – 1998 Annual Meeting. Session HSA1/002 - Identification of Model Parameters in groundwater hydrology. Poster presentation and published abstract. Nice, April 1998.

Documented, rather than 'estimated' transport rates, should be required input to ground water management and wellhead protection programs. However, such data may not always be rigorously input to regional model aquifer scales. Uncertainty in applying 'point-source' data, the perceived 'high' cost of getting it and scaling factors are reasons its importance may have been overlooked. Failing, however, to include such 'reality' causes misleading technical bias commonly compounded by continued misapplication. Legislated controls have also largely failed to address differences in (colloid) particles and dissolved chemicals transport. Many management and well-intentioned protection strategies have relied on simple flow assumptions and 'rule-of-thumb' regional values to predict velocities and transport. Model sizes are frequently too large to be effective. Multiple tracer test results over distances up to 100m in three 'managed' and significant porous media aquifers in Switzerland suggest localized channels of 10m to 50 m width limited the injected fluorescent and colloidal tracer migration. Velocity, based on first detection, was between about 40m/d and 250m/d for both tracer types, about twice the velocity to peak particle concentrations. Uranine peaks were from 50 to 100 percent slower than colloid peaks. Neither regional nor site-specific geology nor hydrogeology identified, beforehand, these channels with their rapid migration potential. Perhaps equal effort should be taken to appropriately scale models to match the input specificity and to develop pertinent local protection and management strategies rather than focus on basin or catchment-size models whose scale may not adequately reflect heterogeneity in transport potential. The distinct tracer behavior is significant as input to models of contaminant transport scenarios.

22. Ronald Kozel, Keith **Kennedy**, Jurg Zobrist and Pierre Rossi, 1998, **REDOX CONDITION HETEROGENEITY AND ITS INFLUENCE ON CHEMICAL, MICROBIOLOGICAL AND HYDRODYNAMIC CHARACTERISTICS IN A WASTE PLUME (W. SWITZERLAND)**. European Geophysical Society – 1998 Annual Meeting. Session HSA8/03 - Redox processes in aquifers. Oral presentation and published abstract. Nice, April 1998.

Diverse and widespread reduced conditions exist in the Seeland aquifer (70 km<sup>2</sup>), an important public water supply. To the SE, these are mainly due to decades of seepage, rich in degradable organics, from unlined discharge sites at a sugar refinery. Studies in the 1950s and 1970s showed an extended plume of reduced groundwater deteriorating water supplies. The aquifer consists of about 20 m of inter-layered gravel, sand, silt and clay which are of lacustrine and fluvio-glacial origin. This heterogeneity makes it difficult to determine exact horizontal/vertical plume extent, its Redox conditions and the transport of contaminants DOC, Fe, Mn and K. Recent regional surveys show limited rehabilitation from the infiltrating Alte Aare River. In 1997, the Redox chemistry and the microbial activity were evaluated in 10 wells at a field site about 1 km from the original ponds. Redox conditions varied strongly even over the limited distance of 70 m and between the shallow and deeper aquifer layers. Eh varied from -180 mV to +61 mV and dissolved oxygen from 5 to 25 % saturation. Oxygen added to observation wells caused rapid indigenous iron and manganese oxidizing bacterial growth (*Siderocapsa* sp., *Gallionella* sp., *Leptothrix* sp.) and high-permeability (10-2 m/s) gravel plugged in several hours. Ongoing work will document the iron, manganese, sulfate and bacterial species and investigate the mechanism of pathway clogging.

23. Keith **Kennedy**, Robert Mosé, Pierre Rossi and Imre Müller, 1998, **COMPARATIVE BEHAVIOR OF BIOCOLLOID AND CHEMICAL MASS TRANSPORT IN HETEROGENEOUS POROUS MEDIA**. European Geophysical Society – 1998 Annual Meeting. Session HSA1/03 – Reactive mass transport: experimental studies of chemical, colloid and biological processes. Oral presentation and published abstract. Nice, April 1998.

Carefully documented colloid/solute mass transport behavior can develop better predictive tools and understanding of particulate, particulate-assisted and dissolved constituent contaminant migration/reaction processes. Our initial objective was to compare flow path and mass transport rates of biocolloids and dissolved solutes in heterogeneous porous media. This was done with repeated measurements in a well-defined physical tank model (MARCEAUs). Five different biocolloids (Marine-H4, H6, H40, Non-marine-T7, Psf2 bacteriophages) were variably co-mingled with both salt and uranine thereby providing a uniform input signal. The 1mx1mx6m tank with three different-permeability sands arranged discretely in 1080 'boxes' (10cmx10cmx40cm) has scale and pore-size conditions similar to some current field sites. MARCEAU's advantage was a controllable gradient, temperature and chemistry. Salt, a 'conventional' tracer, was monitored on-line and continuously at about 360 nodes and gave a base with which to compare colloid and uranine response. This was measured at six 'wells' in three of the tank's distinct transport fields. Results showed a remarkably similar salt and uranine transport that was consistent throughout the tank. Double peaks were clear evidence of multiple pathway contributions in the longer path, lower permeability areas. Biocolloid behavior differed. Marine phages were consistent and throughout with similar breakthrough time to salt in high permeability areas. The subdued second response suggested more mass reaction and selectivity in pathway routing. Results provide a base for comparative colloid/solute transport modeling and related reactive process studies.

24. Keith **Kennedy**, 2000, **BACTERIOPHAGES AS PARTICLE MIGRATION INDICATORS IN SUBSURFACE ENVIRONMENTS**. IAHS sponsored conference – Tracers and Modeling in Hydrogeology, IAHS Red Book, **262**, 151-158. Invited guest speaker - Oral presentation and published paper. Liege, May 2000.

Bacteriophages can assist characterize hydrogeological settings and document contaminant behavior. They emulate viral and facilitated/assisted transport. Field research was done in Switzerland in peri-glacial aquifers under natural gradients to 65 m and in a granodiorite shear zone to 1.7 m. Phages had distinct and quantifiable breakthrough. Particle first arrivals were similar to solutes. Phage  $C/C_{0-max}$  was notably earlier than solute  $Tr/Tr_{0-max}$ . Typical retardation factors (RF) were from 0.2 to 0.9. In the porous media, transport rates were from 40 to 440 m day<sup>-1</sup>, phage relative breakthroughs (RBs) was from 0.005 to 72 % and  $C/C_{0-max}$  from  $3e^{-10}$  to  $4e^{-4}$ . Three shear zone dipole tests illustrated an open path, as well as fault gouge dominated, responses. RBs were from 0.15 to 41 % and  $C/C_{0-max}$  from  $7e^{-4}$  to  $2e^{-1}$ . Low particle attenuation was in channels discovered in thin (<2 m) aquifer layers and in one direction in the shear zone.

## Appendix A2 for Chapter 2

### Laboratory Tank Model – MARCEAUS Fluid Mechanics Institute, ULP- Strasbourg, FR

Appendix A2-1 Test 1 Data

Appendix A2-2 Test 2 Data

Appendix A2-3 Test 3 Data

Appendix A2-4 Test 4 Data

Appendix A2-5 Test 5 Data

Appendix A2-6 Test 6 Data

Appendix A2-7 Tank Model Illustrations  
(after Ruch, 1992)

APPENDIX 2-1 Strasbourg Test 1 Data

| STR1 Start: 19-Mar-97 13:15 |      |          |      |         |      | STR1 End: 20-Feb-97 03:24 |      |          |      |         |      |
|-----------------------------|------|----------|------|---------|------|---------------------------|------|----------|------|---------|------|
| Sample Date/Time            | (h)  | (pfu/ml) |      | (us/cm) |      | Sample Date/Time          | (h)  | (pfu/ml) |      | (us/cm) |      |
| DS LOCATION:                | TSI  | H40      | H4   | EC      | d EC | P2 LOCATION               | TSI  | H40      | H4   | EC      | d EC |
| 3/19/1997 12:50             | -0.4 | 0        | 0    | 661     |      | 3/19/1997 12:50           | -0.4 | 0        | 0    | 706     |      |
| 3/19/1997 15:15             | 2.0  | 0        | 0    | 664     |      | 3/19/1997 17:15           | 4.0  | 55       | 5    | 709     | 3    |
| 3/19/1997 16:15             | 3.0  | 5950     | 885  | 742     | 78   | 3/19/1997 18:15           | 5.0  | 1215     | 60   | 761     | 55   |
| 3/19/1997 17:15             | 4.0  | 9500     | 440  | 1055    | 391  | 3/19/1997 18:45           | 5.5  | 1000     | 88   | 828     | 122  |
| 3/19/1997 17:45             | 4.5  | 2950     | 246  | 1105    | 441  | 3/19/1997 19:15           | 6.0  | 800      | 94   | 935     | 229  |
| 3/19/1997 18:15             | 5.0  | 3050     | 440  | 1240    | 576  | 3/19/1997 19:45           | 6.5  | 2000     | 316  | 1117    | 411  |
| 3/19/1997 18:45             | 5.5  | 2110     | 665  | 1330    | 666  | 3/19/1997 20:15           | 7.0  | 2650     | 338  | 1329    | 623  |
| 3/19/1997 19:15             | 6.0  | 1555     | 430  | 1487    | 823  | 3/19/1997 20:45           | 7.5  | 1600     | 281  | 1486    | 780  |
| 3/19/1997 19:45             | 6.5  | 1200     | 400  | 1521    | 857  | 3/19/1997 21:15           | 8.0  | 2000     | 97   | 1644    | 938  |
| 3/19/1997 20:30             | 7.2  | 1075     | 300  | 1587    | 923  | 3/19/1997 21:45           | 8.5  | 1450     | 106  | 1762    | 1056 |
| 3/19/1997 20:45             | 7.5  | 930      | 116  | 1514    | 850  | 3/19/1997 22:15           | 9.0  | 1550     | 91   | 1899    | 1193 |
| 3/19/1997 21:15             | 8.0  | 446      | 150  | 1408    | 744  | 3/19/1997 22:45           | 9.5  | 1090     | 85   | 1995    | 1289 |
| 3/19/1997 21:45             | 8.5  | 166      | 12   | 1172    | 508  | 3/19/1997 23:15           | 10.0 | 925      | 56   | 2080    | 1374 |
| 3/19/1997 22:15             | 9.0  |          |      | 1046    | 382  | 3/20/1997 00:15           | 11.0 | 410      |      | 2110    | 1404 |
| 3/19/1997 22:45             | 9.5  |          |      |         |      | 3/20/1997 00:45           | 11.5 | 370      | 14   | 1987    | 1281 |
| 3/19/1997 23:15             | 10.0 |          |      | 856     | 192  | 3/20/1997 01:15           | 12.0 | 213      | na   | 1775    | 1069 |
| 3/20/1997 00:15             | 11.0 | 71       | 5    | 861     | 197  | 3/20/1997 01:45           | 12.5 | 168      | 6    | 1445    | 739  |
| 3/20/1997 01:15             | 12.0 | 79       | 3    | 788     | 124  | 3/20/1997 02:15           | 13.0 | 175      |      | 1187    | 481  |
| 3/20/1997 03:15             | 14.0 | 50       | 0    | 735     | 71   | 3/20/1997 02:45           | 13.5 | 147      | 4    | 1012    | 306  |
|                             |      |          |      |         |      | 3/20/1997 03:15           | 14.0 | 105      |      | 869     | 163  |
| IW LOCATION:                | TSI  | H40      | H4   | EC      | d EC | P3 LOCATION               | TSI  | H40      | H4   | EC      | d EC |
| 3/19/1997 12:50             | -0.4 | 0        | 0    | 660     |      | 3/19/1997 12:50           | -0.4 | 0        | 0    | 693     |      |
| 3/19/1997 13:35             | 0.3  | 64       | 7    | 709     | 49   | 3/19/1997 14:15           | 1.0  |          |      | 710     |      |
| 3/19/1997 13:55             | 0.7  | 6300     | 425  | 705     | 45   | 3/19/1997 15:15           | 2.0  | 0        | 0    | 699     | 6    |
| 3/19/1997 14:15             | 1.0  | 38500    | 4700 | 1058    | 398  | 3/19/1997 15:35           | 2.3  | 170      | 13   | 707     | 14   |
| 3/19/1997 14:35             | 1.3  | 44000    | 4450 | 1557    | 897  | 3/19/1997 15:55           | 2.7  | 9000     | 965  | 774     | 81   |
| 3/19/1997 14:55             | 1.7  | 22000    | 1950 | 1967    | 1307 | 3/19/1997 16:15           | 3.0  | 13000    | 1020 | 1391    | 698  |
| 3/19/1997 15:15             | 2.0  | 9600     | 530  | 2180    | 1520 | 3/19/1997 16:35           | 3.3  | 9500     | 410  | 1927    | 1234 |
| 3/19/1997 16:15             | 3.0  | 790      | 10   | 2370    | 1710 | 3/19/1997 16:55           | 3.7  | 3650     | 105  | 2180    | 1487 |
| 3/19/1997 17:15             | 4.0  | 90       | 1    | 2350    | 1690 | 3/19/1997 17:15           | 4.0  | 1505     | 45   | 2260    | 1567 |
| 3/19/1997 18:15             | 5.0  | 26       | 0    | 1700    | 1040 | 3/19/1997 17:45           | 4.5  | 865      | 15   | 2280    | 1587 |
| 3/19/1997 18:45             | 5.5  |          | 7    |         |      | 3/19/1997 18:15           | 5.0  | 430      | 6    | 2310    | 1617 |
| 3/19/1997 19:15             | 6.0  |          |      | 741     | 81   | 3/19/1997 18:45           | 5.5  | 235      | 4    | 2330    | 1637 |
| 3/19/1997 20:30             | 7.2  | 8        | 0    | 713     | 53   | 3/19/1997 19:15           | 6.0  | 211      | 1    | 2330    | 1637 |
| 3/19/1997 21:15             | 8.0  |          |      | 692     | 32   | 3/19/1997 20:30           | 7.2  | 222      | 5    | 1705    | 1012 |
| 3/19/1997 22:15             | 9.0  | 6        | 0    | 662     | 2    | 3/19/1997 21:15           | 8.0  | 66       |      | 1000    | 307  |
| 3/19/1997 22:45             | 9.5  |          |      |         |      | 3/19/1997 22:15           | 9.0  | 37       | 0    | 810     | 117  |
| 3/19/1997 23:15             | 10.0 |          |      | 690     | 30   | 3/19/1997 22:45           | 9.5  |          |      |         |      |
| 3/20/1997 03:15             | 14.0 | 3        | 0    | 707     | 47   | 3/19/1997 23:15           | 10.0 |          |      | 752     | 59   |
|                             |      |          |      |         |      | 3/20/1997 00:15           | 11.0 |          |      | 752     | 59   |
|                             |      |          |      |         |      | 3/20/1997 01:15           | 12.0 | 17       | 0    | 722     | 29   |
|                             |      |          |      |         |      | 3/20/1997 02:15           | 13.0 |          |      |         |      |
|                             |      |          |      |         |      | 3/20/1997 03:15           | 14.0 | 17       | 0    | 725     | 32   |
| P1 LOCATION                 | TSI  | H40      | H4   | EC      | d EC | P4 LOCATION               | TSI  | H40      | H4   | EC      | d EC |
| 3/19/1997 12:50             | -0.4 | 0        | 0    | 707     |      | 3/19/1997 12:50           | -0.4 | 0        | 0    | 711     |      |
| 3/19/1997 15:15             | 2.0  | 0        | 0    | 707     |      | 3/19/1997 15:15           | 2.0  | 0        | 0    | 711     |      |
| 3/19/1997 16:15             | 3.0  | 3        | 0    | 709     |      | 3/19/1997 16:15           | 3.0  |          |      | 721     | 10   |
| 3/19/1997 16:35             | 3.3  | 47       | 6    | 707     | 0    | 3/19/1997 17:15           | 4.0  | 50       | 14   | 724     | 13   |
| 3/19/1997 16:55             | 3.7  | 395      | 61   | 708     | 1    | 3/19/1997 18:15           | 5.0  | 400      | 8    | 744     | 33   |
| 3/19/1997 17:15             | 4.0  | 165      | 195  | 728     | 21   | 3/19/1997 18:45           | 5.5  | 1080     | 106  | 847     | 136  |
| 3/19/1997 17:45             | 4.5  | 248      | 365  | 787     | 80   | 3/19/1997 19:15           | 6.0  | 1720     | 74   | 946     | 235  |
| 3/19/1997 18:15             | 5.0  | 4050     | 520  | 914     | 207  | 3/19/1997 19:45           | 6.5  | 1420     | 112  | 1114    | 403  |
| 3/19/1997 18:45             | 5.5  | 4600     | 1185 | 1124    | 417  | 3/19/1997 20:30           | 7.2  | 1160     | 85   | 1163    | 452  |
| 3/19/1997 19:15             | 6.0  | 4550     | 790  | 1444    | 737  | 3/19/1997 20:45           | 7.5  | 1300     | 99   | 1255    | 544  |
| 3/19/1997 20:15             | 7.0  | 1700     | 300  | 1785    | 1078 | 3/19/1997 21:15           | 8.0  | 905      | 67   | 1342    | 631  |
| 3/19/1997 21:15             | 8.0  | 625      | 65   | 2010    | 1303 | 3/19/1997 21:45           | 8.5  | 685      | 61   | 1427    | 716  |
| 3/19/1997 22:15             | 9.0  | 470      | 57   | 1932    | 1225 | 3/19/1997 22:15           | 9.0  | 700      | 42   | 1502    | 791  |
| 3/19/1997 22:45             | 9.5  |          |      |         |      | 3/19/1997 22:45           | 9.5  | 440      | 41   | 1603    | 892  |
| 3/19/1997 23:15             | 10.0 | 580      | 59   | 1626    | 919  | 3/19/1997 23:15           | 10.0 | 370      | 28   | 1781    | 1070 |
| 3/20/1997 00:15             | 11.0 | 325      | 38   | 1343    | 636  | 3/20/1997 00:15           | 11.0 | 263      |      | 1832    | 1121 |
| 3/20/1997 01:15             | 12.0 |          |      | 1055    | 348  | 3/20/1997 00:45           | 11.5 | 188      | 10   | 1846    | 1135 |
| 3/20/1997 02:15             | 13.0 | 106      | 7    | 891     | 184  | 3/20/1997 01:15           | 12.0 | 126      |      | 1783    | 1072 |
| 3/20/1997 02:15             | 13.0 |          | 2    |         |      | 3/20/1997 01:45           | 12.5 | 112      | 7    | 1672    | 961  |
| 3/20/1997 02:45             | 13.5 |          | 4    | 847     | 140  | 3/20/1997 02:15           | 13.0 | 74       |      | 1520    | 809  |
| 3/20/1997 03:15             | 14.0 | 108      | 4    | 817     | 110  | 3/20/1997 02:45           | 13.5 | 87       | 5    | 1331    | 620  |
|                             |      |          |      |         |      | 3/20/1997 03:15           | 14.0 | 68       | na   | 1326    | 615  |

APPENDIX 2-1 Strasbourg Test 1 Data

| STR1 Start: 19-Mar-97 13:15 |      |          |      |         |      | STR1 End: 20-Feb-97 03:24                                   |      |          |       |         |      |
|-----------------------------|------|----------|------|---------|------|-------------------------------------------------------------|------|----------|-------|---------|------|
| Sample DateTime             | (h)  | (pfu/ml) |      | (us/cm) |      | Sample DateTime                                             | (h)  | (pfu/ml) |       | (us/cm) |      |
| PW LOCATION                 | TSI  | H40      | H4   | EC      | d EC | US LOCATION                                                 | TSI  | H40      | H4    | EC      | d EC |
| 3/19/1997 12:50             | -0.4 | 0        | 0    | 701     |      | 3/19/1997 12:50                                             | -0.4 | 0        | 0     | 684     |      |
| 3/19/1997 14:15             | 1.0  | 1        | 0    | 669     |      | 3/19/1997 13:35                                             | 0.3  | 105590   | 26500 | 1990    | 1306 |
| 3/19/1997 14:35             | 1.3  | 0        | 0    | 656     |      | End Phage injection from separate source after about 1 hour |      |          |       |         |      |
| 3/19/1997 14:55             | 1.7  | 4100     | 420  | 696     | 27   | 3/19/1997 14:15                                             | 1.0  | 3550     | 22    | 2350    | 1666 |
| 3/19/1997 15:15             | 2.0  | 43000    | 4950 | 929     | 260  | 3/19/1997 15:15                                             | 2.0  | 49       | 1     | 2370    | 1686 |
| 3/19/1997 15:35             | 2.3  | 37500    | 4000 | 1694    | 1025 | 3/19/1997 16:15                                             | 3.0  | 14       | 0     | 2370    | 1686 |
| 3/19/1997 15:55             | 2.7  | 10500    | 800  | 2160    | 1491 | 3/19/1997 17:15                                             | 4.0  | 6        | 0     | 2370    | 1686 |
| 3/19/1997 16:15             | 3.0  | 3500     | 95   | 2280    | 1611 | 3/19/1997 18:45                                             | 5.5  |          |       |         |      |
| 3/19/1997 16:35             | 3.3  | 1650     | 39   | 2370    | 1701 | 3/19/1997 19:15                                             | 6.0  | 3        | 0     | 619     | 619  |
| 3/19/1997 16:55             | 3.7  | 985      | 33   | 2370    | 1701 | 3/19/1997 21:15                                             | 8.0  | 0        | 0     | 674     | stop |
| 3/19/1997 17:15             | 4.0  | 505      | 19   | 2370    | 1701 | 3/19/1997 22:45                                             | 9.5  |          |       |         |      |
| 3/19/1997 17:45             | 4.5  | 255      | 8    | 2380    | 1711 | 3/19/1997 23:15                                             | 10.0 | 0        | 0     | 622     |      |
| 3/19/1997 18:15             | 5.0  | 210      | 7    | 2370    | 1701 | 3/20/1997 01:15                                             | 12.0 |          |       | 713     |      |
| 3/19/1997 18:45             | 5.5  |          |      |         |      | 3/20/1997 03:15                                             | 14.0 | 0        | 0     | 705     |      |
| 3/19/1997 19:15             | 6.0  | 116      | 7    | 2300    | 1631 |                                                             |      |          |       |         |      |
| 3/19/1997 20:15             | 7.0  |          |      | 822     | 153  |                                                             |      |          |       |         |      |
| 3/19/1997 20:45             | 7.5  | 19       | 1    | 707     | 38   |                                                             |      |          |       |         |      |
| 3/19/1997 21:15             | 8.0  |          |      | 680     | 11   |                                                             |      |          |       |         |      |
| 3/19/1997 21:45             | 8.5  | 20       | 1    | 690     | 21   |                                                             |      |          |       |         |      |
| 3/19/1997 22:15             | 9.0  |          |      | 679     | 10   |                                                             |      |          |       |         |      |
| 3/19/1997 22:45             | 9.5  |          |      |         |      |                                                             |      |          |       |         |      |
| 3/19/1997 23:15             | 10.0 | 13       | 1    | 687     | 18   |                                                             |      |          |       |         |      |
| 3/20/1997 01:15             | 12.0 |          |      | 714     | 45   |                                                             |      |          |       |         |      |
| 3/20/1997 03:15             | 14.0 | 10       | 0    | 708     | 39   |                                                             |      |          |       |         |      |

# APPENDIX A2-2 Strasbourg Test 2 Data

| STR2 START: 21-Mar-97 08:00 |      |          |      |       |      | STR2 END: 21-Mar-97 21:30 |      |          |     |       |      |
|-----------------------------|------|----------|------|-------|------|---------------------------|------|----------|-----|-------|------|
| Sample Date/Time            | (h)  | (pfu/ml) |      | us/cm |      | Sample Date/Time          | (h)  | (pfu/ml) |     | us/cm |      |
| DS LOCATION                 | TSI  | H40      | H6   | EC    | d EC | P2 LOCATION               | TSI  | H40      | H6  | EC    | d EC |
| 21/Mar/97 06:50             | -1.2 | 28       | 0    | 644   | 0    | 21/Mar/97 06:50           | -1.2 | 30       | 0   | 705   | 0    |
| 21/Mar/97 09:00             | 1.0  | 23       | 0    | 713   | 69   | 21/Mar/97 09:40           | 1.7  |          |     | 707   | 2    |
| 21/Mar/97 09:40             | 1.7  | 16       | 0    | 699   | 55   | 21/Mar/97 12:00           | 4.0  | 33       | 0   | 708   | 3    |
| 21/Mar/97 10:40             | 2.7  | 75       | 5    | 714   | 70   | 21/Mar/97 12:20           | 4.3  | 38       | 1   | 715   | 10   |
| 21/Mar/97 11:40             | 3.7  | 910      | 128  | 1120  | 476  | 21/Mar/97 12:40           | 4.7  | 75       | 4   | 745   | 40   |
| 21/Mar/97 12:20             | 4.3  | 1900     | 229  | 1672  | 1028 | 21/Mar/97 13:00           | 5.0  | 195      | 12  | 780   | 75   |
| 21/Mar/97 13:00             | 5.0  | 2340     | 380  | 1900  | 1256 | 21/Mar/97 13:30           | 5.5  | 280      | 19  | 844   | 139  |
| 21/Mar/97 13:30             | 5.5  | 2135     | 530  | 2040  | 1396 | 21/Mar/97 14:00           | 6.0  | 360      | 27  | 924   | 219  |
| 21/Mar/97 14:00             | 6.0  | 3250     | 605  | 2150  | 1506 | 21/Mar/97 14:30           | 6.5  | 630      | 41  | 1102  | 397  |
| 21/Mar/97 14:30             | 6.5  | 3150     | 810  | 2200  | 1556 | 21/Mar/97 15:00           | 7.0  | 700      | 65  | 1281  | 576  |
| 21/Mar/97 15:00             | 7.0  | 2550     | 505  | 2070  | 1426 | 21/Mar/97 15:30           | 7.5  | 790      | 82  | 1446  | 741  |
| 21/Mar/97 16:00             | 8.0  | 935      | 170  | 1537  | 893  | 21/Mar/97 16:00           | 8.0  | 815      | 86  | 1597  | 892  |
| 21/Mar/97 17:00             | 9.0  | 1070     | 225  | 1254  | 610  | 21/Mar/97 16:30           | 8.5  | 830      | 86  | 1728  | 1023 |
| 21/Mar/97 19:30             | 11.5 | 60       | 23   | 798   | 154  | 21/Mar/97 17:00           | 9.0  | 750      | 78  | 1825  | 1120 |
| 21/Mar/97 21:30             | 13.5 | 87       | 15   | 738   | 94   | 21/Mar/97 17:30           | 9.5  | 870      | 89  | 1967  | 1262 |
|                             |      |          |      |       |      | 21/Mar/97 21:00           | 13.0 | 430      | 50  | 1152  | 447  |
|                             |      |          |      |       |      | 21/Mar/97 21:30           | 13.5 | 300      | 42  | 971   | 266  |
| IW LOCATION                 | TSI  | H40      | H6   | EC    | d EC | P3 LOCATION               | TSI  | H40      | H6  | EC    | d EC |
| 21/Mar/97 06:50             | -1.2 | 2        | 0    | 659   | 0    | 21/Mar/97 06:50           | -1.2 | 5        | 0   | 713   | 0    |
| 21/Mar/97 08:10             | 0.2  | 1        | 0    | 667   | 8    | 21/Mar/97 09:00           | 1.0  |          |     | 716   | 3    |
| 21/Mar/97 08:20             | 0.3  | 0        | 0    | 701   | 42   | 21/Mar/97 10:00           | 2.0  | 7        | 0   | 713   | 0    |
| 21/Mar/97 08:30             | 0.5  | 11       | 10   | 665   | 6    | 21/Mar/97 10:20           | 2.3  | 5        | 0   | 671   | -42  |
| 21/Mar/97 08:45             | 0.8  | 194      | 160  | 654   | -5   | 21/Mar/97 10:40           | 2.7  | 238      | 12  | 796   | 83   |
| 21/Mar/97 09:00             | 1.0  | 1855     | 1310 | 1171  | 512  | 21/Mar/97 11:00           | 3.0  | 1020     | 60  | 1341  | 628  |
| 21/Mar/97 09:20             | 1.3  | 3650     | 1475 | 1644  | 985  | 21/Mar/97 11:20           | 3.3  | 1460     | 86  | 1834  | 1121 |
| 21/Mar/97 09:40             | 1.7  | 3700     | 1945 | 1993  | 1334 | 21/Mar/97 11:40           | 3.7  | 2045     | 96  | 2120  | 1407 |
| 21/Mar/97 10:40             | 2.7  | 4250     | 1320 | 2290  | 1631 | 21/Mar/97 12:00           | 4.0  | 1940     | 98  | 2210  | 1497 |
| 21/Mar/97 11:00             | 3.0  | 3000     | 1515 | 2310  | 1651 | 21/Mar/97 12:20           | 4.3  | 1640     | 93  | 2250  | 1537 |
| 21/Mar/97 11:20             | 3.3  | 4000     | 1990 | 2310  | 1651 | 21/Mar/97 12:40           | 4.7  | 1390     | 85  | 2260  | 1547 |
| 21/Mar/97 12:00             | 4.0  | 4500     | 1520 | 2300  | 1641 | 21/Mar/97 13:00           | 5.0  | 1735     | 80  | 2270  | 1557 |
| 21/Mar/97 12:20             | 4.3  | 4000     | 2160 | 2310  | 1651 | 21/Mar/97 13:30           | 5.5  | 1670     | 123 | 2270  | 1557 |
| 21/Mar/97 12:40             | 4.7  | 6000     | 1960 | 2190  | 1531 | 21/Mar/97 14:00           | 6.0  | 2655     | 181 | 2250  | 1537 |
| 21/Mar/97 13:00             | 5.0  | 4000     | 1065 | 1672  | 1013 | 21/Mar/97 14:30           | 6.5  | 3005     | 280 | 2260  | 1547 |
| 21/Mar/97 13:30             | 5.5  | 1305     | 285  | 1025  | 366  | 21/Mar/97 15:00           | 7.0  | 1560     | 104 | 1957  | 1244 |
| 21/Mar/97 14:00             | 6.0  | 273      | 47   | 778   | 119  | 21/Mar/97 16:00           | 8.0  | 132      | 13  | 957   | 244  |
| 21/Mar/97 14:30             | 6.5  | 30       | 11   | 737   | 78   | 21/Mar/97 16:30           | 8.5  | 78       | 10  | 855   | 142  |
| 21/Mar/97 15:00             | 7.0  | 16       | 5    | 717   | 58   | 21/Mar/97 17:00           | 9.0  |          | 3   | 826   | 113  |
| 21/Mar/97 16:00             | 8.0  |          |      | 709   | 50   | 21/Mar/97 17:30           | 9.5  |          |     | 775   | 62   |
| 21/Mar/97 17:00             | 9.0  |          |      | 705   | 46   | 21/Mar/97 18:30           | 10.5 | 41       | 2   | 684   | -29  |
| 21/Mar/97 19:30             | 11.5 | 5        | 3    | 704   | 45   | 21/Mar/97 19:30           | 11.5 |          |     | 725   | 12   |
| 21/Mar/97 21:30             | 13.5 |          |      | 700   | 41   | 21/Mar/97 21:30           | 13.5 | 18       | 1   | 715   | 2    |
| P1 LOCATION                 | TSI  | H40      | H6   | EC    | d EC | P4 LOCATION               | TSI  | H40      | H6  | EC    | d EC |
| 21/Mar/97 06:50             | -1.2 | 48       | 0    | 718   | 0    | 21/Mar/97 06:50           | -1.2 | 23       | 0   | 707   | 0    |
| 21/Mar/97 09:40             | 1.7  |          |      | 722   | 4    | 21/Mar/97 09:40           | 1.7  |          |     | 710   | 3    |
| 21/Mar/97 10:40             | 2.7  |          |      | 719   | 1    | 21/Mar/97 11:20           | 3.3  |          | 0   | 710   | 3    |
| 21/Mar/97 11:00             | 3.0  | 38       | 0    | 721   | 3    | 21/Mar/97 11:40           | 3.7  | 26       | 0   | 714   | 7    |
| 21/Mar/97 11:20             | 3.3  | 27       | 1    | 726   | 8    | 21/Mar/97 12:00           | 4.0  | 31       | 0   | 715   | 8    |
| 21/Mar/97 11:40             | 3.7  | 40       | 2    | 727   | 9    | 21/Mar/97 12:20           | 4.3  | 22       | 2   | 718   | 11   |
| 21/Mar/97 12:00             | 4.0  | 80       | 10   | 747   | 29   | 21/Mar/97 12:40           | 4.7  | 33       | 2   | 722   | 15   |
| 21/Mar/97 12:20             | 4.3  | 137      | 17   | 768   | 50   | 21/Mar/97 13:00           | 5.0  | 39       | 7   | 730   | 23   |
| 21/Mar/97 12:40             | 4.7  | 273      | 39   | 820   | 102  | 21/Mar/97 13:30           | 5.5  | 80       | 11  | 761   | 54   |
| 21/Mar/97 13:00             | 5.0  | 465      | 54   | 908   | 190  | 21/Mar/97 14:00           | 6.0  | 150      | 27  | 827   | 120  |
| 21/Mar/97 14:00             | 6.0  | 1405     | 133  | 1466  | 748  | 21/Mar/97 14:30           | 6.5  | 230      | 41  | 928   | 221  |
| 21/Mar/97 15:00             | 7.0  | 2025     | 251  | 1872  | 1154 | 21/Mar/97 15:00           | 7.0  | 280      | 42  | 1021  | 314  |
| 21/Mar/97 16:00             | 8.0  | 2615     | 430  | 2070  | 1352 | 21/Mar/97 15:30           | 7.5  | 290      | 42  | 1134  | 427  |
| 21/Mar/97 16:30             | 8.5  | 2670     | 570  | 2040  | 1322 | 21/Mar/97 16:00           | 8.0  | 310      | 40  | 1237  | 530  |
| 21/Mar/97 17:00             | 9.0  | 2680     | 505  | 1975  | 1257 | 21/Mar/97 16:30           | 8.5  | 410      | 55  | 1328  | 621  |
| 21/Mar/97 17:30             | 9.5  | 1710     | 415  | 1749  | 1031 | 21/Mar/97 17:00           | 9.0  | 340      | 49  | 1394  | 687  |
| 21/Mar/97 18:30             | 10.5 | 1085     | 177  | 1485  | 767  | 21/Mar/97 17:30           | 9.5  | 375      | 44  | 1492  | 785  |
| 21/Mar/97 19:30             | 11.5 | 420      | 85   | 1116  | 398  | 21/Mar/97 18:30           | 10.5 | 250      | 27  | 1639  | 932  |
| 21/Mar/97 21:30             | 13.5 | 170      | 38   | 805   | 87   | 21/Mar/97 19:30           | 11.5 | 290      | 32  | 1776  | 1069 |
|                             |      |          |      |       |      | 21/Mar/97 20:00           | 12.0 | 155      | 32  | 1782  | 1075 |
|                             |      |          |      |       |      | 21/Mar/97 20:30           | 12.5 | 156      |     | 1748  | 1041 |
|                             |      |          |      |       |      | 21/Mar/97 21:00           | 13.0 | 130      | 7   | 1635  | 928  |
|                             |      |          |      |       |      | 21/Mar/97 21:30           | 13.5 | 119      |     | 1456  | 749  |

# APPENDIX A2-2 Strasbourg Test 2 Data

| STR2 START: 21-Mar-97 08:00 |         |          |      |       |      | STR2 END: 21-Mar-97 21:30 |      |          |       |       |      |
|-----------------------------|---------|----------|------|-------|------|---------------------------|------|----------|-------|-------|------|
| Sample DateTime             | (h)     | (pfu/ml) |      | us/cm |      | Sample DateTime           | (h)  | (pfu/ml) |       | us/cm |      |
| P4 LOCATION                 | TSI     | H40      | H6   | EC    | d EC | US LOCATION               | TSI  | H40      | H6    | EC    | d EC |
| 21/Mar/97 06:50             | -1.2    | 23       | 0    | 707   | 0    | 21/Mar/97 06:50           | -1.2 | 0        | 0     | 641   | 0    |
| 21/Mar/97 09:40             | 1.7     |          |      | 710   | 3    | 22/Mar/97 08:00           | 0.0  | 0        | 0     |       |      |
| 21/Mar/97 11:20             | 3.3     |          | 0    | 710   | 3    | 21/Mar/97 08:10           | 0.2  | 5685     | 16000 | 1453  | 812  |
| 21/Mar/97 11:40             | 3.7     | 26       | 0    | 714   | 7    | 21/Mar/97 08:20           | 0.3  | 9500     | 28400 | 1911  | 1270 |
| 21/Mar/97 12:00             | 4.0     | 31       | 0    | 715   | 8    | 21/Mar/97 08:30           | 0.5  | 14500    | 37500 | 2160  | 1519 |
| 21/Mar/97 12:20             | 4.3     | 22       | 2    | 718   | 11   | 21/Mar/97 08:45           | 0.8  | 8000     | 33500 | 2250  | 1609 |
| 21/Mar/97 12:40             | 4.7     | 33       | 2    | 722   | 15   | 21/Mar/97 09:00           | 1.0  | 11500    | 28000 | 2300  | 1659 |
| 21/Mar/97 13:00             | 5.0     | 39       | 7    | 730   | 23   | 21/Mar/97 09:40           | 1.7  | 6500     | 24500 | 2320  | 1679 |
| 21/Mar/97 13:30             | 5.5     | 80       | 11   | 761   | 54   | 21/Mar/97 10:00           | 2.0  | 9500     | 14000 | 2320  | 1679 |
| 21/Mar/97 14:00             | 6.0     | 150      | 27   | 827   | 120  | 21/Mar/97 11:00           | 3.0  | 13000    | 22000 | 2320  | 1679 |
| 21/Mar/97 14:30             | 6.5     | 230      | 41   | 928   | 221  | 21/Mar/97 11:40           | 3.7  | 11000    | 29000 | 2310  | 1669 |
| 21/Mar/97 15:00             | 7.0     | 280      | 42   | 1021  | 314  | 21/Mar/97 12:00           | 4.0  | 9600     | 25300 | 2090  | 1449 |
| 21/Mar/97 15:30             | 7.5     | 290      | 42   | 1134  | 427  | 21/Mar/97 12:20           | 4.3  | 1270     | 3980  | 1145  | 504  |
| 21/Mar/97 16:00             | 8.0     | 310      | 40   | 1237  | 530  | 21/Mar/97 12:40           | 4.7  | 81       | 395   | 800   | 159  |
| 21/Mar/97 16:30             | 8.5     | 410      | 55   | 1328  | 621  | 21/Mar/97 13:00           | 5.0  | 13       | 12    | 741   | 100  |
| 21/Mar/97 17:00             | 9.0     | 340      | 49   | 1394  | 687  | 21/Mar/97 14:00           | 6.0  | 3        | 1     | 706   | 65   |
| 21/Mar/97 17:30             | 9.5     | 375      | 44   | 1492  | 785  | 21/Mar/97 15:00           | 7.0  | 2        | 2     | 697   | 56   |
| 21/Mar/97 18:30             | 10.5    | 250      | 27   | 1639  | 932  | 21/Mar/97 16:00           | 8.0  |          |       | 718   | 77   |
| 21/Mar/97 19:30             | 11.5    | 290      | 32   | 1776  | 1069 | 21/Mar/97 17:00           | 9.0  |          |       | 694   | 53   |
| 21/Mar/97 20:00             | 12.0    | 155      | 32   | 1782  | 1075 | 21/Mar/97 19:30           | 11.5 | 1        | 0     | 702   | 61   |
| 21/Mar/97 20:30             | 12.5    | 156      |      | 1748  | 1041 | 21/Mar/97 21:30           | 13.5 |          |       | 691   | 50   |
| 21/Mar/97 21:00             | 13.0    | 130      | 7    | 1635  | 928  |                           |      |          |       |       |      |
| 21/Mar/97 21:30             | 13.5    | 119      |      | 1456  | 749  |                           |      |          |       |       |      |
| PW LOCATION                 | TSI (h) | H40      | H6   | EC    | d EC |                           |      |          |       |       |      |
| 21/Mar/97 06:50             | -1.2    | 1        |      | 684   | 0    |                           |      |          |       |       |      |
| 21/Mar/97 08:20             | 0.3     |          | 0    | 709   | 25   |                           |      |          |       |       |      |
| 21/Mar/97 09:20             | 1.3     | 2        | 0    | 696   | 12   |                           |      |          |       |       |      |
| 21/Mar/97 09:40             | 1.7     | 153      | 20   | 663   | -21  |                           |      |          |       |       |      |
| 21/Mar/97 10:00             | 2.0     | 4690     | 2000 | 2200  | 1516 |                           |      |          |       |       |      |
| 21/Mar/97 10:20             | 2.3     | 3035     | 700  | 1674  | 990  |                           |      |          |       |       |      |
| 21/Mar/97 10:40             | 2.7     | 3850     | 695  | 2160  | 1476 |                           |      |          |       |       |      |
| 21/Mar/97 11:00             | 3.0     | 5150     | 680  | 2260  | 1576 |                           |      |          |       |       |      |
| 21/Mar/97 11:20             | 3.3     | 3500     | 675  | 2290  | 1606 |                           |      |          |       |       |      |
| 21/Mar/97 11:40             | 3.7     | 3600     | 620  | 2330  | 1646 |                           |      |          |       |       |      |
| 21/Mar/97 12:20             | 4.3     | 3950     | 620  | 2320  | 1636 |                           |      |          |       |       |      |
| 21/Mar/97 13:00             | 5.0     | 4775     | 935  | 2310  | 1626 |                           |      |          |       |       |      |
| 21/Mar/97 14:00             | 6.0     | 4000     | 475  | 1966  | 1282 |                           |      |          |       |       |      |
| 21/Mar/97 15:00             | 7.0     | 48       | 19   | 798   | 114  |                           |      |          |       |       |      |
| 21/Mar/97 16:00             | 8.0     | 20       |      | 713   | 29   |                           |      |          |       |       |      |
| 21/Mar/97 17:00             | 9.0     |          | 7    | 707   | 23   |                           |      |          |       |       |      |
| 21/Mar/97 19:30             | 11.5    | 7        |      | 703   | 19   |                           |      |          |       |       |      |
| 21/Mar/97 21:30             | 13.5    |          | 4    | 709   | 25   |                           |      |          |       |       |      |

# APPENDIX 2-3 Strasbourg Test 3 Data

| STR3 START: 27-Apr-97 08:00 |      |          |       |       |         |      | STR3 END: 28-Apr-97 09:15 |      |          |      |       |         |      |
|-----------------------------|------|----------|-------|-------|---------|------|---------------------------|------|----------|------|-------|---------|------|
| Sample TimeDate             | (h)  | (pfu/ml) |       | (ppb) | (us/cm) |      | Sample TimeDate           | (h)  | (pfu/ml) |      | (ppb) | (us/cm) |      |
| US LOCATION                 | TSI  | H40      | H6    | UR    | EC      | d EC | P1 LOCATION               | TSI  | H40      | H6   | UR    | EC      | d EC |
| 27-Apr-97 07:20             | -0.7 | 0        | 0     | 0.4   | 695     |      | 27-Apr-97 07:20           | -0.7 | 2        | 0    | 1     | 697     |      |
| 27-Apr-97 08:10             | 0.2  | 3000     | 12000 | 460   | 2560    | 1865 | 27-Apr-97 11:00           | 3.0  | 0        | 0    | 1     | 699     |      |
| 27-Apr-97 08:20             | 0.3  | 4700     | 22500 | 661   | 3360    | 2665 | 27-Apr-97 11:20           | 3.3  | 2        | 1    | 0.4   | 696     |      |
| 27-Apr-97 08:30             | 0.5  | 5600     | 25500 | 736   | 3770    | 3075 | 27-Apr-97 11:40           | 3.7  | 5        | 2    | 1     | 700     | 3    |
| 27-Apr-97 09:00             | 1.0  | 5500     | 29500 | 865   | 4340    | 3645 | 27-Apr-97 11:55           | 3.9  | 11       | 3    | 1     | 705     | 8    |
| 27-Apr-97 09:20             | 1.3  | 6100     | 34000 | 871   | 4400    | 3705 | 27-Apr-97 12:20           | 4.3  | 20       | 9    | 5     | 730     | 33   |
| 27-Apr-97 10:00             | 2.0  | 7450     | 36000 | 902   | 4440    | 3745 | 27-Apr-97 12:40           | 4.7  | 50       | 23   | 8     | 757     | 60   |
| 27-Apr-97 10:40             | 2.7  | 6100     | 33000 | 907   | 4430    | 3735 | 27-Apr-97 13:00           | 5.0  | 55       | 45   | 15    | 799     | 102  |
| 27-Apr-97 11:55             | 3.9  | 5950     | 27500 | 888   | 4440    | 3745 | 27-Apr-97 13:00           | 5.0  | 50       | 30   | 13    | 792     | 95   |
| 27-Apr-97 12:20             | 4.3  | 2495     | 8800  | 358   | 2120    | 1425 | 27-Apr-97 14:00           | 6.0  | 350      | 260  | 77    | 1213    | 516  |
| 27-Apr-97 12:40             | 4.7  | 1200     | 3630  | 158   | 1324    | 629  | 27-Apr-97 15:00           | 7.0  | 750      | 460  | 326   | 2230    | 1533 |
| 27-Apr-97 13:00             | 5.0  | 325      | 1550  | 56    | 933     | 238  | 27-Apr-97 16:00           | 8.0  | 2400     | 1075 | 583   | 3100    | 2403 |
| 27-Apr-97 14:00             | 6.0  | 38       | 94    | 4     | 712     | 17   | 27-Apr-97 17:00           | 9.0  | 2450     | 1495 | 729   | 3730    | 3033 |
| 27-Apr-97 15:00             | 7.0  | 4        | 19    | 1     | 696     | 1    | 27-Apr-97 18:00           | 10.0 | 2305     | 1015 | 713   | 3520    | 2823 |
| 27-Apr-97 16:00             | 8.0  | 4        | 15    | 0     | 693     |      | 27-Apr-97 19:00           | 11.0 | 1250     | 660  | 654   | 3060    | 2363 |
| 28-Apr-97 02:00             | 18.0 | 0        | 1     | 0     | 692     |      | 27-Apr-97 21:00           | 13.0 | 570      | 235  | 220   | 1275    | 578  |
| DS LOCATION                 |      |          |       |       |         |      | 27-Apr-97 21:00           | 13.0 | 490      | 175  | 229   | 1326    | 629  |
| 27-Apr-97 07:20             | -0.7 | 0        | 1     |       | 696     |      | 27-Apr-97 23:00           | 15.0 | 95       | 55   | 57    | 813     | 116  |
| 27-Apr-97 08:00             | 0.0  | 0        | 0     |       | 695     |      | 28-Apr-97 01:00           | 17.0 | 84       | 25   | 19    | 705     | 8    |
| 27-Apr-97 09:40             | 1.7  |          | 0     | 0.3   | 698     |      | 28-Apr-97 02:00           | 18.0 | 47       | 21   | 8     | 698     | 1    |
| 27-Apr-97 10:00             | 2.0  | 0        |       | 0.3   | 695     |      | P2 LOCATION               |      |          |      |       |         |      |
| 27-Apr-97 10:20             | 2.3  | 1        | 0     | 0.2   | 689     |      | 27-Apr-97 07:20           | -0.7 | 1        | 0    | 0     | 693     |      |
| 27-Apr-97 10:40             | 2.7  | 2        | 0     | 0.4   | 696     | 2    | 27-Apr-97 11:55           | 3.9  | 20       | 7    | 3     | 720     | 27   |
| 27-Apr-97 11:00             | 3.0  | 11       | 5     | 1     | 700     | 6    | 27-Apr-97 12:20           | 4.3  | 74       | 19   | 11    | 757     | 64   |
| 27-Apr-97 11:20             | 3.3  | 120      | 29    | 15    | 785     | 91   | 27-Apr-97 12:40           | 4.7  | 85       | 49   | 20    | 820     | 127  |
| 27-Apr-97 11:40             | 3.7  | 185      | 45    | 27    | 886     | 192  | 27-Apr-97 12:40           | 4.7  | 115      | 52   | 24    | 856     | 163  |
| 27-Apr-97 11:55             | 3.9  | 220      | 55    | 42    | 997     | 303  | 27-Apr-97 13:00           | 5.0  | 200      | 72   | 42    | 945     | 252  |
| 27-Apr-97 12:20             | 4.3  | 505      | 185   | 160   | 1545    | 851  | 27-Apr-97 13:30           | 5.5  | 470      | 230  | 95    | 1240    | 547  |
| 27-Apr-97 12:40             | 4.7  | 500      | 285   | 290   | 2130    | 1436 | 27-Apr-97 14:00           | 6.0  | 870      | 350  | 177   | 1737    | 1044 |
| 27-Apr-97 13:00             | 5.0  | 1900     | 520   | 389   | 2620    | 1926 | 27-Apr-97 14:30           | 6.5  | 1005     | 515  | 265   | 2260    | 1567 |
| 27-Apr-97 13:30             | 5.5  | 1800     | 655   | 491   | 3000    | 2306 | 27-Apr-97 15:00           | 7.0  | 1845     | 880  | 431   | 3140    | 2447 |
| 27-Apr-97 14:00             | 6.0  | 1700     | 790   | 543   | 3170    | 2476 | 27-Apr-97 15:30           | 7.5  | 1500     | 920  | 566   | 3470    | 2777 |
| 27-Apr-97 14:30             | 6.5  | 1900     | 960   | 577   | 3360    | 2666 | 27-Apr-97 16:00           | 8.0  | 2135     | 1155 | 641   | 3610    | 2917 |
| 27-Apr-97 15:00             | 7.0  | 2100     | 935   | 625   | 3540    | 2846 | 27-Apr-97 17:00           | 9.0  | 1100     | 900  | 629   | 3530    | 2837 |
| 27-Apr-97 15:30             | 7.5  | 1900     | 805   | 589   | 3440    | 2746 | 27-Apr-97 17:00           | 9.0  | 1200     | 820  | 623   | 3510    | 2817 |
| 27-Apr-97 16:00             | 8.0  | 1835     | 830   | 513   | 2810    | 2116 | 27-Apr-97 18:00           | 10.0 | 1210     | 445  | 580   | 3320    | 2627 |
| 27-Apr-97 16:30             | 8.5  | 2150     | 830   | 396   | 2180    | 1486 | 27-Apr-97 19:00           | 11.0 | 530      | 375  | 548   | 3250    | 2557 |
| 27-Apr-97 17:00             | 9.0  | 925      | 310   | 226   | 1459    | 765  | 27-Apr-97 20:00           | 12.0 | 395      | 239  | 595   | 3170    | 2477 |
| 27-Apr-97 17:40             | 9.7  | 550      | 140   | 142   | 1143    | 449  | 27-Apr-97 21:00           | 13.0 | 480      | 155  | 415   | 1986    | 1293 |
| 27-Apr-97 18:00             | 10.0 | 280      | 111.5 | 102   | 996     | 302  | 27-Apr-97 22:00           | 14.0 | 275      | 118  | 195   | 1242    | 549  |
| 27-Apr-97 19:00             | 11.0 | 180      | 62    | 57    | 838     | 144  | 27-Apr-97 23:00           | 15.0 | 177      | 62   | 88    | 869     | 176  |
| 27-Apr-97 20:00             | 12.0 | 69       | 25    | 27    | 739     | 45   | 28-Apr-97 00:00           | 16.0 | 78       | 33   | 42    | 745     | 52   |
| 27-Apr-97 21:00             | 13.0 | 58       | 24    | 23    | 734     | 40   | 28-Apr-97 01:00           | 17.0 | 56       | 34   | 21    | 702     | 9    |
| 27-Apr-97 22:00             | 14.0 | 38       | 13    | 9     | 703     | 9    | 28-Apr-97 02:00           | 18.0 | 57       | 30   | 10    | 695     | 2    |
| 27-Apr-97 23:00             | 15.0 | 28       | 11    | 6     | 698     | 4    | 28-Apr-97 02:00           | 18.0 | 61       | 25   | 8     | 695     | 2    |
| 28-Apr-97 00:00             | 16.0 | 33       | 11    | 6     | 700     | 6    | 28-Apr-97 09:15           | 25.2 | 29       | 9    | 2     | 696     | 3    |
| 28-Apr-97 02:00             | 18.0 | 11       | 6     | 3     | 697     | 3    | P3 LOCATION               |      |          |      |       |         |      |
| IW LOCATION                 |      |          |       |       |         |      | 27-Apr-97 07:20           | -0.7 | 1        | 0    | 0     | 697     |      |
| 27-Apr-97 07:20             | -0.7 | 0        | 0     | 0     | 695     | 695  | 27-Apr-97 10:20           | 2.3  | 0        | 0    | 0     | 692     |      |
| 27-Apr-97 08:20             | 0.3  | 1        | 0     | 0     | 695     |      | 27-Apr-97 10:40           | 2.7  | 45       | 20   | 4     | 726     | 34   |
| 27-Apr-97 08:30             | 0.5  | 5        | 10    | 0.7   | 690     |      | 27-Apr-97 11:00           | 3.0  | 515      | 60   | 165   | 1565    | 873  |
| 27-Apr-97 08:45             | 0.8  | 540      | 635   | 91    | 1073    | 378  | 27-Apr-97 11:20           | 3.3  | 1575     | 248  | 462   | 2780    | 2088 |
| 27-Apr-97 09:00             | 1.0  | 1965     | 1820  | 341   | 2060    | 1365 | 27-Apr-97 11:40           | 3.7  | 1750     | 500  | 656   | 3550    | 2858 |
| 27-Apr-97 09:20             | 1.3  | 3050     | 2550  | 566   | 2980    | 2285 | 27-Apr-97 11:55           | 3.9  | 2115     | 460  | 737   | 3860    | 3168 |
| 27-Apr-97 09:40             | 1.7  | 4250     | 3160  | 743   | 3730    | 3035 | 27-Apr-97 12:40           | 4.7  | 2900     | 530  | 826   | 4180    | 3488 |
| 27-Apr-97 10:20             | 2.3  | 5200     | 2800  | 866   | 4290    | 3595 | 27-Apr-97 13:00           | 5.0  | 2875     | 910  | 838   | 4210    | 3518 |
| 27-Apr-97 11:00             | 3.0  | 5900     | 3750  | 886   | 4390    | 3695 | 27-Apr-97 13:30           | 5.5  | 2600     | 645  | 856   | 4250    | 3558 |
| 27-Apr-97 11:55             | 3.9  | 5500     | 2850  | 881   | 4400    | 3705 | 27-Apr-97 14:00           | 6.0  | 3450     | 630  | 856   | 4260    | 3568 |
| 27-Apr-97 12:20             | 4.3  | 5400     | 3365  | 885   | 4400    | 3705 | 27-Apr-97 14:30           | 6.5  | 2500     | 655  | 854   | 4280    | 3588 |
| 27-Apr-97 12:40             | 4.7  | 4350     | 2800  | 829   | 4150    | 3455 | 27-Apr-97 15:00           | 7.0  | 2480     | 605  | 776   | 3780    | 3088 |
| 27-Apr-97 13:00             | 5.0  | 3150     | 1990  | 579   | 2980    | 2285 | 27-Apr-97 15:30           | 7.5  | 2000     | 510  | 613   | 3010    | 2318 |
| 27-Apr-97 13:30             | 5.5  | 1290     | 725   | 271   | 1714    | 1019 | 27-Apr-97 16:00           | 8.0  | 1120     | 165  | 406   | 2200    | 1508 |
| 27-Apr-97 14:00             | 6.0  | 580      | 260   | 109   | 1074    | 379  | 27-Apr-97 17:00           | 9.0  | 330      | 50   | 208   | 1447    | 755  |
| 27-Apr-97 14:30             | 6.5  | 165      | 105   | 39    | 834     | 139  | 27-Apr-97 19:00           | 11.0 | 95       | 8    | 82    | 967     | 275  |
| 27-Apr-97 15:00             | 7.0  | 64       | 40    | 11    | 725     | 30   | 28-Apr-97 02:00           | 18.0 | 8        | 2    | 9     | 720     | 28   |
| 27-Apr-97 16:00             | 8.0  | 26       | 9     | 3     | 698     | 3    |                           |      |          |      |       |         |      |
| 27-Apr-97 21:00             | 13.0 | 12       | 5     | 1     | 694     |      |                           |      |          |      |       |         |      |
| 28-Apr-97 02:00             | 18.0 | 6        | 4     | 1     | 698     | 3    |                           |      |          |      |       |         |      |

# APPENDIX 2-3 Strasbourg Test 3 Data

| STR3 START: 27-Apr-97 08:00 |      |          |     |       |         |      | STR3 END: 28-Apr-97 09:15 |      |          |       |       |         |      |
|-----------------------------|------|----------|-----|-------|---------|------|---------------------------|------|----------|-------|-------|---------|------|
| Sample TimeDate             | (h)  | (pfu/ml) |     | (ppb) | (us/cm) |      | Sample TimeDate           | (h)  | (pfu/ml) |       | (ppb) | (us/cm) |      |
| P4 LOCATION                 | TSI  | H40      | H6  | UR    | EC      | d EC | PW LOCATION               | TSI  | H40      | H6    | UR    | EC      | d EC |
| 27-Apr-97 07:20             | -0.7 | 2        | 0   | 0     | 695     |      | 27-Apr-97 07:20           | -0.7 | 0.001    | 0.001 | 0     | 693     |      |
| 27-Apr-97 11:40             | 3.7  | 18       | 2   | 3     | 717     | 22   | 27-Apr-97 09:20           | 1.3  | 0.001    | 1     | 0     | 667     |      |
| 27-Apr-97 11:55             | 3.9  | 31       | 11  | 5     | 724     | 29   | 27-Apr-97 09:40           | 1.7  | 65       | 27.5  | 6     | 721     | 28   |
| 27-Apr-97 12:20             | 4.3  | 41       | 11  | 11    | 758     | 63   | 27-Apr-97 10:00           | 2.0  | 650      | 240   | 101   | 1191    | 498  |
| 27-Apr-97 12:40             | 4.7  | 76       | 29  | 16    | 790     | 95   | 27-Apr-97 10:20           | 2.3  | 2800     | 550   | 413   | 2520    | 1827 |
| 27-Apr-97 13:00             | 5.0  | 558      | 35  | 23    | 835     | 140  | 27-Apr-97 10:40           | 2.7  | 3050     | 1260  | 671   | 3600    | 2907 |
| 27-Apr-97 13:30             | 5.5  | 201      | 64  | 42    | 942     | 247  | 27-Apr-97 11:20           | 3.3  | 4650     | 1650  | 861   | 4330    | 3637 |
| 27-Apr-97 14:00             | 6.0  | 360      | 141 | 79    | 1230    | 535  | 27-Apr-97 11:55           | 3.9  | 4900     | 2035  | 882   | 4400    | 3707 |
| 27-Apr-97 14:30             | 6.5  | 480      | 320 | 144   | 1619    | 924  | 27-Apr-97 13:00           | 5.0  | 5400     | 2300  | 878   | 4400    | 3707 |
| 27-Apr-97 15:00             | 7.0  | 595      | 365 |       | 2030    | 1335 | 27-Apr-97 13:30           | 5.5  | 5250     | 2150  | 885   | 4400    | 3707 |
| 27-Apr-97 15:00             | 7.0  | 725      | 280 | 239   | 2090    | 1395 | 27-Apr-97 14:00           | 6.0  | 3950     | 1400  | 783   | 3930    | 3237 |
| 27-Apr-97 15:30             | 7.5  | 840      | 370 | 283   | 2270    | 1575 | 27-Apr-97 14:30           | 6.5  | 3050     | 1050  | 484   | 2460    | 1767 |
| 27-Apr-97 16:00             | 8.0  | 625      | 445 | 354   | 2470    | 1775 | 27-Apr-97 15:00           | 7.0  | 650      | 190   | 128   | 1081    | 388  |
| 27-Apr-97 16:30             | 8.5  | 635      | 465 | 382   | 2550    | 1855 | 27-Apr-97 16:00           | 8.0  | 55       | 21    | 25    | 730     | 37   |
| 27-Apr-97 17:00             | 9.0  | 465      | 340 | 380   | 2500    | 1805 | 27-Apr-97 21:00           | 13.0 | 9        | 3     | 3     | 695     | 2    |
| 27-Apr-97 17:40             | 9.7  | 415      | 280 | 350   | 2370    | 1675 | 28-Apr-97 02:00           | 18.0 | 7.5      | 2.5   | 1     | 698     | 5    |
| 27-Apr-97 18:00             | 10.0 | 325      | 195 | 344   | 2340    | 1645 |                           |      |          |       |       |         |      |
| 27-Apr-97 19:00             | 11.0 | 250      | 145 | 363   | 2460    | 1765 |                           |      |          |       |       |         |      |
| 27-Apr-97 20:00             | 12.0 | 340      | 180 | 430   | 2730    | 2035 |                           |      |          |       |       |         |      |
| 27-Apr-97 21:00             | 13.0 | 155      | 92  | 474   | 2740    | 2045 |                           |      |          |       |       |         |      |
| 27-Apr-97 22:00             | 14.0 | 105      | 78  | 475   | 2480    | 1785 |                           |      |          |       |       |         |      |
| 27-Apr-97 23:00             | 15.0 | 165      | 50  | 358   | 1865    | 1170 |                           |      |          |       |       |         |      |
| 27-Apr-97 23:20             | 15.3 | 65       | 55  | 304   | 1650    | 955  |                           |      |          |       |       |         |      |
| 27-Apr-97 23:40             | 15.7 | 104      | 50  | 266   | 1470    | 775  |                           |      |          |       |       |         |      |
| 28-Apr-97 00:00             | 16.0 | 58       | 41  | 210   | 1269    | 574  |                           |      |          |       |       |         |      |
| 28-Apr-97 01:00             | 17.0 | 64       | 21  | 99    | 891     | 196  |                           |      |          |       |       |         |      |
| 28-Apr-97 02:00             | 18.0 | 35       | 16  | 27    | 714     | 19   |                           |      |          |       |       |         |      |
| 28-Apr-97 09:15             | 25.2 | 8        | 6   | 3     | 697     | 2    |                           |      |          |       |       |         |      |

# APPENDIX 2-4 Strasbourg Test 4

| STR4 START: 28-Jun-97 08:00 |      |          |      |       |       |         |      | STR4 END: 29-Jun-97 12:00 |      |          |      |    |       |         |      |
|-----------------------------|------|----------|------|-------|-------|---------|------|---------------------------|------|----------|------|----|-------|---------|------|
| Sample TimeDate             | (h)  | (pfu/ml) |      |       | (ppb) | (us/cm) |      | Sample TimeDate           | (h)  | (pfu/ml) |      |    | (ppb) | (us/cm) |      |
| US LOCATION                 | TSI  | H40      | Psf2 | T7    | UR    | EC      | d EC | P2 LOCATION               | TSI  | H40      | Psf2 | T7 | UR    | EC      | d EC |
| 28/Jun/97 7:10              | -0.8 | 0        | 0    | 0     | 0.5   | 704     |      | 28/Jun/97 7:10            | -0.8 |          |      |    | 0.3   | 711     |      |
| 28/Jun/97 7:40              | -0.3 |          |      |       | 0.6   | 712     |      | 28/Jun/97 7:40            | -0.3 |          |      |    | 0.3   | 713     |      |
| 28/Jun/97 8:05              | 0.1  | 1725     | 73   | 375   | 69    | 1904    | 1192 | 28/Jun/97 10:20           | 2.3  |          |      |    | 0.3   | 713     |      |
| 28/Jun/97 8:15              | 0.2  | 4850     | 290  | 4550  | 168   | 3120    | 2408 | 28/Jun/97 10:40           | 2.7  | 0        |      |    | 0.3   | 712     |      |
| 28/Jun/97 8:30              | 0.5  | 6150     | 415  | 6000  | 226   | 3860    | 3148 | 28/Jun/97 11:00           | 3.0  | 1        |      |    | 0.2   | 713     |      |
| 28/Jun/97 9:00              | 1.0  | 7000     | 555  | 6500  | 259   | 4290    | 3578 | 28/Jun/97 11:20           | 3.3  | 9        |      |    | 0.3   | 717     | 4    |
| 28/Jun/97 9:30              | 1.5  | 7350     | 590  | 4500  | 259   | 4370    | 3658 | 28/Jun/97 11:40           | 3.7  | 13       |      |    | 0.7   | 726     | 13   |
| 28/Jun/97 10:00             | 2.0  | 7050     | 540  | 4500  | 254   | 4380    | 3668 | 28/Jun/97 11:55           | 3.9  | 38       |      |    | 1.3   | 739     | 26   |
| 28/Jun/97 11:00             | 3.0  | 7450     | 455  | 5500  | 243   | 4370    | 3658 | 28/Jun/97 11:55           | 3.9  | 39       |      |    | 2     | 746     | 33   |
| 28/Jun/97 11:55             | 3.9  | 7600     | 590  | 2815  | 243   | 4370    | 3658 | 28/Jun/97 12:40           | 4.7  | 239      |      |    | 10    | 949     | 236  |
| 28/Jun/97 12:10             | 4.2  | 4550     | 62   | 5     | 119   | 2810    | 2098 | 28/Jun/97 13:00           | 5.0  | 485      |      |    | 20    | 1140    | 427  |
| 28/Jun/97 12:30             | 4.5  | 1400     | 1    | 0.001 | 28    | 1453    | 741  | 28/Jun/97 13:30           | 5.5  | 900      |      |    | 42    | 1613    | 900  |
| 28/Jun/97 13:00             | 5.0  | 5        |      |       | 6     | 866     | 154  | 28/Jun/97 14:00           | 6.0  | 1050     |      |    | 71    | 2148    | 1435 |
| 28/Jun/97 13:30             | 5.5  | 0        |      |       | 1     | 743     | 31   | 28/Jun/97 14:30           | 6.5  | 1890     |      |    | 115   | 3050    | 2337 |
| 28/Jun/97 13:35             | 5.6  |          |      |       | 1     |         |      | 28/Jun/97 15:00           | 7.0  | 2600     | 1    |    | 179   | 3560    | 2847 |
| 28/Jun/97 14:00             | 6.0  |          |      |       | 0.6   | 718     | 6    | 28/Jun/97 15:30           | 7.5  | 2350     | 1    |    | 209   | 3750    | 3037 |
| 28/Jun/97 16:00             | 8.0  |          |      |       | 0.4   | 707     |      | 28/Jun/97 16:00           | 8.0  | 2200     |      |    | 215   | 3790    | 3077 |
| 28/Jun/97 20:00             | 12.0 |          |      |       | 0.4   | 712     | 0    | 28/Jun/97 16:30           | 8.5  | 1950     |      |    | 211   | 3780    | 3067 |
| 29/Jun/97 8:00              | 24.0 |          |      |       |       | 709     |      | 28/Jun/97 17:00           | 9.0  | 1625     | 1    |    | 205   | 3730    | 3017 |
| DS LOCATION                 |      |          |      |       |       |         |      | 28/Jun/97 17:30           | 9.5  | 1465     |      |    | 200   | 3660    | 2947 |
| 28/Jun/97 7:10              | -0.8 |          |      |       | 0.4   | 712     |      | 28/Jun/97 18:00           | 10.0 | 1070     | 1    |    | 193   | 3580    | 2867 |
| 28/Jun/97 7:40              | -0.3 |          |      |       | 0.4   | 711     |      | 28/Jun/97 18:30           | 10.5 | 925      |      |    | 193   | 3580    | 2867 |
| 28/Jun/97 10:00             | 2.0  |          |      |       | 0.4   | 712     |      | 28/Jun/97 19:00           | 11.0 | 910      |      |    | 201   | 3530    | 2817 |
| 28/Jun/97 10:20             | 2.3  |          | 1    | 0     | 0.4   | 713     | 1    | 28/Jun/97 20:00           | 12.0 | 685      |      |    | 178   | 2840    | 2127 |
| 28/Jun/97 10:40             | 2.7  | 21       | 0    |       | 1     | 728     | 16   | 28/Jun/97 20:05           | 12.1 | 600      |      |    | 166   | 2610    | 1897 |
| 28/Jun/97 11:00             | 3.0  | 156      | 0    |       | 6     | 853     | 141  | 28/Jun/97 20:30           | 12.5 | 595      |      |    | 119   | 1954    | 1241 |
| 28/Jun/97 11:20             | 3.3  | 450      | 3    |       | 30    | 1359    | 647  | 28/Jun/97 21:00           | 13.0 | 495      |      |    | 68    | 1406    | 693  |
| 28/Jun/97 11:20             | 3.3  | 580      |      |       | 40    | 1544    | 832  | 28/Jun/97 21:30           | 13.5 | 265      |      |    | 43    | 1089    | 376  |
| 28/Jun/97 11:40             | 3.7  | 1180     | 4    |       | 72    | 2050    | 1338 | 28/Jun/97 22:00           | 14.0 | 155      |      |    | 30    | 925     | 212  |
| 28/Jun/97 11:55             | 3.9  | 1240     | 7    | n     | 95    | 2380    | 1668 | 28/Jun/97 22:30           | 14.5 | 90       |      |    | 19    | 809     | 96   |
| 28/Jun/97 12:20             | 4.3  | 1550     | 8    | o     | 123   | 2700    | 1988 | 28/Jun/97 23:00           | 15.0 | 80       |      |    | 12    | 745     | 32   |
| 28/Jun/97 12:40             | 4.7  | 1935     | 8    | n     | 137   | 2910    | 2198 | 29/Jun/97 0:00            | 16.0 | 72       |      |    | 5     | 714     | 1    |
| 28/Jun/97 13:00             | 5.0  | 2150     | 7    | e     | 146   | 3060    | 2348 | 29/Jun/97 1:00            | 17.0 | 68       |      |    | 3     | 710     |      |
| 28/Jun/97 14:00             | 6.0  | 2250     | 6    | d     | 169   | 3470    | 2758 | 29/Jun/97 3:00            | 19.0 | 45       |      |    | 2     | 714     | 1    |
| 28/Jun/97 15:00             | 7.0  | 2800     | 1    | e     | 182   | 3720    | 3008 | 29/Jun/97 8:00            | 24.0 | 31       |      |    | 0.9   | 715     | 2    |
| 28/Jun/97 15:30             | 7.5  | 2000     | 2    | t     | 169   | 3520    | 2808 | 29/Jun/97 12:00           | 28.0 | 19       |      |    | 0.5   | 716     | 3    |
| 28/Jun/97 16:00             | 8.0  | 1550     | 1    | e     | 134   | 2850    | 2138 | P4 LOCATION               |      |          |      |    |       |         |      |
| 28/Jun/97 16:30             | 8.5  | 1450     | 1    | c     | 92    | 2170    | 1458 | 28/Jun/97 7:10            | -0.8 |          |      |    | 0.1   | 707     |      |
| 28/Jun/97 16:45             | 8.7  |          |      | d     | 85    | 2080    | 1368 | 28/Jun/97 7:30            | -0.5 |          |      |    | 0.3   | 712     |      |
| 28/Jun/97 17:00             | 9.0  | 710      |      |       | 68    | 1801    | 1089 | 28/Jun/97 10:20           | 2.3  |          |      |    | 0.3   | 710     |      |
| 28/Jun/97 17:40             | 9.7  | 495      |      |       | 55    | 1524    | 812  | 28/Jun/97 10:40           | 2.7  | 0        |      |    | 0.3   | 711     |      |
| 28/Jun/97 18:00             | 10.0 | 450      | 1    |       | 43    | 1356    | 644  | 28/Jun/97 11:00           | 3.0  | 4        |      |    | 0.4   | 711     |      |
| 28/Jun/97 19:00             | 11.0 | 260      |      |       | 30    | 1125    | 413  | 28/Jun/97 11:20           | 3.3  | 7        |      |    | 0.6   | 718     | 6    |
| 28/Jun/97 20:00             | 12.0 | 164      |      |       | 22    | 996     | 284  | 28/Jun/97 11:40           | 3.7  | 24       |      |    | 1.3   | 734     | 22   |
| 28/Jun/97 22:00             | 14.0 | 78       |      |       | 11    | 843     | 131  | 28/Jun/97 11:55           | 3.9  | 33       |      |    | 2     | 750     | 38   |
| 29/Jun/97 0:00              | 16.0 | 35       |      |       | 6     | 769     | 57   | 28/Jun/97 12:20           | 4.3  | 60       |      |    | 3     | 783     | 71   |
| 29/Jun/97 3:00              | 19.0 | 14       |      |       | 3     | 725     | 13   | 28/Jun/97 12:40           | 4.7  | 102      |      |    | 5     | 830     | 118  |
| 29/Jun/97 8:00              | 24.0 | 9        |      |       | 1     | 717     | 5    | 28/Jun/97 12:45           | 4.7  | 110      |      |    | 6     | 849     | 137  |
| 29/Jun/97 12:00             | 28.0 | 5        |      |       | 0.8   | 719     | 7    | 28/Jun/97 13:00           | 5.0  | 161      |      |    | 8     | 905     | 193  |
| PW LOCATION                 |      |          |      |       |       |         |      | 28/Jun/97 13:30           | 5.5  | 370      |      |    | 17    | 1208    | 496  |
| 28/Jun/97 7:10              | -0.8 | 0        |      |       | 0.2   | 703     |      | 28/Jun/97 14:00           | 6.0  | 580      |      |    | 30    | 1610    | 898  |
| 28/Jun/97 7:40              | -0.3 |          |      |       | 0.3   | 710     |      | 28/Jun/97 14:30           | 6.5  | 860      |      |    | 53    | 2120    | 1408 |
| 28/Jun/97 9:00              | 1.0  | 0        | 0    |       | 0.3   | 707     |      | 28/Jun/97 15:00           | 7.0  | 1005     |      |    | 71    | 2420    | 1708 |
| 28/Jun/97 9:15              | 1.2  | 0        |      |       | 0.2   | 703     |      | 28/Jun/97 15:30           | 7.5  | 885      |      |    | 90    | 2650    | 1938 |
| 28/Jun/97 9:30              | 1.5  | 18       | 0    |       | 0.2   | 720     | 14   | 28/Jun/97 16:00           | 8.0  | 800      |      |    | 97    | 2780    | 2068 |
| 28/Jun/97 9:45              | 1.7  | 298      | 0    |       | 8     | 918     | 212  | 28/Jun/97 16:30           | 8.5  | 695      |      |    | 105   | 2810    | 2098 |
| 28/Jun/97 10:00             | 2.0  | 990      | 7    | n     | 30    | 1394    | 688  | 28/Jun/97 17:00           | 9.0  | 680      |      |    | 99    | 2740    | 2028 |
| 28/Jun/97 10:20             | 2.3  | 3300     | 48   | o     | 146   | 3130    | 2424 | 28/Jun/97 17:30           | 9.5  | 655      |      |    | 97    | 2660    | 1948 |
| 28/Jun/97 10:40             | 2.7  | 4700     | 104  | n     | 228   | 4010    | 3304 | 28/Jun/97 18:00           | 10.0 | 425      |      |    | 93    | 2630    | 1918 |
| 28/Jun/97 11:20             | 3.3  | 4900     | 200  | e     | 254   | 4310    | 3604 | 28/Jun/97 18:30           | 10.5 | 350      |      |    | 96    | 2660    | 1948 |
| 28/Jun/97 11:55             | 3.9  | 5900     | 245  | d     | 255   | 4350    | 3644 | 28/Jun/97 19:00           | 11.0 | 360      |      |    | 97    | 2760    | 2048 |
| 28/Jun/97 12:40             | 4.7  | 6350     | 205  | e     | 248   | 4350    | 3644 | 28/Jun/97 20:00           | 12.0 | 290      |      |    | 115   | 2890    | 2178 |
| 28/Jun/97 13:00             | 5.0  | 5350     | 215  | t     | 246   | 4340    | 3634 | 28/Jun/97 20:30           | 12.5 | 216      |      |    | 118   | 2820    | 2108 |
| 28/Jun/97 13:30             | 5.5  | 5550     | 145  | c     | 241   | 4330    | 3624 | 28/Jun/97 21:00           | 13.0 | 194      |      |    | 114   | 2630    | 1918 |
| 28/Jun/97 14:00             | 6.0  | 4800     | 105  | t     | 219   | 3990    | 3284 | 28/Jun/97 21:30           | 13.5 | 172      |      |    | 105   | 2300    | 1588 |
| 28/Jun/97 14:30             | 6.5  | 2450     | 1    | e     | 60    | 1891    | 1185 | 28/Jun/97 22:00           | 14.0 | 157      |      |    | 89    | 1960    | 1248 |
| 28/Jun/97 15:00             | 7.0  | 120      | 1    | d     | 18    | 994     | 288  | 28/Jun/97 22:30           | 14.5 | 137      |      |    | 70    | 1620    | 908  |
| 28/Jun/97 15:30             | 7.5  | 32       | 1    |       | 6     | 767     | 61   | 28/Jun/97 23:00           | 15.0 | 105      |      |    | 51    | 1329    | 617  |
| 28/Jun/97 16:00             | 8.0  | 15       | 1    |       | 3     | 723     | 17   | 28/Jun/97 23:30           | 15.5 | 85       |      |    | 34    | 1080    | 368  |
| 28/Jun/97 18:00             | 10.0 | 6        | 1    |       | 1     | 709     | 3    | 29/Jun/97 0:00            | 16.0 | 72       |      |    | 20    | 891     | 179  |
| 28/Jun/97 20:00             | 12.0 | 5        | 0    |       | 0.6   | 709     | 3    | 29/Jun/97 0:30            | 16.5 | 54       |      |    | 12    | 784     | 72   |
| 28/Jun/97 22:00             | 14.0 | 5        | 0    |       | 0.4   | 712     | 6    | 29/Jun/97 1:00            | 17.0 | 52       |      |    | 7     | 734     | 22   |
| 29/Jun/97 1:00              | 17.0 | 3        | 0    |       | 0.3   | 703     |      | 29/Jun/97 3:00            | 19.0 | 32       |      |    | 2     | 711     | -1   |
|                             |      |          |      |       |       |         |      | 29/Jun/97 8:00            | 24.0 | 16       |      |    | 0.8   | 710     | -2   |
|                             |      |          |      |       |       |         |      | 29/Jun/97 12:00           | 28.0 | 14       |      |    | 0.6   | 710     | -2   |

# APPENDIX 2-5 Strasbourg Test 5 Data

| STR 5 Start: 22-Jun-98 17:00 |          |        |         |      |      | STR 5 End: 23-Jun-98 18:00 |          |       |         |      |       |
|------------------------------|----------|--------|---------|------|------|----------------------------|----------|-------|---------|------|-------|
| (h)                          | (pfu/ml) | (ppb)  | (us/cm) |      |      | (h)                        | (pfu/ml) | (ppb) | (us/cm) |      |       |
| US LOCATION                  |          |        |         |      |      | P2 LOCATION                |          |       |         |      |       |
| TSI                          | H40      | MS2    | UR      | EC   | d EC | TSI                        | H40      | MS2   | UR      | EC   | d EC  |
| 0.0                          | -        | -      | -       |      |      | -0.2                       | 0.1      | 0.1   | 0.72    | 702  |       |
| 0.1                          | 1,905    | 2,350  | 38.4    | 1380 | 677  | 3.5                        | 0.1      | 0.1   | 0.54    | 708  | 6     |
| 0.2                          | 5,050    | 17,000 | 125.9   | 2360 | 1657 | 3.9                        | 0.5      | 0.1   | 0.49    |      |       |
| 0.5                          | 5,900    | 19,500 | 202.4   | 3080 | 2377 | 4.1                        | 1        | 2.5   | 0.51    | 708  | 6     |
| 1.0                          | 9,350    | 34,000 | 267.5   | 3590 | 2887 | 4.2                        | 6.5      | 12    | 0.54    |      |       |
| 1.7                          | 10,500   | 40,000 | 283.7   | 3740 | 3037 | 4.5                        | 19.5     | 60    | 0.80    | 729  | 27    |
| 2.5                          | 14,500   | 32,000 | 287.4   | 3730 | 3027 | 5.0                        | 93       | 212   | 4       | 790  | 88    |
| 3.5                          | 7,500    | 29,500 | 289.1   | 3740 | 3037 | 5.5                        | 195      | 575   | 11      | 902  | 200   |
| 3.9                          | 9,500    | 40,000 | 289.5   | 3750 | 3047 | 6.0                        | 320      | 1050  | 18      | 1017 | 315   |
| 4.1                          | 8,450    | 32,500 | 237.9   | 3230 | 2527 | 6.5                        | 395      | 1395  | 29      | 1160 | 458   |
| 4.2                          | 4,750    | 16,500 | 145.4   | 2390 | 1687 | 7.0                        | 575      | 2050  | 40      | 1337 | 635   |
| 4.5                          | 3,100    | 3,500  | 63.3    | 1509 | 806  | 7.5                        | 705      | 1600  | 53      | 1615 | 913   |
| 5.0                          | 218      | 1      | 13.4    | 984  | 281  | 8.0                        | 1200     | 2000  | 70      | 1919 | 1,217 |
| 5.5                          | 0        | 0      | 3.6     | 783  | 80   | 8.5                        | 900      | 2050  | 86      | 2180 | 1,478 |
| 6.0                          | 0        | 0      | 1.2     | 733  | 30   | 9.0                        | 1050     | 1850  | 111     | 2390 | 1,688 |
| 7.0                          | 0        | 0      | 0.2     | 706  | 3    | 9.5                        | 1450     | 2450  | 131     | 2530 | 1,828 |
| 8.0                          | 0        | 0      | 0.1     | 703  | 0    | 10.0                       | 1400     | 2080  | 144     | 2610 | 1,908 |
| 12.0                         | 0        | 0      | 0.1     | 704  | 1    | 10.5                       | 1050     | 1850  | 153     | 2670 | 1,968 |
| 18.0                         | 0        | 0      | 0.1     | 711  | 8    | 11.0                       | 1300     | 1700  | 157     | 2680 | 1,978 |
| 24.0                         | 0        | 0      | 0.1     | 704  | 1    | 11.5                       | 1300     | 850   | 158     | 2650 | 1,948 |
| PW LOCATION                  |          |        |         |      |      | 12.0                       | 1150     | 1200  | 151     | 2560 | 1,858 |
| TSI                          | H40      | MS2    | UR      | EC   | d EC | 12.5                       | 1500     | 1750  | 150     | 2490 | 1,788 |
| -0.2                         | 0.1      | 0.1    | VALUES  |      | 705  | 13.0                       | 1000     | 850   | 146     | 2420 | 1,718 |
| 0.0                          |          |        | 0.3     |      |      | 13.5                       | 1150     | 1050  | 146     | 2330 | 1,628 |
| 1.3                          | 0.1      | 0.1    | 0.3     | 706  | 1    | 14.0                       | 1400     | 1000  | 141     | 2150 | 1,448 |
| 2.0                          | 0.1      | 0.1    | 0.3     | 707  | 2    | 14.5                       | 1200     | 1185  | 132     | 1980 | 1,278 |
| 2.5                          | 89       | 71     | 2       | 756  | 51   | 15.0                       | 900      | 925   | 110     | 1788 | 1,086 |
| 3.0                          | 530      | 1,125  | 16      | 1140 | 435  | 16.0                       | 510      | 570   | 72      | 1402 | 700   |
| 3.5                          | 2,395    | 8,550  | 134     | 2760 | 2055 | 17.0                       | 340      | 460   | 54      | 1223 | 521   |
| 4.1                          | 3,550    | 12,500 | 234     | 3460 | 2755 | 18.0                       | 295      | 320   | 43      | 1063 | 361   |
| 4.5                          | 4,250    | 19,500 | 261     | 3710 | 3005 | 19.0                       | 220      | 190   | 31      | 920  | 218   |
| 5.0                          | 5,000    | 18,500 | 265     | 3780 | 3075 | 20.0                       | 140      | 122   | 20      | 815  | 113   |
| 5.5                          | 4,750    | 24,500 | 285     | 3800 | 3095 | 21.0                       | 70       | 71.5  | 12      | 752  | 50    |
| 6.0                          | 5,100    | 20,500 | 285     | 3810 | 3105 | 22.0                       | 49.5     | 56    | 6       | 722  | 20    |
| 6.5                          | 4,250    | 20,000 | 280     | 3800 | 3095 | 23.0                       | 34       | 39    | 4       | 709  | 7     |
| 7.0                          | 10,250   | 14,500 | 233     | 3090 | 2385 | 24.0                       | 21       | 30    | 2       | 705  | 3     |
| 7.5                          | 7,150    | 6,250  | 92      | 1625 | 920  | 25.0                       | 25.5     | 13    | 2       |      |       |
| 8.0                          | 3,700    | 1,750  | 36      | 1017 | 312  |                            |          |       |         |      |       |
| 8.5                          | 500      | 765    | 17      | 844  | 139  |                            |          |       |         |      |       |
| 9.5                          | 85       | 290    | 5       | 765  | 60   |                            |          |       |         |      |       |
| 10.0                         | 90       | 190    | 4       | 726  | 21   |                            |          |       |         |      |       |
| 11.0                         | 55       | 118    | 2       | 710  | 5    |                            |          |       |         |      |       |
| 12.0                         | 46       | 88     | 2       | 704  |      |                            |          |       |         |      |       |
| 14.0                         | 35       | 72     | 1       |      |      |                            |          |       |         |      |       |
| 18.0                         | 26       | 32     | 1       |      |      |                            |          |       |         |      |       |
| 24.0                         | 21       | 19     | 0.4     |      |      |                            |          |       |         |      |       |

APPENDIX 2-5 Strasbourg Test 5 Data

| STR 5 Start: 22-Jun-98 17:00 |          |       |         |      |       | STR 5 End: 23-Jun-98 18:00 |          |       |         |      |       |
|------------------------------|----------|-------|---------|------|-------|----------------------------|----------|-------|---------|------|-------|
| (h)                          | (pfu/ml) | (ppb) | (us/cm) |      |       | (h)                        | (pfu/ml) | (ppb) | (us/cm) |      |       |
| P4 LOCATION                  |          |       |         |      |       | DS LOCATION                |          |       |         |      |       |
| TSI                          | H40      | MS2   | UR      | EC   | d EC  | TSI                        | H40      | MS2   | UR      | EC   | d EC  |
| -0.2                         | 0.1      | 0.1   | 1       | 702  |       | 2.5                        | 0        | 0     | 1       | 711  | 1     |
| 3.0                          | 0.1      | 0.1   | 1       | 705  | 3     | 3.5                        | 19       | 15    | 1       | 711  | 1     |
| 3.5                          | 0.5      | 0.1   | 1       | 705  | 3     | 4.1                        | 395      | 1100  | 13      | 882  | 172   |
| 3.9                          | 3        | 7     | 1       |      |       | 4.5                        | 1010     | 2600  | 45      | 1508 | 798   |
| 4.1                          | 12       | 20    | 1       | 708  | 6     | 5.0                        | 1800     | 3950  | 86      | 1915 | 1,205 |
| 4.2                          | 53       | 65    | 1       |      |       | 5.5                        | 2150     | 7000  | 116     | 2150 | 1,440 |
| 4.5                          | 75       | 135   | 2       | 770  | 68    | 6.0                        | 2300     | 7980  | 132     | 2280 | 1,570 |
| 5.0                          | 300      | 485   | 9       | 880  | 178   | 7.0                        | 2800     | 7550  | 170     | 2670 | 1,960 |
| 5.5                          | 410      | 650   | 20      | 1004 | 302   | 8.0                        | 3250     | 4000  | 191     | 2850 | 2,140 |
| 6.0                          | 530      | 1350  | 28      | 1093 | 391   | 8.5                        | 3800     | 10050 | 176     | 2690 | 1,980 |
| 6.5                          | 640      | 1400  | 36      | 1205 | 503   | 9.0                        | 4150     | 6900  | 156     | 2430 | 1,720 |
| 7.0                          | 820      | 1900  | 45      | 1345 | 643   | 10.0                       | 1950     | 3500  | 114     | 1900 | 1,190 |
| 7.5                          | 790      | 2300  | 57      | 1522 | 820   | 11.0                       | 900      | 1815  | 80      | 1529 | 819   |
| 8.0                          | 945      | 1750  | 70      | 1699 | 997   | 12.0                       | 600      | 990   | 56      | 1268 | 558   |
| 8.5                          | 1150     | 1650  | 78      | 1780 | 1,078 | 14.0                       | 180      | 505   | 28      | 949  | 239   |
| 9.0                          | 1850     | 1565  | 87      | 1904 | 1,202 | 16.0                       | 95       | 245   | 15      | 834  | 124   |
| 9.5                          | 1350     | 1600  | 97      | 1970 | 1,268 | 18.0                       | 60       | 160   | 10      | 780  | 70    |
| 10.0                         | 1100     | 1950  | 105     | 2040 | 1,338 | 19.0                       | 81       | 75    | 7       | 761  | 51    |
| 10.5                         | 1350     | 1850  | 110     | 2070 | 1,368 | 20.0                       | 54       | 85    | 6       | 748  | 38    |
| 11.0                         | 1250     | 1600  | 111     | 2030 | 1,328 | 21.0                       | 41       | 58    | 5       | 740  | 30    |
| 11.5                         | 1140     | 1800  | 107     | 1930 | 1,228 | 22.0                       | 39       | 45    | 4       | 734  | 24    |
| 12.0                         | 950      | 1250  | 98      | 1821 | 1,119 | 23.0                       | 35       | 42    | 3       | 728  | 18    |
| 12.5                         | 1095     | 805   | 90      | 1680 | 978   | 24.0                       | 24       | 29    | 3       | 722  | 12    |
| 13.0                         | 700      | 635   | 80      | 1614 | 912   |                            |          |       |         |      |       |
| 13.5                         | 710      | 535   | 72      | 1551 | 849   |                            |          |       |         |      |       |
| 14.0                         | 605      | 555   | 67      | 1539 | 837   |                            |          |       |         |      |       |
| 14.5                         | 515      | 460   | 66      | 1561 | 859   |                            |          |       |         |      |       |
| 15.0                         | 510      | 530   | 68      | 1621 | 919   |                            |          |       |         |      |       |
| 15.7                         | 580      | 525   | 71      | 1705 | 1,003 |                            |          |       |         |      |       |
| 16.0                         | 510      | 575   | 76      | 1748 | 1,046 |                            |          |       |         |      |       |
| 16.5                         | 695      | 450   | 81      | 1789 | 1,087 |                            |          |       |         |      |       |
| 17.0                         | 575      | 340   | 88      | 1800 | 1,098 |                            |          |       |         |      |       |
| 17.5                         | 640      | 485   | 95      | 1764 | 1,062 |                            |          |       |         |      |       |
| 18.0                         | 760      | 410   | 97      | 1716 | 1,014 |                            |          |       |         |      |       |
| 18.5                         | 830      | 385   | 96      | 1623 | 921   |                            |          |       |         |      |       |
| 19.0                         | 665      | 295   | 93      | 1505 | 803   |                            |          |       |         |      |       |
| 19.5                         | 640      | 355   | 83      | 1343 | 641   |                            |          |       |         |      |       |
| 20.0                         | 655      | 290   | 72      | 1218 | 516   |                            |          |       |         |      |       |
| 20.5                         | 465      | 255   | 59      | 1084 | 382   |                            |          |       |         |      |       |
| 21.0                         | 335      | 180   | 45      | 972  | 270   |                            |          |       |         |      |       |
| 21.5                         | 215      | 120   | 33      | 878  | 176   |                            |          |       |         |      |       |
| 22.0                         | 215      | 108   | 24      | 817  | 115   |                            |          |       |         |      |       |
| 23.0                         | 80       | 59    | 13      | 741  | 39    |                            |          |       |         |      |       |
| 24.0                         | 51       | 37    | 7       | 713  | 11    |                            |          |       |         |      |       |
| 25.0                         | 29       | 16    | 4       |      |       |                            |          |       |         |      |       |

APPENDIX 2-6 Strasbourg Test 6 Data

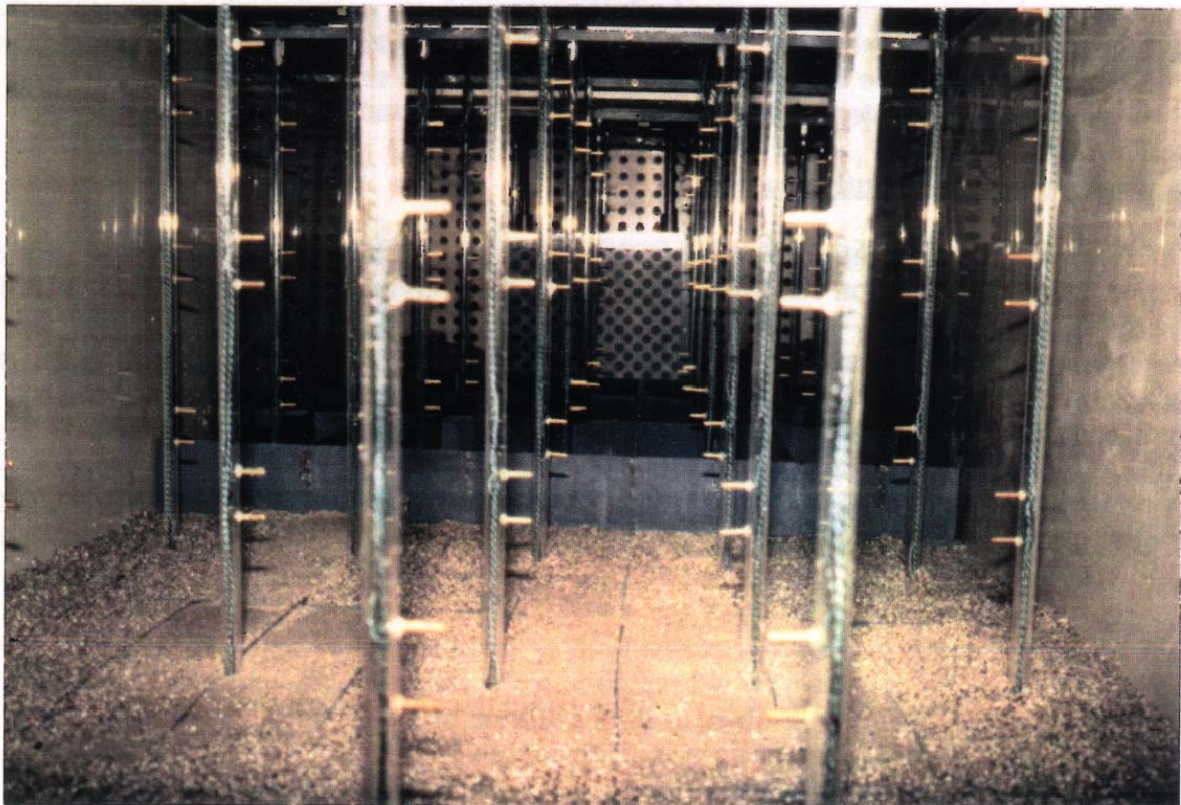
| STR 6 Start: 29-Jun-98 14:00 |          |       |      | STR 6 End: 30-Jun-98 16:00 |          |        |     |             |     |      |      |
|------------------------------|----------|-------|------|----------------------------|----------|--------|-----|-------------|-----|------|------|
| (h)                          | (pfu/ml) | (ppb) |      | (h)                        | (pfu/ml) | (ppb)  |     |             |     |      |      |
| US LOCATION                  |          |       |      | PW LOCATION                |          |        |     | P4 LOCATION |     |      |      |
| TSI                          | H40      | MS2   | UR   | TSI                        | H40      | MS2    | UR  | TSI         | H40 | MS2  | UR   |
| -0.2                         | 0.1      | 0.001 | 0.13 | -0.2                       |          |        | 0   | -0.2        |     |      | 0.14 |
| 0.0                          |          |       | 0.13 | 0.0                        | -        | -      | 0   | 0.0         |     |      |      |
| 0.1                          | 1,600    | 500   | 38   | 1.7                        | 2        |        | 0   | 3.0         | 4   | 0.1  | 0.14 |
| 0.3                          | 4,700    | 4500  | 131  | 2.0                        | 1        | 0      | 0   | 3.5         | 7   | 1    | 0.14 |
| 0.5                          | 7,150    | 10500 | 195  | 2.5                        | 160      | 9      | 3   | 3.9         | 19  | 7    | 0.24 |
| 1.0                          | 8,950    | 17000 | 243  | 3.0                        | 1,240    | 112    | 50  | 4.1         | 35  | 28   | 0.53 |
| 1.7                          | 9,850    | 20000 | 269  | 3.5                        | 2,100    | 5,300  | 145 | 4.3         | 44  | 57   | 1.04 |
| 2.5                          | 9,750    | 23000 | 269  | 4.1                        | 3,650    | 7,000  | 215 | 4.5         | 87  | 115  | 3    |
| 3.5                          | 9,100    | 21500 | 265  | 4.5                        | 4,150    | 7,500  | 231 | 5.0         | 270 | 320  | 10   |
| 3.9                          | 9,450    | 19500 | 266  | 5.0                        | 3,400    | 10,000 | 252 | 5.5         | 305 | 535  | 19   |
| 4.1                          | 8,300    | 4000  | 266  | 5.5                        | 2,750    | 9,000  | 248 | 6.0         | 405 | 845  | 26   |
| 4.3                          | 5,500    | 5900  | 136  | 6.0                        | 4,100    | 8,500  | 252 | 6.5         | 535 | 980  | 33   |
| 4.5                          | 2,900    | 2000  | 60   | 6.5                        | 3,500    | 7,000  | 251 | 7.0         | 525 | 700  | 39   |
| 5.0                          | 225      | 0.1   | 12   | 7.0                        | 2,750    | 4,500  | 196 | 7.5         | 560 | 850  | 47   |
| 6.0                          | 0        | 0.1   | 1    | 7.5                        | 1,450    | 1,250  | 85  | 8.0         | 540 | 1000 | 55   |
| P2 LOCATION                  |          |       |      | 8.0                        | 670      | 650    | 33  | 8.5         | 800 | 1090 | 61   |
| TSI                          | H40      | MS2   | UR   | 8.5                        | 395      | 265    | 14  | 9.0         | 700 | 850  | 62   |
| 4.5                          | 4        | 1     | 0    | 9.0                        | 315      | 140    | 8   | 9.5         | 615 | 550  | 63   |
| 5.0                          | 10       | 12    | 0    | 9.5                        | 243      | 145    | 5   | 10.0        | 600 | 500  | 66   |
| 5.5                          | 15       | 15    | 1    | 10.0                       | 184      | 115    | 4   | 10.5        | 600 | 500  | 68   |
| 6.0                          | 40       | 35    | 3    | 11.0                       | 124      | 55     | 2   | 11.0        | 500 | 350  | 71   |
| 6.5                          | 55       | 70    | 7    | 12.0                       | 103      | 55     | 2   | 11.5        | 380 | 400  | 73   |
| 7.0                          | 55       | 130   | 12   | 14.0                       | 79       | 38     | 1   | 12.0        | 405 | 350  | 73   |
| 7.5                          | 110      | 150   | 19   | 18.0                       | 50       | 37     | 1   | 12.5        | 360 | 250  | 73   |
| 8.0                          | 225      | 200   | 27   | 26.0                       | 34       | 15     | 0   | 13.0        | 280 | 200  | 71   |
| 8.5                          | 255      | 200   | 38   | DS LOCATION                |          |        |     | 13.5        | 245 | 220  | 69   |
| 9.0                          | 250      | 200   | 52   | TSI                        | H40      | MS2    | UR  | 14.0        | 215 | 190  | 69   |
| 9.5                          | 450      | 150   | 69   | -0.2                       |          |        | 0.1 | 14.5        | 255 | 140  | 70   |
| 10.0                         | 480      | 650   | 89   | 2.0                        | 3        | 0.1    | 0.1 | 15.0        | 195 | 120  | 72   |
| 10.5                         | 600      | 300   | 106  | 2.5                        | 3        | 0.5    | 0.1 | 15.5        | 210 | 175  | 74   |
| 11.0                         | 650      | 200   | 121  | 3.0                        | 4        | 0.1    | 0.1 | 16.0        | 195 | 95   | 77   |
| 11.5                         | 665      | 300   | 133  | 3.5                        | 12       | 2.5    | 0.2 | 16.5        | 180 | 80   | 80   |
| 12.0                         | 735      | 342   | 146  | 4.1                        | 155      | 95     | 2   | 17.0        | 205 | 80   | 81   |
| 12.5                         | 700      | 400   | 150  | 4.5                        | 285      | 300    | 10  | 17.5        | 155 | 55   | 80   |
| 13.0                         | 450      | 210   | 146  | 5.0                        | 600      | 1250   | 32  | 18.0        | 125 | 75   | 75   |
| 13.5                         | 350      | 190   | 138  | 5.5                        | 900      | 1850   | 69  | 18.5        | 170 | 35   | 70   |
| 14.0                         | 300      | 165   | 126  | 6.0                        | 1,900    | 2350   | 100 | 19.0        | 90  | 55   | 62   |
| 14.5                         | 350      | 75    | 112  | 6.5                        | 1,550    | 3400   | 126 | 19.5        | 90  | 60   | 51   |
| 15.0                         | 355      | 65    | 96   | 7.0                        | 1,300    | 4950   | 147 | 20.0        | 95  | 60   | 44   |
| 16.0                         | 290      | 60    | 65   | 7.5                        | 2,450    | 4450   | 164 | 20.5        | 60  | 20   | 36   |
| 17.0                         | 160      | 20    | 45   | 8.0                        | 1,900    | 2200   | 175 | 21.0        | 115 | 15   | 27   |
| 18.0                         | 205      | 10    | 32   | 8.5                        | 1,550    | 3200   | 174 | 21.5        | 75  | 45   | 21   |
| 19.0                         | 90       | 5     | 22   | 9.0                        | 1,500    | 3250   | 159 | 22.0        | 50  | 10   | 15   |
| 20.0                         | 85       | 10    | 15   | 9.5                        | 1,100    | 1350   | 133 | 23.0        | 41  | 11   | 9    |
| 21.0                         | 68       | 13    | 10   | 10.0                       | 965      | 1150   | 109 | 24.0        | 40  | 13.5 | 5    |
| 22.0                         | 60       | 11    | 6    | 11.0                       | 455      | 650    | 72  | 25.0        | 27  | 19   | 3    |
| 24.0                         | 39       | 4     | 3    | 12.0                       | 385      | 320    | 48  | 26.0        | 32  | 16   | 2    |
| 25.0                         | 37       | 4     | 2    | 13.0                       | 320      | 320    | 34  |             |     |      |      |
| 26.0                         | 35       | 0     | 2    | 14.0                       | 140      | 180    | 27  |             |     |      |      |
|                              |          |       |      | 15.0                       | 115      | 90     | 22  |             |     |      |      |
|                              |          |       |      | 16.0                       | 106      | 75     | 16  |             |     |      |      |
|                              |          |       |      | 18.0                       | 60       | 55     | 9   |             |     |      |      |
|                              |          |       |      | 19.0                       | 64       | 30     | 8   |             |     |      |      |
|                              |          |       |      | 20.0                       | 55       | 37.5   | 6   |             |     |      |      |
|                              |          |       |      | 21.0                       | 51       | 43.5   | 5   |             |     |      |      |
|                              |          |       |      | 22.0                       | 40       | 26     | 4   |             |     |      |      |
|                              |          |       |      | 24.0                       | 33       | 25.5   | 3   |             |     |      |      |
|                              |          |       |      | 26.0                       | 35       | 25     | 2   |             |     |      |      |

## Appendix A2-7

### Figure A2-7-1 Tank interior with Electrical Conductivity probes

(after M. Ruch, 1992)

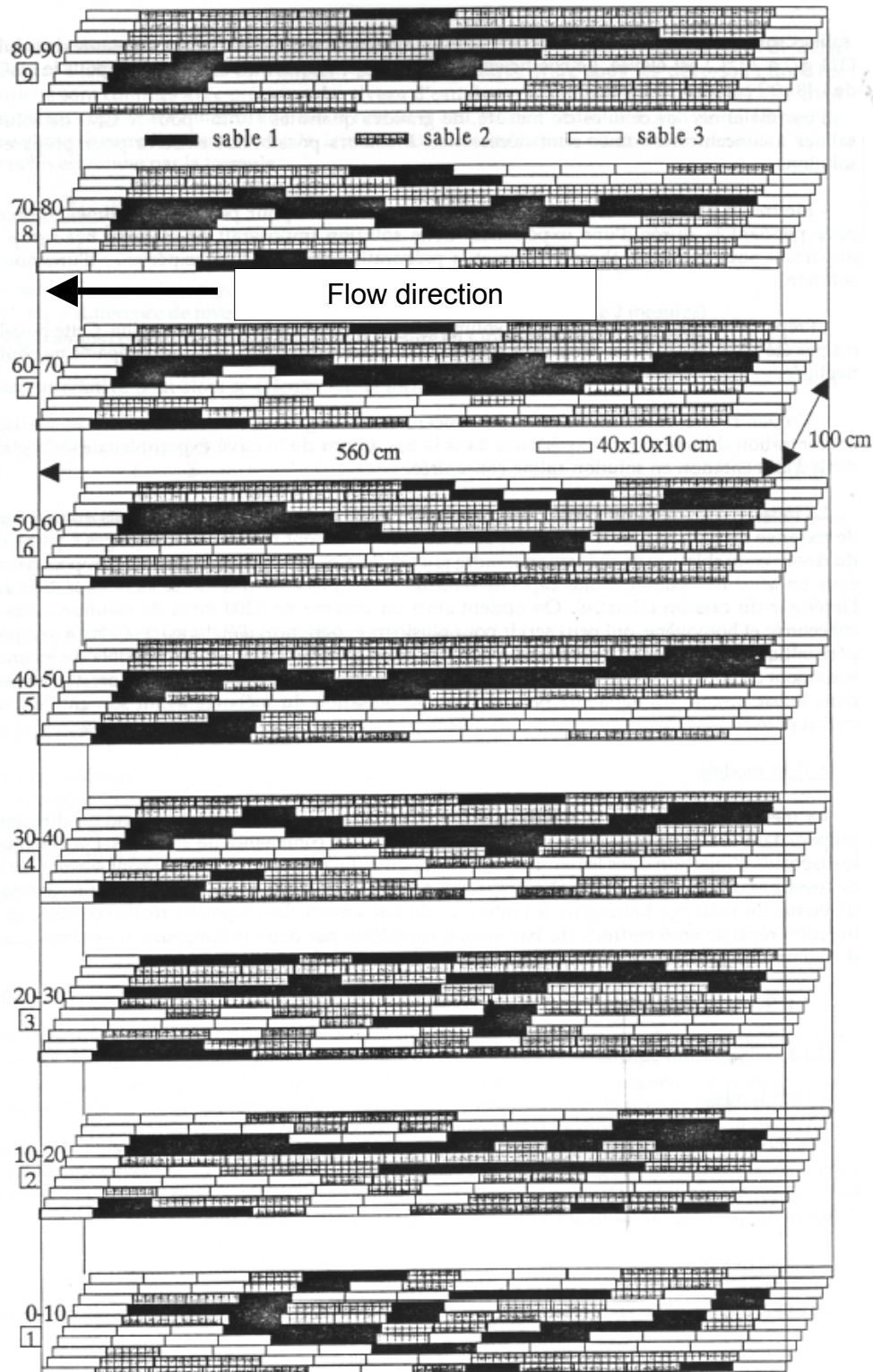
This photograph taken from Ruch, 1992 illustrates the layout of the approximately 350 electrical conductivity probes located within the tank itself that are used to monitor and document the migration of conductivity as it changes throughout the tank during tracer tests.



## Appendix A2-7 Figure A2-7-2 Sand layer layout in MARCEAUS

(after M. Ruch, 1992)

This layered diagram taken from Ruch, 1992 illustrates the variability in hydraulic conductivity in the sand placed in cells throughout the tank.



## Appendix A3 for Chapter 3

### Bois de Finges (BdF) Field Site Canton Valais, Switzerland

Appendix A3-1 BdF Test 1 Data

Appendix A3-2 BdF Test 2 Data

Appendix A3-3 BdF Test 3 Data

Appendix A3-4 BdF Test 4 Data

# APPENDIX A3-1 Bois de Finges Test 1 Data

| BDF1 - Start<br>11/17/1997 09:32 | (h)  | WELL 11B |             | WELL 11C |           | WELL 11D |           |
|----------------------------------|------|----------|-------------|----------|-----------|----------|-----------|
|                                  |      | (ppb)    | (pfu/ml)    | (ppb)    | (pfu/ml)  | (ppb)    | (pfu/ml)  |
| Sample DateTime                  | TSI  | URANINE  | Phage H40   | URANINE  | Phage H40 | URANINE  | Phage H40 |
| 17-Nov-97 09:50                  | 0.3  | 36,253   | 331,000,000 |          | 0         |          | 0         |
| 17-Nov-97 10:00                  | 0.5  | 7,590    | 69,300,000  | 0.6      | 0         | 1        | 0         |
| 17-Nov-97 10:30                  | 1.0  | 712      | 6,500,000   | 0.7      | 0         | 1        | 0         |
| 17-Nov-97 10:45                  | 1.2  | 438      | 4,000,000   | 0.7      |           | 1        |           |
| 17-Nov-97 11:00                  | 1.5  | 307      | 2,800,000   | 0.7      | 0         | 1        | 0         |
| 17-Nov-97 11:15                  | 1.7  | 227      | 1,950,000   | 0.6      | 0         | 1        |           |
| 17-Nov-97 11:30                  | 2.0  | 82       | 850,000     | 0.6      | 2         | 1        | 13        |
| 17-Nov-97 11:45                  | 2.2  |          |             | 0.7      | 14        | 1        |           |
| 17-Nov-97 12:00                  | 2.5  | 42       | 235,000     | 0.7      | 66        | 1        | 6,800     |
| 17-Nov-97 12:15                  | 2.7  |          |             | 0.6      | 175       | 3        | 21,500    |
| 17-Nov-97 12:30                  | 3.0  | 22       | 65,000      | 0.7      | 595       | 8        | 65,500    |
| 17-Nov-97 12:45                  | 3.2  |          |             | 0.8      | 1,190     | 17       | 116,500   |
| 17-Nov-97 13:00                  | 3.5  | 16       | 55,000      | 0.7      | 1,605     | 28       | 159,500   |
| 17-Nov-97 13:15                  | 3.7  |          |             | 1.3      | 2,750     | 46       | 163,000   |
| 17-Nov-97 13:30                  | 4.0  | 12       | 30,000      | 1.5      | 3,600     | 63       | 202,500   |
| 17-Nov-97 13:45                  | 4.2  |          |             | 1.8      | 3,350     | 82       | 229,000   |
| 17-Nov-97 14:00                  | 4.5  |          |             | 1.9      | 1,850     | 102      | 207,000   |
| 17-Nov-97 14:20                  | 4.8  | 8        | 22,500      | 2.3      | 2,000     | 128      | 187,000   |
| 17-Nov-97 14:40                  | 5.1  |          |             | 2.4      | 1,415     | 151      | 176,000   |
| 17-Nov-97 15:00                  | 5.5  | 5        | 13,500      | 2.5      | 880       | 165      | 122,500   |
| 17-Nov-97 15:20                  | 5.8  |          |             | 2.7      | 825       | 182      | 85,500    |
| 17-Nov-97 15:40                  | 6.1  |          |             | 2.9      | 625       | 186      | 93,000    |
| 17-Nov-97 16:00                  | 6.5  | 4        | 8,000       | 3.0      | 390       | 196      | 57,000    |
| 17-Nov-97 16:20                  | 6.8  |          |             | 2.8      | 325       | 188      | 57,000    |
| 17-Nov-97 16:40                  | 7.1  |          |             | 3.1      | 200       | 184      | 43,500    |
| 17-Nov-97 17:00                  | 7.5  |          | 3,150       | 3.2      | 207       | 177      | 23,000    |
| 17-Nov-97 17:30                  | 8.0  |          |             | 3.1      | 112       | 165      | 17,000    |
| 17-Nov-97 18:00                  | 8.5  |          |             | 3.1      | 81        | 151      | 10,000    |
| 17-Nov-97 18:30                  | 9.0  |          |             | 3.3      | 59        | 140      | 8,100     |
| 17-Nov-97 19:00                  | 9.5  | 3        |             | 3.4      | 32        | 128      | 5,750     |
| 17-Nov-97 20:30                  | 11.0 |          |             | 3.7      | 17        | 98       | 3,100     |
| 17-Nov-97 23:00                  | 13.5 | 1        |             | 4.1      | 10        | 67       | 2,450     |
| 18-Nov-97 02:00                  | 16.5 |          |             | 3.5      | 1         | 45       | 1,455     |
| 18-Nov-97 06:30                  | 21.0 | 1        |             | 3.6      | 12        | 26       | 1,100     |
| 18-Nov-97 09:00                  | 23.5 | 1        |             | 3.1      | 18        | 21       | 890       |
| 18-Nov-97 12:00                  | 26.5 | 1        | 102         | 2.6      | 13        | 16       | 900       |
| 18-Nov-97 15:00                  | 29.5 | 1        | 77          | 2.3      | 8         | 13       | 705       |
| 18-Nov-97 19:00                  | 33.5 |          |             | 1.9      | 6         | 9        |           |
| 18-Nov-97 23:00                  | 37.5 | 1        | 206         | 1.6      | 9         | 7        |           |
| 19-Nov-97 03:00                  | 41.5 |          |             | 1.4      | 8         | 6        |           |
| 19-Nov-97 07:00                  | 45.5 | 1        | 251         | 1.3      | 4         | 4        |           |
| 19-Nov-97 12:00                  | 50.5 |          |             |          | 5         |          |           |
| 19-Nov-97 16:00                  | 54.5 |          |             |          | 4         |          |           |

APPENDIX A3-2 Bois de Finges Test 2 Data

| BDF2 Start:     |      | WELL 11B |          |        | WELL 11C |          |     | WELL 11D |          |       |
|-----------------|------|----------|----------|--------|----------|----------|-----|----------|----------|-------|
| 30-Mar-98 07:55 | (h)  | (ppb)    | (pfu/ml) |        | (ppb)    | (pfu/ml) |     | (ppb)    | (pfu/ml) |       |
| Sample DateTime | TSI  | UR       | H40      | MS2    | UR       | H40      | MS2 | UR       | H40      | MS2   |
| 30-Mar-98 07:25 | -0.5 | 0.09     | 0        | 0      | 0.08     | 0        | 0   | 0.08     | 1.5      | 0     |
| 30-Mar-98 07:55 | 0.0  |          |          |        |          |          |     |          |          |       |
| 30-Mar-98 08:00 | 0.1  | 2.E+05   | 2.E+08   | 7.E+07 |          |          |     |          |          |       |
| 30-Mar-98 08:00 | 0.1  | 3.E+05   |          |        |          |          |     |          |          |       |
| 30-Mar-98 08:00 | 0.1  | 3.E+05   |          |        |          |          |     |          |          |       |
| 30-Mar-98 08:00 | 0.1  | 3.E+05   |          |        |          |          |     |          |          |       |
| 30-Mar-98 08:00 | 0.1  | 3.E+05   |          |        |          |          |     |          |          |       |
| 30-Mar-98 08:15 | 0.3  | 3.E+04   | 3.E+07   | 1.E+07 |          |          |     |          |          |       |
| 30-Mar-98 08:30 | 0.6  | 4.E+03   | 4.E+06   | 9.E+05 | 0.15     |          |     | 0.06     |          |       |
| 30-Mar-98 09:00 | 1.1  | 1.E+03   | 1.E+06   | 3.E+05 | 0.10     | 0        | 0   | 0.08     | 1        | 0     |
| 30-Mar-98 09:00 | 1.1  | 2.E+03   |          |        |          |          |     |          |          |       |
| 30-Mar-98 09:00 | 1.1  | 2.E+03   |          |        |          |          |     |          |          |       |
| 30-Mar-98 09:15 | 1.3  |          |          |        |          |          |     | 0.08     |          |       |
| 30-Mar-98 09:20 | 1.4  | 589      | 4.E+05   | 1.E+05 |          |          |     |          |          |       |
| 30-Mar-98 09:20 | 1.4  | 869      |          |        |          |          |     |          |          |       |
| 30-Mar-98 09:20 | 1.4  | 858      |          |        |          |          |     |          |          |       |
| 30-Mar-98 09:30 | 1.6  |          |          |        |          | 0        | 0   | 0.14     | 2        | 0     |
| 30-Mar-98 09:40 | 1.8  | 382      | 2.E+05   | 5.E+04 |          |          |     |          |          |       |
| 30-Mar-98 09:40 | 1.8  | 403      |          |        |          |          |     |          |          |       |
| 30-Mar-98 09:40 | 1.8  | 403      |          |        |          |          |     |          |          |       |
| 30-Mar-98 09:45 | 1.8  |          |          |        |          |          |     |          |          |       |
| 30-Mar-98 10:00 | 2.1  | 170      | 7.E+04   | 2.E+04 | 0.10     | 0        | 0   | 0.08     | 1.5      | 0     |
| 30-Mar-98 10:15 | 2.3  |          |          |        |          |          |     | 0.09     |          |       |
| 30-Mar-98 10:20 | 2.4  | 120      | 4.E+04   | 1.E+04 |          |          |     |          |          |       |
| 30-Mar-98 10:30 | 2.6  |          |          |        | 0.08     | 0        | 0   | 0.09     | 2.5      | 0     |
| 30-Mar-98 10:40 | 2.8  | 86       | 3.E+04   | 6.E+03 |          |          |     |          |          |       |
| 30-Mar-98 10:45 | 2.8  |          |          |        |          |          |     |          |          |       |
| 30-Mar-98 11:00 | 3.1  | 52       | 2.E+04   | 4.E+03 | 0.10     | 0        | 0   |          |          |       |
| 30-Mar-98 11:15 | 3.3  |          |          |        |          | 0        | 0.5 |          |          |       |
| 30-Mar-98 11:30 | 3.6  | 33       | 7.E+03   | 2.E+03 | 0.11     | 3        | 1   |          |          |       |
| 30-Mar-98 11:45 | 3.8  |          |          |        |          | 3.5      | 0.5 | 0.11     | 515      | 6     |
| 30-Mar-98 12:00 | 4.1  | 25       | 4.E+03   | 1.E+03 | 0.11     | 11.5     | 0.5 | 0.30     | 175      | 15    |
| 30-Mar-98 12:15 | 4.3  |          |          |        | 0.13     | 11.5     | 3   | 0.62     | 215      | 41    |
| 30-Mar-98 12:30 | 4.6  | 18       | 3.E+03   | 1.E+03 | 0.14     | 15       | 4   | 1.46     | 650      | 79.5  |
| 30-Mar-98 12:45 | 4.8  |          |          |        | 0.19     | 24       | 2.5 | 2.61     | 825      | 109   |
| 30-Mar-98 13:00 | 5.1  | 16       | 4.E+03   | 1.E+03 | 0.23     | 27.5     | 4.5 | 4.79     | 1620     | 200.5 |
| 30-Mar-98 13:20 | 5.4  |          |          |        | 0.30     | 67.5     | 5.5 | 9.05     | 2385     | 196   |
| 30-Mar-98 13:30 | 5.6  |          |          |        |          |          |     |          |          |       |
| 30-Mar-98 13:40 | 5.8  |          |          |        | 0.42     | 54.5     | 6   | 15.45    | 3250     | 240   |
| 30-Mar-98 14:00 | 6.1  | 9.11     | 1750     | 1100   | 0.46     | 78.5     | 7.5 | 24.85    | 4800     | 280   |
| 30-Mar-98 14:20 | 6.4  |          |          |        | 0.72     | 125      | 7.5 | 36.24    | 6000     | 330   |
| 30-Mar-98 14:30 | 6.6  |          |          |        |          |          |     |          |          |       |
| 30-Mar-98 14:40 | 6.8  |          |          |        | 0.72     | 82.5     | 2.5 | 52.08    | 4500     | 420   |
| 30-Mar-98 15:00 | 7.1  | 7.72     | 965      | 685    | 0.78     | 64.5     | 5.5 | 66.73    | 5500     | 235   |
| 30-Mar-98 15:30 | 7.6  |          |          |        | 1.21     | 91.5     | 5   |          |          |       |
| 30-Mar-98 16:00 | 8.1  |          |          |        | 1.80     | 56.5     | 4   |          |          |       |
| 30-Mar-98 16:30 | 8.6  |          |          |        | 1.64     | 55.5     | 2.5 | 158.02   | 10000    | 240   |
| 30-Mar-98 16:45 | 8.8  |          |          |        |          |          |     | 171.29   | 9000     | 270   |
| 30-Mar-98 17:00 | 9.1  | 4.59     | 575      | 800    | 1.82     | 37.5     | 3.5 | 181.49   | 6000     | 170   |
| 30-Mar-98 17:20 | 9.4  |          |          |        |          |          |     | 203.17   | 3000     | 165   |
| 30-Mar-98 17:30 | 9.6  |          |          |        | 2.03     | 43       | 3.5 |          |          |       |
| 30-Mar-98 17:40 | 9.8  |          |          |        |          |          |     | 223.96   | 4750     | 175   |
| 30-Mar-98 18:00 | 10.1 |          |          |        | 1.94     | 24.5     | 2   | 228.91   | 4650     | 130   |
| 30-Mar-98 18:45 | 10.8 |          |          |        | 2.11     | 14.5     | 0.5 | 256.04   | 2800     | 67    |
| 30-Mar-98 19:30 | 11.6 |          |          |        | 2.46     | 3        | 0.5 | 274.26   | 1740     | 54    |
| 30-Mar-98 20:00 | 12.1 | 2.02     | 228.5    | 360    |          |          |     |          |          |       |
| 30-Mar-98 20:15 | 12.3 |          |          |        | 2.75     | 6        | 0   | 259.21   | 1115     | 39.5  |
| 30-Mar-98 21:00 | 13.1 |          |          |        | 3.47     | 3.5      | 0.5 | 262.97   | 700      | 24.5  |
| 30-Mar-98 22:00 | 14.1 | 1.46     | 149      | 260    | 4.00     | 4.5      | 0   | 251.49   | 380      | 22.5  |
| 30-Mar-98 23:30 | 15.6 |          |          |        | 5.09     | 1        | 0   | 229.31   | 345      | 18    |
| 31-Mar-98 01:00 | 17.1 |          |          |        | 6.24     | 1.5      | 0.5 | 202.77   | 205.5    | 12    |
| 31-Mar-98 03:00 | 19.1 |          |          |        | 7.55     | 0.5      | 1   | 177.03   | 140.5    | 11.5  |
| 31-Mar-98 05:30 | 21.6 |          |          |        | 8.42     | 0        | 0   | 144.36   | 126      | 9     |
| 31-Mar-98 08:00 | 24.1 | 0.44     | 40.5     | 140    | 9.03     | 1        | 0   | 120.99   | 90       | 9     |
| 31-Mar-98 10:30 | 26.6 |          |          |        | 8.50     | 0.5      | 0   | 102.18   | 62.5     | 7.5   |
| 31-Mar-98 13:30 | 29.6 |          |          |        | 8.74     | 0.5      | 0   | 83.56    | 73.5     | 2.5   |
| 31-Mar-98 16:45 | 32.8 |          |          |        | 7.84     | 0.5      | 0   | 71.39    | 63       | 4.5   |
| 31-Mar-98 20:15 | 36.3 | 0.37     | 76.5     | 245    | 7.47     | 0.5      | 0   | 54.06    | 57       | 3.5   |
| 1-Apr-98 00:30  | 40.6 |          |          |        | 6.34     | 0        | 0   | 43.37    | 54       | 1.5   |
| 1-Apr-98 05:30  | 45.6 |          |          |        | 5.35     | 0        | 0   | 32.87    | 50       | 3.5   |
| 1-Apr-98 11:00  | 51.1 | 0.22     | 23.5     | 43.5   |          |          |     | 25.27    | 33.5     | 2.5   |
| 1-Apr-98 11:15  | 51.3 |          | 24       | 31.5   | 4.46     | 0        | 0   |          |          |       |
| 1-Apr-98 13:30  | 53.6 |          |          |        | 4.08     | 0        | 0   | 22.38    | 40       | 2.5   |
| 1-Apr-98 16:30  | 56.6 |          |          |        | 3.61     | 0        | 0   | 19.70    | 38       | 4     |
| 1-Apr-98 22:30  | 62.6 |          |          |        | 2.92     | 0        | 0   | 14.95    | 35.5     | 2     |
| 2-Apr-98 07:00  | 71.1 | 0.14     | 26       | 54.5   | 2.14     | 0.5      | 0   | 9.98     | 34.5     | 1     |
| 2-Apr-98 12:15  | 76.3 |          |          |        | 1.78     | 1        | 0   | 8.12     | 25.5     | 3.5   |
| 2-Apr-98 16:30  | 80.6 |          |          |        | 1.50     | 0        | 0   | 7.13     | 24       | 1     |
| 2-Apr-98 21:15  | 85.3 |          |          |        | 1.41     | 0        | 0   | 6.32     | 30.5     | 2     |
| 3-Apr-98 08:00  | 96.1 |          |          |        | 1.04     | 0        | 0   | 4.16     | 23.5     | 1.5   |

APPENDIX 3-3 Bois de Finges Test 3 Data

| BDF3 Start:<br>10/Aug/98 10:30 |      | WELL 11B |          | WELL 11C |          | WELL 11D |          |
|--------------------------------|------|----------|----------|----------|----------|----------|----------|
|                                | (h)  | (ppb)    | (pfu/ml) | (ppb)    | (pfu/ml) | (ppb)    | (pfu/ml) |
| Sample DateTime                | TSI  | UR       | H40      | UR       | H40      | UR       | H40      |
| 10-Aug-98 10:15                | -0.2 |          | 0.001    | 1        | 1        |          | 2.5      |
| 10-Aug-98 10:40                | 0.2  | 40500    | 1.6E+08  |          |          |          |          |
| 10-Aug-98 10:50                | 0.3  |          | 5.8E+07  |          |          |          |          |
| 10-Aug-98 11:00                | 0.5  | 60000    | 2.1E+08  | 0.1      | 0        | 0.1      | 1.5      |
| 10-Aug-98 11:15                | 0.8  | 41167    | 1.6E+08  | 0.1      | 1        | 0.1      | 1        |
| 10-Aug-98 11:30                | 1.0  | 36833    | 1.0E+08  | 0.1      | 2        | 0.1      | 0.5      |
| 10-Aug-98 11:45                | 1.3  | 433      | 1.5E+06  | 0.1      | 2        | 0.1      | 1        |
| 10-Aug-98 12:00                | 1.5  | 64       | 1.9E+05  | 0.1      | 3        | 0.1      | 16.5     |
| 10-Aug-98 12:15                | 1.7  |          |          | 0.1      | 23       | 0.2      | 505      |
| 10-Aug-98 12:30                | 2.0  | 10       | 3.0E+04  | 0.1      | 111      | 1.3      | 3100     |
| 10-Aug-98 12:45                | 2.3  |          |          | 0.2      | 380      | 4        | 17000    |
| 10-Aug-98 13:00                | 2.5  |          |          | 0.5      | 675      | 11       | 23500    |
| 10-Aug-98 13:20                | 2.8  |          |          | 1.2      | 1400     | 33       | 41500    |
| 10-Aug-98 13:30                | 3.0  | 2.6      | 9000     |          |          |          |          |
| 10-Aug-98 13:40                | 3.2  |          |          | 1.9      | 1750     | 56       | 45500    |
| 10-Aug-98 14:00                | 3.5  |          |          | 3.1      | 2150     | 94       | 53000    |
| 10-Aug-98 14:20                | 3.8  |          |          | 4.0      | 2200     | 121      | 40000    |
| 10-Aug-98 14:40                | 4.2  |          |          | 5.4      | 1400     | 140      | 23500    |
| 10-Aug-98 15:00                | 4.5  | 0.8      | 1950     | 6.1      | 865      | 147      | 19000    |
| 10-Aug-98 15:30                | 5.0  |          |          | 6.4      | 475      | 142      | 10000    |
| 10-Aug-98 16:00                | 5.5  |          |          | 7.4      | 215      | 117      | 4500     |
| 10-Aug-98 16:30                | 6.0  |          |          | 7.7      | 180      | 100      | 2750     |
| 10-Aug-98 17:00                | 6.5  |          |          | 7.4      | 81       | 86       | 2250     |
| 10-Aug-98 17:30                | 7.0  |          |          | 7.1      | 59       | 69       | 1800     |
| 10-Aug-98 18:00                | 7.5  | 0.3      | 1170     | 7.1      | 49       | 59       | 1100     |
| 10-Aug-98 18:30                | 8.0  |          |          | 7.4      | 28       | 47       | 1300     |
| 10-Aug-98 19:00                | 8.5  |          |          | 7.1      | 36       | 40       | 1000     |
| 10-Aug-98 20:00                | 9.5  |          |          | 6.1      | 16       | 30       | 650      |
| 10-Aug-98 21:00                | 10.5 |          |          | 5.7      | 10       | 21       | 525      |
| 10-Aug-98 22:30                | 12.0 |          |          | 4.5      | 9        | 15       | 415      |
| 11-Aug-98 00:00                | 13.5 | 0.2      | 69       |          | 8        |          | 365      |
| 11-Aug-98 00:30                | 14.0 |          |          | 3.4      |          | 9        |          |
| 11-Aug-98 02:30                | 16.0 |          |          | 2.8      | 6        | 7        | 231.5    |
| 11-Aug-98 05:00                | 18.5 |          |          | 1.8      | 3        | 4        | 193.5    |
| 11-Aug-98 08:00                | 21.5 |          |          | 1.5      | 4        | 3        | 152      |
| 11-Aug-98 10:30                | 24.0 | 0.1      | 37       |          |          | 2.4      | 77.5     |
| 11-Aug-98 11:25                | 24.9 |          |          | 1.1      | 5        |          |          |
| 11-Aug-98 13:00                | 26.5 |          |          | 0.8      | 5        | 1.9      | 56       |
| 11-Aug-98 17:30                | 31.0 |          |          | 0.7      | 2.0      | 1.4      | 59       |
| 11-Aug-98 21:30                | 35.0 |          |          | 0.6      | 1.0      | 1.1      | 52       |

# APPENDIX A3-4 Bois de Finges Test 4 Data

| BDF4 Start:     |       | WELL 11A | WELL 11C                              |                                           | WELL 11D |          |
|-----------------|-------|----------|---------------------------------------|-------------------------------------------|----------|----------|
| 12/Aug/98 07:00 | (h)   | (pfu/ml) | (ppb)                                 | (pfu/ml)                                  | (ppb)    | (pfu/ml) |
| Sample DateTime | TSI   | H4       | UR                                    | H4                                        | UR       | H4       |
| 12/Aug/98 06:50 | (0.2) |          |                                       | 0.001                                     |          | 0        |
| 12/Aug/98 07:00 | 0.0   | 0.001    | 0.37                                  |                                           | 0.67     |          |
| 12/Aug/98 07:10 | 0.2   | 1        | Uranine Data<br>from<br>downhole unit | Sampled too<br>late for initial<br>values |          |          |
| 12/Aug/98 07:20 | 0.3   | 8        |                                       |                                           |          |          |
| 12/Aug/98 07:30 | 0.5   | 21       |                                       |                                           |          |          |
| 12/Aug/98 07:45 | 0.8   | 28       |                                       |                                           |          |          |
| 12/Aug/98 08:00 | 1.0   | 42       |                                       |                                           |          |          |
| 12/Aug/98 08:15 | 1.3   |          | 94                                    | 1.8E+05                                   | 0.66     |          |
| 12/Aug/98 08:30 | 1.5   | 20       | 273                                   | 4.7E+05                                   | 0.68     | 0        |
| 12/Aug/98 08:40 | 1.7   |          | 277                                   | 5.4E+05                                   | 0.57     |          |
| 12/Aug/98 08:45 | 1.8   |          | 240                                   | 7.7E+05                                   |          |          |
| 12/Aug/98 08:50 | 1.8   |          |                                       |                                           | 0.67     | 0        |
| 12/Aug/98 09:00 | 2.0   | 8        | 189                                   | 7.6E+05                                   |          |          |
| 12/Aug/98 09:10 | 2.2   |          | 145                                   | 6.6E+05                                   | 0.68     |          |
| 12/Aug/98 09:15 | 2.3   |          | 117                                   | 6.8E+05                                   |          |          |
| 12/Aug/98 09:20 | 2.3   |          |                                       |                                           | 0.62     |          |
| 12/Aug/98 09:30 | 2.5   | 14       | 99                                    | 6.0E+05                                   |          |          |
| 12/Aug/98 09:40 | 2.7   |          | 72                                    | 4.2E+05                                   | 0.60     |          |
| 12/Aug/98 09:45 | 2.8   |          | 64                                    | 2.0E+05                                   |          |          |
| 12/Aug/98 09:50 | 2.8   |          |                                       |                                           | 0.62     | 0        |
| 12/Aug/98 10:00 | 3.0   |          | 51                                    | 1.8E+05                                   |          |          |
| 12/Aug/98 10:15 | 3.3   |          | 42                                    | 1.4E+05                                   | 0.60     |          |
| 12/Aug/98 10:30 | 3.5   |          | 32                                    | 7.6E+04                                   | 0.63     | 0        |
| 12/Aug/98 10:45 | 3.8   |          | 23                                    | 3.8E+04                                   | 0.57     |          |
| 12/Aug/98 11:00 | 4.0   | 4.00     | 19                                    |                                           | 0.54     | 0        |
| 12/Aug/98 11:10 | 4.2   |          |                                       | 3.3E+04                                   | 0.57     |          |
| 12/Aug/98 11:20 | 4.3   |          | 14                                    |                                           |          |          |
| 12/Aug/98 11:40 | 4.7   |          | 12                                    | 1.9E+04                                   | 0.54     | 0        |
| 12/Aug/98 12:00 | 5.0   | 3.00     | 10                                    | 1.6E+04                                   | 0.55     |          |
| 12/Aug/98 12:30 | 5.5   |          | 8                                     | 1.2E+04                                   | 0.60     | 0        |
| 12/Aug/98 13:00 | 6.0   |          | 6                                     | 7,200                                     | 0.54     |          |
| 12/Aug/98 13:30 | 6.5   |          | 5                                     | 5,950                                     | 0.52     | 0        |
| 12/Aug/98 14:00 | 7.0   |          | 4                                     | 3,600                                     | 0.50     |          |
| 12/Aug/98 14:45 | 7.8   |          | 3.4                                   | 2,650                                     | 0.52     | 0        |
| 12/Aug/98 15:30 | 8.5   |          | 2.9                                   | 1,900                                     | 0.46     |          |
| 12/Aug/98 16:45 | 9.8   |          | 2.3                                   | 3,035                                     | 0.44     | 0        |
| 12/Aug/98 17:15 | 10.3  |          | 1.9                                   | 3,575                                     |          |          |
| 12/Aug/98 19:00 | 12.0  |          |                                       |                                           | 0.41     | 0        |
| 12/Aug/98 23:00 | 16.0  |          | 1.2                                   | 1595                                      | 0.40     | 0        |
| 13/Aug/98 07:30 | 24.5  |          | 0.7                                   | 1175                                      | 0.33     | 0        |
| 13/Aug/98 17:30 | 34.5  |          | 0.4                                   | 655                                       | 0.27     | 0        |
|                 |       |          |                                       | 525                                       |          | 0        |

# Appendix A4 for Chapter 4

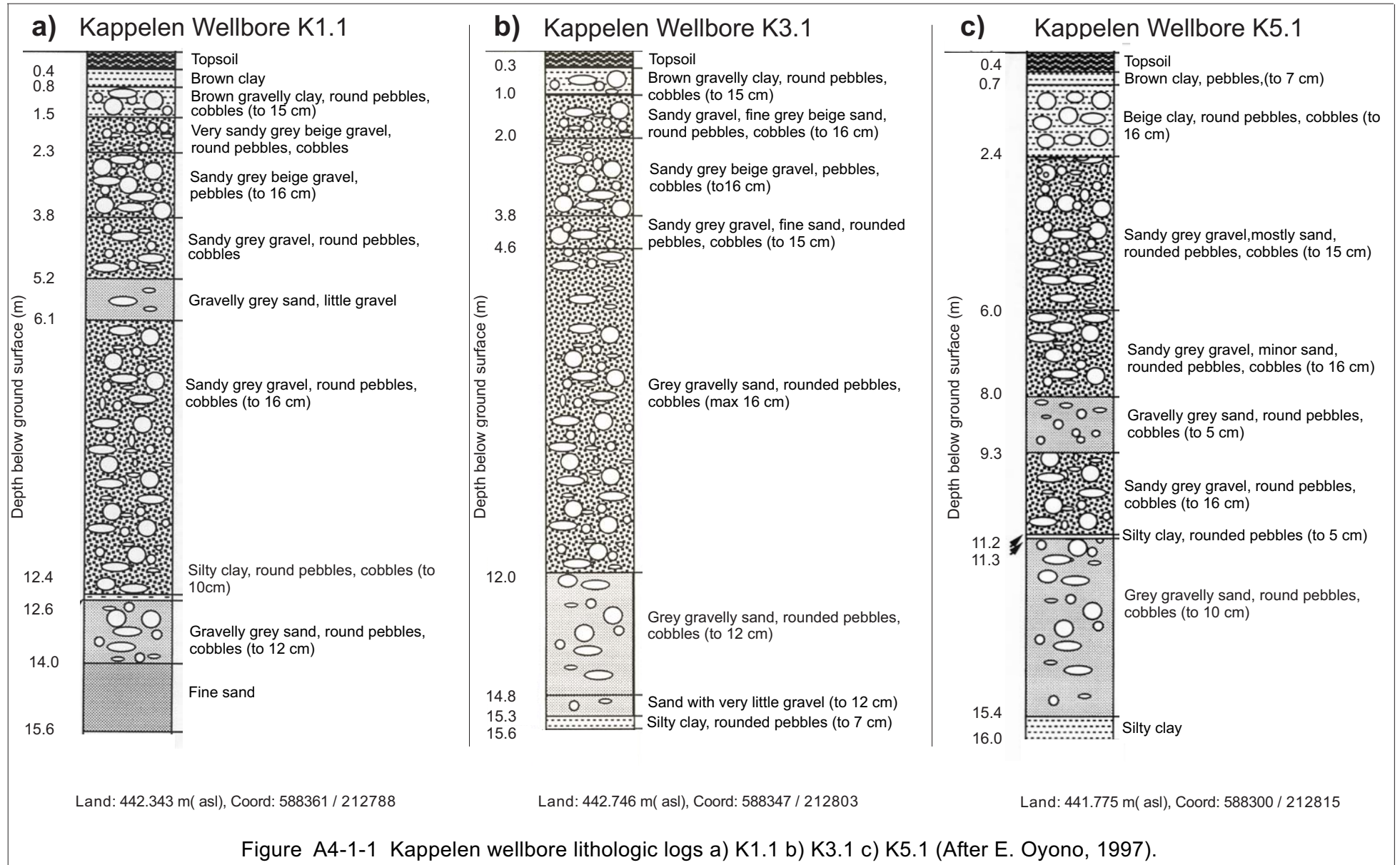
## Kappelen Field Site

Canton Bern, Switzerland

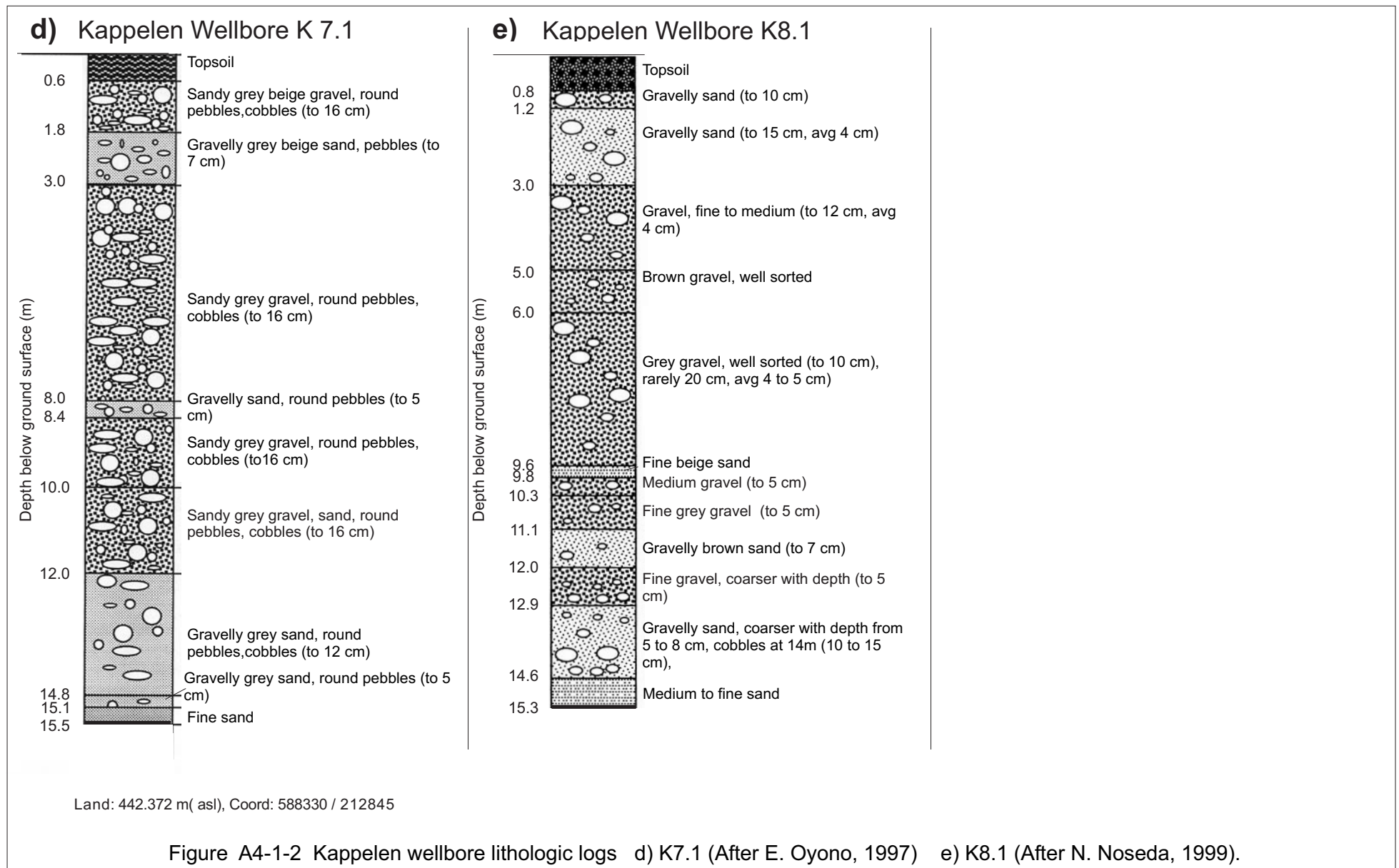
Appendix A4-1 Kappelen Lithologic Logs

Appendix A4-2 Kappelen Tracer Data (Tests 3,7)

## Appendix A4-1 Kappelen Lithologic Logs



## Appendix A4-1 Kappelen Lithologic Logs



APPENDIX A4-2-1 Kappelen Test 3 Data

| KAP3 Start:      |       | K2.1  |          | K3.1  |          | K4.1  |          | K5.1  | K6.1  |          | K7.1  |          |
|------------------|-------|-------|----------|-------|----------|-------|----------|-------|-------|----------|-------|----------|
| 01-May-97 23:00  | (h)   | (ppb) | (pfu/ml) | (ppb) | (pfu/ml) | (ppb) | (pfu/ml) | (ppb) | (ppb) | (pfu/ml) | (ppb) | (pfu/ml) |
| Sample Date Time | TSI   | UR    | H40      | UR    | H40      | UR    | H40      | UR    | UR    | H40      | UR    | H40      |
| 01-May-97 23:00  | 0.0   |       |          |       |          |       |          |       |       |          |       |          |
| 02-May-97 08:40  | 9.7   |       |          | 1.9   |          |       |          |       |       |          |       |          |
| 02-May-97 09:00  | 10.0  |       | 0        | 2.0   |          |       |          |       |       |          |       |          |
| 02-May-97 09:30  | 10.5  |       |          | 2.0   | 0.5      |       |          |       |       |          |       |          |
| 02-May-97 10:00  | 11.0  |       |          | 2.1   | 0.5      |       |          |       |       |          |       |          |
| 02-May-97 10:20  | 11.3  |       |          | 2.2   | 2.0      |       |          |       |       |          |       |          |
| 02-May-97 10:40  | 11.7  |       |          | 2.3   | 5.0      |       |          |       |       |          |       |          |
| 02-May-97 11:00  | 12.0  |       | 0        | 2.6   | 18.5     |       |          |       |       |          |       |          |
| 02-May-97 11:20  | 12.3  |       | 0        | 2.6   | 20.5     |       |          |       |       |          |       |          |
| 02-May-97 11:30  | 12.5  | 1.0   |          | 2.8   |          |       |          |       |       |          |       |          |
| 02-May-97 11:40  | 12.7  |       |          | 3.3   | 15.5     |       |          |       |       |          |       |          |
| 02-May-97 12:00  | 13.0  | 1.0   | 1        | 4.1   | 32.5     | 0.8   |          |       |       |          |       |          |
| 02-May-97 12:20  | 13.3  | 1.0   |          | 5.2   | 25.0     | 0.7   |          |       |       |          |       |          |
| 02-May-97 12:40  | 13.7  |       | 1        | 6.8   | 150      | 0.5   |          |       |       |          |       |          |
| 02-May-97 12:50  | 13.8  | 0.9   |          |       |          |       |          |       |       |          |       |          |
| 02-May-97 13:00  | 14.0  |       |          | 9.0   | 200      | 0.7   |          |       |       |          |       |          |
| 02-May-97 13:20  | 14.3  | 1.0   | 10       |       |          |       |          |       |       |          |       |          |
| 02-May-97 13:30  | 14.5  |       |          | 13.1  | 330      | 0.6   |          |       |       |          |       |          |
| 02-May-97 13:45  | 14.7  | 1.1   | 9        |       |          |       |          |       |       |          |       |          |
| 02-May-97 14:00  | 15.0  |       |          | 15.7  | 360      | 0.6   |          |       |       |          |       |          |
| 02-May-97 14:15  | 15.2  | 1.4   | 34       |       |          |       |          |       |       |          |       |          |
| 02-May-97 14:30  | 15.5  | 1.5   | 17       | 24.2  |          | 0.5   |          |       |       |          |       |          |
| 02-May-97 14:45  | 15.8  | 1.8   | 27       |       |          |       |          |       |       |          |       |          |
| 02-May-97 15:00  | 16.0  | 1.9   | 26       | 31.8  | 630      | 0.5   |          |       |       |          |       |          |
| 02-May-97 15:15  | 16.2  | 2.1   |          |       |          |       |          |       |       |          |       |          |
| 02-May-97 15:30  | 16.5  | 2.3   |          | 42.4  |          | 0.7   |          |       |       |          |       |          |
| 02-May-97 16:00  | 17.0  | 3.2   | 290      | 46.7  | 310      | 0.6   |          |       |       |          |       |          |
| 02-May-97 16:30  | 17.5  | 4.4   |          | 60.4  |          | 0.6   |          |       |       |          |       |          |
| 02-May-97 17:00  | 18.0  | 5.8   | 70       | 67.6  | 560      | 0.7   |          |       |       |          |       |          |
| 02-May-97 18:00  | 19.0  | 9.8   | 265      | 80.4  |          | 0.7   |          |       |       |          |       |          |
| 02-May-97 18:20  | 19.3  |       |          | 100.4 | 540      |       |          |       |       |          |       |          |
| 02-May-97 19:00  | 20.0  | 14.5  |          | 112.4 |          | 0.8   |          |       |       |          |       |          |
| 02-May-97 19:20  | 20.3  | 16.1  |          |       |          |       |          |       |       |          |       |          |
| 02-May-97 20:00  | 21.0  | 21.5  | 160      | 136.3 | 335      | 0.8   |          |       |       |          |       |          |
| 02-May-97 21:00  | 22.0  | 29.6  | 410      | 149.6 | 345      | 1.7   |          |       |       |          |       |          |
| 03-May-97 00:00  | 25.0  | 53.8  | 200      | 179.1 | 143      | 1.7   |          |       |       |          |       |          |
| 03-May-97 04:00  | 29.0  | 95.6  | 350      | 202.7 | 163      | 1.6   |          |       |       |          |       |          |
| 03-May-97 08:00  | 33.0  | 119.6 | 260      | 201.1 | 133      | 1.6   |          |       |       |          |       |          |
| 03-May-97 10:45  | 35.7  | 129.0 | 300      | 194.7 | 113      | 1.8   |          |       |       |          |       |          |
| 03-May-97 13:00  | 38.0  | 137.2 | 550      | 177.2 | 105      | 0.9   |          |       |       |          |       |          |
| 03-May-97 15:00  | 40.0  | 146.9 | 360      | 167.9 | 39.5     | 0.7   |          |       |       |          |       |          |
| 03-May-97 16:00  | 41.0  | 151.9 |          | 159.2 |          | 0.7   |          |       |       |          |       |          |
| 03-May-97 17:00  | 42.0  | 153.8 | 305      | 154.8 | 34.0     | 0.7   |          |       |       |          |       |          |
| 03-May-97 18:00  | 43.0  | 151.1 |          | 142.7 |          | 0.6   |          |       |       |          |       |          |
| 03-May-97 19:00  | 44.0  | 146.3 | 135      | 135.2 | 16.5     |       |          |       | 1.2   | 5        |       |          |
| 03-May-97 20:00  | 45.0  | 144.9 |          | 127.4 |          |       |          |       | 1.2   |          |       |          |
| 03-May-97 21:00  | 46.0  | 146.1 | 122      | 124.9 | 2.0      |       |          |       | 1.2   |          |       |          |
| 03-May-97 23:00  | 48.0  | 143.4 | 150      | 105.7 | 11.5     |       |          |       | 1.7   | 7        |       |          |
| 04-May-97 03:00  | 52.0  | 146.3 | 58       | 93.7  | 11.5     |       |          |       | 2.0   | 6        |       |          |
| 04-May-97 08:30  | 57.5  | 144.3 | 41       | 76.5  | 20.0     |       |          |       | 2.9   | 7        |       |          |
| 04-May-97 13:30  | 62.5  | 130.9 | 40       | 59.8  | 7.0      |       |          |       | 4.0   | 19       |       |          |
| 04-May-97 22:15  | 71.3  | 121.1 | 34       | 46.9  | 6.0      |       |          |       | 6.3   | 12       |       |          |
| 05-May-97 07:30  | 80.5  | 109.8 | 48       | 34.9  | 10.5     |       |          |       | 9.0   | 10.5     |       |          |
| 05-May-97 10:30  | 83.5  |       |          |       |          |       |          |       |       |          | 1.2   |          |
| 05-May-97 19:30  | 92.5  | 88.5  | 6        | 23.3  | 3.0      |       |          | 3.4   | 12.1  | 9        | 2.3   |          |
| 06-May-97 15:00  | 112.0 | 67.9  | 24       | 12.7  | 6.5      |       | 1        | 1.9   | 15.7  | 1.5      | 1.4   | positive |
| 07-May-97 08:00  | 129.0 | 53.9  | 21       | 8.6   |          |       | 5        | 1.8   | 15.8  | 1        | 1.4   |          |
| 08-May-97 10:30  | 155.5 | 29.0  | 7        |       | 2.5      |       |          |       |       |          |       |          |
| 08-May-97 13:30  | 158.5 |       |          |       |          |       |          | 2.4   | 15.5  |          | 2.0   |          |
| 08-May-97 17:00  | 162.0 |       |          | 17.4  | 17.5     |       |          |       |       |          |       |          |
| 10-May-97 10:30  | 203.5 |       |          | 13.2  | 22.5     |       |          | 2.1   | 12.2  |          | 3.1   |          |

Appendix A4-2-2 Kappelen Test 7 Sample Data

| KAP7 Start:     |      | K1.2  |          | K2.2  | K3.2  |          |         | K4.2  |          | K7.2  |
|-----------------|------|-------|----------|-------|-------|----------|---------|-------|----------|-------|
| 12-Jul-98 12:15 | (h)  | (ppb) | (pfu/ml) | (ppb) | (ppb) | (pfu/ml) |         | (ppb) | (pfu/ml) | (ppb) |
| Sample DateTime | TSI  | UR    | H40      | H40   | UR    | H40      | H4      | UR    | H40      | UR    |
| 12-Jul-98 12:15 | 0.0  |       |          | 0     |       |          |         |       | 0        |       |
| 12-Jul-98 12:25 | 0.2  |       | 6.0E+09  |       |       |          |         |       |          |       |
| 12-Jul-98 12:35 | 0.3  |       | 9.0E+09  |       |       |          |         |       |          |       |
| 12-Jul-98 12:55 | 0.7  |       | 1.5E+10  |       |       |          |         |       |          |       |
| 12-Jul-98 13:15 | 1.0  |       | 1.5E+10  |       |       |          |         |       |          |       |
| 12-Jul-98 13:35 | 1.3  |       | 1.6E+10  |       |       |          |         |       |          |       |
| 12-Jul-98 13:45 | 1.5  |       | 1.5E+10  |       |       |          |         |       |          |       |
| 12-Jul-98 13:50 | 1.6  |       | 1.4E+10  |       |       |          |         |       |          |       |
| 12-Jul-98 14:00 | 1.8  |       | 1.0E+10  |       |       |          |         |       |          |       |
| 12-Jul-98 14:15 | 2.0  |       | 6.1E+09  |       |       |          |         |       |          |       |
| 12-Jul-98 14:30 | 2.3  |       | 4.3E+09  |       |       |          |         |       |          |       |
| 12-Jul-98 15:00 | 2.8  |       | 2.5E+09  |       |       |          |         |       |          |       |
| 12-Jul-98 16:00 | 3.8  |       | 8.3E+08  |       |       |          |         |       | 0        |       |
| 12-Jul-98 17:00 | 4.8  |       | 3.4E+08  |       |       |          |         |       |          |       |
| 12-Jul-98 18:00 | 5.8  |       | 8.9E+07  |       |       |          |         |       |          |       |
| 12-Jul-98 19:00 | 6.8  |       | 7.3E+07  |       |       |          |         |       |          |       |
| 12-Jul-98 20:00 | 7.8  | 840   | 3.1E+07  |       |       |          |         | 0.3   |          |       |
| 12-Jul-98 21:00 | 8.8  |       | 9.7E+06  |       |       |          |         | 0.2   | 0        |       |
| 12-Jul-98 22:00 | 9.8  | 296   | 3.1E+06  |       |       |          |         | 0.2   | 0        |       |
| 12-Jul-98 23:00 | 10.8 |       |          |       |       |          |         | 0.2   | 0.5      |       |
| 13-Jul-98 00:00 | 11.8 | 107   | 5.9E+05  | 0     | 0.5   | 2        |         | 0.2   | 12       |       |
| 13-Jul-98 01:00 | 12.8 |       |          | 0     | 0.6   | 13       |         | 0.2   | 71       |       |
| 13-Jul-98 02:00 | 13.8 | 40    | 1.2E+05  |       | 0.6   | 47       |         | 0.3   | 208      |       |
| 13-Jul-98 03:00 | 14.8 |       |          | 0     | 0.7   | 103      |         | 0.3   | 510      |       |
| 13-Jul-98 04:00 | 15.8 | 19    | 5.6E+04  |       | 0.8   | 290      |         | 0.8   | 660      |       |
| 13-Jul-98 05:00 | 16.8 |       |          | 0     | 1.7   | 750      |         | 0.5   | 670      |       |
| 13-Jul-98 06:00 | 17.8 | 11    | 2.1E+04  | 0.500 | 3.4   | 800      |         | 0.8   | 560      |       |
| 13-Jul-98 07:00 | 18.8 |       |          | 0     | 6.7   | 1,100    |         | 1.1   | 390      |       |
| 13-Jul-98 08:00 | 19.8 |       |          | 0     | 11.6  | 660      |         | 1.3   | 275      |       |
| 13-Jul-98 09:00 | 20.8 | 7     | 4.1E+03  | 0     | 19    | 745      |         | 1.6   | 204      |       |
| 13-Jul-98 10:00 | 21.8 |       |          | 0     | 29    | 1,200    |         | 1.8   | 119      |       |
| 13-Jul-98 11:00 | 22.8 |       |          | 0     | 46    | 1,540    | 0       | 2.0   | 82       |       |
| 13-Jul-98 12:00 | 23.8 | 4     | 5.1E+03  | 0     | 64    | 995      | 0       | 2.3   | 60       |       |
| 13-Jul-98 12:35 | 24.3 |       |          |       |       |          | 7.5E+08 |       |          |       |
| 13-Jul-98 12:55 | 24.7 |       |          |       |       |          | 6.8E+08 |       |          |       |
| 13-Jul-98 13:00 | 24.8 |       |          | 0.5   | 92    | 4,932    |         | 2.4   | 45       |       |
| 13-Jul-98 13:15 | 25.0 |       |          |       |       |          | 6.7E+08 |       |          |       |
| 13-Jul-98 13:35 | 25.3 |       |          |       |       |          | 6.3E+08 |       |          |       |
| 13-Jul-98 13:45 | 25.5 |       |          |       |       |          | 6.7E+08 |       |          |       |
| 13-Jul-98 13:50 | 25.6 |       |          |       |       |          | 3.6E+08 |       |          |       |
| 13-Jul-98 14:00 | 25.8 |       |          | 0.5   | 120   | 11,082   | 2.0E+08 | 2.7   | 23       |       |
| 13-Jul-98 14:30 | 26.3 |       |          |       |       |          | 9.0E+07 |       |          |       |
| 13-Jul-98 15:00 | 26.8 |       |          | 0.5   | 150   | 18,830   | 6.8E+07 | 2.9   | 23       |       |
| 13-Jul-98 16:00 | 27.8 |       |          | 0.5   | 180   | 16,388   | 6.5E+07 | 3.2   | 13       |       |
| 13-Jul-98 17:00 | 28.8 | 2.43  | 5.4E+03  | 2.5   | 210   | 11,746   | 3.3E+07 | 3.4   | 15       |       |
| 13-Jul-98 18:00 | 29.8 |       |          | 4.0   | 240   | 11,969   | 7.7E+06 | 3.8   | 15       |       |
| 13-Jul-98 19:30 | 31.3 |       |          | 2.0   | 275   | 6,642    | 3.5E+06 | 4.1   | 6        |       |
| 13-Jul-98 21:00 | 32.8 |       |          | 1.5   | 310   | 4,249    | 6.5E+05 | 4.4   | 9        |       |
| 13-Jul-98 22:30 | 34.3 |       |          | 0.5   | 338   | 2,637    | 7.1E+04 | 4.7   | 9        | 0.3   |
| 14-Jul-98 00:00 | 35.8 |       |          | 1.5   | 360   | 1,691    | 3000    | 5.1   | 8        | 0.2   |
| 14-Jul-98 02:00 | 37.8 |       |          | 0.5   | 383   | 971      | 420     | 5.3   | 7        | 0.3   |
| 14-Jul-98 04:00 | 39.8 |       |          | 1.0   | 407   | 820      | 27      | 5.6   | 7        | 0.3   |
| 14-Jul-98 06:00 | 41.8 |       |          | 1.0   | 418   | 480      | 14      | 5.9   | 6        | 0.3   |
| 14-Jul-98 08:00 | 43.8 |       |          | 1.0   | 427   | 375      | 3       | 6.0   | 6        | 0.2   |
| 14-Jul-98 09:00 | 44.8 | 1.42  | 1170     |       |       |          |         |       |          |       |
| 14-Jul-98 10:00 | 45.8 |       |          |       |       |          |         |       |          | 0.3   |
| 14-Jul-98 11:00 | 46.8 |       |          | 0.5   | 457   | 190      | 6       | 6.3   | 5        |       |
| 14-Jul-98 12:00 | 47.8 |       |          |       | 461   | 195      | 6       |       |          | 0.3   |

Appendix A4-2-2 Kappelen Test 7 Sample Data

| KAP7 Start:     |       | K1.2  |          | K2.2  | K3.2  |          |     | K4.2  |          | K7.2  |
|-----------------|-------|-------|----------|-------|-------|----------|-----|-------|----------|-------|
| 12-Jul-98 12:15 | (h)   | (ppb) | (pfu/ml) | (ppb) | (ppb) | (pfu/ml) |     | (ppb) | (pfu/ml) | (ppb) |
| Sample DateTime | TSI   | UR    | H40      | H40   | UR    | H40      | H4  | UR    | H40      | UR    |
| 14-Jul-98 14:00 | 49.8  |       |          | 0.5   | 472   | 180      | 4   | 6.3   | 5        | 0.3   |
| 14-Jul-98 17:00 | 52.8  |       |          |       | 492   | 107      | 1.0 |       |          | 0.2   |
| 14-Jul-98 18:00 | 53.8  |       |          | 0     |       | 159      | 1.0 |       | 5        |       |
| 14-Jul-98 20:00 | 55.8  |       |          |       | 522   | 109      | 2.5 | 6.7   |          | 0.3   |
| 14-Jul-98 23:00 | 58.8  |       |          | 0     | 540   | 106      | 1.0 | 6.7   | 2        | 0.5   |
| 15-Jul-98 02:00 | 61.8  |       |          |       |       |          |     |       |          | 0.2   |
| 15-Jul-98 05:00 | 64.8  |       |          | 0     | 560   | 118      | 1.5 | 7.0   | 4        | 0.3   |
| 15-Jul-98 09:00 | 68.8  |       |          |       | 570   | 52       | 0.5 |       |          | 0.3   |
| 15-Jul-98 13:00 | 72.8  |       |          | 0     | 580   | 55       | 0   | 7.8   | -        | 0.2   |
| 15-Jul-98 15:00 | 74.8  |       |          |       | 583   | 89       | 0   | 8.2   | 1        |       |
| 15-Jul-98 17:00 | 76.8  |       |          |       |       |          |     | 8.2   | 1        | 0.4   |
| 15-Jul-98 17:30 | 77.3  |       |          |       | 586   | 27       | 0   |       |          |       |
| 15-Jul-98 18:00 | 77.8  | 0.74  | 2350     |       |       |          |     |       |          | 0.3   |
| 15-Jul-98 19:00 | 78.8  |       |          |       | 586   | 31       | 0   |       |          |       |
| 15-Jul-98 21:00 | 80.8  |       |          |       | 591   | 63       | 0.5 | 8.4   | 3        | 0.3   |
| 15-Jul-98 23:00 | 82.8  |       |          |       | 598   | 46       | 0.5 | 8.4   |          |       |
| 16-Jul-98 01:00 | 84.8  |       |          |       | 564   | 66       | 3   | 8.5   | 3        |       |
| 16-Jul-98 03:00 | 86.8  |       |          |       |       |          |     |       |          | 0.3   |
| 16-Jul-98 05:30 | 89.3  |       |          |       | 553   | 67       | 6   | 8.6   | 1        | 0.3   |
| 16-Jul-98 07:00 | 90.8  |       |          |       | 534   | 58       | 4   |       |          |       |
| 16-Jul-98 08:00 | 91.8  |       | 1205     | 0     |       |          |     |       |          |       |
| 16-Jul-98 09:00 | 92.8  |       |          |       | 541   | 63       | 5   | 8.5   | 1        | 0.2   |
| 16-Jul-98 11:00 | 94.8  |       |          |       | 537   | 28       | 0   | 8.5   |          | 0.3   |
| 16-Jul-98 13:00 | 96.8  |       |          |       | 552   | 24       | 0.5 | 8.2   | 1        | 0.4   |
| 16-Jul-98 15:00 | 98.8  |       |          |       | 553   | 32       | 0.5 | 8.1   |          | 0.3   |
| 16-Jul-98 17:00 | 100.8 |       |          |       | 554   | 38       | 0.5 | 8.0   | 2        | 0.3   |
| 16-Jul-98 18:00 | 101.8 | 0.57  | 1170     |       |       |          |     |       |          |       |
| 16-Jul-98 18:30 | 102.3 |       |          | 0     |       |          |     |       |          |       |
| 16-Jul-98 19:00 | 102.8 |       |          |       | 553   | 21       | 2.5 |       |          |       |
| 16-Jul-98 21:00 | 104.8 |       |          |       | 534   | 36       | 1   |       |          | 0.3   |
| 17-Jul-98 01:30 | 109.3 |       |          |       | 542   | 47       | 16  |       |          |       |
| 17-Jul-98 06:00 | 113.8 |       |          |       | 551   | 44       | 7   |       |          | 0.2   |
| 17-Jul-98 10:00 | 117.8 |       |          |       |       | 74       | 68  |       | 2        |       |
| 17-Jul-98 14:00 | 121.8 |       |          |       | 497   | 9        | 0   | 6.2   |          | 0.3   |
| 17-Jul-98 15:30 | 123.3 | 0.55  | 350      | 0     |       |          |     |       |          |       |
| 17-Jul-98 16:00 | 123.8 |       |          |       | 492   | 6        | 2   | 6.2   | 2        | 0.2   |
| 17-Jul-98 18:00 | 125.8 |       |          |       | 481   | 13       | 6   |       |          | 0.2   |
| 17-Jul-98 22:00 | 129.8 |       |          |       | 465   | 10       | 0   | 6.0   | 1        | 0.3   |
| 18-Jul-98 02:00 | 133.8 |       |          |       | 449   | 12       | 0.5 |       |          | 0.3   |
| 18-Jul-98 08:30 | 140.3 |       |          |       | 419   | 12       | 0   | 6.1   | 2        | 0.2   |
| 18-Jul-98 12:00 | 143.8 |       | 760      |       | 407   | 14       | 0   | 6.2   |          | 0.3   |
| 18-Jul-98 18:00 | 149.8 |       |          |       | 387   | 14       | 0   | 6.5   | 2        | 0.3   |
| 19-Jul-98 00:00 | 155.8 |       |          |       | 364   | 26       | 1   |       |          | 0.3   |
| 19-Jul-98 09:00 | 164.8 |       |          |       | 333   | 11       | 1   | 6.7   | 2        | 0.4   |
| 19-Jul-98 15:00 | 170.8 |       |          |       | 256   | 70       | 90  |       |          | 0.4   |
| 20-Jul-98 00:00 | 179.8 |       |          |       | 281   | 25       |     |       |          | 0.5   |
| 20-Jul-98 07:00 | 186.8 |       |          |       | 187   | 19       | 24  | 5.8   | 1        | 0.5   |
| 20-Jul-98 14:00 | 193.8 |       |          |       | 241   | 6        | 13  | 5.5   | 0        | 0.6   |
| 21-Jul-98 00:00 | 203.8 |       |          |       | 244   | 16       | 19  |       |          | 0.7   |
| 21-Jul-98 09:00 | 212.8 |       |          |       | 228   | 50       | 37  | 6.1   | 0        | 0.7   |
| 21-Jul-98 14:00 | 217.8 | 0.49  | 2350     |       | 225   | 8        | 1   | 6.4   |          | 0.7   |
| 21-Jul-98 22:00 | 225.8 |       |          |       | 209   | 92       | 68  |       |          | 0.8   |
| 22-Jul-98 12:00 | 239.8 |       |          |       | 195   | 9        | 0   | 5.8   |          | 0.9   |
| 22-Jul-98 22:30 | 250.3 |       |          |       | 184   | 24       | 2   | 5.4   |          | 0.9   |
| 23-Jul-98 09:30 | 261.3 |       |          |       |       |          |     | 4.7   |          | 1.0   |
| 23-Jul-98 14:00 | 265.8 |       |          |       |       |          |     |       |          |       |
| 23-Jul-98 22:00 | 273.8 |       |          |       |       |          |     |       |          |       |
| 24-Jul-98 09:00 | 284.8 |       |          |       |       |          |     | 4.1   |          | 1.2   |

# Appendix A5 for Chapter 5

## Wilerwald Field Site

Canton Bern, Switzerland

Appendix A5-1 Test 4 Sample Uranine and Phage Data

Appendix A5-2 Test 4 Continuous Uranine Data

Appendix A5-3 Test 4 Fluorescent Microsphere Data  
(provided by S. Niehren, ETH-Zurich, 1998)

# APPENDIX A5-1 WW4 Sample Uranine and Phage Data

| WW4 Start:      |       | WELL C6 |          |       |       | WELL CD |          |      |      | WELL D7 |          |      |     |
|-----------------|-------|---------|----------|-------|-------|---------|----------|------|------|---------|----------|------|-----|
| 26-Aug-97 15:05 | (h)   | (ppb)   | (pfu/ml) |       |       | (ppb)   | (pfu/ml) |      |      | (ppb)   | (pfu/ml) |      |     |
| Sample DateTime | TSI   | UR      | H40      | Psf2  | MS2   | UR      | H40      | Psf2 | MS2  | UR      | H40      | Psf2 | MS2 |
| 26-Aug-97 15:10 | 0.1   |         | 5        |       |       |         |          |      |      |         |          |      |     |
| 26-Aug-97 15:20 | 0.3   |         | 10       |       |       |         |          |      |      |         |          |      |     |
| 26-Aug-97 15:30 | 0.4   |         | 5        |       |       |         |          |      |      |         |          |      |     |
| 26-Aug-97 15:40 | 0.6   |         | 3        |       |       |         |          |      |      |         |          |      |     |
| 26-Aug-97 15:50 | 0.8   |         | 5        |       |       |         |          |      |      |         |          |      |     |
| 26-Aug-97 16:00 | 0.9   |         | 385      | 0     | 0     |         |          |      |      |         | 1        |      |     |
| 26-Aug-97 16:10 | 1.1   |         | 40.5     | 0     | 0     |         |          |      |      |         |          |      |     |
| 26-Aug-97 16:20 | 1.3   | 2       | 55       | 6     | 13    |         |          |      |      |         |          |      |     |
| 26-Aug-97 16:30 | 1.4   | 9       | 735      | 150   | 540   |         |          |      |      |         |          |      |     |
| 26-Aug-97 16:40 | 1.6   | 48      | 8000     | 2150  | 5400  |         |          |      |      |         |          |      |     |
| 26-Aug-97 16:50 | 1.8   | 231     | 14000    | 8000  | 8000  |         |          |      |      |         |          |      |     |
| 26-Aug-97 17:00 | 1.9   | 511     | 39500    | 8000  | 21000 |         |          |      |      |         | 1        |      |     |
| 26-Aug-97 17:15 | 2.2   | 830     | 57500    | 12000 | 32000 |         |          |      |      |         |          |      |     |
| 26-Aug-97 17:35 | 2.5   | 912     | 50000    | 11000 | 23000 |         |          |      |      |         |          |      |     |
| 26-Aug-97 17:45 | 2.7   | 867     | 35000    | 7000  | 20500 |         |          |      |      |         |          |      |     |
| 26-Aug-97 18:00 | 2.9   | 763     | 19500    | 3500  | 8500  | 0.6     |          |      |      | 0.6     | 1        |      |     |
| 26-Aug-97 18:15 | 3.2   | 618     | 20000    | 2700  | 7250  |         |          |      |      |         |          |      |     |
| 26-Aug-97 18:30 | 3.4   | 530     | 20000    | 2150  | 6450  | 0.5     |          |      |      | 0.4     |          |      |     |
| 26-Aug-97 18:45 | 3.7   | 466     | 11500    | 1210  | 3050  |         |          |      | 0    |         |          |      |     |
| 26-Aug-97 19:00 | 3.9   | 381     | 9500     | 945   | 2060  | 0.4     |          |      | 0    | 0.5     | 1        |      |     |
| 26-Aug-97 19:30 | 4.4   | 279     | 4000     | 505   | 1020  | 0.4     | 0        | 0    | 0.5  | 0.5     |          |      |     |
| 26-Aug-97 20:00 | 4.9   | 211     | 1650     | 245   | 635   | 0.9     | 12       | 3    | 9    | 0.9     | 0.5      |      |     |
| 26-Aug-97 20:30 | 5.4   | 167     | 1280     | 185   | 380   | 0.9     | 50       | 7    | 19   | 0.4     |          |      |     |
| 26-Aug-97 21:00 | 5.9   | 136     | 800      | 95    | 250   | 3       | 350      | 45   | 74   | 0.4     | 0.5      | 0    | 0   |
| 26-Aug-97 21:30 | 6.4   | 109     | 515      | 150   | 225   | 6       | 875      | 62   | 145  | 0.5     | 0.5      | 0    | 0   |
| 26-Aug-97 22:00 | 6.9   | 91      | 440      | 95    | 160   | 14      | 1105     | 73   | 220  | 0.5     | 6        | 0.5  | 2   |
| 26-Aug-97 22:30 | 7.4   | 75      | 300      | 46    | 86    | 26      | 2120     | 150  | 365  | 0.5     | 13       | 3    | 3   |
| 26-Aug-97 23:00 | 7.9   | 64      | 290      | 58    | 86    | 41      | 2925     | 165  | 580  |         | 230      | 25   | 24  |
| 26-Aug-97 23:30 | 8.4   | 55      | 320      | 55    | 94    | 57      | 4500     | 190  | 470  | 3       | 495      | 28   | 43  |
| 27-Aug-97 00:00 | 8.9   | 49      | 300      | 45    | 66    | 76      | 4200     | 175  | 445  | 8       | 880      | 65   | 94  |
| 27-Aug-97 00:30 | 9.4   | 44      | 175      | 46    | 53    | 91      | 4800     | 145  | 390  | 18      | 1845     | 90   | 245 |
| 27-Aug-97 01:00 | 9.9   | 38      | 93       | 33    | 50    | 109     | 4750     | 180  | 370  | 36      | 4250     | 195  | 310 |
| 27-Aug-97 02:00 | 10.9  | 30      | 79       | 28    | 48    | 137     | 4300     | 250  | 265  | 96      | 4400     | 255  | 410 |
| 27-Aug-97 03:00 | 11.9  | 27      | 68       | 35    | 41    | 177     | 4450     | 175  | 210  | 173     | 7500     | 125  | 310 |
| 27-Aug-97 04:00 | 12.9  | 21      | 61       | 26    | 36    | 191     | 3650     | 170  | 185  | 252     | 6000     | 135  | 245 |
| 27-Aug-97 05:00 | 13.9  | 20      | 55       | 22    | 34    | 215     | 3500     | 120  | 101  | 306     | 4200     | 125  | 73  |
| 27-Aug-97 06:00 | 14.9  | 16      | 47       | 16    | 28    | 244     | 2700     | 95   | 94   | 384     | 2400     | 65   | 43  |
| 27-Aug-97 08:00 | 16.9  | 13      | 40       | 11    | 22    | 294     | 2100     | 45   | 80   | 413     | 1377     | 37   | 37  |
| 27-Aug-97 10:00 | 18.9  | 11      | 35       | 9     | 27    | 266     | 1445     | 34   | 57   | 426     | 610      | 18   | 17  |
| 27-Aug-97 12:00 | 20.9  | 9       | 24       | 7     | 21    | 296     | 1100     | 19   | 29   | 389     | 375      | 5    | 13  |
| 27-Aug-97 14:00 | 22.9  |         |          |       |       | 289     | 715      | 16   | 33   | 363     | 233      | 6    | 7   |
| 27-Aug-97 16:00 | 24.9  | 6       | 21       | 8     | 15    | 287     | 598      | 11   | 22   | 336     | 126      | 6    | 4   |
| 27-Aug-97 18:00 | 26.9  | 5       |          |       |       | 287     | 335      | 8    | 16   | 309     | 91       | 5    | 8   |
| 27-Aug-97 20:00 | 28.9  | 4       | 21       | 7     | 14    | 278     | 310      | 5    | 16   | 282     | 58       | 3    | 3   |
| 27-Aug-97 21:00 | 29.9  | 4       |          |       |       | 265     | 240      | 7    | 14   | 272     | 68       | 2    | 5   |
| 27-Aug-97 22:00 | 30.9  | 4       |          |       |       | 263     | 200      | 4    | 11   | 254     | 56       | 2    | 4   |
| 28-Aug-97 00:00 | 32.9  | 4       | 24       | 3     | 10    | 263     | 140      | 3    | 9    | 234     | 68       | 1    | 4   |
| 28-Aug-97 04:00 | 36.9  |         |          |       |       | 241     | 123      | 2    | 3    | 201     | 58       | 2    | 6   |
| 28-Aug-97 08:00 | 40.9  | 3       | 16       | 1.5   | 6     | 219     | 87       | 1    | 5    | 165     | 45       | 3    | 3   |
| 28-Aug-97 12:00 | 44.9  | 2       |          |       |       | 203     | 78       | 3    | 6    | 141     | 49       | 4    | 3   |
| 28-Aug-97 16:00 | 48.9  | 2       |          |       |       | 183     | 73       | 1.5  | 3.5  | 129     | 65       | 1    | 1   |
| 28-Aug-97 20:00 | 52.9  |         |          |       |       | 174     | 60       | 0    | 3.5  | 114     | 31       | 2    | 2   |
| 29-Aug-97 00:00 | 56.9  | 2       | 24       | 1     | 4.5   | 162     | 51       | 0.5  | 3    | 99      | 44       | 2    | 2   |
| 29-Aug-97 06:00 | 62.9  |         |          |       |       | 136     | 45       | 0    | 3    | 85      | 36       | 0.5  | 1   |
| 29-Aug-97 12:00 | 68.9  |         | 23       | 1     | 3.5   | 126     | 8        | 2    | 5    | 73      | 26       | 1    | 1   |
| 29-Aug-97 20:00 | 76.9  |         |          |       |       | 119     | 29       | 0    | 0.5  | 69      | 17       | 1.5  | 1.5 |
| 30-Aug-97 00:00 | 80.9  |         | 9        | 1.5   | 4     | 105     | 23       | 0    | 4    | 57      | 17       | 0.5  | 0.5 |
| 30-Aug-97 08:00 | 88.9  |         |          |       |       | 94      | 20       | 0    | 1.5  | 48      | 11       | 0    | 2   |
| 30-Aug-97 16:00 | 96.9  |         | 5        | 1     | 3     | 86      | 19       | 0.3  | 0.5  | 45      | 15       | 0    | 0.5 |
| 31-Aug-97 00:00 | 104.9 |         | 6        | 0     | 3     | 79      | 18       | 1    | 0    | 41      | 14       | 1    | 0.5 |
| 31-Aug-97 10:00 | 114.9 |         | 5        | 2     | 1.5   | 65      | 17       | 0.5  | 1.5  | 32      | 11       | 0.5  | 0   |
| 31-Aug-97 20:00 | 124.9 |         | 8        | 0     | 3     |         | 15       | 0.5  | 2.25 |         | 11       | 0.3  | 1   |
| 01-Sep-97 06:00 | 134.9 |         | 7        | 1     | 2.5   | 52      | 19       | 0.5  | 1    | 23      | 11       | 0.5  | 0.5 |
| 01-Sep-97 19:00 | 147.9 |         | 8        | 0.5   | 2     |         | 12       | 0    | 0.5  |         | 10       | 0    | 2   |
| 02-Sep-97 10:00 | 162.9 |         | 5        | 0     | 3     |         | 14       | 0    | 1    |         | 5        | 0    | 2   |
| 03-Sep-97 13:30 | 190.4 | 0.7     | 12       | 1.5   | 3.5   | 27      | 8        | 0.5  | 1    | 11      | 6        | 0    | 0.5 |
| 08-Sep-97 12:30 | 309.4 | 0.6     |          |       |       | 12      |          |      |      | 4       |          |      |     |

Appendix 5-2-1 Well C6 Continuous Uranine Data

| (h)  | (ppb) | (h) | (ppb) | (h) | (ppb) | (h)  | (ppb) | (h)  | (ppb) | (h)  | (ppb) | (h)  | (ppb) | (h)        | (ppb) |
|------|-------|-----|-------|-----|-------|------|-------|------|-------|------|-------|------|-------|------------|-------|
| TSI  | UR    | TSI | UR    | TSI | UR    | TSI  | UR    | TSI  | UR    | TSI  | UR    | TSI  | UR    | TSI        | UR    |
| -3.5 | 0.2   | 0.7 | 0.2   | 4.7 | 241.7 | 8.7  | 50.2  | 12.7 | 21.5  | 16.7 | 12.4  | 20.7 | 8.2   | 24.7       | 5.9   |
| -3.4 | 0.2   | 0.7 | 0.2   | 4.7 | 233.4 | 8.7  | 49.3  | 12.7 | 21.2  | 16.7 | 12.2  | 20.7 | 8.2   | 24.7       | 5.9   |
| -3.3 | 0.3   | 0.8 | 0.3   | 4.8 | 224.4 | 8.8  | 48.4  | 12.8 | 21.0  | 16.8 | 12.2  | 20.8 | 8.0   | 24.8       | 5.9   |
| -3.3 | 0.3   | 0.9 | 0.7   | 4.9 | 216.3 | 8.9  | 2.5   | 12.9 | 20.7  | 16.9 | 12.0  | 20.9 | 8.0   | 24.9       | 5.8   |
| -3.2 | 0.3   | 0.9 | 1.1   | 4.9 | 208.6 | 8.9  | 24.1  | 12.9 | 20.5  | 16.9 | 12.0  | 20.9 | 7.9   | 24.9       | 5.8   |
| -3.1 | 0.3   | 1.0 | 0.5   | 5.0 | 201.0 | 9.0  | 45.6  | 13.0 | 20.3  | 17.0 | 11.9  | 21.0 | 8.0   | 25.0       | 5.8   |
| -3.1 | 0.2   | 1.1 | 0.5   | 5.1 | 194.5 | 9.1  | 44.7  | 13.1 | 20.1  | 17.1 | 11.7  | 21.1 | 8.0   | 25.1       | 5.8   |
| -3.0 | 0.2   | 1.1 | 0.5   | 5.1 | 187.5 | 9.1  | 43.8  | 13.1 | 19.9  | 17.1 | 11.7  | 21.1 | 7.8   | 25.1       | 5.7   |
| -2.9 | 0.2   | 1.2 | 0.5   | 5.2 | 181.0 | 9.2  | 43.1  | 13.2 | 19.6  | 17.2 | 2.6   | 21.2 | 7.7   | 25.2       | 5.7   |
| -2.9 | 0.2   | 1.3 | 0.7   | 5.3 | 175.0 | 9.3  | 42.3  | 13.3 | 19.4  | 17.3 | 7.0   | 21.3 | 7.7   | 25.3       | 5.6   |
| -2.8 | 0.3   | 1.3 | 0.9   | 5.3 | 169.1 | 9.3  | 41.7  | 13.3 | 19.2  | 17.3 | 11.5  | 21.3 | 7.7   | 25.3       | 5.6   |
| -2.7 | 0.3   | 1.4 | 2.2   | 5.4 | 163.5 | 9.4  | 41.0  | 13.4 | 19.1  | 17.4 | 11.4  | 21.4 | 7.6   | 25.4       | 5.6   |
| -2.7 | 0.3   | 1.5 | 6.7   | 5.5 | 158.2 | 9.5  | 40.3  | 13.5 | 18.9  | 17.5 | 11.3  | 21.5 | 7.6   | 25.5       | 5.6   |
| -2.6 | 0.2   | 1.5 | 19.4  | 5.5 | 153.3 | 9.5  | 39.7  | 13.5 | 18.6  | 17.5 | 11.2  | 21.5 | 7.5   | 25.5       | 5.5   |
| -2.5 | 0.1   | 1.6 | 47.7  | 5.6 | 148.6 | 9.6  | 39.1  | 13.6 | 18.5  | 17.6 | 11.1  | 21.6 | 7.5   | 25.6       | 5.5   |
| -2.5 | 0.2   | 1.7 | 97.1  | 5.7 | 143.6 | 9.7  | 38.4  | 13.7 | 18.3  | 17.7 | 11.1  | 21.7 | 7.5   | 25.7       | 5.5   |
| -2.4 | 0.3   | 1.7 | 174.2 | 5.7 | 139.1 | 9.7  | 37.8  | 13.7 | 18.1  | 17.7 | 11.0  | 21.7 | 7.5   | 25.7       | 5.4   |
| -2.3 | 0.2   | 1.8 | 273.6 | 5.8 | 135.1 | 9.8  | 37.3  | 13.8 | 17.9  | 17.8 | 10.9  | 21.8 | 7.5   | 25.8       | 5.5   |
| -2.3 | 0.3   | 1.9 | 386.6 | 5.9 | 131.1 | 9.9  | 36.6  | 13.9 | 17.7  | 17.9 | 10.8  | 21.9 | 7.3   | 25.9       | 5.5   |
| -2.2 | 0.3   | 1.9 | 500.9 | 5.9 | 127.3 | 9.9  | 36.1  | 13.9 | 17.7  | 17.9 | 10.7  | 21.9 | 7.3   | 25.9       | 2.6   |
| -2.0 | 0.6   | 2.0 | 608.6 | 6.0 | 123.7 | 10.0 | 35.8  | 14.0 | 17.5  | 18.0 | 10.7  | 22.0 | 7.3   | 26.0       | 4.0   |
| -1.9 | 0.5   | 2.1 | 700.9 | 6.1 | 120.3 | 10.1 | 35.2  | 14.1 | 17.2  | 18.1 | 10.6  | 22.1 | 7.3   | 26.1       | 5.4   |
| -1.9 | 0.5   | 2.1 | 775.0 | 6.1 | 117.3 | 10.1 | 34.6  | 14.1 | 17.1  | 18.1 | 10.5  | 22.1 | 7.2   | 26.1       | 5.3   |
| -1.8 | 0.3   | 2.2 | 830.7 | 6.2 | 114.0 | 10.2 | 34.1  | 14.2 | 17.0  | 18.2 | 10.5  | 22.2 | 7.2   | End Record |       |
| -1.7 | 0.3   | 2.3 | 869.3 | 6.3 | 110.7 | 10.3 | 33.6  | 14.3 | 16.8  | 18.3 | 10.3  | 22.3 | 7.2   |            |       |
| -1.7 | 0.4   | 2.3 | 892.7 | 6.3 | 107.6 | 10.3 | 33.1  | 14.3 | 16.6  | 18.3 | 10.3  | 22.3 | 7.2   |            |       |
| -1.6 | 0.3   | 2.4 | 896.7 | 6.4 | 104.9 | 10.4 | 32.7  | 14.4 | 16.5  | 18.4 | 10.2  | 22.4 | 7.1   |            |       |
| -1.5 | 0.3   | 2.5 | 900.7 | 6.5 | 102.1 | 10.5 | 32.2  | 14.5 | 16.4  | 18.5 | 10.2  | 22.5 | 7.0   |            |       |
| -1.5 | 0.3   | 2.5 | 891.7 | 6.5 | 99.4  | 10.5 | 31.7  | 14.5 | 16.2  | 18.5 | 10.1  | 22.5 | 6.9   |            |       |
| -1.4 | 0.4   | 2.6 | 872.4 | 6.6 | 96.7  | 10.6 | 31.3  | 14.6 | 16.0  | 18.6 | 10.1  | 22.6 | 6.9   |            |       |
| -1.3 | 0.4   | 2.7 | 851.5 | 6.7 | 94.3  | 10.7 | 30.8  | 14.7 | 15.8  | 18.7 | 10.0  | 22.7 | 6.9   |            |       |
| -1.3 | 0.4   | 2.7 | 828.1 | 6.7 | 92.1  | 10.7 | 30.5  | 14.7 | 15.7  | 18.7 | 10.0  | 22.7 | 6.8   |            |       |
| -1.2 | 0.4   | 2.8 | 799.9 | 6.8 | 89.8  | 10.8 | 30.1  | 14.8 | 15.6  | 18.8 | 9.8   | 22.8 | 6.8   |            |       |
| -1.1 | 0.4   | 2.9 | 770.7 | 6.9 | 87.4  | 10.9 | 29.6  | 14.9 | 15.5  | 18.9 | 9.8   | 22.9 | 6.9   |            |       |
| -1.1 | 0.3   | 2.9 | 741.0 | 6.9 | 85.5  | 10.9 | 29.2  | 14.9 | 15.3  | 18.9 | 9.7   | 22.9 | 6.8   |            |       |
| -1.0 | 0.3   | 3.0 | 711.7 | 7.0 | 83.6  | 11.0 | 28.9  | 15.0 | 15.1  | 19.0 | 9.6   | 23.0 | 6.7   |            |       |
| -0.9 | 0.3   | 3.1 | 658.6 | 7.1 | 81.5  | 11.1 | 28.5  | 15.1 | 15.1  | 19.1 | 9.5   | 23.1 | 6.7   |            |       |
| -0.9 | 0.3   | 3.1 | 654.1 | 7.1 | 79.7  | 11.1 | 28.2  | 15.1 | 15.0  | 19.1 | 9.4   | 23.1 | 6.7   |            |       |
| -0.8 | 0.3   | 3.2 | 624.8 | 7.2 | 77.9  | 11.2 | 27.8  | 15.2 | 14.9  | 19.2 | 9.4   | 23.2 | 6.6   |            |       |
| -0.7 | 0.3   | 3.3 | 596.9 | 7.3 | 76.1  | 11.3 | 27.4  | 15.3 | 14.7  | 19.3 | 9.3   | 23.3 | 6.6   |            |       |
| -0.7 | 0.3   | 3.3 | 570.9 | 7.3 | 74.5  | 11.3 | 27.1  | 15.3 | 14.5  | 19.3 | 9.3   | 23.3 | 6.6   |            |       |
| -0.6 | 0.3   | 3.4 | 544.5 | 7.4 | 73.0  | 11.4 | 26.8  | 15.4 | 14.4  | 19.4 | 9.3   | 23.4 | 6.4   |            |       |
| -0.5 | 0.3   | 3.5 | 521.1 | 7.5 | 71.2  | 11.5 | 26.4  | 15.5 | 14.3  | 19.5 | 9.2   | 23.5 | 6.4   |            |       |
| -0.5 | 0.3   | 3.5 | 497.9 | 7.5 | 69.7  | 11.5 | 26.0  | 15.5 | 14.2  | 19.5 | 9.1   | 23.5 | 6.4   |            |       |
| -0.4 | 0.3   | 3.6 | 475.7 | 7.6 | 68.3  | 11.6 | 25.7  | 15.6 | 14.1  | 19.6 | 9.0   | 23.6 | 6.4   |            |       |
| -0.3 | 0.3   | 3.7 | 454.7 | 7.7 | 66.8  | 11.7 | 25.4  | 15.7 | 13.9  | 19.7 | 9.0   | 23.7 | 6.3   |            |       |
| -0.3 | 0.4   | 3.7 | 434.7 | 7.7 | 65.4  | 11.7 | 25.3  | 15.7 | 13.8  | 19.7 | 8.9   | 23.7 | 6.3   |            |       |
| -0.2 | 0.3   | 3.8 | 416.0 | 7.8 | 63.9  | 11.8 | 24.8  | 15.8 | 13.7  | 19.8 | 8.9   | 23.8 | 6.3   |            |       |
| -0.1 | 0.3   | 3.9 | 398.1 | 7.9 | 62.8  | 11.9 | 24.4  | 15.9 | 13.6  | 19.9 | 8.8   | 23.9 | 6.3   |            |       |
| -0.1 | 0.1   | 3.9 | 380.8 | 7.9 | 61.4  | 11.9 | 24.2  | 15.9 | 13.4  | 19.9 | 8.8   | 23.9 | 6.2   |            |       |
| 0.0  | 0.2   | 4.0 | 365.4 | 8.0 | 60.3  | 12.0 | 24.0  | 16.0 | 13.3  | 20.0 | 8.7   | 24.0 | 6.2   |            |       |
| 0.1  | 0.3   | 4.1 | 349.1 | 8.1 | 59.1  | 12.1 | 23.8  | 16.1 | 13.2  | 20.1 | 8.6   | 24.1 | 6.1   |            |       |
| 0.1  | 0.3   | 4.1 | 334.1 | 8.1 | 58.1  | 12.1 | 23.5  | 16.1 | 13.1  | 20.1 | 8.6   | 24.1 | 6.1   |            |       |
| 0.2  | 0.3   | 4.2 | 320.3 | 8.2 | 56.8  | 12.2 | 23.2  | 16.2 | 13.1  | 20.2 | 8.5   | 24.2 | 6.1   |            |       |
| 0.3  | 0.3   | 4.3 | 307.3 | 8.3 | 56.2  | 12.3 | 22.9  | 16.3 | 12.9  | 20.3 | 8.4   | 24.3 | 6.1   |            |       |
| 0.3  | 0.3   | 4.3 | 295.4 | 8.3 | 55.3  | 12.3 | 22.7  | 16.3 | 12.9  | 20.3 | 8.4   | 24.3 | 6.0   |            |       |
| 0.4  | 0.3   | 4.4 | 283.7 | 8.4 | 54.4  | 12.4 | 22.4  | 16.4 | 12.7  | 20.4 | 8.4   | 24.4 | 6.0   |            |       |
| 0.5  | 0.1   | 4.5 | 272.7 | 8.5 | 53.2  | 12.5 | 22.1  | 16.5 | 12.6  | 20.5 | 8.4   | 24.5 | 6.0   |            |       |
| 0.5  | 0.2   | 4.5 | 261.5 | 8.5 | 52.1  | 12.5 | 21.8  | 16.5 | 12.6  | 20.5 | 8.3   | 24.5 | 5.9   |            |       |
| 0.6  | 0.3   | 4.6 | 251.5 | 8.6 | 51.0  | 12.6 | 21.6  | 16.6 | 12.4  | 20.6 | 8.2   | 24.6 | 5.9   |            |       |

# APPENDIX A5-2-2 Well CD Continuous Uranine Data

| (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)        | (ppb) |
|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|------------|-------|
| TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI        | UR    |
| 117.38 | 58.0  | 121.32 | 55.4  | 125.32 | 53.2  | 129.32 | 51.0  | 133.32 | 48.7  | 137.32 | 46.3  | 141.32 | 44.1  | 145.32 | 41.9  | 149.32 | 40.5  | 153.32 | 38.8  | 157.32 | 37.6  | 161.32     | 35.6  |
| 117.45 | 58.2  | 121.38 | 55.4  | 125.38 | 53.1  | 129.38 | 51.0  | 133.38 | 48.6  | 137.38 | 46.4  | 141.38 | 44.0  | 145.38 | 41.8  | 149.38 | 40.6  | 153.38 | 38.8  | 157.38 | 37.7  | 161.38     | 35.6  |
| 117.52 | 58.2  | 121.45 | 55.4  | 125.45 | 53.1  | 129.45 | 51.0  | 133.45 | 48.6  | 137.45 | 46.3  | 141.45 | 44.0  | 145.45 | 41.8  | 149.45 | 40.5  | 153.45 | 39.0  | 157.45 | 37.5  | 161.45     | 35.5  |
| 117.58 | 58.2  | 121.52 | 55.3  | 125.52 | 53.0  | 129.52 | 51.0  | 133.52 | 48.7  | 137.52 | 46.3  | 141.52 | 44.0  | 145.52 | 41.8  | 149.52 | 40.5  | 153.52 | 38.9  | 157.52 | 37.5  | 161.52     | 35.5  |
| 117.65 | 58.1  | 121.58 | 55.1  | 125.58 | 53.0  | 129.58 | 50.9  | 133.58 | 48.6  | 137.58 | 46.2  | 141.58 | 43.9  | 145.58 | 41.8  | 149.58 | 40.3  | 153.58 | 38.8  | 157.58 | 37.4  | 161.58     | 35.7  |
| 117.72 | 58.1  | 121.65 | 55.1  | 125.65 | 52.9  | 129.65 | 50.8  | 133.65 | 48.6  | 137.65 | 46.2  | 141.65 | 44.0  | 145.65 | 41.7  | 149.65 | 40.2  | 153.65 | 38.8  | 157.65 | 37.5  | 161.65     | 35.6  |
| 117.78 | 58.0  | 121.72 | 55.0  | 125.72 | 52.9  | 129.72 | 50.8  | 133.72 | 48.5  | 137.72 | 46.1  | 141.72 | 43.9  | 145.72 | 41.6  | 149.72 | 40.4  | 153.72 | 38.7  | 157.72 | 37.5  | 161.72     | 35.5  |
| 117.85 | 58.0  | 121.78 | 55.0  | 125.78 | 52.9  | 129.78 | 50.7  | 133.78 | 48.5  | 137.78 | 46.1  | 141.78 | 43.8  | 145.78 | 41.7  | 149.78 | 40.3  | 153.78 | 38.7  | 157.78 | 37.3  | 161.78     | 35.5  |
| 117.92 | 57.8  | 121.85 | 55.0  | 125.85 | 52.8  | 129.85 | 50.7  | 133.85 | 48.5  | 137.85 | 46.1  | 141.85 | 43.8  | 145.85 | 41.6  | 149.85 | 40.3  | 153.85 | 38.7  | 157.85 | 37.4  | 161.85     | 35.5  |
| 117.98 | 57.8  | 121.92 | 54.9  | 125.92 | 52.8  | 129.92 | 50.7  | 133.92 | 48.4  | 137.92 | 46.1  | 141.92 | 43.8  | 145.92 | 41.7  | 149.92 | 40.3  | 153.92 | 38.7  | 157.92 | 37.2  | 161.92     | 35.5  |
| 118.05 | 57.8  | 121.98 | 54.8  | 125.98 | 52.8  | 129.98 | 50.7  | 133.98 | 48.5  | 137.98 | 46.1  | 141.98 | 43.7  | 145.98 | 41.5  | 149.98 | 40.2  | 153.98 | 38.7  | 157.98 | 37.3  | 161.98     | 35.5  |
| 118.12 | 57.8  | 122.05 | 54.7  | 126.05 | 52.7  | 130.05 | 50.6  | 134.05 | 48.4  | 138.05 | 46.0  | 142.05 | 43.7  | 146.05 | 41.6  | 150.05 | 40.3  | 154.05 | 38.7  | 158.05 | 37.2  | 162.05     | 35.4  |
| 118.18 | 57.7  | 122.12 | 54.7  | 126.12 | 52.8  | 130.12 | 50.6  | 134.12 | 48.3  | 138.12 | 45.9  | 142.12 | 43.7  | 146.12 | 41.5  | 150.12 | 40.3  | 154.12 | 38.7  | 158.12 | 37.2  | 162.12     | 35.5  |
| 118.25 | 57.7  | 122.18 | 54.7  | 126.18 | 52.7  | 130.18 | 50.6  | 134.18 | 48.2  | 138.18 | 45.9  | 142.18 | 43.6  | 146.18 | 41.5  | 150.18 | 40.4  | 154.18 | 38.7  | 158.18 | 37.2  | 162.18     | 35.4  |
| 118.32 | 57.6  | 122.25 | 54.7  | 126.25 | 52.7  | 130.25 | 50.5  | 134.25 | 48.3  | 138.25 | 45.9  | 142.25 | 43.5  | 146.25 | 41.5  | 150.25 | 40.3  | 154.25 | 38.8  | 158.25 | 37.3  | 162.25     | 35.4  |
| 118.38 | 57.6  | 122.32 | 54.7  | 126.32 | 52.6  | 130.32 | 50.6  | 134.32 | 48.2  | 138.32 | 45.8  | 142.32 | 43.5  | 146.32 | 41.2  | 150.32 | 40.3  | 154.32 | 38.9  | 158.32 | 37.2  | 162.32     | 35.3  |
| 118.45 | 57.5  | 122.38 | 54.6  | 126.38 | 52.6  | 130.38 | 50.4  | 134.38 | 48.2  | 138.38 | 45.7  | 142.38 | 43.5  | 146.38 | 41.3  | 150.38 | 40.3  | 154.38 | 38.7  | 158.38 | 37.1  | 162.38     | 35.4  |
| 118.52 | 57.5  | 122.45 | 54.6  | 126.45 | 52.6  | 130.45 | 50.4  | 134.45 | 48.1  | 138.45 | 45.7  | 142.45 | 43.5  | 146.45 | 41.4  | 150.45 | 40.2  | 154.45 | 38.8  | 158.45 | 37.1  | 162.45     | 35.3  |
| 118.58 | 57.4  | 122.52 | 54.6  | 126.52 | 52.5  | 130.52 | 50.4  | 134.52 | 48.1  | 138.52 | 45.7  | 142.52 | 43.5  | 146.52 | 41.4  | 150.52 | 40.0  | 154.52 | 38.6  | 158.52 | 37.1  | 162.52     | 35.3  |
| 118.65 | 57.2  | 122.58 | 54.5  | 126.58 | 52.5  | 130.58 | 50.3  | 134.58 | 48.0  | 138.58 | 45.5  | 142.58 | 43.4  | 146.58 | 41.4  | 150.58 | 40.1  | 154.58 | 38.7  | 158.58 | 37.0  | 162.58     | 35.3  |
| 118.72 | 57.1  | 122.65 | 54.5  | 126.65 | 52.4  | 130.65 | 50.3  | 134.65 | 48.0  | 138.65 | 45.5  | 142.65 | 43.3  | 146.65 | 41.4  | 150.65 | 40.1  | 154.65 | 38.5  | 158.65 | 37.1  | 162.65     | 35.2  |
| 118.78 | 57.1  | 122.72 | 54.5  | 126.72 | 52.4  | 130.72 | 50.3  | 134.72 | 48.0  | 138.72 | 45.5  | 142.72 | 43.2  | 146.72 | 41.3  | 150.72 | 40.0  | 154.72 | 38.7  | 158.72 | 37.0  | 162.72     | 35.2  |
| 118.85 | 57.1  | 122.78 | 54.3  | 126.78 | 52.4  | 130.78 | 50.2  | 134.78 | 48.0  | 138.78 | 45.4  | 142.78 | 43.1  | 146.78 | 41.3  | 150.78 | 40.0  | 154.78 | 38.5  | 158.78 | 37.1  | 162.78     | 35.2  |
| 118.92 | 57.1  | 122.85 | 54.3  | 126.85 | 52.3  | 130.85 | 50.2  | 134.85 | 48.0  | 138.85 | 45.5  | 142.85 | 43.2  | 146.85 | 41.3  | 150.85 | 40.1  | 154.85 | 38.5  | 158.85 | 37.0  | 162.85     | 35.1  |
| 118.98 | 57.0  | 122.92 | 54.3  | 126.92 | 52.3  | 130.92 | 50.2  | 134.92 | 47.8  | 138.92 | 45.5  | 142.92 | 43.3  | 146.92 | 41.3  | 150.92 | 40.0  | 154.92 | 38.5  | 158.92 | 36.9  | End Record |       |
| 119.05 | 57.0  | 122.98 | 54.3  | 126.98 | 52.2  | 130.98 | 50.2  | 134.98 | 47.6  | 138.98 | 45.4  | 142.98 | 43.2  | 146.98 | 41.2  | 150.98 | 40.0  | 154.98 | 38.5  | 158.98 | 36.9  |            |       |
| 119.12 | 57.0  | 123.05 | 54.3  | 127.05 | 52.3  | 131.05 | 50.0  | 135.05 | 47.5  | 139.05 | 45.3  | 143.05 | 43.2  | 147.05 | 41.2  | 151.05 | 40.0  | 155.05 | 38.4  | 159.05 | 36.9  |            |       |
| 119.18 | 56.9  | 123.12 | 54.2  | 127.12 | 52.2  | 131.12 | 50.1  | 135.12 | 47.5  | 139.12 | 45.2  | 143.12 | 43.1  | 147.12 | 41.2  | 151.12 | 39.9  | 155.12 | 38.3  | 159.12 | 36.9  |            |       |
| 119.25 | 56.9  | 123.18 | 54.2  | 127.18 | 52.2  | 131.18 | 50.0  | 135.18 | 47.5  | 139.18 | 45.2  | 143.18 | 43.0  | 147.18 | 41.2  | 151.18 | 39.9  | 155.18 | 38.3  | 159.18 | 36.9  |            |       |
| 119.32 | 56.8  | 123.25 | 54.1  | 127.25 | 52.1  | 131.25 | 50.0  | 135.25 | 47.5  | 139.25 | 45.2  | 143.25 | 43.0  | 147.25 | 41.1  | 151.25 | 40.1  | 155.25 | 38.2  | 159.25 | 36.9  |            |       |
| 119.38 | 56.7  | 123.32 | 54.1  | 127.32 | 52.1  | 131.32 | 50.0  | 135.32 | 47.5  | 139.32 | 45.2  | 143.32 | 43.0  | 147.32 | 41.1  | 151.32 | 39.9  | 155.32 | 38.1  | 159.32 | 36.8  |            |       |
| 119.45 | 56.7  | 123.38 | 54.1  | 127.38 | 52.1  | 131.38 | 49.8  | 135.38 | 47.5  | 139.38 | 45.2  | 143.38 | 43.0  | 147.38 | 41.1  | 151.38 | 39.8  | 155.38 | 38.0  | 159.38 | 36.8  |            |       |
| 119.52 | 56.7  | 123.45 | 54.0  | 127.45 | 52.0  | 131.45 | 49.9  | 135.45 | 47.4  | 139.45 | 45.1  | 143.45 | 43.0  | 147.45 | 41.1  | 151.45 | 39.8  | 155.45 | 38.1  | 159.45 | 36.7  |            |       |
| 119.58 | 56.6  | 123.52 | 54.1  | 127.52 | 52.0  | 131.52 | 49.8  | 135.52 | 47.4  | 139.52 | 45.1  | 143.52 | 42.9  | 147.52 | 41.1  | 151.52 | 39.9  | 155.52 | 38.1  | 159.52 | 36.7  |            |       |
| 119.65 | 56.6  | 123.58 | 54.0  | 127.58 | 51.9  | 131.58 | 49.8  | 135.58 | 47.4  | 139.58 | 45.1  | 143.58 | 42.8  | 147.58 | 41.0  | 151.58 | 39.8  | 155.58 | 38.1  | 159.58 | 36.7  |            |       |
| 119.72 | 56.4  | 123.65 | 53.9  | 127.65 | 51.8  | 131.65 | 49.7  | 135.65 | 47.3  | 139.65 | 45.0  | 143.65 | 42.8  | 147.65 | 41.0  | 151.65 | 39.7  | 155.65 | 38.1  | 159.65 | 36.6  |            |       |
| 119.78 | 56.4  | 123.72 | 53.8  | 127.72 | 51.8  | 131.72 | 49.7  | 135.72 | 47.3  | 139.72 | 45.0  | 143.72 | 42.9  | 147.72 | 41.0  | 151.72 | 39.6  | 155.72 | 38.0  | 159.72 | 36.6  |            |       |
| 119.85 | 56.4  | 123.78 | 53.8  | 127.78 | 51.7  | 131.78 | 49.7  | 135.78 | 47.2  | 139.78 | 44.9  | 143.78 | 42.8  | 147.78 | 40.9  | 151.78 | 39.5  | 155.78 | 38.0  | 159.78 | 36.6  |            |       |
| 119.92 | 56.3  | 123.85 | 53.8  | 127.85 | 51.6  | 131.85 | 49.6  | 135.85 | 47.2  | 139.85 | 44.9  | 143.85 | 42.7  | 147.85 | 40.9  | 151.85 | 39.4  | 155.85 | 38.1  | 159.85 | 36.5  |            |       |
| 119.98 | 56.3  | 123.92 | 53.7  | 127.92 | 51.6  | 131.92 | 49.5  | 135.92 | 47.1  | 139.92 | 44.9  | 143.92 | 42.7  | 147.92 | 40.9  | 151.92 | 39.4  | 155.92 | 38.1  | 159.92 | 36.5  |            |       |
| 120.05 | 56.2  | 123.98 | 53.8  | 127.98 | 51.6  | 131.98 | 49.5  | 135.98 | 47.1  | 139.98 | 44.8  | 143.98 | 42.6  | 147.98 | 40.8  | 151.98 | 39.4  | 155.98 | 38.1  | 159.98 | 36.4  |            |       |
| 120.12 | 56.2  | 124.05 | 53.8  | 128.05 | 51.6  | 132.05 | 49.5  | 136.05 | 47.0  | 140.05 | 44.8  | 144.05 | 42.6  | 148.05 | 41.1  | 152.05 | 39.4  | 156.05 | 38.1  | 160.05 | 36.5  |            |       |
| 120.18 | 56.2  | 124.12 | 53.7  | 128.12 | 51.6  | 132.12 | 49.5  | 136.12 | 47.1  | 140.12 | 44.8  | 144.12 | 42.6  | 148.12 | 41.0  | 152.12 | 39.4  | 156.12 | 38.0  | 160.12 | 36.5  |            |       |
| 120.25 | 56.1  | 124.18 | 53.8  | 128.18 | 51.6  | 132.18 | 49.6  | 136.18 | 47.0  | 140.18 | 44.7  | 144.18 | 42.6  | 148.18 | 40.9  | 152.18 | 39.3  |        |       |        |       |            |       |

### APPENDIX A5-2-3 Well D7 Continuous Uranine Data

| (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)    | (ppb) | (h)        | (ppb)  | (h)    | (ppb)  | (h)    | (ppb) |
|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|--------|-------|------------|--------|--------|--------|--------|-------|
| TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI    | UR    | TSI        | UR     | TSI    | UR     | TSI    | UR    |
| 117.82 | 29.8  | 121.82 | 28.2  | 125.82 | 26.9  | 129.82 | 25.7  | 133.82 | 24.4  | 137.82 | 23.2  | 141.82 | 21.9  | 145.82 | 20.7  | 149.82 | 19.7  | 153.82 | 19.0  | 157.82 | 18.1  | 161.82     | 17.2   | 165.82 | 16.3   | 169.82 | 15.4  |
| 117.88 | 30.2  | 121.88 | 28.2  | 125.88 | 26.9  | 129.88 | 25.7  | 133.88 | 24.4  | 137.88 | 23.1  | 141.88 | 21.8  | 145.88 | 20.7  | 149.88 | 19.8  | 153.88 | 18.9  | 157.88 | 18.1  | 161.88     | 17.4   | 165.88 | 16.5   | 169.88 | 15.6  |
| 117.95 | 30.2  | 121.95 | 28.3  | 125.95 | 27.0  | 129.95 | 25.6  | 133.95 | 24.4  | 137.95 | 23.2  | 141.95 | 21.8  | 145.95 | 20.7  | 149.95 | 19.6  | 153.95 | 18.9  | 157.95 | 18.1  | 161.95     | 17.3   | 165.95 | 16.4   | 169.95 | 15.5  |
| 118.02 | 30.1  | 122.02 | 28.2  | 126.02 | 26.9  | 130.02 | 25.6  | 134.02 | 24.3  | 138.02 | 23.1  | 142.02 | 21.9  | 146.02 | 20.7  | 150.02 | 19.8  | 154.02 | 18.9  | 158.02 | 18.1  | 162.02     | 17.3   | 166.02 | 16.5   | 170.02 | 15.6  |
| 118.08 | 30.2  | 122.08 | 28.3  | 126.08 | 26.9  | 130.08 | 25.6  | 134.08 | 24.3  | 138.08 | 23.2  | 142.08 | 21.7  | 146.08 | 20.6  | 150.08 | 19.7  | 154.08 | 18.8  | 158.08 | 18.1  | 162.08     | 17.2   | 166.08 | 16.6   | 170.08 | 15.7  |
| 118.15 | 30.2  | 122.15 | 28.1  | 126.15 | 26.9  | 130.15 | 25.6  | 134.15 | 24.3  | 138.15 | 23.1  | 142.15 | 21.8  | 146.15 | 20.6  | 150.15 | 19.7  | 154.15 | 18.9  | 158.15 | 18.1  | 162.15     | 17.2   | 166.15 | 16.7   | 170.15 | 15.8  |
| 118.22 | 30.1  | 122.22 | 28.1  | 126.22 | 26.8  | 130.22 | 25.5  | 134.22 | 24.2  | 138.22 | 23.0  | 142.22 | 21.7  | 146.22 | 20.6  | 150.22 | 19.7  | 154.22 | 18.8  | 158.22 | 18.0  | 162.22     | 17.2   | 166.22 | 16.8   | 170.22 | 15.9  |
| 118.28 | 30.1  | 122.28 | 28.1  | 126.28 | 26.8  | 130.28 | 25.5  | 134.28 | 24.3  | 138.28 | 22.9  | 142.28 | 21.8  | 146.28 | 3.1   | 150.28 | 19.7  | 154.28 | 18.9  | 158.28 | 18.0  | 162.28     | 17.1   | 166.28 | 16.9   | 170.28 | 16.0  |
| 118.35 | 30.1  | 122.35 | 28.0  | 126.35 | 26.7  | 130.35 | 25.5  | 134.35 | 24.2  | 138.35 | 23.0  | 142.35 | 21.6  | 146.35 | 20.6  | 150.35 | 19.7  | 154.35 | 18.7  | 158.35 | 18.0  | 162.35     | 17.2   | 166.35 | 17.0   | 170.35 | 16.1  |
| 118.42 | 30.1  | 122.42 | 28.0  | 126.42 | 26.6  | 130.42 | 25.4  | 134.42 | 24.3  | 138.42 | 22.9  | 142.42 | 21.7  | 146.42 | 20.6  | 150.42 | 19.7  | 154.42 | 18.8  | 158.42 | 18.1  | 162.42     | 17.2   | 166.42 | 17.1   | 170.42 | 16.2  |
| 118.48 | 30.0  | 122.48 | 27.9  | 126.48 | 26.6  | 130.48 | 25.4  | 134.48 | 24.1  | 138.48 | 23.0  | 142.48 | 21.6  | 146.48 | 20.5  | 150.48 | 19.7  | 154.48 | 18.8  | 158.48 | 18.0  | 162.48     | 17.2   | 166.48 | 17.2   | 170.48 | 16.3  |
| 118.55 | 29.9  | 122.55 | 28.0  | 126.55 | 26.5  | 130.55 | 25.4  | 134.55 | 24.2  | 138.55 | 22.9  | 142.55 | 21.7  | 146.55 | 20.4  | 150.55 | 19.7  | 154.55 | 18.7  | 158.55 | 17.9  | 162.55     | 17.3   | 166.55 | 17.3   | 170.55 | 16.4  |
| 118.62 | 29.9  | 122.62 | 27.9  | 126.62 | 26.6  | 130.62 | 25.5  | 134.62 | 24.1  | 138.62 | 22.9  | 142.62 | 21.6  | 146.62 | 20.5  | 150.62 | 19.7  | 154.62 | 18.7  | 158.62 | 18.0  | 162.62     | 17.0   | 166.62 | 17.4   | 170.62 | 16.5  |
| 118.68 | 30.0  | 122.68 | 27.9  | 126.68 | 26.6  | 130.68 | 25.4  | 134.68 | 24.1  | 138.68 | 22.9  | 142.68 | 21.6  | 146.68 | 20.5  | 150.68 | 19.6  | 154.68 | 18.8  | 158.68 | 18.0  | 162.68     | 17.1   | 166.68 | 17.4   | 170.68 | 16.6  |
| 118.75 | 29.9  | 122.75 | 27.8  | 126.75 | 26.5  | 130.75 | 25.4  | 134.75 | 24.1  | 138.75 | 22.8  | 142.75 | 21.5  | 146.75 | 20.5  | 150.75 | 19.6  | 154.75 | 18.8  | 158.75 | 17.9  | 162.75     | 17.0   | 166.75 | 17.5   | 170.75 | 16.7  |
| 118.82 | 29.8  | 122.82 | 27.9  | 126.82 | 26.5  | 130.82 | 25.3  | 134.82 | 24.1  | 138.82 | 22.9  | 142.82 | 21.5  | 146.82 | 20.5  | 150.82 | 19.5  | 154.82 | 18.7  | 158.82 | 18.0  | 162.82     | 17.1   | 166.82 | 17.5   | 170.82 | 16.8  |
| 118.88 | 29.8  | 122.88 | 27.9  | 126.88 | 26.5  | 130.88 | 25.3  | 134.88 | 24.0  | 138.88 | 22.9  | 142.88 | 21.5  | 146.88 | 20.5  | 150.88 | 19.5  | 154.88 | 18.7  | 158.88 | 17.9  | End Record | 166.88 | 17.5   | 170.88 | 16.9   |       |
| 118.95 | 29.8  | 122.95 | 27.9  | 126.95 | 26.5  | 130.95 | 25.3  | 134.95 | 24.0  | 138.95 | 22.8  | 142.95 | 21.5  | 146.95 | 20.4  | 150.95 | 19.6  | 154.95 | 18.7  | 158.95 | 17.8  |            | 166.95 | 17.6   | 170.95 | 17.0   |       |
| 119.02 | 29.8  | 123.02 | 28.0  | 127.02 | 26.5  | 131.02 | 25.3  | 135.02 | 24.0  | 139.02 | 22.9  | 143.02 | 21.5  | 147.02 | 20.4  | 151.02 | 19.6  | 155.02 | 18.7  | 159.02 | 17.8  | 163.02     | 17.2   | 167.02 | 17.6   | 171.02 | 17.1  |
| 119.08 | 29.8  | 123.08 | 27.9  | 127.08 | 26.5  | 131.08 | 25.3  | 135.08 | 24.0  | 139.08 | 22.8  | 143.08 | 21.5  | 147.08 | 20.5  | 151.08 | 19.6  | 155.08 | 18.6  | 159.08 | 17.9  | 163.08     | 17.3   | 167.08 | 17.7   | 171.08 | 17.2  |
| 119.15 | 29.7  | 123.15 | 27.9  | 127.15 | 26.4  | 131.15 | 25.3  | 135.15 | 24.0  | 139.15 | 22.8  | 143.15 | 21.4  | 147.15 | 20.4  | 151.15 | 19.4  | 155.15 | 18.7  | 159.15 | 17.8  | 163.15     | 17.4   | 167.15 | 17.8   | 171.15 | 17.3  |
| 119.22 | 29.9  | 123.22 | 28.0  | 127.22 | 26.4  | 131.22 | 25.2  | 135.22 | 24.0  | 139.22 | 22.7  | 143.22 | 21.4  | 147.22 | 20.3  | 151.22 | 19.5  | 155.22 | 18.7  | 159.22 | 17.9  | 163.22     | 17.5   | 167.22 | 17.9   | 171.22 | 17.4  |
| 119.28 | 29.6  | 123.28 | 27.8  | 127.28 | 26.5  | 131.28 | 25.3  | 135.28 | 23.9  | 139.28 | 22.6  | 143.28 | 21.4  | 147.28 | 20.3  | 151.28 | 19.5  | 155.28 | 18.8  | 159.28 | 17.8  | 163.28     | 17.6   | 167.28 | 18.0   | 171.28 | 17.5  |
| 119.35 | 29.6  | 123.35 | 27.9  | 127.35 | 26.3  | 131.35 | 25.2  | 135.35 | 23.9  | 139.35 | 22.7  | 143.35 | 21.3  | 147.35 | 20.4  | 151.35 | 19.5  | 155.35 | 18.6  | 159.35 | 17.9  | 163.35     | 17.7   | 167.35 | 18.1   | 171.35 | 17.6  |
| 119.42 | 29.6  | 123.42 | 27.9  | 127.42 | 26.4  | 131.42 | 25.2  | 135.42 | 23.9  | 139.42 | 22.7  | 143.42 | 21.4  | 147.42 | 20.3  | 151.42 | 19.5  | 155.42 | 18.6  | 159.42 | 17.8  | 163.42     | 17.8   | 167.42 | 18.2   | 171.42 | 17.7  |
| 119.48 | 29.6  | 123.48 | 27.9  | 127.48 | 26.5  | 131.48 | 25.1  | 135.48 | 23.8  | 139.48 | 22.6  | 143.48 | 21.3  | 147.48 | 20.3  | 151.48 | 19.4  | 155.48 | 18.7  | 159.48 | 17.8  | 163.48     | 17.9   | 167.48 | 18.3   | 171.48 | 17.8  |
| 119.55 | 29.5  | 123.55 | 27.8  | 127.55 | 26.4  | 131.55 | 25.1  | 135.55 | 23.9  | 139.55 | 22.6  | 143.55 | 21.3  | 147.55 | 20.3  | 151.55 | 19.5  | 155.55 | 18.6  | 159.55 | 17.8  | 163.55     | 18.0   | 167.55 | 18.4   | 171.55 | 17.9  |
| 119.62 | 29.5  | 123.62 | 27.8  | 127.62 | 26.4  | 131.62 | 25.1  | 135.62 | 23.9  | 139.62 | 22.6  | 143.62 | 21.3  | 147.62 | 20.4  | 151.62 | 19.4  | 155.62 | 18.5  | 159.62 | 17.7  | 163.62     | 18.1   | 167.62 | 18.5   | 171.62 | 18.0  |
| 119.68 | 29.5  | 123.68 | 27.8  | 127.68 | 26.4  | 131.68 | 25.0  | 135.68 | 23.8  | 139.68 | 22.5  | 143.68 | 21.3  | 147.68 | 20.3  | 151.68 | 19.4  | 155.68 | 18.5  | 159.68 | 17.8  | 163.68     | 18.2   | 167.68 | 18.6   | 171.68 | 18.1  |
| 119.75 | 29.4  | 123.75 | 27.7  | 127.75 | 26.3  | 131.75 | 25.0  | 135.75 | 23.9  | 139.75 | 22.5  | 143.75 | 21.3  | 147.75 | 20.3  | 151.75 | 19.3  | 155.75 | 18.6  | 159.75 | 17.8  | 163.75     | 18.3   | 167.75 | 18.7   | 171.75 | 18.2  |
| 119.82 | 29.3  | 123.82 | 27.7  | 127.82 | 26.4  | 131.82 | 25.0  | 135.82 | 23.8  | 139.82 | 22.5  | 143.82 | 21.3  | 147.82 | 20.3  | 151.82 | 19.4  | 155.82 | 18.6  | 159.82 | 17.7  | 163.82     | 18.4   | 167.82 | 18.8   | 171.82 | 18.3  |
| 119.88 | 29.4  | 123.88 | 27.7  | 127.88 | 26.3  | 131.88 | 25.1  | 135.88 | 23.7  | 139.88 | 22.4  | 143.88 | 21.2  | 147.88 | 20.4  | 151.88 | 19.3  | 155.88 | 18.4  | 159.88 | 17.6  | 163.88     | 18.5   | 167.88 | 18.9   | 171.88 | 18.4  |
| 119.95 | 29.2  | 123.95 | 27.6  | 127.95 | 26.3  | 131.95 | 25.0  | 135.95 | 23.7  | 139.95 | 22.4  | 143.95 | 21.2  | 147.95 | 20.2  | 151.95 | 19.3  | 155.95 | 18.5  | 159.95 | 17.6  | 163.95     | 18.6   | 167.95 | 19.0   | 171.95 | 18.5  |
| 120.02 | 29.4  | 124.02 | 27.6  | 128.02 | 26.2  | 132.02 | 25.0  | 136.02 | 23.7  | 140.02 | 22.4  | 144.02 | 21.2  | 148.02 | 20.5  | 152.02 | 19.3  | 156.02 | 18.5  | 160.02 | 17.7  | 164.02     | 18.7   | 168.02 | 19.1   | 172.02 | 18.6  |
| 120.08 | 29.3  | 124.08 | 27.7  | 128.08 | 26.2  | 132.08 | 25.0  | 136.08 | 23.7  | 140.08 | 22.4  | 144.08 | 21.2  | 148.08 | 20.2  | 152.08 | 19.2  | 156.08 | 18.4  | 160.08 | 17.6  | 164.08     | 18.8   | 168.08 | 19.2   | 172.08 | 18.7  |
| 120.15 | 29.3  | 124.15 | 27.7  | 128.15 | 26.2  | 132.15 | 24.9  | 136.15 | 23.7  | 140.15 | 22.3  | 144.15 | 21.2  | 148.15 | 20.2  | 152.15 | 19.2  | 156.15 | 18.4  | 160.15 | 17.5  | 164.15     | 18.9   | 168.15 | 19.3   | 172.15 | 18.8  |
| 120.22 | 29.3  | 124.22 | 27.6  | 128.22 | 26.2  | 132.22 | 24.9  | 136.22 | 23.7  | 140.22 | 22.5  | 144.22 | 21.1  | 148.22 | 20.1  | 152.22 | 19.3  | 156.22 | 18.5  | 160.22 | 17.7  | 164.22     | 19.0   | 168.22 | 19.4   | 172.22 | 18.9  |
| 120.28 | 29.1  | 124.28 | 27.6  | 128.28 | 26.2  | 132.28 | 24.9  | 136.28 | 23.6  | 140.28 | 22.3  | 144.28 | 21.1  | 148.28 | 20.2  | 152.28 | 19.2  | 156.28 | 18.5  | 160.28 | 17.6  | 164.28     | 19.1   | 168.28 | 19.5   | 172.28 | 19.0  |
| 120.35 | 29.2  | 124.35 | 27.6  | 128.35 | 26.2  | 132.35 | 24.8  | 136.35 | 23.6  | 140.35 | 22.3  | 144.35 | 21.1  | 148.35 | 20.2  | 152.35 | 19.3  | 156.35 | 18.4  | 160.35 | 17.7  | 164.35     | 19.2   | 168.35 | 19.6   | 172.35 | 19.1  |
| 120.42 | 29.1  | 124.42 | 27.7  | 128.42 | 26.2  | 132.42 | 24.8  | 136.42 | 23.7  | 140.42 | 22.3  | 144.42 | 21.1  | 148.42 | 20.1  | 152.42 | 19.1  | 156.42 | 18.4  | 160.42 | 17.6  | 164.42     | 19.3   | 168.42 | 19.7   | 172.42 | 19.2  |
| 120.48 | 28.9  | 124.48 | 27.5  | 128.48 | 26.0  | 132.48 | 24.8  | 136.48 | 23.7  | 140.48 | 22.3  | 144.48 | 21.0  | 148.48 | 20.1  | 152.48 | 19.1  | 156.48 | 18.4  | 160.48 | 17.5  | 164.48     | 19.4   | 168.48 | 19.8   | 172.48 | 19.3  |
| 120.55 | 3.4   | 124.55 | 27.4  | 128.55 | 26.0  | 132.55 | 24.8  | 136.55 | 23.6  | 140.55 | 22.3  | 144.55 | 21.1  | 148.55 | 20.1  | 152.55 | 19.1  | 156.55 | 18.4  | 160.55 | 17.6  | 164.55     | 19.5   | 168.55 | 19.9   | 172.55 | 19.4  |
| 120.62 | 3.4   | 124.62 | 27.4  | 128.62 | 26.0  | 132.62 | 24.8  | 136.62 | 23.6  | 140.62 |       |        |       |        |       |        |       |        |       |        |       |            |        |        |        |        |       |

# Appendix A5-3-1 Well C6 Microsphere Data

(Method: see S. Niehren, 1998)

| Site:Wilerwald Well:C6 Test start:26Aug97 |                  |              |                  |              |                  |              |                  |              |                  |              |                 |              |                 |                              |                 |
|-------------------------------------------|------------------|--------------|------------------|--------------|------------------|--------------|------------------|--------------|------------------|--------------|-----------------|--------------|-----------------|------------------------------|-----------------|
| 1                                         |                  | 2            |                  | 3            |                  | 4            |                  | 5            |                  | 6            |                 | 7            |                 | 8                            |                 |
| TSI<br>(min)                              | Sphere/<br>10 ml | TSI<br>(min) | Sphere/<br>10 ml | TSI<br>(min) | Sphere/<br>10 ml | TSI<br>(min) | Sphere/<br>10 ml | TSI<br>(min) | Sphere/<br>10 ml | TSI<br>(min) | Sphere/<br>1 ml | TSI<br>(min) | Sphere/<br>1 ml | TSI<br>(min)                 | Sphere/<br>1 ml |
| -9                                        | 0                | 54           | 0                | 117          | 9                | 180          | 46               | 243          | 1                | 306          | 2               | 369          | 0               | 432                          | 0               |
| -8                                        | 0                | 55           | 0                | 118          | 32               | 181          | 54               | 244          | 0                | 307          | 0               | 370          | 0               | 433                          | 0               |
| -7                                        | 0                | 56           | 0                | 119          | 30               | 182          | 33               | 245          | 2                | 308          | 3               | 371          | 0               | 434                          | 0               |
| -6                                        | 0                | 57           | 0                | 120          | 20               | 183          | 33               | 246          | 2                | 309          | 2               | 372          | 1               | 435                          | 2               |
| -5                                        | 0                | 58           | 0                | 121          | 20               | 184          | 30               | 247          | 2                | 310          | 0               | 373          | 0               | 436                          | 0               |
| -4                                        | 0                | 59           | 0                | 122          | 17               | 185          | 24               | 248          | 14               | 311          | 2               | 374          | 0               | 437                          | 0               |
| -3                                        | 0                | 60           | 0                | 123          | 31               | 186          | 34               | 249          | 4                | 312          | 4               | 375          | 0               | 438                          | 1               |
| -2                                        | 2                | 61           | 0                | 124          | 29               | 187          | 13               | 250          | 3                | 313          | 0               | 376          | 0               | 439                          | 0               |
| -1                                        | 0                | 62           | 0                | 125          | 17               | 188          | 20               | 251          | 4                | 314          | 2               | 377          | 0               | 440                          | 0               |
| 0                                         | 0                | 63           | 0                | 126          | 32               | 189          | 10               | 252          | 1                | 315          | 4               | 378          | 0               | 441                          | 0               |
| 1                                         | 0                | 64           | 0                | 127          | 31               | 190          | 17               | 253          | 3                | 316          | 3               | 379          | 4               | 442                          | 0               |
| 2                                         | 0                | 65           | 0                | 128          | 28               | 191          | 13               | 254          | 2                | 317          | 0               | 380          | 2               | 443                          | 2               |
| 3                                         | 0                | 66           | 0                | 129          | 37               | 192          | 13               | 255          | 4                | 318          | 0               | 381          | 0               | 444                          | 0               |
| 4                                         | 0                | 67           | 0                | 130          | 40               | 193          | 28               | 256          | 3                | 319          | 0               | 382          | 1               | 445                          | 2               |
| 5                                         | 0                | 68           | 0                | 131          | 34               | 194          | 11               | 257          | 4                | 320          | 5               | 383          | 4               | 446                          | 0               |
| 6                                         | 0                | 69           | 3                | 132          | 23               | 195          | 16               | 258          | 4                | 321          | 0               | 384          | 4               | 447                          | 0               |
| 7                                         | 1                | 70           | 1                | 133          | 41               | 196          | 9                | 259          | 2                | 322          | 1               | 385          | 2               | 448                          | 0               |
| 8                                         | 0                | 71           | 0                | 134          | 24               | 197          | 10               | 260          | 0                | 323          | 1               | 386          | 2               | 449                          | 0               |
| 9                                         | 0                | 72           | 1                | 135          | 40               | 198          | 9                | 261          | 1                | 324          | 1               | 387          | 0               | 450                          | 2               |
| 10                                        | 0                | 73           | 0                | 136          | 48               | 199          | 9                | 262          | 1                | 325          | 0               | 388          | 2               | 451                          | 0               |
| 11                                        | 0                | 74           | 0                | 137          | 29               | 200          | 6                | 263          | 14               | 326          | 1               | 389          | 0               | 452                          | 0               |
| 12                                        | 0                | 75           | 0                | 138          | 53               | 201          | 6                | 264          | 1                | 327          | 4               | 390          | 0               | 453                          | 0               |
| 13                                        | 1                | 76           | 0                | 139          | 48               | 202          | 7                | 265          | 1                | 328          | 0               | 391          | 0               | 454                          | 0               |
| 14                                        | 0                | 77           | 0                | 140          | 45               | 203          | 10               | 266          | 3                | 329          | 0               | 392          | 0               | 455                          | 0               |
| 15                                        | 0                | 78           | 0                | 141          | 56               | 204          | 14               | 267          | 4                | 330          | 1               | 393          | 0               | 456                          | 0               |
| 16                                        | 0                | 79           | 0                | 142          | 46               | 205          | 9                | 268          | 7                | 331          | 2               | 394          | 0               | 457                          | 0               |
| 17                                        | 1                | 80           | 0                | 143          | 39               | 206          | 3                | 269          | 5                | 332          | 0               | 395          | 0               | 458                          | 1               |
| 18                                        | 0                | 81           | 1                | 144          | 48               | 207          | 11               | 270          | 8                | 333          | 0               | 396          | 18              | 459                          | 8               |
| 19                                        | 5                | 82           | 3                | 145          | 38               | 208          | 3                | 271          | 1                | 334          | 0               | 397          | 0               | 460                          | 0               |
| 20                                        | 0                | 83           | 0                | 146          | 36               | 209          | 2                | 272          | 0                | 335          | 0               | 398          | 1               | 461                          | 0               |
| 21                                        | 0                | 84           | 0                | 147          | 23               | 210          | 4                | 273          | 0                | 336          | 15              | 399          | 2               | 462                          | 0               |
| 22                                        | 0                | 85           | 0                | 148          | 44               | 211          | 6                | 274          | 1                | 337          | 0               | 400          | 6               | 463                          | 0               |
| 23                                        | 1                | 86           | 0                | 149          | 37               | 212          | 7                | 275          | 0                | 338          | 1               | 401          | 0               | 464                          | 0               |
| 24                                        | 2                | 87           | 0                | 150          | 35               | 213          | 4                | 276          | 0                | 339          | 1               | 402          | 4               | 465                          | 0               |
| 25                                        | 2                | 88           | 0                | 151          | 47               | 214          | 2                | 277          | 1                | 340          | 0               | 403          | 4               | 466                          | 0               |
| 26                                        | 4                | 89           | 0                | 152          | 29               | 215          | 5                | 278          | 1                | 341          | 0               | 404          | 3               | Sampling<br>rate:<br>1ml/min |                 |
| 27                                        | 0                | 90           | 0                | 153          | 34               | 216          | 6                | 279          | 3                | 342          | 0               | 405          | 3               |                              |                 |
| 28                                        | 2                | 91           | 1                | 154          | 21               | 217          | 2                | 280          | 0                | 343          | 0               | 406          | 2               |                              |                 |
| 29                                        | 5                | 92           | 1                | 155          | 27               | 218          | 11               | 281          | 1                | 344          | 0               | 407          | 0               |                              |                 |
| 30                                        | 0                | 93           | 0                | 156          | 21               | 219          | 7                | 282          | 0                | 345          | 2               | 408          | 0               |                              |                 |
| 31                                        | 0                | 94           | 2                | 157          | 17               | 220          | 15               | 283          | 3                | 346          | 1               | 409          | 0               |                              |                 |
| 32                                        | 0                | 95           | 2                | 158          | 41               | 221          | 9                | 284          | 1                | 347          | 0               | 410          | 0               |                              |                 |
| 33                                        | 0                | 96           | 0                | 159          | 19               | 222          | 7                | 285          | 2                | 348          | 1               | 411          | 0               |                              |                 |
| 34                                        | 1                | 97           | 0                | 160          | 23               | 223          | 5                | 286          | 2                | 349          | 2               | 412          | 0               |                              |                 |
| 35                                        | 0                | 98           | 1                | 161          | 17               | 224          | 1                | 287          | 4                | 350          | 0               | 413          | 0               |                              |                 |
| 36                                        | 2                | 99           | 0                | 162          | 11               | 225          | 7                | 288          | 3                | 351          | 0               | 414          | 0               |                              |                 |
| 37                                        | 0                | 100          | 4                | 163          | 26               | 226          | 2                | 289          | 1                | 352          | 0               | 415          | 0               |                              |                 |
| 38                                        | 0                | 101          | 8                | 164          | 27               | 227          | 8                | 290          | 1                | 353          | 0               | 416          | 0               |                              |                 |
| 39                                        | 0                | 102          | 5                | 165          | 21               | 228          | 6                | 291          | 1                | 354          | 7               | 417          | 0               |                              |                 |
| 40                                        | 3                | 103          | 5                | 166          | 26               | 229          | 2                | 292          | 0                | 355          | 2               | 418          | 0               |                              |                 |
| 41                                        | 0                | 104          | 9                | 167          | 15               | 230          | 6                | 293          | 0                | 356          | 3               | 419          | 1               |                              |                 |
| 42                                        | 3                | 105          | 12               | 168          | 17               | 231          | 5                | 294          | 1                | 357          | 0               | 420          | 0               |                              |                 |
| 43                                        | 0                | 106          | 10               | 169          | 17               | 232          | 3                | 295          | 0                | 358          | 0               | 421          | 0               |                              |                 |
| 44                                        | 0                | 107          | 9                | 170          | 18               | 233          | 7                | 296          | 0                | 359          | 0               | 422          | 0               |                              |                 |
| 45                                        | 0                | 108          | 16               | 171          | 39               | 234          | 3                | 297          | 15               | 360          | 1               | 423          | 0               |                              |                 |
| 46                                        | 0                | 109          | 8                | 172          | 40               | 235          | 3                | 298          | 11               | 361          | 0               | 424          | 2               |                              |                 |
| 47                                        | 0                | 110          | 10               | 173          | 91               | 236          | 3                | 299          | 1                | 362          | 0               | 425          | 0               |                              |                 |
| 48                                        | 0                | 111          | 16               | 174          | 62               | 237          | 2                | 300          | 5                | 363          | 0               | 426          | 0               |                              |                 |
| 49                                        | 0                | 112          | 16               | 175          | 80               | 238          | 9                | 301          | 4                | 364          | 9               | 427          | 0               |                              |                 |
| 50                                        | 0                | 113          | 20               | 176          | 67               | 239          | 10               | 302          | 0                | 365          | 9               | 428          | 0               |                              |                 |
| 51                                        | 0                | 114          | 30               | 177          | 77               | 240          | 2                | 303          | 4                | 366          | 0               | 429          | 0               |                              |                 |
| 52                                        | 0                | 115          | 18               | 178          | 68               | 241          | 9                | 304          | 2                | 367          | 0               | 430          | 0               |                              |                 |
| 53                                        | 3                | 116          | 17               | 179          | 53               | 242          | 5                | 305          | 2                | 368          | 3               | 431          | 0               |                              |                 |

# Appendix A5-3-2 Well D7 Microsphere Data

(Method: see S. Niehren, 1998)

| Site: Wilerwald WELL:D7 Test start: 26Aug97 |                  |              |                  |              |                  |              |                  |              |                  |                               |                  |
|---------------------------------------------|------------------|--------------|------------------|--------------|------------------|--------------|------------------|--------------|------------------|-------------------------------|------------------|
| 1                                           |                  | 2            |                  | 3            |                  | 4            |                  | 5            |                  | 6                             |                  |
| TSI<br>(min)                                | Sphere/<br>10 ml | TSI<br>(min) | Sphere/<br>10 ml | TSI<br>(min) | Sphere/<br>10 ml | TSI<br>(min) | Sphere/<br>10 ml | TSI<br>(min) | Sphere/<br>10 ml | TSI<br>(min)                  | Sphere/<br>10 ml |
| 530                                         | 172              | 1122         | 84               | 1725         | 38               | 2322         | 11               | 2912         | 8                | 3895                          | 8                |
| 540                                         | 183              | 1132         | 110              | 1735         | 21               | 2332         | 15               | 2922         | 29               | 3905                          | 5                |
| 550                                         | 204              | 1142         | 82               | 1745         | 28               | 2342         | 24               | 2932         | 15               | 3915                          | 6                |
| 560                                         | 252              | 1152         | 71               | 1755         | 17               | 2352         | 16               | 2942         | 9                | 3925                          | 5                |
| 570                                         | 273              | 1162         | 84               | 1765         | 29               | 2362         | 11               | 2952         | 4                | 3935                          | 9                |
| 580                                         | 254              | 1172         | 73               | 1775         | 32               | 2372         | 15               | 2962         | 16               | 3945                          | 21               |
| 590                                         | 314              | 1182         | 60               | 1785         | 74               | 2382         | 7                | 2972         | 20               | 3955                          | 5                |
| 600                                         | 265              | 1192         | 54               | 1795         | 37               | 2392         | 8                | 2982         | 9                | 3965                          | 11               |
| 610                                         | 220              | 1202         | 76               | 1805         | 33               | 2402         | 5                | 2992         | 11               | 3975                          | 12               |
| 620                                         | 265              | 1212         | 60               | 1815         | 36               | 2412         | 14               | 3002         | 8                | 3985                          | 6                |
| 630                                         | 300              | 1222         | 67               | 1825         | 25               | 2422         | 4                | 3012         | 4                | 3995                          | 13               |
| 640                                         | 311              | 1232         | 70               | 1835         | 27               | 2432         | 26               | 3025         | 12               | 4005                          | 4                |
| 650                                         | 353              | 1242         | 74               | 1845         | 30               | 2442         | 23               | 3035         | 4                | 4015                          | 10               |
| 660                                         | 331              | 1252         | 85               | 1855         | 24               | 2452         | 15               | 3045         | 21               | 4025                          | 16               |
| 670                                         | 288              | 1262         | 50               | 1865         | 44               | 2462         | 24               | 3055         | 10               | 4035                          | 6                |
| 680                                         | 285              | 1272         | 56               | 1875         | 22               | 2472         | 25               | 3065         | 9                | 4045                          | 15               |
| 690                                         | 350              | 1282         | 46               | 1885         | 19               | 2492         | 21               | 3075         | 4                | 4055                          | 9                |
| 700                                         | 377              | 1292         | 55               | 1895         | 14               | 2502         | 14               | 3085         | 8                | 4065                          | 6                |
| 710                                         | 321              | 1302         | 70               | 1905         | 42               | 2512         | 20               | 3095         | 19               | 4075                          | 9                |
| 720                                         | 304              | 1312         | 74               | 1915         | 38               | 2522         | 11               | 3105         | 6                | 4085                          | 17               |
| 730                                         | 330              | 1322         | 45               | 1925         | 29               | 2532         | 11               | 3115         | 14               | 4095                          | 10               |
| 740                                         | 297              | 1332         | 53               | 1935         | 26               | 2542         | 6                | 3125         | 9                | 4105                          | 8                |
| 750                                         | 287              | 1342         | 42               | 1945         | 33               | 2552         | 18               | 3135         | 7                | 4115                          | 6                |
| 760                                         | 291              | 1352         | 99               | 1955         | 19               | 2562         | 8                | 3145         | 11               | 4125                          | 12               |
| 770                                         | 295              | 1362         | 55               | 1982         | 77               | 2572         | 10               | 3155         | 13               | 4135                          | 6                |
| 780                                         | 279              | 1372         | 53               | 1992         | 17               | 2582         | 16               | 3165         | 6                | 4145                          | 4                |
| 790                                         | 296              | 1382         | 48               | 2002         | 54               | 2592         | 8                | 3175         | 1                | 4155                          | 7                |
| 800                                         | 254              | 1392         | 39               | 2012         | 10               | 2602         | 10               | 3185         | 9                | 4165                          | 9                |
| 810                                         | 275              | 1402         | 47               | 2022         | 14               | 2612         | 6                | 3195         | 10               | 4175                          | 7                |
| 820                                         | 274              | 1412         | 42               | 2032         | 30               | 2622         | 17               | 3205         | 8                | 4185                          | 16               |
| 830                                         | 233              | 1422         | 40               | 2042         | 26               | 2632         | 21               | 3215         | 8                | 4195                          | 14               |
| 840                                         | 235              | 1432         | 28               | 2052         | 18               | 2642         | 10               | 3225         | 10               | 4205                          | 10               |
| 850                                         | 241              | 1442         | 44               | 2062         | 40               | 2652         | 22               | 3235         | 21               | 4215                          | 14               |
| 860                                         | 197              | 1452         | 67               | 2072         | 28               | 2662         | 7                | 3245         | 8                | 4225                          | 6                |
| 870                                         | 259              | 1462         | 30               | 2082         | 24               | 2672         | 9                | 3255         | 8                | 4235                          | 5                |
| 880                                         | 178              | 1472         | 63               | 2092         | 18               | 2682         | 15               | 3265         | 18               | 4245                          | 12               |
| 890                                         | 191              | 1482         | 48               | 2102         | 17               | 2692         | 11               | 3275         | 8                | 4255                          | 7                |
| 900                                         | 185              | 1492         | 35               | 2112         | 17               | 2702         | 9                | 3285         | 11               | 4265                          | 6                |
| 910                                         | 181              | 1502         | 50               | 2122         | 27               | 2712         | 18               | 3295         | 22               | 4275                          | 13               |
| 920                                         | 172              | 1512         | 38               | 2132         | 29               | 2722         | 8                | 3305         | 5                | 4285                          | 11               |
| 930                                         | 185              | 1545         | 55               | 2142         | 25               | 2732         | 8                | 3315         | 7                | Sampling<br>rate:<br>1 ml/min | END              |
| 940                                         | 180              | 1555         | 24               | 2152         | 17               | 2742         | 8                | 3325         | 9                |                               |                  |
| 950                                         | 166              | 1565         | 48               | 2162         | 18               | 2752         | 13               | 3335         | 6                |                               |                  |
| 960                                         | 161              | 1575         | 46               | 2172         | 21               | 2762         | 12               | 3345         | 10               |                               |                  |
| 970                                         | 148              | 1585         | 35               | 2182         | 12               | 2772         | 6                | 3355         | 12               |                               |                  |
| 980                                         | 155              | 1595         | 36               | 2192         | 14               | 2782         | 9                | 3365         | 7                |                               |                  |
| 990                                         | 115              | 1605         | 37               | 2202         | 31               | 2792         | 10               | 3775         | 14               |                               |                  |
| 1012                                        | 129              | 1615         | 27               | 2212         | 7                | 2802         | 9                | 3785         | 1                |                               |                  |
| 1022                                        | 114              | 1625         | 33               | 2222         | 13               | 2812         | 4                | 3795         | 3                |                               |                  |
| 1032                                        | 96               | 1635         | 41               | 2232         | 10               | 2822         | 15               | 3805         | 2                |                               |                  |
| 1042                                        | 100              | 1645         | 30               | 2242         | 15               | 2832         | 13               | 3815         | 17               |                               |                  |
| 1052                                        | 91               | 1655         | 48               | 2252         | 14               | 2842         | 10               | 3825         | 11               |                               |                  |
| 1062                                        | 70               | 1665         | 23               | 2262         | 22               | 2852         | 11               | 3835         | 10               |                               |                  |
| 1072                                        | 99               | 1675         | 49               | 2272         | 12               | 2862         | 23               | 3845         | 3                |                               |                  |
| 1082                                        | 103              | 1685         | 33               | 2282         | 13               | 2872         | 8                | 3855         | 8                |                               |                  |
| 1092                                        | 109              | 1695         | 41               | 2292         | 9                | 2882         | 7                | 3865         | 8                |                               |                  |
| 1102                                        | 127              | 1705         | 44               | 2302         | 11               | 2892         | 8                | 3875         | 1                |                               |                  |
| 1112                                        | 112              | 1715         | 40               | 2312         | 14               | 2902         | 17               | 3885         | 5                |                               |                  |

# Abstract

Breakthrough responses of biocolloid particles, specifically bacteriophages (phages), were compared to those of more classical tracer solutes, salt and uranine, during transport in heterogeneous, highly permeable granular porous media. Research focused on phage performance and characterization in a 6 m laboratory tank model and at three ground water field sites in gravel aquifer settings (Switzerland).

The MARCEAUS tank facility (Univ. Louis Pasteur, Inst. Fluid Mechanics, Strasbourg) with dimensions of 1 m by 1 m by 6 m was used to distinguish flow path variability and rank mass transport rate/recovery of six different bacteriophages (Marine-H4, H6, H40, Non-marine-T7, Psf2, MS2) in a moderately heterogeneous sandy porous media. Phages were co-mingled with salt and uranine and input with a consistent (~4 h) step signal. Testing was done under controllable gradient, temperature and chemistry conditions. Salt levels were monitored in-situ in real time with 353 conductivity probes. Phage and uranine samples were taken from six internal and the outflow locations. Salt and uranine transport responses were similar. Phage recovery, normalized to the solutes' recovery (relative breakthrough - RB) changed according to phage type, location within the tank's heterogeneity and the test water's ionic strength. RB decreased consistently when EC reduced from 3700 to 700  $\mu\text{S cm}^{-1}$ . RB ranged from 5 to 94% (H40), 3 to 59% (MS2), 4 to 38% (H4), 0 to 33% (Psf2) and 0.3 to 11% (H6). Total recovery varying at the outflow was 28 to 51% (H40-5 tests), 25 to 31% (MS2-2 tests), 24% (H4), 2 to 4% (H6-2 tests), and 1% (Psf2). T7 was never detected. Velocity, based on first detection and normalized to flow rate varied with path heterogeneity from 0.17 to 0.27 in lower permeability multiple paths and was 0.32 to 0.37 in channel paths. Double solute peaks reflected multiple contributions along longer and differing pathways but the phages had either no, or a subdued double, response illustrating higher attachment and more restrictive pathways.

In Bois de Finges (BdF), six tracer tests were done under high, moderate and low water level conditions using two injection points and five monitoring wells to 22 m distances. Contaminant migration in this highly permeable gravel setting along the Rhone River Valley was documented using three phages and uranine so as to be able to evaluate particle transport and ground water protection rationale. Solute and biocolloid transport rates to 22 m were from about 100 to 500  $\text{m d}^{-1}$ , up to 40-fold faster than predicted using the conventional flow/effective porosity calculation approach. In one well, a 1 m vertical heterogeneity dominated transport in 12 m of gravel-dominated aquifer. Biocolloids were consistently detected earlier likely due to lower than solute detection limits. Maximum biocolloid concentrations (pseudo-peaks) commonly occurred prior to solute peaks with colloid removal processes rather than media properties control particle breakthrough responses and patterns. Phage H40 RBs were typically 1.5 to 4%, commonly 10-fold greater than MS2. In a thin lens situation, solute maximum occurred marginally before H4 phage maximum, the only instance of this occurring during this research. As well, phage H4 RB exceeded 72% illustrating negligible particle attenuation to 10 m distance. Detected transport directions were up to 90 degrees from measured gradient estimates. This degree of evident heterogeneity, the high particulate RB and uncertainty in different flow system locations demonstrated detailed field testing is critical to realistic ground water protection strategies for particulate contaminants.

At Wilerwald (WW), two colloid types (bacteriophages H40, MS2, Psf2 and 1  $\mu\text{m}$  fluorescent microsphere) were injected with uranine. The site's well-channelled heterogeneity had previously been documented with surface geophysics. Injection was done into a preferential flow layer from 4 to 6 m deep with sampling in three wells to 64 m. Prototype instruments were successfully applied under field conditions to provide direct inline measurement of the microspheres (laser detection) and uranine (spectrofluorometry) allowing an optimized sample strategy and minimized test duration and analytical scope. Tracer resolutions were 0.1 microspheres  $\text{mL}^{-1}$ , 0.5 to 1 ppb (uranine) and 0.5 pfu  $\text{mL}^{-1}$  (phages). The four colloids had consistent initial detection at two 'channel' and one 'off-channel' well and particulate velocity was 165 to 285  $\text{m d}^{-1}$ . There was no conclusive evidence of preferential colloid advection. Solute velocity (peak value) in the channel decreased from 135  $\text{m d}^{-1}$  at 14 m to 80  $\text{m d}^{-1}$  at 64 m. To the off channel well at 34 m, it was 40  $\text{m d}^{-1}$ . Colloid maximums (pseudo-peaks) occurred similarly at each well, 1.25- to 2.5-fold earlier than uranine peaks. Phage RBs were high at 14 m (16 to 41%) reflecting relatively open, large and clay-free pores and pathways. They decreased to 0.2 to 0.3% (MS2, Psf2) and 3% (H40) at both 34 and 64 m. Microsphere RBs were 9% at both 14 and 64 m. Phage H40 had 2- to 10-fold higher RB than MS2 and Psf2. Colloid maximum dimensionless concentrations (MDC) were typically 2.5 to 3 orders of magnitude lower than uranine. A dormancy effect for suspended sediment-attached H40 was unexpectedly observed two years after its last use. A numerical interpretation of the response at 64 m was done by matching the data using a 1-D finite difference code (CoTrans), a model based on solute dual porosity zones (mobile/immobile) with particle reversible sorption mechanisms. By matching the colloid breakthrough, the wider range of dispersivity determined by matching the solute data was limited to 2.5 to 3.5 m.

Characterization at Kappelen (KAP) involved development and testing of a new well field installed in a 15 to 20 m deep gravel-dominated deposit located in the prolific Seeland aquifer. Initially, reconnaissance surface geophysics (VLF-EM, RMT) was used to define the nature and extent of the fluvial-glacial gravel aquifer over a 6  $\text{km}^2$  area. Localized RMT work further identified the preferred site in a flat forested area with an underlying silty aquitard. Two piezometers and 16 wells were installed over a 120 by 120 m area at depths in both lower (~10 to 15 m) and upper (~4 to 9 m) zones where the relatively low hydraulic gradient ranged from 0.0005 to 0.001, the hydraulic conductivity varied from  $1\text{e}^{-04}$  to  $1\text{e}^{-02}$   $\text{m s}^{-1}$  and there were partially confined conditions documented in the lower zone. Seven tracer tests were done over 15 months. Bacteriophages and uranine responses were well characterized to 20 m although both zones responded with dissimilar transport direction and velocity. To 60 m, the H40 phage and uranine were only detected at one well in each zone well enough to characterize the component behavior. Phage and solute initial detection times were not sufficiently different to distinguish if particles exhibited preferential advective transport. Solute velocity (peak time) ranged from about 14 to 21 and 3 to 7  $\text{m d}^{-1}$  in the lower and upper zones, respectively. Phage transport velocities (first detection/arrival) were 3- to 8-fold higher. Phage retardation factors were from 0.2 to 0.9 reflecting that phage maximum values commonly occurred 2- to 5-fold earlier than uranine consistent with a diminishing mass attenuated along transport paths. Phage H40 RB was from 0.005% (lower zone - 60 m) to 0.01 to 0.02% (upper and lower zone - 20 m). REDOX conditions in the aquifer caused well bacterial biofouling and likely account for the phage H40 RB at KAP being 10- to 100- fold lower than at BdF and WW.

Biocolloid particle response varied with setting heterogeneity, site geochemistry, matrix mineralogy and microbiology in addition to individual biocolloid properties. Off channel locations exhibited 3- to 10-fold lower recovery than channels. Bacteriophage recovery (RB) ranking in the tank was H40 = or > MS2 > H4 >> H6 >>> Psf2 >>>> T7 and in the field was H40 >> MS2 = Psf2 ~ = or > H4. H40 RBs were 4- to 16-fold higher than for MS2. Where concurrently injected, each bacteriophage was typically detected in the same initial sample implying equal phage advective rates despite their wide recovery range. Phages were not always detected ahead of solutes but in any case, earlier phage detection may directly result from their lower detection levels than solutes rather than from preferential advective flow. Times at which phage maximum concentrations (pseudo-peak) occur at a sampling point can be strongly influenced by attenuation processes along their pathways and therefore, particle maximum concentration should not be comparable to the peaks for solutes. With one exception, phage maximums occurred earlier than solute peaks. However, the phage pseudo-peak timing, as reflected by calculated RF values, was not indicative of the wider variability in recovery/attenuation. On the other hand, RB and MDC values for phage responses were consistent. These parameters should be used for comparative recovery evaluations. Internal circulation provided a continuously mixed system opposite contributing intervals and an effective sampling approach with undisturbed flow dynamics. Real-time on-line solute and microsphere measurement methods and the reduced laboratory phage results feedback greatly improved field execution. Combined use of both solute and particles as tracers increased subsurface definition particularly in strongly heterogeneous settings and provided a consistent basis for evaluating intra-phage behavior when concurrent phage injection cannot be carried out. Particle migration velocity based on particle initial detection time (10 to 45  $\text{m d}^{-1}$  at KAP, 175 to 300  $\text{m d}^{-1}$  at WW, BdF) were 3- to 8-fold higher than the corresponding velocity calculated by applying the conventional approach of using the solute tracer breakthrough peak time. The coherent analytical results together with appropriately applied recovery evaluation methods illustrate that phages, when concurrently injected and sampled appropriately, can be well characterized despite 20-fold or higher RB ranges. This characterization, which can distinguish among and reflect viral transport processes, is recommended in more large-scale aquifer settings to continue development and validation of effective predictive particle transport modeling and related tools.