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Attenuated poliovirus bacteriophage and bromide transport through a coarse-grained aquifer western Montana

Quinn T. Kiley The University of Montana

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Attenuated Poliovirus, Bacteriophage, and Bromide Transport Through a Coarse-Grained Aquifer, Western Montana.

by

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B.S., Washington & Lee University, Lexington, Virginia

Presented in partial fulfillment of the requirements for the

degree of Master of Science.

University of Montana

April, 1997

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Quinn T. Kiley, M.S., April 1997 Geology

Attenuated Poliovirus, Bacteriophage, and Bromide Transport Through a Coarse-Grained Aquifer, Western Montana.

Chairman: Dr. William W. Woessner

Abstract WWW 5-19-97

Microbial contamination of groundwater supply wells causes 50% of the outbreaks associated with waterborne diseases each year. The transport of the bacteriophages MS2, PRD1, \varnothing X174, the attenuated enterovirus poliovirus type-1 **(CHAT strain), and bromide in a cold water, sand and gravel aquifer was studied under natural gradient conditions near Missoula, MT. The average transport velocity for bromide was 25-30m/d. Bacteriophages were observed at concentrations of 10^ PFU/ml 40.5m from the injection well. After 8 hours of transport approximately 97% of the injected attenuated poliovirus and 35-79% of the bacteriophages adsorbed to the aquifer material. Although adsorption occurs, a portion of the viruses appears to act conservatively creating breakthrough curves similar to bromide, though with long tails. Virus were persistent, as seeded viruses were observed 185 days after injection.**

Acknowledgments

I would like to tharik the National Water Resources Institute and the US EPA for their joint funding of this research. The Montana State Fish, Wildlife, and Parks Department was instrumental in allowing us access to the Erskine site, without which none of this work would have been possible.

My utmost thanks goes to Bill Woessner for guiding me throughout this project He gave me a controlling hand at the field site, but was wary of whether or not everything was "under control". Bill's editing, seemingly continuous, I hope has resulted in clear document that truly expresses the breadth and scope of the work we did at the Erskine site. Dan DeBorde, who kept us hydrogeologists from losing sight of the virological aspect of the study, and whose work in the laboratory with PhD candidate Pat Ball was both laborious, productive, and efficient. Johnnie Moore who served on the committee and in the midst of all of his other commitments was willing to help me through my tenure in Missoula.

I would be doing a great injustice if I did not mention the legions of students who aided me in the field and the laboratory, sampling and analyzing, and instrumenting the sight. Lynn Biegelsen, who was always ready for another tracer test. Loreene Skeel and Judy Fitzner who helped me wade through the bureaucracy and stay afloat for two years.

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1. Introduction

Microbial contamination of groundwater supplies causes over half the waterborne disease outbreaks in the United States (Keswick and Gerba, 1980). Wellhead protection from microbial contamination, especially viruses, has been a major topic of research in recent years (Welling et al, 1975; Mathess and Pekdeger, 1981; Pekdeger and Mathess, 1983; Bitton et al, 1984; Yates et al, 1985; Jansons et al, 1989a,b; Bales et al, 1995; Rossi et al, 1994). These studies have led to a greater understanding of the physical and chemical factors controlling the transport and survival of viruses in groundwater. Temperature, pH, adsorption, and dispersion have been identified as major controls of virus fate and transport. Lower groundwater temperature allows for greater persistence of viruses (Yahya et al, 1993; Yates and Yates, 1987). Groundwater pH has been reported to influence virus adsorption. Viruses more readily adsorb to sediments when the groundwater pH is less than 5 and adsorb less effectively when the pH is greater than 5 (Goyal and Gerba, 1979; Bales et al, 1993). In addition to the varying chemical characteristics in an aquifer, one virus strain will adsorb more strongly to an aquifer matrix than another under identical conditions because of the differences in viral surface properties (Goyal and Gerba, 1979). The mechanism of adsorption as described by Gerba(1984) results from the virus adsorbing ions onto its surface layer and then affixing to an oppositely charged medium, the aquifer material The lowering of pH decreases the thickness of the layer of ions, allowing van der Waals forces to effectively bond the virus to the dispersive medium (Gerba, 1984).

Though these basic transport and survival processes have been documented for indicator bacteriophages and some strains of poliovirus in laboratory settings, relatively few multiple virus seeding experiments have been conducted at the field scale (Alhajjar et al, 1987; Jansons et al, 1989a,b; Bales et al, 1989; Bales et al, 1995, Rossi et al, 1994). Unfortunately, field assessments often include insufficient hydrogeologic data to allow reasonable transferability of study results to similar hydrogeologic settings. In addition, research completed in well characterized sand and gravel dominated aquifers is costly (weeks of sampling and complex assay procedures). As a result, well documented virus plumes and peaks have been limited to about a 15m travel distance (Bales et ai, 1995). These limitations have forced regulators assessing the adequacy of existing and proposed set back distances used to protect groundwater supply wells to extrapolate the available data by applying poorly calibrated predictive models (HydroGeoLogic, 1994a, 1994b; Yates and Yates, 1989; Macler, 1995; Macler and Pontius, 1995; U.S. EPA, 1994).

This work documents the behavior of four viruses seeded into a well characterized cold water, highly conductive, unconfined aquifer. The experiment design and site conditions allowed for: 1) rapid collection of tracer data (72 hr.) , 2) control of virus inactivation, 3) detailed resolution of the virus plumes and peak travel times. The hydrogeologic setting was chosen to represent a '"worst case" scenario for virus transport through unfractured porous media, permitting direct observation of transport over the suggested 30m separation between a virus source and a water supply well. The field experiment included the simultaneous injection of the bacteriophages MS2, PRDl, and \varnothing X174, and attenuated poliovirus type-1 (CHAT strain). The CHAT strain of polio is attenuated and not pathogenic. It is similar to the Sabin live vaccine in that it is alive and infectious, but has been altered so as to not cause the disease poliomyelitis. The migration of the viral plume through the sampling well network was monitored for 72hrs. Virus transport was observed over a distance of 40 m, with a 6 log reduction in the titer over that distance.

2.0 Methods

2.1 Site Description

The study was conducted in the grassland flood plain of the Clark Fork River at the Erskine Fishing Access near Missoula, MT. (Figure 1). The shallow, unconfined, flood plain aquifer contains clast supported cobbles and gravel with a medium- to coarsegrained sand matrix to a depth of 6m, where the aquifer material fines and becomes predominantly sand. The hydrologie properties were determined from tracer tests and aquifer tests (Table 1). The water table varied between 2.1 to 2.5m below ground surface. The 10°C groundwater is a calcium bicarbonate type (Appendix B).

2.2 Field Methods

An area of 240m by 285m was instrumented with 89 monitoring wells and 10 staff gauges in low lying areas and sloughs (Appendix C). Seven tracer tests using bromide and rhodamine-wt were used in conjunction with water table maps constructed from monthly head measurements to determine the south westerly flow path in the vicinity of injection well 14 (Appendix D). The multilevel monitoring well network was designed such that the tracer would pass through the arcs of multilevel monitoring wells at distances of 7.5, 19.5, 30, and 40.5 m from injection well 14 (Figure 2). Each multilevel monitoring well was built with 0.5cm diameter high-density polyethylene (HDPE) tubing affixed to a 1.3cm diameter PVC pipe. These sampling ports are 1.8, 2.7, 3.6, and 4.5m below the surface. The tubing

Figure 1. Erskine Research Site near Missoula, MT.

Figure 2. Sampling well network consisting of 20 multilevel wells (M) in arcs 7.5, 19.5, 30, and 40.5m from injection well 14.

Table 2. initial concentration of injected tracers

was perforated over 5cm and screened with nylon mesh (Appendix C). Flexible tubing was dedicated to each piece of HDPE for use with a peristaltic pump.

The multiple virus seeding was preceded one week by a bromide tracer test. In both tests 18.9 liters of groundwater from a background well up gradient from the injection well were used to create the tracer solution. The solution was gravity drained into injection well 14 over a period of 10 to 12 minutes. Injection well 14 is a 3.18cm diameter steel sand point screened from 2.1 to 2.7 m. Initial concentrations of the tracers injected are shown in Table 2. Prior to virus injection, the use of the selected viruses was approved by the University Biohazards Committee, Missoula City-County Health Department, Montana Department of Environmental Quality, and Region 8 EPA. In addition, a Montana Environmental Impact Statement was submitted at the request of the land steward, Montana Department of Fish, Wildlife, and Parks.

Sampling for the tracer experiments covered a 36 hr period for bromide, and a 72 hr period for the virus seeding. Samples were collected with peristaltic pumps from 14 and all 20 multilevel monitoring well ports at the 2.7, 3.6, and 4.5m depths, which corresponded to 0.6, 1.5, and 2.4m below the water table. The sampling schedule was designed to capture expected peak arrivals at each arc of wells. Wells were sampled from expected lowest concentration to expected highest concentration to further reduce the risk of cross contamination. Bromide samples were collected in HDPE 50 ml bottles, filtered $(0.45 \,\mu m)$ and analyzed using a standard ion chromatography technique (Pfaff, 1993). An analytical error of 2% was calculated for the ion chromatography technique used. Bromide concentrations were reported in mg/l to an instrument detection limit of 0.01 mg/l. Virus samples were collected in sterile 50 ml polypropylene tubes, immediately

placed on ice, and transported in ice-filled coolers to the laboratory where they were stored at 4° C.

2.3 Analytical Methods

The coliphages MS2, PRD1, and \varnothing X174 were assayed using host bacteria specific to each virus. A single layer assaying method was employed to assay all three coliphages because of its relative simplicity and efficiency (Adams, 1959). The single agar procedure was performed as follows: 1) host cultures were grown to mid-log phase and placed on ice to quench any further growth; 2) 1 ml of host bacteria was added to 10 ml. of sample (groundwater) and placed in a 37° C water bath for 3 to 5 minutes; 3) 11ml. of soft agar was added to the sample and bacteria mixture; 4) 10ml. of the mixture was plated onto each of two 100mm petri dishes. After the agar sets the dishes were inverted in a 37°C incubator. The titer in plaque forming units per milliliter (PFU/ml) was then determined by counting the number of plaques on the plates. The detection limits for the assay of the bacteriophages is 1 virus in 10ml of sample.

Although not reported in the majority of previously published virus transport papers, there is significant error associated with the infectious assay for bacteriophages. Analysis of 10 duplicate samples from a single sampling port permitted error calculations to confidence levels of 95% . A minimum error of 15% was calculated for the assay of bacteriophages. Error was calculated using the standard method for examinations of water and waste water (Eaton et al, 1995).

Prior to assay for attenuated poliovirus, 5 to 7ml. of field sample were filtered through a 0A5 micron filter and diluted in ELAH at a 1:1 dilution. These samples were stored in 15 mJ polypropylene tubes at -70° C. The use of controls showed that this procedure had no detrimental effects on the virus recovery and did not lower the titer.

The attenuated poliovirus was assayed on 3 to 5 day old Buffalo Oreen Monkey Kidney (BGM) cells that were grown in 25 cm² tissue culture flasks (Smith and Gerba, 1982). The cells were prepared for the assay with the proper adjustments made to compensate for the difference in the volumes of tissue culture flasks. One ml of sample, diluted one to one with ELAH containing antibiotics without calf serum, was added to BGM cells. The inoculum was exposed to the BGM host cells for 90 minutes at room temperature to initiate viral attachment. The 1 ml inoculum was then removed and 10ml. of an agar-medium overlay was added to the flasks. The agar-medium overlay was held in a 41° C water bath during use. After the overlay was added, the flasks were covered to protect them from light and allowed to harden before they were inverted and put in a $37[°]$ C incubator. The flasks were monitored for five days, with plaques counted on a daily basis. The titer was then determined when plaque development was complete. The detection limit of this method is 1 virus in 2ml of sample.

Analytical errors were calculated for the infectious assay of attenuated poliovirus with the same methods used for the bacteriophages. Minimum error is not known, but an estimated minimum of 20% is used here.

A mass balance was performed using the 8 hour data for the virus and bromide plumes. There was no tracer detected at the 3.6m sampling port, 1.5m below the water table. An area bounded by two lines of known concentration was calculated. The concentration of that area was the average of the known concentration boundaries delineating the area. Using an estimated aquifer porosity of 0.20 and assuming the plume was 0.9m thick, the amount of tracer in aqueous phase was determined (Johnson, 1992).

3. Results

A bromide tracer was injected at the water table using well 14 on September 22, 1996. The viruses MS2, PRD1, ØX174, and attenuated poliovirus type-1 (CHAT strain) were also injected at the water table, one week later on October 2, 1996, using well 14 The plume centers for both injections passed through wells M2, M7, M14, and M17. The transport of viruses through groundwater is controlled by all the hydrologie properties of the aquifer, and the sorptive nature of the virus itself. The viruses moving through the aquifer that are not adsorbed onto the aquifer material are affected by mechanical dispersion. The longitudinal dispersivity was determined to be 0.42m using a Peclet number of 18 based on the breakthrough data for well M2, located 7.5m from well 14 (Sauty, 1980). Transverse spreading properties were not calculated.

Plume sizes and peak concentrations varied partly as a function of initial concentration. The plumes for all viruses and Br⁻ showed slight vertical migration, with a maximum of 1.8m over 30m of horizontal transport. The lowest sampling port (4.5m depth) was generally below the plume and served to establish a vertical zero concentration boundary. The 2hr sampling frequency and well locations permitted identification of plume distribution, peak arrivals, and determination of transport rates.

Previously observed dispersion of bromide and virus tracers and their resulting distribution and concentrations at this site suggested sampling over a 36hr period for bromide and a 72hr period for viruses would capture the peak arrivals throughout the sampling network. Virus inactivation was determined to be insignificant in this aquifer over the short duration of the test. A vial filled with groundwater from the site and a known concentration of seeded virus was immersed in an unused well for the duration of the experiment. No change in concentration over the 72hr experiment was detectable.

The concentration of virus injected into 14 declined more rapidly than bromide over time (Figure3). The concentration of bromide declined one log in 28hr, where the poliovirus concentration dropped one log in 5hr. The bacteriophage concentrations declined one log in 15-20hr

The sampling plan effectively captured the tracer concentrations as the plumes moved through each arc of wells, and away from 14 (Figure 3). Peak arrival times at a given well were similar for the four viruses. The bromide peak appears to arrive after the virus peaks during the first 7.5m of transport (Table 3, Figure 4). Due to the error associated with measuring tracer concentrations, peak identification can be difficult. At monitoring well M2 definable virus peaks were observed, but the peak arrival time for bromide cannot be accurately identified. Analysis of breakthrough curve data collected at well M2 suggest that poliovirus is transported faster than bromide and the bacteriophages. The peak arrival of attenuated poliovirus occurs two hours prior to the arrival of the bromide and bacteriophage peaks. Peak arrival times for each tracer could not be distinguished due the over lap of error bars at maximum concentrations at wells M7 and M14 (Figure 5, 6). Therefore, a range of transport rates for the peaks was calculated at these wells (Table 3). A similar approach was used to interpret peak arrivals at well $M17$. Trace concentrations of bromide and attenuated poliovirus were sporadically detected in the wells at the 30m and 40.5m arcs, but breakthrough curves could not be constructed due to paucity of data (Figures 6, 7).

Figure 3. Concentration reduction with time for injection well I4. Virus concentrations in PFU/ml, bromide concentrations in mg/l.

Table 3. Transport velocities (m/d) calculated from breakthrough curves

Figure 4. Breakthrough curves for well M-2,0.6m below water table, 7.5m from 14. Virus concentrations in PFU/ml, bromide concentrations in mg/l.

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Figure 5. Breakthrough curves for well M-7,0.6m below water table, 19.5m from 14. Virus concentrations in PFU/ml, bromide concentrations in mg/l.

Figure 6. Breakthrough curves for well M-14, 0.6m below water table, 30m from I4. Virus concentrations in PFU/ml, bromide concentration in mg/l.

Figure 7. Breakthrough curves for well M-17, 0.6m below water table, 40.5m from I4. Virus concentrations in PFU/ml, bromide concentration in ppm.

Plume sizes and shapes differed between viruses mainly in relation to initial concentrations. Virus plumes exceeding 40m in length and 16m in width were observed throughout the well network at the end of the 72hr sampling period (Appendix D). The plumes follow the same flow path and had similar distributions across the well network. The PRDl plume can be used to represent the distribution of viruses for comparison to the bromide plumes (Figures 8,9). Although little vertical plume migration was observed, an areal plume was defined at a depth of 3.6m (Figure 10). The 72 hr data was used to develop the cross-sections for MS2, PRD1, and \varnothing X174 at their greatest distribution through the well field. The similarity of the cross-sections is such that they can be represented by the PRDl plume (Figure 11). Concentrations at the 2.7m ports, 0.6m below the water table, are higher than those at the 3 .6m ports, 1.5m below the water table, with the exception of those measured at well M13. The highest observed concentrations are at the injection well throughout the experiment.

Plumes were plotted from the data collected 8 hours after injection (Table 4; Figures 12-16). The bromide plume covered an area of 51.8m^2 and represented 87% of the total bromide injected. The MS2 plume detected 8hrs after injection was much larger than the plumes for the other tracers, $1270.6m^2$. The 8hr MS2 plume represents 64.2% of the total MS2 injected, this apparent conservative behavior may be the cause of the large plume. The 8hr PRD1 plume, covering 62.7m^2 and representing 24.4% of the initial virus injected, is much smaller than the MS2 plume. \emptyset X174 is similar to PRD1 in that its plume, 71.1m^2 , represents 21.2% of the initial amount injected suggesting a greater portion of the injectate was adsorbed. The attenuated poliovirus was apparently adsorbed at a faster rate than the other viruses. Only 3% of the poliovirus injected is in aqueous

Figure 8. 72hr PRD-1 plume 0.6m below water table from 10/2/96 virus seeding experiment. Groundwater is flowing from east to west.

Figure 9. 36hr bromide plume 0.6m below water table from 9/22/96 tracer experiment. Groundwater is flowing from east to west

Figure 10. 72hr PRD-1 plume 1.5m below water table from 10/2/96 virus seeding experiment. Groundwater is flowing from east to west Figure 10.

Distance from Injection Well (m)

Figure 11. 72hr PRD-1 plume cross-section from 10/2/96 virus seeding experiment. Flow direction is to the west

Table 4. Percent of tracer adsorbed and in the aqueous phase

Figure 1Z 8hr Bromide plume at 9ft from 9/20/96 tracer test, concentration in mg/l. Flow direction is to the west

Figure 13. 8hr MS2 plume at 9ft depth from 10/2/96 seeding experiment. Concentration in PFU/ml, **flow direction to the west.**

Figure 1 4 8hr PRDl plume at 9ft depth from 10/2/96 seeding experiment Concentration in PFU/ml, flow direction to the west.

Figure15.8hr PhiX174 plume at 9ft depth from KV2/96 seeding experiment Concentrations in PFLVmi, flow direction to the west.

Figure 16. 8hr Pollvirus plume at 9ft depth from 10/2/96 seeding experiment. Concentrations in PFU/mi, fiow direction to the west

phase 8 hours after injection. The larger plume size, $109.4m^2$, may be a result of the difference between assay techniques used for poliovirus and those used for the bacteriophages.

4.0 Discussion

The highest concentrations of tracers were measured in wells I4, M2, M7, M14, and M17. Tracer tests and aquifer tests performed in the well field suggest that there is zone of extremely high hydraulic conductivity, 13,500m/d, intersecting the injection well 14 and monitoring wells M2 and M7. The cause of this zone could be a very coarsegrained buried channel or gravel bar deposit. The wide range of hydraulic conductivity derived from aquifer tests and the depositional environment suggests a heterogeneous flow field. Flow through the sampling network is controlled by a coarse-grained zone of high hydraulic conductivity. This high velocity zone creates a preferential flow path through the sampling network. Such zones are characteristic of high energy, gravel deposits in this region (Miller, 1991; Smith, 1992).

4.1 Comparison of Virus and Bromide Distribution

While bromide and viruses follow the same fiow path; virus plumes are detected over areas much greater than the bromide plume. This is in part a function of our ability to resolve bromide and virus plumes. In an effort to avoid density effects, bromide was injected a 10^3 mg/l and detectable to 0.1 mg/l. Bacteriophages were injected at 10^7 - 10^{10} PFU/ml and detectable to 0.1 PFU/ml, and poliovirus was injected at 10^6 PFU/ml and detectable to 0.5 PFU/ml. This suggests that the use of bromide as a predictive tracer for viral contamination may not be appropriate. The use of bromide to predict virus transport would most likely underestimate the areal extent of viral contamination. However, this study illustrates the utility of the use of bromide to predict virus flow paths and peak transport rates.

4.2 Conservative Virus Sub-Population

Based on breakthrough curve analyses, a portion of the injected viruses were observed to be traveling at average rates similar to the conservative bromide ion. This group of virus have not been retarded by adsorbing to the aquifer material, and appear to behave conservatively. The reasons for this conservative behavior are unknown, but there are several possibilities. There may be a genetic sub-population of viruses that express their genetic differences in their protein coats, yielding different adsorptive properties. This sub-population may be less likely to adsorb to the aquifer material and thus are transported in a conservative manner (Goyal and Gerba, 1979). Another possibility is that the portion of viruses that moves conservatively down gradient may be adsorbing to colloidal material in the groundwater. The viruses could then "piggy back" through the aquifer. These viruses would not be adsorbing differently than those attached to the aquifer material, but would appear to be acting conservatively.

4.3 Comparison of Transport Rates

Virus peaks appear to move at or faster than the average groundwater flow velocity as defined by bromide. This phenomenon has been observed by other workers. Bales et al (1989) documented the bacteriophages MS2 and *f2* traveling at 1.6 to 1.9 times the velocity of conservative tracers through sand columns in the laboratory. Bales et al (1995) reported bromide and PRDl moving at the same rate in a sand and gravel aquifer. If the viruses represented by the peaks identified in the breakthrough curves are behaving conservatively and moving faster than the bromide, further explanation is needed. A difference in effective flow path length for viruses and bromide could account for this disparity. Tortuosity (T) is the relative difference between the observed straight line flow path (L) and the actual interpore flow path (L_e) , such that $T = L_e L$ (Fetter, 1993). *L* is the same for bromide and viruses, however *Le* may be quite different. Bromide is an ion 1.96 angstroms in diameter, and therefore it is subject to flow through tortuous pathways and pore sizes down to the molecular level. Viruses are between 20 and 300nm in diameter and are subject to pore size exclusion (Pekdeger and Mathess, 1983). Some pores that the bromide ion can enter are smaller than the diameter of viruses. The effect of pore size exclusion on an individual virus is macropore flow and lower tortuosity. Pore size exclusion, or filtration, has been identified as a major control of microbial flow (Wood and Ehrlich, 1978; Pekdeger and Mathess, 1983). Viruses flow is thus concentrated through larger pores that may have a shorter effective flow path length (L_e) . This would result in the virus peak arriving before the bromide peak If viruses are adsorbed onto colloidal material, they too would be affected by pore size exclusion.

The hypothesis of pore size exclusion is based on the premise that viruses are indeed being transported through the aquifer faster than the average groundwater flow velocity as defined with bromide. By plotting error bars on the breakthrough curves it becomes obvious that distinct transport velocities cannot be differentiated. Responsible reporting of the data results in ranges of transport velocities that overlap, and therefore one cannot assert that the rates are any different (Table 3).

The exception to this statement is the comparison of transport rates for bromide and attenuated poliovirus. Poliovirus peaks arrive before bromide peaks at wells M2 and M7, 7.5 and 19.5m from injection well 14. The use of standard solute transport analysis would result in a calculated average transport rate for polioviruses that is faster than that calculated for bromide. A plausible mechanism for this faster transport has been previously discussed, however pore size exclusion should affect all the viruses not just poliovirus. Although the attenuated poliovirus peak does arrive before the bromide peak, an alternative explanation is that the poliovirus transport is not actually faster than bromide transport.

Poliovirus adsorbs more readily than the other viruses, as represented by relative concentration plots and mass balances (Figure 17). The breakthrough curves for poliovirus also indicate different adsorptive properties for poliovirus. The tailing effect observed for the bacteriophages is not present in the polio curve. A sharp decline in concentration after the peaks suggests that the poliovirus is adsorbing more completely to the aquifer material than the bacteriophages, and the adsorbed mass of poliovirus is not desorbing as fast as the mass of adsorbed bacteriophages. The strong adsorptive characteristics of attenuated poliovirus manifests itself in the peaks identified in the breakthrough curves. The high percentage of poliovirus adsorbed to the aquifer material and the rapid rate at which it adsorbs limits the amount of attenuated poliovirus in the aqueous phase. If bromide concentration is being affected only by mechanical dispersion, then the differences in plots of C/Co for bromide and poliovirus are due to the rate of poliovirus adsorption. This rate, expressed as C/Co vs. time and plotted as negative values for clarity, is illustrated by the adsorption function in Figure 18. The non-linear rate of poliovirus adsorption vs. Its transport rate would result in a truncation of the breakthrough curve shifting the peak towards the left. The resulting earlier peak will be misinterpreted as an overall faster rate of transport. Without better resolution of virus

Figure 17. Plot of relative concentration (C/Co) v. distance from injection well 14,0.6m below water table in wells 14, M2, M7, M14, and M17.

Figure 18. Relative concentration of attenuated poliovirus and bromide over time, observed at well M2. The adsorption funtion is the difference between the two curves.

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breakthrough curves, it seems more likely the poliovirus is moving at an average rate typical of the bacteriophages.

5.0 Conclusion

A preferential flow path identified in the sampling network appears to result from a coarse-grained zone of high hydraulic conductivity In addition to allowing average transport rate over 30m/d the cold groundwater negated virus die off, allowing it to remain infectious for at least 185 days in the system.

The use of four viruses and bromide to evaluate virus behavior and transport in a sand and gravel aquifer has yielded some interesting findings: 1) the average rate of transport for a portion of seeded virus is as fast as the average groundwater flow velocity defined with bromide, 2) the adsorption and desorption of viruses at different rates may affect observed virus peak arrival times; 3) to properly interpret virus transport the error inherent in infectious assays must be analyzed and reported; 4) each virus demonstrated different adsorptive properties. But perhaps most importantly, the research at the Erskine site has defined the difference between peak arrival times and solute transport rates in respect to viruses. The application of standard solute transport analysis to determine solute transport rates may be inappropriate for virus transport. The use of the breakthrough curve to calculate average transport rate for the solute assumes that the peak represents the average transport of the entire mass. That peak represents a portion of the total virus injected, and in the case of poliovirus it may not properly represent its rate of transport.

This "worst-case" scenario at the Erskine research site documents viruses being transported at faster rates and higher concentrations over distance than has been previously reported. A portion of viruses seeded into this groundwater system moved at an average rate of over 30 m/d. Long tails seen in breakthrough data imply re-release of sorbed virus for large periods of time. This re-release of sorbed viruses affects the virus peaks and contributes to long term survival of the viruses seeded into this system. Hydrogeologically based natural disinfection distances (source well separation distances) would need to exceed the traditional 30m values in this coarse-grained system. The results further suggest that the use of bromide to assess the threat posed by viral contamination would insufficiently represent virus transport in a coarse-grained aquifer.

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Appendix A

Viruses and Health Risks

Viruses are microorganisms, 20 to 300nm in diameter, composed of a genetic core containing RNA or DNA and surrounded by a protein coat, with more complex viruses encased in lipids (Levine, 1992). Viruses can only reproduce in living host cells that they have infected. Viruses can infect animals, plants, and bacteria (bacteriophages). Bacteriophages, first identified by Frederick Twort in 1912, infect and reproduce in bacteria. Three bacteriophages were injected into the aquifer to study virus transport in groundwater. The "phages" pose no threat to human health because they are infectious to bacteria, not animal cells.

Attenuated poliovirus type-1 (CHAT strain) was also used as a viral tracer An attenuated virus is still infectious, but does not produce a pathology or disease in an infected organism. The strain used in this experiment is similar to, but weaker than, the Sabin live vaccine. Because this attenuated \irus is still infectious, cautions were taken in the field to limit exposure to virus laden groundwater. All groundwater pumped during the experiment was collected and chlorinated on site with chlorine bleach.

Modeling

Several attempts to model virus transport have been made (Mills et al, 1991; Alhajjar et al, 1988; Tim and Mostaghimi, 1991). These models have had limited success. Mills et al (1991) developed a colloid transport model, COMET. Viruses range in size from 20 to 300nm, well within the range of colloids, and the model COMET although directed towards solid waste, is still applicable to viruses. Mills work despite being only a few years old, points out the lack of knowledge about colloidal attachment to solids. This is important because attachment is believed to be a major control of virus and colloid transport. COMET deals primarily with predicting the transport of contaminants adsorbed onto colloids, and the mechanisms used in the models may affect viruses and their transport in water. Therefore, COMET may be useful for predicting virus transport. A stochastic model focused on biological tracers was developed by Alhajjar et al (1988). The researchers hoped to use indicator bacteria fecal streptococci and total and fecal coliforms as indicators for the presence of viruses. Field studies demonstrated that these indicators did not travel or survive in a similar fashion to poliovirus, which was also introduced into the system. This study is important because it illustrates the fact that viruses behave differently than other biological tracers. Modeling efforts must specifically geared towards viruses for them to be accurate. \WOTRANS, CANVAS, and VIRALT are models specific to virus transport and use numeric solutions to model virus laden waste water percolating through soils (Tim and Mostaghimi, 1991; HydrGeoLogic, 1994a, 1994b). These models, although designed for virus transport are severely limited by the lack of knowledge about how viruses are transported in varying hydrogeologic settings. Very few common characteristics have been identified that can be applied to different virus types, in fact the behavior of a single virus type may vary from system to system. .

Appendix B

Water Table Variations

The Erskine Fishing Access near Frenchtown, MT. lies in the flood plain of the Clark Fork River. The close proximity of the site to a major river results in a shallow water table that is under the influence of the river stage. To monitor the water table fluctuations, determine direction of flow, and observe the surface water influence on the shallow aquifer, water levels were measured periodically during the study in 44 wells and 10 staff gauges. The wells and staff gauges are noted on each potentiometric map, and can be seen in Figure Bl. A typical potentiometric map generated from these measurements illustrates a westerly flow direction and low gradient (Figure B2). The combination of a continuous water level recorder and periodic water level measurements produced a hydrograph for the Erskine site (Figure B3). Water level measurements were taken from November 1995 to September 1996 and are relative to a 100ft elevation datum on the surface $(Table B1)$.

Hydrologie Properties

The hydrologie properties of the aquifer were derived by two methods including bromide tracer tests and aquifer tests. The tracer tests are described in Appendix D. The data from the September 1996 bromide tracer tests were used to calculate hydraulic conductivity (K) using the equation:

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$$
K = VI/n
$$

where V is velocity, \boldsymbol{n} is estimated porosity, and \boldsymbol{I} is measured hydraulic gradient (Table B2). Aquifer test data was subjected to time-drawdown, recovery, and steady state analysis. Steady state calculations used a version of the Thiem equation:

$$
K = [Q log(r_2/r_1)] + [1.366(h_2^2 - h_1^2)]
$$

where *K* is in m/d, *Q* is pumping rate in m^3/d , r_1 and h_1 are the radial distance in meters and the head in meters measured from the bottom of the aquifer, during pumping, for a near monitoring well, and r_2 and h_2 are for a distant monitoring well (Table B2) (Driscoll, 1986). This equation was used for steady state data from the pumping of well W1 and W2. Another version of the Thiem equation from Driscoll (1986) was used to analyze steady state drawdown in the pumping well for well WO and W3:

$$
K = [Q \log(R/r)] + [1.366(H^{2} - h^{2})]
$$

where the variables are as described above and H is the static head in meters measured from the bottom of the aquifer, *h* is the head in meters measured from the bottom of the aquifer while pumping, *R* is the radius of the cone of depression (estimated to be 30m) and *r* is the radius of the pumping well, all in meters (Table B2).

Time-drawdown data was analyzed using Driscoll's version of the Theis equation rearranged to yield *K* in m/d:

$$
K = (0.183 \text{ Q}) \div (\Delta s \text{ b})
$$

where *As* is drawdown in meters over one log-cycle of time, and *b* is aquifer thickness in meter (Table B3-6, Figures B4-24).

The final analysis on this pumping data focused on water level recovery in the pumping wells $W1$, $W2$, and $W3$. Using the Theis concepts, Driscoll's equation is as follows.

 $K = (0.183 \text{ Q}) \div (s-s^2) b$

where (s-s[']) is the difference between the pumping water level and the recovered water level in meters.

The results of these analyses indicate a heterogeneous flow field over the study site. However, the correlation of the calculated K from the tracer data and the aquifer test at Wl suggest that the hydraulic conductivity between the injection well 14 and Wl is approximately 13,200 m/d. In an attempt to generalize the hydrologie properties of the site, the results from all methods of calculation were pooled. The average *K* over the entire site is 4,000 m/d, with a median value of 1,000 m/d. The *K* calculated for the area from 14 to Wl is likely a zone of high conductivity, closer to the maximum for the site 13,000 m/d than the minimum of 120 m/d.

Figure B1: Wells and staff gauges used in water level measurments

Figure B2. Potentiometric map for 9/24/96 with flow lines, 0.05 countour interval relative to a 100 ft datum.

Figure B3. Hydrograph illustrating yearly water table fluctuation at Erskine site.

Table B1. Water levels measured at the Erskine Site, 11/95 to 3/97

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Table B2. Hydraulic conductivity calculated using steady state analysis.

Hydraulic conductivity calculated from bromide tracer test on 09/20/96.

Hydraulic conductivity calculated in a monitoring well using the steady state Thiem equation.

Pumping W1

Pumping W2

Hydraulic conductivity calculated in the pumping

well using the Thiem equation.

Hydraulic conductivity calculated with the Theis

equation using recovery data.

Table B2. Hydraulic conductivity calculated using steady state analysis.

Figure B4. Time-drawdown plot observed in W1, pumping W1.

Figure B5. Time-drawdown plot observed in SP1, pumping W1.

Figure B6. Time-drawdown plot observed in M7, pumping W1.

Figure B7. Time-drawdown plot observed in M8, pumping W1.

Figure B8. Time-drawdown plot observed in M9, pumping W1.

Figure B9. Time-drawdown plot observed in M5, pumping W1.

Figure B10. Time-drawdown plot observed in M6, pumping W1.

Figure B11. Time-drawdown plot observed in SP16, pumping W1.

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Figure B12. Time-drawdown plot observed in SP24, pumping W1.

Figure B13. Time-drawdown plot observed in W2, pumping W1.

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Figure B14. Time-drawdown plot observed in pumping well W2.

Figure B15. Time-drawdown plot observed in SP24, pumping W2.

Figure B16. Time-drawdown plot observed in SP3, pumping W2.

Figure B17. Time-drawdown plot observed in W1, pumping W2.

Figure B18. Time-drawdown plot observed in M10, pumping W2.

Figure B20. Time-drawdown plot observed in M12, pumping W2.

Figure B21. Time-drawdown plot observed in M13, pumping W2.

Figure B22. Time-drawdown plot observed in M14, pumping W2.

Figure B23. Time-drawdown plot observed in M15, pumping in W2.

Table B6. Time-drawdown data from aquifer test of W3.

Figure B24. Time-drawdown plot observed in pumping well W3.

Appendix C

Site Instrumentation

The Erskine study site was instrumented in several stages using 5 different well designs and various placement strategies (Figure Cl). The initial wells with the designation *EE* were installed to determine the general flow direction, depth to water, and to identify the aquifer material. These wells were installed in June 1995 using a 11.43cm diameter- hollow stem auger, and are located as in Figure C2. The *EE* wells are constructed of 5cm diameter PVC pipe and screened from 3 to 4.5m. The elevation of the top of the casing for each of the *EE* wells, and the elevation of their screened interval are relative to a 100 ft surface elevation datum (Table Cl). The potentiometric map produced from water levels in the *EE* wells indicated a westerly groundwater flow direction.

A tracer field with 36 single level wells was constructed, the wells were driven with a jack-hammer to a depth of $2.7\text{-}3m$ (Figure C1) (Table C1). A 1.9cm steel pipe was driven into the ground and where possible a 1.27cm diameter PVC pipe was inserted into the steel pipe, and the steel pipe was extracted leaving a 1.27cm diameter PVC monitoring well in place. These wells were designated with a P . A row of injection wells was installed with a jack-hammer. The injection wells, designated I , were made from 4.4 cm diameter steel pipe in 0.9m lengths, male threaded at both ends, joined by couplings. Two 0.9m sections of pipe were attached to a 0.75m sand point, screened over 0.6m. Seven tracer tests were performed in the well field (Appendix D).

To develop a more accurate knowledge of the flow path and plumes originating from the injection wells during tracer experiments, and further document the potentiometric surface, 24 sand points were installed with a Geoprobe®. These wells, labeled *SP,* were of similar construction to the injection wells. Two 0.9m lengths of 3.18cm diameter steel pipe were attached with couplings to a 0.9m sand point of the same diameter. The sand points are screened over the entire 0.9m length (Table Cl). The addition of the *SP* wells completed the Phase 1 well network and provided a more accurate measurement of the water table (Figure C1) (Appendix A). Although tracer movement was observed in more detail in these wells, they also identified the need to install a network of multilevel wells before conducting an extensive four virus seeding experiment.

The multilevel wells, M, consisted of a 3m and 1.8m lengths of 1.27cm diameter PVC pipe attached with a PVC coupling and glue. The down hole end of this PVC was perforated for 5cm and wrapped in a screen fashioned from fine mesh paint strainers. Three lengths of 0.5cm diameter HDPE tubing, 2.1, 3.0, 3.9, and 5.1m were attached with steel wire to this main stem of PVC. The HDPE tubing was perforated and screened in the same manner as the PVC piping, over 5cm and covered with screen. To implant the multilevel sampler a drive rod was pushed to a depth of 6.75m. An interior drive rod was extracted and the multilevel sampler was inserted into the outer casing to a depth of 6.75m. The outer casing was then extracted from the hole, taking care to hold the multilevel sampler in place. The assemblage was designed to leave 0.3m of the well out of the ground positioning the sampling ports at 1.8, 2.7, 3.6, and 4.5m below the surface. Upon installing the multilevel well, a 4.8m length of HDPE tubing was inserted down the inside of the 1.27cm PVC stem to facilitate sampling. Nineteen multilevel wells were installed in arcs 7.5, 19.5, 30, and 40.5m, and one placed 0.45m from the injection well.

This Phase 2 well network was used for monitoring the transport of four viruses in a seeding experiment (Figure C2).

Four production wells were installed for use in aquifer tests and later forced gradient tracer tests. These wells, W , are 10.2cm diameter, 1.8m long blank steel casing attached to a 3m long, 40 slot steel screen. A well was placed up gradient to serve as a background well, at 19.5 and 30m in the Phase 2 well network, and down and cross gradient out of the known flow field.

All the wells sampled in tracer experiments had HDPE tubing dedicated to it for sampling purposes. Staff gauges were installed in low lying areas and the slough running to the south of the site to monitor surface water influences on the water table (Figure Cl). The relative elevations of the top of the well casing, top and bottom of the screened interval, or sampling ports; and instrument construction details are listed in Table Cl.

Figure C1. Well design at the Erskine site.

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Figure C2. Phase 1 Well Network at the Erskine Study Site.

Table C1. Instrument Description 89

Instrument Construction Data Sheet Screen Screen Screen Screen

Table C1. Instrument Description and the number of $\frac{90}{2}$

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Figure C3. Phase 2 Well Network consisting og Multilevel and Production Wells

Appendix D

Rhodamine-wt

Tracer tests were the primary investigative tool used in the early stages of this research (Table Dl). The fluorescent dye rhodamine-wt was used during construction of the Phase 1 well network primarily to identify flow paths from the injection wells (Figure Dl). The injectate consisted of 50 to 100ml. of liquid concentrate added to 5 gallons of deionized water. Peak concentrations were observed and transport rates calculated. Rhodamine-wt and its analysis with a fluorimeter is inexpensive and quick, and for this reason it was used 5 times to determine flow paths and rates. Rhodamine-wt is an organic dye and adheres to, or stains, the aquifer material. This adsorptive process retards the transport of the tracer and underestimates average groundwater flow velocities. Another concerns in using rhodamine-wt is its organic nature. Because it is organic and bioavailabe it could possibly affect the survival of viruses once they are seeded into the system. To avoid this possibility, rhodamine-wt was not used immediately before virus seeding experiments. In general, rhodamine-wt is considered one of the most useful dyes for water tracing, and it was successfully used for that purpose in this study (Smart and Laidlaw, 1977).

Sodium Bromide

Sodium bromide, NaBr, is used as a tracer by many hydrogeologists due to its conservative nature (Davis et al, 1980). Bromide occurs naturally at low concentrations in some groundwater systems, but is not detectable at the Erskine site $(\leq 0.1$ mg/l). Bromide

was used to determine flow direction, average groundwater velocity, and hydrologie properties of the aquifer (Figure D2) (Table B2). To avoid density effects observed in the laboratory by Isotok et al (1995) and in field investigations by LeBlanc et al (1991), bromide was injected in concentrations ranging from 1000 to 1500 mg/1. Bromide effectively offers 4 orders of magnitude of resolution when analyzed using ion chromatography, and the high hydraulic conductivity at the Erskine site resulted in rapid dilution of the bromide plume. Due to dilution, the highest peak measured beyond the injection well was on the order of $10¹$ mg/l. Low analytical sensitivity in bromide detection caused an underestimation of plume size and transport distance.

Viruses

Viruses were seeded on three separate occasions and their transport in the groundwater was monitored. To limit the risk associated with viruses, nonpathogenic viruses were used for this study. That is to say that the viruses used would not cause disease in humans. The bacteriophage MS2 was used in all three experiments. A bacteriophage is a virus that infects and reproduces only in bacteria. Unlike bromide and rhodamine-wt, MS2 offers highly sensitive analysis and large travel distances. MS2 was used to study the fate and transport of viruses in a groundwater system. The seeding of MS2 documented that its flow path was the same as bromide and rhodamine-wt. Because the analysis of viruses are very sensitive, one virus must be present in 10ml of sample to be detected, and the high concentrations injected, 10^{10} PFU/ml, the plumes could be identified over a larger breadth and width than other tracers. In order to compare the behavior of different viruses in the same groundwater system the third and final virus seeding included not only MS2, but the bacteriophages PRD1 and \emptyset X174, as well as poliovirus type-J (CHAT strain). The CHAT strain of polio is attenuated and not pathogenic. It is similar to the Sabin live vaccine in that it is alive and infectious, but has been altered so as to not cause the disease poliomyelitis.

The use of viruses, and other microbial tracers, is advantageous because of the high resolution they provide. These tracers are ineffective for determining hydrologic properties because of their large size, at 20-3OOnm in diameter they are subject to pore size exclusion, and sorptive nature they travel at rates other than the average groundwater flow velocity. When used in conjunction with conservative tracers like bromide, virus transport can be quantified relative to the conservative agent.

Summary of Tracer and Seeding Experiments Performed

One bromide and 2 rhodamine-wt tracer tests were conducted in December, 1995. These tests defined flow path variability from the injection locations, but sampling intervals were insufficient for the determination of aquifer properties. A tracer test was performed in March, 1996, with both rhodamine-wt and bromide injected into separate injection wells using a 6 to 12 hr sampling interval. Two weeks later a second bromide tracer test, with 1 to 11 hr sampling intervals, defined the flow path through the well system and was used to design a sampling schedule for a seeding experiment using the bacteriophage MS2. The preferential flow path from injection well 14 observed during this tracer test validated the early potentiometric maps.

The March 1996 MS2 seeding experiment was a test run for future multiple virus seeding experiments **(Figure D3).** However, the large measurable variability of virus concentrations revealed the need for further instrumentation. Twenty-four, 3.2cm steel sand points, screened from 1.8-2.7m, were installed throughout the field site completing a Phase 1 well network.

Further use of rhodamine-wt and bromide injected in well 14 during June and July, 1996 confirmed the need for an extensive multilevel sampling network. Twenty multilevel samplers with sampling ports at 1.8,2.7,3.6, and 4.5m were driven into the aquifer with a Geoprobe®. After installing the first 11 multilevel samplers, a MS2 seeding experiment was conducted to determine additional well locations for the Phase 2 well network (Figure D4). MS2 was used because of its high resolution, with a detectable 11 orders of magnitude of concentration.

Upon completion of the multilevel monitoring wells, a bromide tracer test was performed. This bromide tracer test confirmed the flow path from injection well 14, and yielded the best measurement of hydrologie properties from a tracer test (Table 1). A 72hr plume was detected throughout the network of sampling wells, over an area exceeding 76.6m² (851ft²) 2ft below the water table (Table D2; Figure D5-D6). The plume was not detected below the 3.6m sampling port. The results of this test also provided a conservative transport comparison to the extensive virus seeding experiment that followed. MS2, \emptyset X174, PRD1, and poliovirus type-1 (CHAT strain) were seeded and the rate of transport and plume distribution were observed. At the 2.7m (9ft) depth the MS2, PRD1, and \varnothing X174 the plumes covered area of 357.1m² (1190.3ft²), 267m² (890ft²), and 237m² (790ft²), respectively. The initial concentrations of the tracers varied by orders of magnitude in the injected volume (Table 2). The difference in plume size for each of the viruses seeded reflect the injected volume and the behavior of the viruses in the groundwater system. Plumes were detected and plotted at the 2.7 and 3.6m

depths for MS2, PRD1, and \emptyset X174, and the 9ft depth for polio (Tables D3-6) (Figure $D7-13$).

Longitudinal cross-sections were plotted for the bacteriophages using the wells along the main flow path, I4, M2, M7, M14, and M17. The similarities between these cross-sections illustrates a downward vertical gradient 30m (100ft) from 14 (Figure D14- 16). The three dimensional perspective provided by combining the plume maps and the cross-sections suggests that the plume is narrow and contained during the first 19.5m (65ft) of transport, expanding volumetrically beyond 30m (100ft).

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Table D1. Brief summary of all tracer tests at the Erskine site.

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Figure D1. Rhodamine-wt plumes as detected in Phase 1 well network, flow direction to the west.

Figure D2. Bromide plumes detected in the Phase 1 well network, flow direction to the west.

Figure D3. 60hr plume from 3/28/96 MS2 seeding experiment. Concentrations in PFU/ml, flow direction to the west.

Figure D4. Plume from 8/22/96 MS2 seeding experiment. Concentrations in Log PFU/ml, flow direction to the west.

Figure D5. Bromide Plume at depth of 9ft from 9/20/96 tracer test. Concentration is in mg/l, flow direction is to the west.

Figure D6. Bromide plume at depth of 12ft from 9/20/96 tracer test. Concentration is in mg/l, flow direction is to the west.

Table D2. Tracer test data form bromide injection into well I4, September 20, 1996.

Figure D7.72hr MS2 Plume at 9ft depth from 1Q/2/96 seeding experiment Concentration In PFU/ml, flow direction to the west.

Figure D8.72hr MS2 plume at 12ft depth from 10/2^96 seeding experiment Concentrations in PFU/ml flow direction to the west.

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Figure DIO. 72hr PRD-1 plume at 12ft depth from 10/2/96 seeding experiment Concentration in PFU/ml, flow direction to west

Tracer test data from PRD1 injected into well I4, $^{\rm 120}$ *1,*1996.

flow direction to the west

Figure D12 72hr Phi X174 plume at 12ft depth from 1W2/96 seeding experiment Concentration in PFLVml, flow direction to the west

Table D5. Tracer test data from \varnothing X174 injected into well I4, 128 **October 2, 1996.**

Figure D13. 72hr Poliovirus Plume at 9ft depth from 10/2/96 seeding experiment. Concentrations in PFU/ml, flow direction to the west

Table D6. Tracer test data from poliovirus type-1 injected into well $14,$ 130 October 2,1996.

Table D6. Tracer test data from poliovirus type-1 injected into well 14, 131 **October 2, 1996.**

Table D6. Tracer test data from poliovirus type-1 injected into well I4, October 2, 1996. 132

Table D6. Tracer test data from poliovirus type-1 injected into well 14, 133 October 2, 1996.

Table D6. Tracer test data from poliovirus type-1 injected into well I4, 134 October 2, 1996.

Table D6. Tracer test data from poliovirus type-1 injected into well I4, 135 **O ctob er 2 , 1996.**

Figure D14, 72hr Cross-section of MS2 plume from 10/2/96 seeding experiment. Concentrations in PFU/ml, flow direction to the west

Distance from Injection Well 14 (m)

Figure D15. 72hr Cross-section of PRD1 from 10/2/96 seeding experiment. Concentrations in PFU/ml, flow direction to the west.

Figure D16. 72hr Cross-section of PRD1 from 10/02/96 seeding experiment. Concentration in PFU/ml, flow direction to the west.