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Movement and Longevity of Viruses in the Subsurface

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Background

In order to assist federal and state decision makers, the Applied Research and Technical Support Branch (ARTSB) of the Ground Water and Ecosystems Restoration Division (GWERD) has, since 1989, developed a series of over 30 Ground Water Issue Papers intended to be brief, state-of-science documents focused on a technical issue of expressed interest and prepared in a concise and readable format. The purpose of this Issue Paper is to discuss some of the conditions under which viral contaminants may survive and be transported in the subsurface, identify sources as well as indicators of viral contamination, outline the effects of hydrogeologic settings on viral movement, and introduce the reader to the current state of virus transport modeling along with an example of modeling applications.

The 1986 Safe Drinking Water Act (SDWA) amendments directed EPA to develop national requirements for drinking water disinfection. The legislation required every public water supply system to disinfect unless it fulfills criteria assuring equivalent protection (Macler, 1996). To provide direction for the regulations associated with "acceptable" health risks to the public (Macler, 1996), EPA established maximum contaminant level goals (MCLGs) for pathogenic microorganisms in drinking water, setting a level of zero for viruses (U.S. EPA, 1989a,b). Due to the various technical and economic considerations involved in monitoring water for these MCLs, a "treatment technique" was proposed to reduce or eliminate viruses (Yates et al., 1990). On June 29, 1989, a Surface Water Treatment Rule (SWTR) was published addressing microbial contamination of drinking water from surface sources, or from ground-water sources directly influenced by surface water, with strict provisions for filtration and disinfection (U.S. EPA, 1989a). On January 14, 2002, a SWTR was promulgated with special emphasis on the protozoan *Cryptosporidium* (U.S. EPA, 2002).

The development of a corresponding rule for ground water, the Ground Water Disinfection Rule (GWDR, later designated as the Ground Water Rule), to meet SDWA requirements began in 1987 and led to a published discussion piece (draft GWDR, U.S. EPA, 1992). The deadline for the GWDR proposal was dependent upon completion of studies of the status of public health with respect to the microbial contamination of ground water, based on studies (Abbaszadegan et al., 1999a,b; Lieberman et al., 1994, 1999) to generate a more careful nationwide picture of the problem. As there are significant differences between ground water and surface water in terms of the type and degree of treatment, the GWDR regulatory workgroup realized that the assessment of vulnerability as a function of site specific conditions (i.e., hydrogeology, land use pattern) was a key element to be addressed (Macler, 1996). Subsequently on May 10, 2000, U.S. EPA proposed "...to require a targeted risk-based regulatory strategy for all ground-water systems addressing risks through a multiple barrier approach that relies on five major components: periodic sanitary surveys of ground water systems requiring the evaluation of eight elements and the identification of significant deficiencies; hydrogeological assessments to identify wells sensitive to fecal contamination; source water monitoring for systems drawing from sensitive wells without treatment or with other indications of risk; a requirement for correction of significant deficiencies and fecal contamination (by eliminating the source of contamination, correcting the significant deficiency, providing an alternative source water, or providing a treatment which achieves at least 99.99 percent (4-log) inactivation or removal of viruses), and compliance monitoring to insure disinfection treatment is reliably operated where it is used." (U.S. EPA 2000)

It should be emphasized that this document is not intended for use in establishing the finalized GWDR or for the interpretation of the results of those investigations. To that end, the reader is referred to the Federal Register (U.S. EPA, 2000).

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Introduction

Over 97 percent of all fresh water on earth is ground water and for over 100 million Americans who rely on ground water as their principal source of potable water (Bitton and Gerba, 1984), over 88 million are served by community water systems and 20 million by non-community water systems (U.S. EPA, 2000). Historically, ground water has been considered a safe source of drinking water which required no treatment. It has long been believed that this valuable resource was protected from surface contamination because the upper soil mantle removed pollutants during percolation (Amundson et al., 1988). It was also believed that, even if contaminated, ground water would be purified through adsorption processes and metabolism of indigenous aquifer microflora (Dizer et al., 1984).

As water demands increase, the possibility of artificially recharging ground water with wastewater or surface water will also increase,

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particularly in states like California, where ground water supplies half of the state's fresh water, and Arizona, where ground water supplies all of the fresh water demand. These activities may result in increasing the concern for waterborne diseases; a concern not unwarranted in lieu of the recent worldwide rise in waterborne diseases and a report by the American Academy of Microbiology (Colwell, 1996) indicating that drinking water is not safe microbiologically.

In the United States alone, the annual number of reported illnesses resulting from contact with waterborne pathogens was estimated to be as low as one million and as high as seven million; and between 1971 and 1982, 51 percent of all waterborne disease outbreaks were due to the consumption of contaminated ground water (Craun, 1985). Macler (1995) estimated that approximately 20-25 percent of the United States' ground-water sources are contaminated with microbial pathogens, including more than 100 types of viruses. A literature review by Craun (1989) indicated that approximately one-half of the surface water and ground-water sources tested contained enteric viruses. Even nine percent of the conventionally treated drinking water (coagulation, sedimentation, filtration, post-filtration disinfection using chlorine/ozone) tested positive for enteric viruses (Gerba and Rose, 1993).

Ground water serves as a water source for 93 percent of the communities in Minnesota, with the most extensive use being from karst topography in the southern half of the state. In this type of geology, cracks, sinkholes, and macropores allow rapid percolation of surface water into ground-water reservoirs. The biological contamination of 18 private rural wells during 16 months of sampling showed that 17 out of 18 wells contained detectable levels of indicator bacteria and coliphages (Amundson et al., 1988).

Although water-transmitted human pathogens include various bacteria, protozoa, helminths, and viruses (Bull et al., 1990), agents of major threat to human health are pathogenic protozoa (*Cryptosporidium* and *Giardia*) and enteroviruses (Schijven and Hassanizadeh, 2000). Despite ample information regarding the fate of viruses in the subsurface, research on the persistency of pathogenic protozoa through passage in soil and ground water is just now emerging (Brush et al., 1999; Harter et al., 2000). In the past it was generally believed that the presence of pathogenic protozoa was confined to surface water. Contrary to that expectation, recent monitoring results from 463 ground-water samples collected at 199 sites in 23 of the 48 contiguous states suggested that up to 50 percent of the ground-water sites were positive for *Cryptosporidium, Giardia*, or both, depending on the parasite and the type of ground-water source (vertical wells, springs, infiltration galleries, and horizontal wells) (Hancock et al., 1998).

Viruses are small, obligate, intracellular parasites that infect and sometimes cause a variety of diseases in animals, plants, bacteria, fungi, and algae. Viruses are colloidal particles, negatively charged at high pH (pH 7), ranging in size from 20 nm to 350 nm. The smallest unit of a mature virus is composed of a core of nucleic acid (RNA or DNA) surrounded by a protein coat. With this unique feature of viral structure and colloidal physicochemical properties, the transport of viruses in soil and ground water can act with a combination of characteristics ranging from solutes, colloids, and microorganisms.

Enteroviruses (see Table 1) are a particularly endemic class of waterborne microorganisms which cause a number of ubiquitous illnesses including diarrhea, gastroenteritis, and meningitis, only to name a few. Included in this group are poliovirus, hepatitis type A (HAV), coxsackievirus A and B, and rotavirus. Although gastroenteritis is the most common disease resulting from these microorganisms (Lukasik et al., 1996), other associated illnesses include hepatitis, typhoid fever, mycobacteriosis, pneumonia, and dermatitis (Bull et al., 1990; Sherris et al., 1990; Levine et al., 1991; Payment et al., 1991). Therefore, in addition to the protection of ground-water resources by adequate set-back distances between the sources of contamination and wells for drinking water, the major concern in water treatment facilities is the removal of pathogens prior to consumption.

Since adsorption to soil particles seems to be a significant cause of virus removal (Schijven and Rietveld, 1996; Schijven et al., 1998) and the same processes are applicable to other water-transmitted pathogens to various degrees (Schijven and Hassanizadeh, 2000), viruses are often selected as conservative models for the transport of major biological contaminants in the subsurface. This selection is based on the knowledge that viruses generally travel greater distances than bacteria (Scheuerman et al., 1987) and protozoa due to their relatively small size (see Figure 1), with variations depending on their degree of inactivation and adsorption characteristics (Keswick and Gerba, 1980; Herbold-Paschke, 1991). It should be pointed out that although the disease which is caused by polioviruses has essentially been eradicated in this country, thereby limiting their presence in the environment, much of the historical data is available for this virus since it was often used in transport models as a marker.

Although it goes without question that the United States has one of the safest public drinking water supplies in the world, current and future challenges - like the emergence of new waterborne diseases, varying water source quality, and increased contamination of ground water - must be met with the best available scientific knowledge.

Sources of Viruses

As shown in Figure 2, there are a number of avenues available for the introduction of viruses to the subsurface (i.e., land disposal of untreated and treated wastewater, land spreading of sludge, septic tanks and sewer lines, and landfill leachates), as well as a number of parameters which affect their migration and survival.

Among these, septic systems may pose a significant chemical as well as biological threat to surface and ground waters. According to Canter and Knox (1984), one trillion gallons of septic-tank waste is released into the subsurface annually. Although phosphate and bacteria are ordinarily removed by soil, nitrate and polioviruses (used as the viral marker) may escape these processes and move through the soil into the ground water (Alhajjar et al., 1988; 1990). The presence of viral particles is even more significant in light of studies that indicate they are not necessarily inactivated in septic tanks (Stramer, 1984) and may move into the ground water where they may survive for long periods of time (Dizer and Hagendorf, 1991; Yates et al., 1985). Gerba and Bitton (1984) isolated viruses from ground-water samples as deep as 30 meters and as far as 100 meters from sewage treatment infiltrate basins. Vaughn et al. (1983) isolated septic tank viruses which had traveled 3.6 meters through the unsaturated zone and 67 meters from the source through the saturated zone. At several other sites enteric viruses have migrated laterally in ground water a few hundred meters (Noell, 1992), and Bales et al. (1993) reported that poliovirus used as the viral indicator was detected from a deep well located more than 1000 meters from the apparent source area.

Substantial amounts of excess sludge, which may contain viruses and other pathogenic microorganisms, are generated from wastewater treatment facilities which use activated sludge

Table 1. Water-transmitted Enteroviruses (modified from Bull et al., 1990)

Group	*Pathogen*	*Disease Contracted*
Enteroviruses	Poliovirus	Meningitis, paralysis, fever
	Echovirus	Meningitis, diarrhea, rash, fever, respiratory disease
	Coxsackievirus A	Meningitis, herpangina, fever, respiratory disease
	Coxsackievirus B	Myocarditis, congenital heart anomalies, pleurodynia, respiratory disease, fever, rash, meningitis
	New enteroviruses (types 68-71)	Meningitis, encephalitis, acute hemorrhagic conjunctivitis, fever, respiratory disease
	Hepatitis type A	
	Enterovirus 72	Infectious hepatitis
	Norwalk virus	Diarrhea, vomiting, fever
	Calcivirus	Gastroenteritis
	Astrovirus	Gastroenteritis
	Reovirus	Not clearly established
	Rotavirus	Diarrhea, vomiting
	Adenovirus	Respiratory disease, eye infections, gastroenteritis
	Snow-Mountain Agent	Gastroenteritis
	Epidemic, non-A, non-B hepatitis	Hepatitis

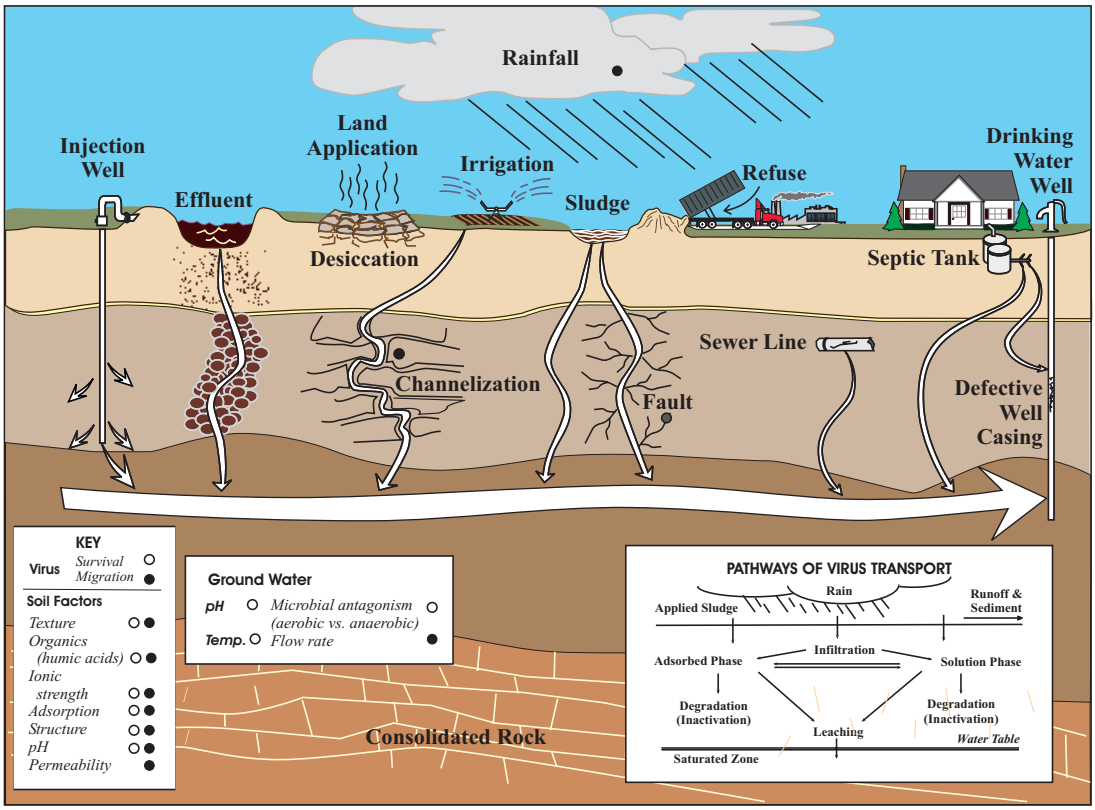

Figure 1. Migration and survival of viruses in the subsurface (modified from Keswick and Gerba, 1980).

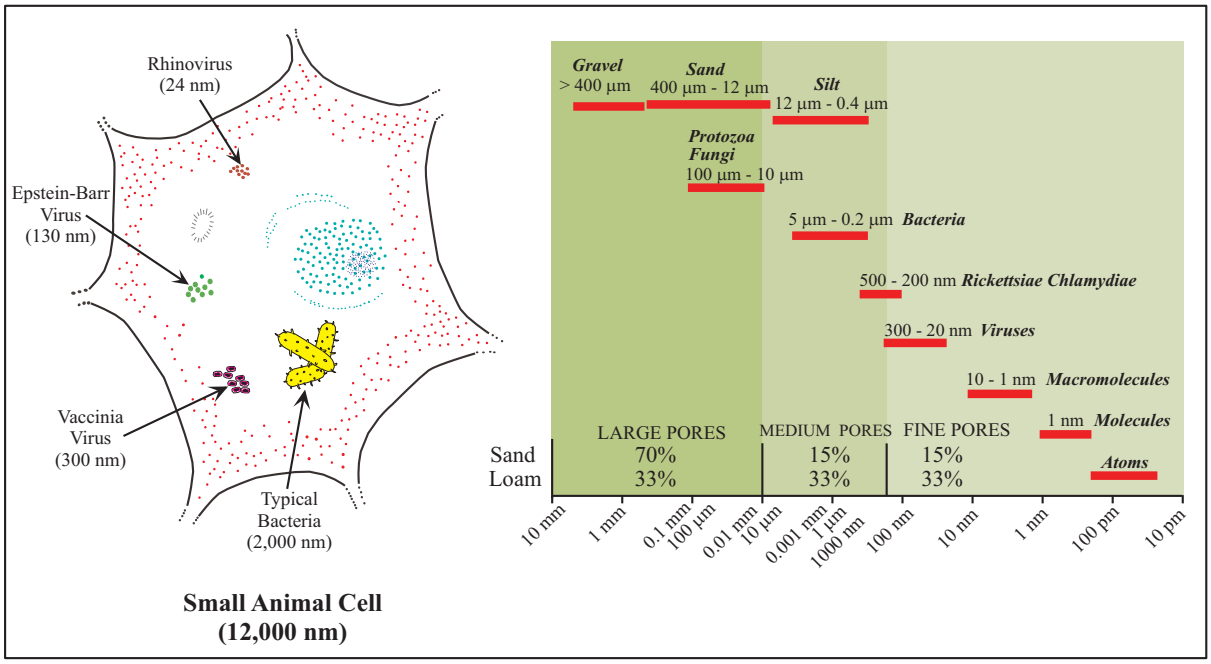

Figure 2. Relative comparison of sizes of microorganisms and molecules with hydraulic equivalent diameters of pore canals (modified from Matthess and Pekdeger, 1981). **Note**: *According to the Soil Science Society of America, definitions of the various grain sizes include: gravel, >2 mm; sand, 2 mm - 50 µm; silt, 50 µm - 2 µm; and clay, <2 µm.*

processes. Since anaerobic sludge digestion is not sufficient for complete viral inactivation, the potential spread of viruses during sludge disposal needs to be considered. Therefore, waste management practices should take into consideration hydraulic loading and contaminant transport characteristics. In this respect, taking advantage of the unsaturated zone as the means for the retention of viruses and source control may become valuable, as was demonstrated by Farrah et al. (1981). It was shown that enteroviruses introduced as tracers were efficiently retained by a sludge soil mixture and were not detected in deep wells located on the sludge disposal site or nearby lagoon. Interestingly, a significant diversity among the enteroviruses toward the sludge soil mixture was seen since, during the initial part of the examination, poliovirus accounted for greater than 90 percent of the viruses in sludge, whereas later, it was determined that echoviruses and coxsackieviruses were the most common enteroviruses identified (Farrah et al., 1981).

Due to the above considerations, the various drinking water standards promulgated since 1975 could be violated by the initial release of treated and untreated wastewater into the environment. Clearly, the microbial concentration of wastewater applied to the land depends on the extent of treatment it receives. For example, in the United States a typical raw sewage contains 7x106 plaque-forming units (PFU)/1,000 liters (Gerba et al., 1975). Primary and secondary wastewater treatment, followed by disinfection, reduces virus concentrations to about 600 PFU/1,000 liters. The application of tertiary treatment followed by disinfection which leads to almost viral free effluent (6PFU/1000 liters) is not a common practice (Vilker et al., 1978).

Indicators of Viruses in Water

Prior to recently obtained data, which indicated a significant risk associated with a low number of enteroviruses in drinking water supplies (Haas, 1983; Haas and Heller, 1990), it was generally believed that coliform bacteria were appropriate and reliable indicators of the sanitary and biological states of aquatic environments. Viral indicators were not used in the past because it was believed that:

- viruses were not normal flora of the intestinal tract, and were excreted only by infected individuals (with the exception of children), usually several orders of magnitude lower than those for coliforms;
- there was a lack of viral detection methods for each of the viral groups of public concern;
- enteric viruses only multiplied within living susceptible cells, and their numbers would be drastically decreased in sewage because of the presence of bacteria, and even further decreased by sewage treatment, dilution, and natural inactivation; and
- although experimentally it had been shown that infection may result from the ingestion of only a very few virus particles, community risk of infection from low level virus contamination has not been determined.

Marzouk et al. (1980) have questioned the validity of bacterial indicators in monitoring the virological quality of water, especially for those countries with high incidence of waterborne illnesses with viral etiology. It has now been established that the bacterial indicator system does not accurately reflect the occurrence of viruses in aquatic environments. Bacterial indicators have a much higher inactivation rate as compared to enteroviruses. Thus the reduction of a bacteria to a safe level by treatment or natural die-off during self purification in natural waters could leave a large number of pathogenic enteric viruses.

Cliver (1971) has proposed the use of human enteroviruses as virological indicators of water and wastewater pollution since they retain their infectious properties for a long period of time. During

the earlier studies, the use of specific enteroviruses, such as polioviruses and/or HAV, has also been proposed due to the frequent isolation of these viruses in sewage contaminated surface waters (Goyal, 1983). It was noted by Payment et al. (1985) that polioviruses can be used as an indicator of enteroviruses on the basis of their persistency.

In practice, the use of HAV as a surrogate for poliovirus was criticized by Metcalf et al. (1978) since hepatitis A is more sensitive to chlorination and would be readily inactivated by water and wastewater treatment. Currently, since the disease which is caused by polioviruses has essentially been eradicated in this country, thereby limiting their presence in sewage, they no longer serve as a natural indicator of sewage contamination. Poliovirus also may not be a suitable index of sewage pollution in those countries where live attenuated poliovirus is used for vaccination (Katzenelson, 1978). Additionally, if used as a single "marker," the transport of poliovirus may be significantly retarded compared to other viruses (Powelson and Gerba, 1993; 1994).

Recognition of the limitations of enteroviruses as the model of viral pollution has led to proposals for using bacteriophages (Stetler, 1984; Havelaar, 1987; Morinigo, 1992.). The phage index offers several advantages because: (1) phage are constant inhabitants of the human intestinal tract; (2) phage are non-invasive to humans; (3) quantitative phage assays are cheap, easy and rapid (Bales et al, 1989; Gerba, 1985); and (4) phage have similar physical properties to enteric viruses (Snowdon and Cliver, 1989). For example, it has been shown that MS-2 is similar to poliovirus in shape and size, 28 nm, and PDR-1 resembles rotavirus in shape and size, 62 nm (Powelson et al., 1990). Both phages survive for long periods of time in ground water and have a low tendency for adsorption to soil surfaces (Yates et al., 1985; Powelson et al., 1990). The use of coliphage as an indicator of water hygiene has been suggested by many investigators (Niemi, 1976; Borrego et al., 1987).

Detection systems have become more specific due to the concern for proliferation of some coliphages in sewage water (Borrego and Cornax, 1990, Snowdon, 1989: Armon and Kott, 1995), and the presence of enteric viruses in the absence of coliphages (Deetz et al., 1984). The use of F-specific phages (Kamiko and Ohgaki, 1992; Nasser and Oman, 1999), and RNA phages of the E morphological groups (Havelaar et al., 1993) has been suggested. These viruses are similar to enteroviruses in morphological characteristics and are only invasive to F-pili carrier bacteria. Adapting the F-specific phages, using *Salmonella typhimurium* WG49 strain (Havelaar et al., 1993) or the combination of fecal streptococci and *E. coli* viruses, has also been proposed as the most promising indicator of remote pollution (Cornax and Morinigo, 1991). Furthermore, in determining the efficiency of a drinking water treatment system, the use of *Clostridium perfringens* and somatic coliphages as indicators of viruses and protozoa cysts has been suggested (Payment and Franco, 1993; Hirata et al., 1991; Geldenhuys and Pretorius, 1989).

Viral Transport and Survival

The ability to determine travel distances and survival times of viruses in the subsurface is crucial for regulatory agencies which are attempting to maintain sources of contamination at sufficient distances from sources of drinking water to protect public health (Keswick, 1982a; Yates et al., 1987). It is a general consensus that the transport of pathogens in the subsurface depends on the extent of their retention on soil particles and their survival. A myriad of studies have been conducted to determine viral transport rates under various experimental conditions. Table 2 summarizes selected studies performed under both laboratory and field conditions. Results shown in Table 2 indicate that solid materials could generally adsorb/retain as much as 95 %, or even more, of the viruses injected into a column. In a column study with breakthrough (Dowd and Pillai, 1997), 79% - 100% of viruses were removed from solution. As in any environmental field investigation, there remains a multiplicity of options with respect to the selection of an appropriate tracer (see Table 2). For example, despite the claim of Yeager and O'Brien (1979) that phages are unsuitable indicators of enterovirus, many others have suggested that phages are easier to work with, and may be more accurately evaluated in quantitative measurements. A thorough review of the earlier literature suggested that polioviruses were used extensively as tracers during transport studies; whereas, the more recent works are focused on bacteriophages.

The major factors which affect viral transport characteristics in the subsurface are provided in Table 3. Among all the factors, temperature appears to be the only well defined parameter causing a predictable effect on viral survival (Yates and Gerba, 1984). A direct relationship between a rise in temperature and viral inactivation rates (K= log inactivated/hr) among various viruses has been suggested (personal communications, C.P. Gerba, 2003). Badawy et al. (1990) stated that during the winter (4-10^{o}C), viral inactivation rates for coliphage, poliovirus, and rotavirus were 0.17, 0.06, and 0.10 per hour, respectively. Whereas, during the summer (36-41^{o}C), the inactivation rates for MS-2, poliovirus, and rotavirus were 0.45, 0.37, and 0.20 per hour, respectively. This study also indicated that viruses may remain viable for 3 to 5 weeks on crops irrigated with sewage effluent; polio and coxsackievirus up to four months on vegetables during commercial and household storage; and up to 30 days on vegetables stored at 4^{o}C. Rhodes et al. (1950), reported a survival of 188 days for poliovirus in river water at 4^{o}C. Interestingly, Blanc and Nasser (1996) reported that HAV survives longer than other enteric viruses at higher temperature. It should be pointed out that this information is based on ambient air. A more direct comparison would be the correlation with temperatures in the subsurface. In this regard the inactivation rates for enteroviruses are 0.06 (10 - 15°C), 0.08 (15 - 20°C), and 0.19 (20 - 25°C).

Microbial ecology may also play an important role in the inactivation of waterborne viruses (Cliver and Herrmann, 1972; Herrmann and Cliver, 1973) especially in surface waters. For example, microbial activity could affect viral survival by the action of proteolytic enzymes of some bacteria (Cliver and Herrmann, 1972) and protozoa (Mose et al., 1970) in destroying the viral capsid protein. In fact, Deng and Cliver (1995) demonstrated rapid inactivation of HAV in the presence of bacteria.

A report by Wellings et al. (1975) claimed that viruses may survive for periods of at least 28 days in ground water. Persistence of enteric viruses in ground water beneath land treatment sites and septic tank discharges has been well documented in a review by Keswick and Gerba (1980) where viral particles were recovered at distances of over 1 kilometer from their source. In an important study performed to monitor viral movement through the soil, Stramer (1984) introduced stool containing poliovirus into septic tanks and detected 220 viral particles per milliliter in a well 53.3 meters away only 12 days after the initial viral introduction. The same author found that the viral particles traveled 4.45 meters per day and persisted for 100 days in ground water after leaving the septic tanks.

Investigations on the persistence of viruses and indicator bacteria in ground water indicate that enteric viruses survive for longer periods of time (Keswick et al., 1982b; Yeager and O'Brien, 1979; and Niemi, 1976) because they are more resistant to environmental conditions (Shuval et al., 1971). To that effect, in an attempt to

Table 2. Virus Transport and Attenuation in the Subsurface

Adsorbent (Depth)	*Influent Loading*	*Effluent Numbers*	*Experimental Conditions*	*Flow Property*	*Virus Removal Capacity*	*Reference*
Virus Attenuation by Laboratory Batch Test						
Activated Carbon	T_4 phage (10^8 PFU/ml)	3.9×10^6 PFU/ml	pH = 6.9, Ionic Strength 0.08 s/l = 1:100 (mg/mL) T = 23 °C	Flask reaction for 24 hours	96% viral removal, removal rate: 0.04 hr^{-1} - 0.8 hr^{-1} in the 1st 12 hrs. and 0.002 hr^{-1} in the 2nd 12 hrs.	Cookson and North (1967)
Sediments, Kaolin, Cellulose, and Carbon Black	QB, fr, MS_2, and T_4 (10^4-10^9 PFU/ml)	10^2-10^8 PFU/ml	pH = 7.2 T = 25 °C	Flask reaction for one hour	Viral adsorption was dependent on surface acidity of the adsorbents	Sakoda et al. (1997)
Virus Transport/Attenuation Through Percolating Laboratory Columns						
5 Soils (30 - 40 cm)	T_2 phage (2×10^7 PFU/ml) T_1 phage (4.8×10^7 PFU/ml)	$< 10^6$ PFU/ml $< 10^6$ PFU/ml	Distilled water and traces of salts pH = 6.3, T = 20 °C	Continuous (0.078-0.313 cm/min)	No virus breakthrough, over 95% viral removal, the highest numbers remained in the top few centimeters of the column	Drewry and Eliasson (1968)
Sandy Forest Soil (20 cm)	*Polio 1* (2×10^7 PFU/ml)	4.8×10^5 PFU/ml	Secondary effluent (pH 7.2) followed by distilled water	Continuous and intermittent	97% viral removal, Polio 1 retention was greater under intermittent flow, breakthrough was only observed with distilled water	Duboise et al. (1976)
Coarse Sand (13 cm)	*Polio 1* (10^5 PFU/ml)	5.0×10^3 PFU/ml 2.5×10^4 PFU/ml	Ground water, sewage effluent pH 8.3, T = 5 °C and 25 °C	Continuous (0.001cm/ min)	No virus breakthrough	Sobsey et al. (1995)
Clay Loam (13 cm)	*Polio 1* (10^5 PFU/ml)	$<0.1 \times 10^3$ PFU/ml (Not detected)	Ground water, sewage effluent pH 4.3, T = 5 and 25 °C	Continuous (Avg = 0.001 cm/min)	No virus breakthrough	Sobsey et al. (1995)
Alluvial aquifer sediments (20 cm)	PRD-1, MS_2 (10^9 PFU/ml)	10^8-10^9 PFU/ml	Ground water and traces of salts pH = 7.3 T = 21 °C	Intermittent (a 2 ml-pulse injection flushed with 6 pore volumes)	79 - 100% removal, breakthrough occurred after 1-2 pore volumes	Dowd and Pillai (1997)
Ottawa sand (10-20 cm)	ϕX-174 bacteriophage	5×10^4 PFU/ml	pH = 7.5 T = 6-9 °C	flow (1.6 -3.4 cm/h)	No breakthrough	Jin et al. (1997)
Field Case Studies of Virus Transport/Attenuation						
Sewage infiltration site soils (silty sand and gravel, 18.3 meters)	f_2 phage (10^5 PFU/ml)	47% of initial loading dropped after 7 hrs.	Settled sewage effluent adjusted to 10^5 PFU/ml	Flow (Avg = 0.6 cm/min)	53% removal, 48 hrs. breakthrough in 18 meter well	Schaub and Sorber (1977)
Flat lands cypress dome soils (sandy with varied clays, 7 meters)	*Enteric viruses: Polio* (71*), *Coxsackie* (75*), *Echo* (30*)	*Polio* (52*), *Coxsackie* (6*), *Echo* (0)	Secondary effluent spray irrigation	Ground water	Removal ratio of 27% for *Polio*, 69% for *Coxsackie*, and100% for *Echo* viruses found in 7 meter deep well after rainfall	Wellings et al. (1975)

* Number of PFU counted in 500 mL sample water.

Table 3. Factors Influencing Virus Fate in Soils

Factor	*Influence on Survival*	*Influence on Migration*
Temperature(	Viruses survive longer at(lower temperatures.(	Unknown.(
Microbial activity(	Some viruses are inactivated(more readily in the presence(of certain microorganisms;(however, adsorption to the(surface of bacteria can be(protective.(	Unknown.(
Moisture content(	Some viruses persist longer(in moist soils than dry soils.(	Generally, virus migration(increases under saturated(flow conditions.(
pH(	Most enteric viruses are(stable over a pH range of 3(to 9; survival may be pro-(longed at near-neutral pH(values.(	Generally, low pH favors(virus adsorption and high(pH results in virus(desorption from soil(particles.(
Salt species and(concentration(	Some viruses are protected(from inactivation by certain(cations; the reverse is also(true.(	Generally, increasing the(concentration of ionic(salts and increasing(cation valencies enhances(virus adsorption.(
Virus association(with soil and other(particulate matter(	In many cases, survival is(prolonged by adsorption to(soil; however, the opposite(has also been observed.(	Virus movement through(the soil is slowed or(prevented by association(with particulates.(
Virus aggregation(	Enhances survival.(	Retards movement.(
Soil properties(	Effects on survival are(probably related to the(degree of virus adsorption.(	Greater virus migration(in coarse-textured soils;(there is a high degree of(virus retention by the(clay fraction of soil.(
Virus type(	Different virus types vary(in their susceptibility to(inactivation by physical,(chemical and biological(factors.(	Virus adsorption to soils(is probably related to(physico-chemical(differences in virus capsid(surfaces.(
Organic matter(	Presence of organic matter(may protect viruses from(inactivation; others have(found that it may reversibly(retard virus infectivity.(	Soluble organic matter(competes with viruses for(adsorption sites on soil(particles.(
Hydraulic conditions(	Unknown.(	Generally, virus migration(increases with increasing(hydraulic loads and flow(rates.(

Modified from Sobsey, 1983.(

monitor the survival of pathogenic microorganisms with ground water collected from a 145-meter deep well in Florida, it was shown that poliovirus type 1 (K=-0.0019) was more stable than *E. coli* or *S. faecalis* (K=0.0012) while coliphage f2 had the highest decay rates. This characteristic is further substantiated by data indicating that both rotaviruses and enteroviruses may be more resistant to chlorination than indicator bacteria (Melnick et al., 1978). In terms of their relative susceptibility, some enteroviruses such as HAV are more stable under adverse environmental conditions than poliovirus 1. The inherent diversity for the longevity of this class of viruses toward factors that affect their survival (i.e., soil type, pH, temperature) is apparent in Table 4. During this assessment, the die-off rate constants were calculated from selected literature which were primarily acquired from ground-water investigations.

The die-off rates in Table 4 represent the time rate of change of the concentration of a microorganism in ground water/soil by assuming the virus die-off follows first-order kinetics. It is noted that die-off rates are also referred to as inactivation, or decay, or survival rates in the literature. Inactivation is a process by which viruses lose their ability to produce progeny (Bitton, 1980; Bitton et al., 1983). Removal rates in solution in batch studies may represent die-off rates of viruses, while removal rates in column or chamber studies may represent attachment/adsorption rates and/or die-off rates (Powelson and Gerba, 1994).

From the case studies examined (i.e., batch, chamber, column and field tests), the following findings were observed.

- Viruses adsorbed on solid surfaces can possess a significantly longer time of activity than viruses suspended in solution. Different inactivation rates in water and on solids were reported. For example, the inactivation at pH 7.2 is 0.055 h^{-1} for *E. coli* phage adsorbed on solids, and is 0.28 h^{-1} for the virus in suspension (Sakoda et al.,1997). However, in many publications the inactivation rates on solids and in solution were not distinguished. The inactivation rates used in studies of virus survival and transport are difficult to interpret.
- Transport of a virus in the subsurface can be controlled by multi-processes, such as advection, dispersion, adsorption, inactivation/decay, etc. Many case studies usually focus on only one or a few processes and ignore others which can be of significance in controlling the transport of viruses.
- Parameters used in transport studies are rarely obtained from independent experiments, and few experiments have been designed to obtain these independent parameters. Examples of the studies developing these independent parameters are Bales et al. (1991) and Dowd et al. (1998).
- Many column studies have been conducted to examine adsorption/inactivation of viruses, but few have been conducted to examine their elution/desorption in columns. Examples of the studies considering these latter processes are Jin et al. (1997), Dowd et al. (1998), and Powelson et al. (1993).
- In many cases, equilibrium adsorption is of little significance, and kinetic sorption with prevailing attachment/sorption appears to control virus removal in the field (e.g., Schijven and Hassanizadeh, 2000).
- Many experimental studies in relation to virus transport have been published; however, relatively few efforts have been made to simulate experimental results. Jin et al. (1997) and Dowd et al. (1998) are two examples of such simulation.

As discussed earlier, viral transport through porous media is controlled by sorption and by inactivation (Bales et al., 1993; 1995; Bitton, 1975; Murray and Laband, 1979). However, adsorption of viruses to soil should not be confused with their inactivation since adsorption is not permanent and can be reversed by the ionic characteristics of the percolating water (Vilker et al., 1978; Bales et al., 1993). Reversible sorption of poliovirus type 1 and coliphage T2 from clay resulted in fully infectious particles (Carlson et al., 1968). Viruses can remain infective after a travel distance of 67 meters vertically and 408 meters horizontally (Keswick and Gerba, 1980). According to Murray and Parks (1980), various forces involved in the attachment of viruses to soil particles may include hydrogen bonding, electrostatic attraction and repulsion, van der Walls forces, and covalent ionic interaction. Bales et al. (1991) demonstrated the importance of solution pH and soil-surface hydrophobicity in attachment and detachment of bacteriophage from solid surfaces. Bales et al. (1993) have shown that low levels of organic matter in porous media can retard viral transport.

Adsorption or release of viruses from soil particles is due to the amphoteric nature of the external viral proteins. Thus, both ionic strength and pH strongly affect the adsorption process (Duboise et al., 1976). Many viruses sorb more strongly in acidic water. Any sharp increase in the pH may enhance the detachment and, therefore, the mobility of the viruses that are attached to the soil matrix. Hydrophobic interactions are also involved in the adsorption of viruses to sands (Dizer et al., 1984). Virus adsorption is significantly influenced by a number of parameters such as the type of virus, soil type, virus load, pH, and salt concentration (Gerba and Bitton, 1984). Although viruses including polio, HAV, reovirus, and coxsackievirus sorb more strongly to clay rather than silt and sand particulate, the extent of sorption of coxsackievirus seems to be limited and without any relationship to the texture of geologic materials. Batch studies with 28 viruses and 9 soil types indicated a wide range of virus adsorption from 0.01 to 99.9 percent (Goyal and Gerba, 1979). The diversity of data reported in the literature makes viral transport modeling difficult (Powelson et al., 1990). According to Yates et al., (1987), modeling capabilities far exceed our current understanding of the behavior of viruses in soil and ground water.

Hydrophobic interactions are apparently also responsible for sorption of viruses at the air-water interface in unsaturated soils (Thompson et al., 1998). Some experimental evidence suggests viruses sorbed at these sites may undergo accelerated inactivation rates (Thompson and Yates, 1999). When viruses are adsorbed to the air-water interface they may be considered to be effectively removed from the transport process (Chu et al., 2001). This is because environmental models have not yet been developed for advection at this surface. Just as virus inactivation may be accelerated at the air-water interface, some have suggested sorption at the solid-water interface may enhance virus longevity (Sim and Chrysiopoulos, 2000). These notions have yet to be rigorously tested experimentally, and as yet, a physical basis for them has not been established.

It should also be noted that most soils have enormous buffering capacity to maintain a pH balance, thereby averting the release of viruses. The soil's organic content can further serve as a retardation factor for some viruses. In general, reoviruses sorb strongly to organic materials as compared to polioviruses and HAV. Vilker et al. (1978) also questioned the results of transport studies based on artificially high initial concentrations of viruses and high water flow rates as compared to those observed in the field (approximately 0.01 cm/min). As expected, the behavior of viruses, as with any other biotic system in the environment is diverse. For example, while Drewry and Eliassen (1968) have shown that percolation through a few meters was sufficient for the removal of viral

Table 4. Die-off Rate Constants (day^{-1}) of Pathogens in the Subsurface

Microorganisms	*Die-off Rate (day^{-1})**	*Environmental Conditions*	*Experimental Methods*	*Reference*
Poliovirus 1	[a] 0.96 [a] 0.52	SW; pH, 8.3; T, 23-27 °C SW; pH, 8.3; T, 4-8 °C	Chamber[#]	O'Brien & Newman (1977)
	0.77 0.21	SW; pH, 7.8; T, 12-20°C GW; pH, 7.8; T, 3-15 °C	Chamber	Keswick et al. (1982b)
	[b] 0.01 [b] 0.02 [b] 0.03	GW; pH, 7.4; T, 10 °C GW; pH, 7.4; T, 20 °C GW; pH, 7.4; T, 30 °C	Batch test	Nasser & Oman (1999)
	0.013 0.07 0.016 0.024	GW saturated loamy soil; T, 10 °C GW saturated loamy soil; T, 23 °C GW saturated sandy soil; T, 10 °C GW saturated sandy soil; T, 23 °C	Batch test	Blanc & Nasser (1996)
	[c]0.51 [c]0.66 [c] > 1.42 [c] > 1.42	GW, sandy soils; pH 8.3; T, 5 °C GW, sandy soils; pH 4.3; T, 25 °C GW, clay loam; pH 8.3; T, 5 °C GW, clay loam; pH 8.3; T, 25 °C	Column test	Sobsey et al. (1995)
Poliovirus 3	1.26	SW; pH, 8.3; T, 23-27 °C	Chamber	O'Brien & Newman (1977)
	1.0	SW; pH, 7.5; T, 9-12 °C	Chamber	Keswick et al. (1982b)
Coxsackievirus A-13 Coxsackievirus B-1	[a] 3.4 0.41	SW; pH, 8.3; T, 23-27 °C SW; pH, 8.3; T, 4-8 °C	Chamber	O'Brien & Newman (1977)
Coxsackievirus B-3	0.19	GW; pH, 7.8; T, 3-15 °C	Chamber	Keswick et al. (1982b)
Coxsackievirus A-9 Coxsackievirus B-3	[b] 2.2 [b] 0.12	Sand-silty soil; pH 7.8, T, 23 °C Sand-silty soil; pH 7.8, T, 23 °C	Batch test	Hurst et al. (1980)
Fecal streptococcus	[b] 0.27	GW; pH, 7.5; T, 9-12 °C	Chamber	McFeters et al. (1974)
	0.23	GW; pH, 7.8; T, 3-15 °C	Chamber	Keswick et al. (1982b)
Fecal Coliforms	[b] 0.45	GW; pH, 7.5; T, 9-12 °C	Chamber	McFeters et al. (1974)
E. coli	0.32	GW; pH, 7.8; T, 3-15 °C	Chamber	Keswick et al. (1982b)
E. coli	[b] 0.001 [b] 0.018 [b] 0.03	GW; pH, 7.4; T, 10 °C GW; pH, 7.4; T, 20 °C GW; pH, 7.4; T, 30 °C	Batch test	Nasser & Oman (1999)
Rotavirus SA-11	0.36	GW; pH, 7.8; T, 3-15 °C	Chamber	Keswick et al. (1982b)
	[b] 0.20	GW; pH, 7.8; T, 23 °C	Batch test	Hurst et al. (1980)
Coliphage f2	0.39	GW; pH, 7.8; T, 3-15 °C	Chamber	Keswick et al. (1982b)
F+ phage	[b] 0.01 [b] 0.02 [b] 0.03	GW; pH, 7.4; T, 10 °C GW; pH, 7.4; T, 20 °C GW; pH, 7.4; T, 30 °C	Batch test	Nasser & Oman (1999)

* as $-\log_{10}$ Ct/Co; GW, Ground Water; SW, Surface Water; BGS, Below ground surface

[a] One log reduction required time (LRT) was used in the reference paper for the inactivation rate.

[b] The values were estimated by curve fitting graphically.

[c] Soil columns (13.3 cm long by 2.5 cm diameter) were each dosed with 13.5 ml of virus-seeded ground water. In 53 days, a total of 16 doses were given to each column. Each dose (13.5 ml) of virus-seeded ground water was kept in a column for about 3.5 days, and then drained. Mean value of the 16 doses was presented in the reference. The values in this table are $\log_{10}$ reduction per day by dividing the mean value by 3.5 (day).

Table 4. continued

Hepatitis A virus	[b] 0.06 [b] 0.016 [b] 0.03	GW; pH, 7.4; T, 10 °C GW; pH, 7.4; T, 20 °C GW; pH, 7.4; T, 30 °C	Batch test	Nasser & Oman (1999)
	0.001 0.01	GW saturated loamy soil; T, 10 °C GW saturated loamy soil; T, 23 °C	Batch test	Blanc & Nasser (1996)
	0.015 0.023	GW saturated sandy soil; T, 10 °C GW saturated sandy soil; T, 23 °C	Batch test	Blanc & Nasser (1996)
	[c]0.42 [c]0.45 [c] > 0.94 [c] > 0.94	GW sandy soils; pH, 8.3; T, 5 °C GW sandy soils; pH, 8.3; T, 25 °C GW clay loam; pH, 8.3; T, 5 °C GW clay loam; pH, 8.3; T, 25 °C	Column test	Sobsey et al. (1995)
MS2 bacteriophage	0.05 0.16	GW saturated loamy soil; T, 10 °C GW saturated loamy soil; T, 23 °C	Batch test	Blanc & Nasser (1996)
	0.12 0.19	GW saturated sandy soil; T, 10 °C GW saturated sandy soil; T, 23 °C	Batch test	Blanc & Nasser (1996)
	0.028 0.053 0.032	N. Carolina GW; pH, 7.9; T,12 °C Arizona GW; pH, 8.2; T, 12 °C New York GW; pH, 7.3; T, 12°C	Batch test	Yates & Gerba (1984)
	[c] > 1.45 [c] > 1.45	Clay loam; pH, 4.3; T, 5 °C Clay loam; pH, 4.3; T, 25 °C	Column test	Sobsey et al. (1995)
	2.24 0.15 0.28	Wetland, 0-3 BGS (m); summer Wetland, 3-70 BGS (m); summer Wetland, 0-70 BGS (m); summer	Field test	Chendorain et al. (1998)
	5.82 0.32 0.57	Wetland, 0-3 BGS (m); winter Wetland, 3-70 BGS (m); winter Wetland, 0-70 BGS (m); winter	Field test	Chendorain et al. (1998)
PRD-1 bacteriophage	0.028 0.026	GW saturated loamy soil; T,10 °C GW saturated loamy soil; T,23 °C	Batch test	Blanc & Nasser (1996)
	0.055 0.034	GW saturated sandy soil; T, 10 °C GW saturated sandy soil; T, 23 °C	Batch test	Blanc & Nasser (1996)
	[d] ≈ 5	Sandy aquifer; pH 5.7; T, 11.5 °C	Field study	Bales et al. (1995)
ϕX-174 bacteriophage	14.2 - 17.3	Ottawa sand saturated with phosphate saline solution (pH = 7.5); T, 6 - 9 °C	Column study	Jin et al. (1997)
MS-2 bacteriophage	0.5 1.8	GW with fresh soil; T, 25 °C GW leached soil; T, 25 °C	Column study	Powelson et al. (1991)
M-1 PRD-1	0 0	Sand (fine-medium grained) Sand (fine-medium grained)	Field study	Bales et al. (1997)

> = virus reduced to limit of detection

[d€] The initial concentration was 1.4×10^7/ml, the breakthrough peak was detected on the 3rd day and the concentration dropped to 50-99 PFU/ml. This is approximately a 5 $\log_{10}$ reduction. Very low concentrations (0.6 - 8 PFU/ml) were detected between the 3rd day to the 24th day.

\# In the chamber test, nucleopore polycarbonate membranes (0.015 mm) were sandwiched between natural rubber gaskets of the plexiglass chamber. Then, the chambers were filled with virus or bacteria suspended in sterile water. The loaded chambers were placed in the bottom of a 10-gallon covered container that had been modified to provide a continuous flow of ground water or in a natural environment. In the chamber test, the loaded chambers were placed in a flowing condition (in a natural stream, in a container with flow, or within a well) while the batch test was conducted in a static condition.

contamination, poliovirus type II used as a "marker" was isolated from a 30 meter deep well located 100 meters from a wastewater drain field in Michigan (Mack et al., 1972). Therefore, current rule-making considerations for the upcoming Ground Water Rule entail a sampling program requirement. Some of the discrepancies in the reported results of these investigations also may be attributed to physical heterogeneity (Harvey et al., 1993) and the earlier methods used for the detection and concentration of enteric viruses which were usually less than 50 percent efficient (Gerba, 1985). The use of reliable current methodologies (i.e., molecular techniques) can, however, minimize the variance between reported and actual numbers. Practices designed to ensure compliance with drinking water standards might more properly rely instead on a cadre of multidisciplinary approaches including predictive models, geological settings that result in viral retention, as well as sampling and analysis. To this end, EPA is aiming, by its proposed Ground Water Rule, to reduce the public health risk related to the ingestion of waterborne pathogens from fecal contamination for a large number of people served by ground water.

In an attempt to demonstrate how to obtain parameters from laboratory experiments which were designed for investigating inactivation and adsorption of viruses, the following case study is offered.

A Case Study

To investigate the influence of inactivation and adsorption mechanisms in water, Rossi and Aragno (1999) presented a batch agitation technique to examine inactivation-adsorption kinetics simultaneously. An initial amount of bacteriophage T7 of about 3×10^5 plaque-forming units (PFU) with and without colloid clay particles was used in the batch study, and the evolution of the amount of bacteriophage was recorded as shown in Figure 3. The inactivation and adsorption mechanisms of viruses in a montmorillonite suspension are mathematically described as:

$$\theta\frac{\delta C}{\delta t}+\rho\frac{\delta S}{\delta t} = \theta\mu_1 C-\rho\mu_s S \quad (1)$$

$$\rho\frac{\delta S}{\delta t} = \theta k_{attach} C-\rho k_{detach} S \quad (2)$$

where:

C is the number of free viruses per unit volume in the aqueous phase,

S is the number of viruses per unit mass of solid in the solid phase,

t is time,

θ is the volume fraction of the aqueous phase,

ρ is solid density in the suspension,

μ_1 and μ_s are the inactivation rate coefficients for free viruses in the aqueous phase and in the attached solid, respectively, and

k_{attach} and k_{detach} are the attachment (adsorption) and detachment (desorption) rate coefficients.

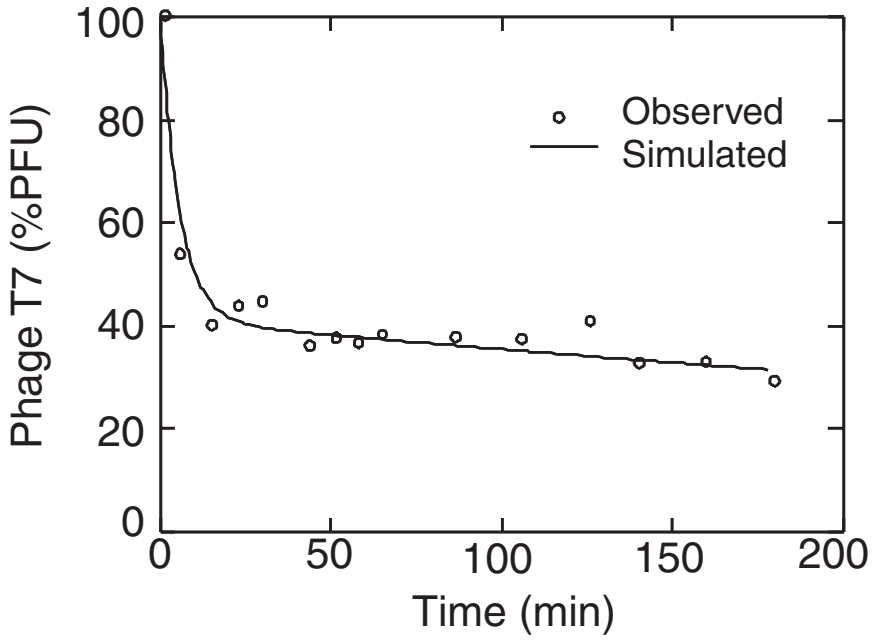

Figure 3. Adsorption and inactivation kinetics of phage T7 in 2.5% montmorilllonite suspension (k_{attach} = 0.10 min^{-1}, k_{detach} = 0.073 min^{-1}, μ_1 = 0.036 min^{-1}, μ_s = 0 min^{-1}). Experimental data are obtained from Rossi and Aragno (1999).

The left-hand side of equation (1) is the time rate of change of viruses in the aqueous phase and in the solid phase, and the right-hand side is the loss of viruses due to inactivation in the aqueous phase and in the solid phase, respectively. Equation (2) states that the time rate of change of the viruses on a solid phase equals the difference between the attachment of viruses from solution to solid and the detachment of the viruses from solid phase to aqueous phase. The system of equations (equations 1 and 2) is solved using a second-order Runge-Kutta algorithm with proper initial conditions when the inactivation rate coefficients and attachment/detachment rate coefficients are known. When experimental data are available, a least-square curve fitting technique is applied to estimate the parameters. Results of Rossi/Aragno parameter estimation indicate that the inactivation rate of viruses on solid phase is not of significance (i.e., $\mu_s = 0$ min^{-1}). The same analyses showed that the other parameters are $k_{attach} = 0.10$ min^{-1}, $k_{detach} = 0.073$ min^{-1}, and $\mu_1 = 0.036$ min^{-1}. In this case, θ is 0.975 and ρ is 0.025 mg/ml. These parameters indicate that virus attachment processes (the term $\theta k_{attach} C$) are much faster than the detachment processes (the term $\rho k_{detach} S$). This is depicted in Figure 3 where the phage T7 concentration dramatically decreases in the early time period.

Effect of Hydrogeologic Settings on Viral Movement

The concentration and loading of viruses and the hydrogeologic setting through which they move will control the potential for viral migration to wells to a much greater extent than biological survivability. A hydrogeologic setting often consists of a soil underlain by unconsolidated deposits of sand, silt, and clay mixtures over rock. The setting further incorporates unsaturated and saturated zones. For purposes of this discussion, the amount of precipitation available to transport the virus through the subsurface will not be considered, although it is recognized that infiltration acts as a transport mechanism as well as a dilution factor.

All other factors being equal, the persistence of viruses at a well, or other source of water, is most likely where saturated flow transports large concentrations of the particles along short flow paths through media which contribute little to attenuation. Although the interrelated processes that control viral movement and persistence in the subsurface are not completely understood (Cadmus Group, et. al., 2000), some of the major hydrogeological factors that can be used to evaluate the potential for viral presence in ground-water wells include:

- transport mechanisms (unsaturated versus saturated flow conditions);
- type of media through which the virus will travel (clays versus sands versus fractured media);
- length of flow path to the extraction point (well); and
- time of travel.

Hydrogeologic settings with shallow water tables are more susceptible to viral transport. Viruses are attenuated or immobilized by processes such as dessication, microbial activity, and stagnation. Further, viruses commonly bind to soil particles, fine-grained materials, and organic matter. The lower transport velocities associated with unsaturated conditions (e.g., move, stop, move cycle) allow these processes more time to occur. If viruses are introduced directly into the water table (such as from leaching tile fields associated with onsite sewage disposal) or if the volume of contaminants can maintain saturated flow conditions (such as in some artificial recharge situations), the potential for contamination is increased (Aller et. al., 1987). Where the viral concentration is high, the probability of contaminant migration is increased regardless of the hydrogeologic setting. Therefore, in hydrogeologic settings with deeper water tables and where contaminants are not introduced into the aquifer through saturated flow conditions, viruses are much less likely to survive being transported to a well.

Hydrogeologic settings with interconnected fractures or large interconnected void spaces that lack fine-grained materials have a greater potential for viral transport and well contamination. Karst aquifers, fractured bedrock and gravel aquifers have been identified in the proposed Ground-Water Rule as sensitive hydrogeologic settings (U.S. EPA, 2000). In these settings, fractures and large void spaces allow rapid transport through the aquifer, thereby reducing the amount of time and particulate contact available for attenuation. Potential interaction with rock walls along fractures is reduced, and contact with fine-grained materials for potential sorption sites is minimal.

Similar to fractured rock aquifers, gravel aquifers with only a small, fine-grained fraction have little potential for viral sorption. However, as the amount of fine-grained material increases, effective grain size decreases, the potential for sorption increases, and travel times decrease. Finer-grained aquifers and aquifers where void spaces are less interconnected or smaller are, therefore, less likely to transport viruses significant distances.

The potential for physical viral removal by filtration also appears to increase as grain size becomes smaller, although the filtration processes are not well understood due to their size. However, filtration of bacteria, which are larger than viruses, has been shown to be an effective removal mechanism.

Hydrogeologic settings where fractures are not as interconnected or where more tortuous flow paths must be followed to reach a well also allow for greater viral removal. For example, in many rock aquifers, ground-water flow follows bedding planes that may result in an elongated, indirect pathway to a well. In other rock aquifers, flow must travel around and through cemented portions of the matrix thereby increasing the flow path. Similarly, sand and gravel aquifers with fine-grained materials in the matrix will have less direct flow paths as the water flows around the finer-grained materials. Generally, it can be stated that tortuosity increases the length of the flow path and decreases the hydraulic conductivity, thus decreasing viral survival. Where finer-grained materials are present or fractures are less interconnected, flow paths are also longer, thereby offering some protection to wells in more permeable units.

Hydrogeologic settings where time of travel is short have a greater potential for viral contamination. Where less permeable units (called aquitards) restrict or reduce vertical flow to underlying aquifers, time of travel is increased. Although inactivation rates have been shown to be extremely variable, time is a major factor affecting virus viability.

Due to the importance of hydrogeologic settings, the proposed Ground Water Rule thoroughly addresses this issue to identify wells that are sensitive to fecal contamination. A component of the proposed Ground Water Rule requires states to perform hydrogeologic assessments for the systems that distribute ground water that is not disinfected (source waters that are not treated to provide 99.99% removal or inactivation of viruses). The states are required to identify sensitive hydrogeologic settings and to perform monitoring for indicators of fecal contamination from sensitive hydrogeologic settings (see U.S. EPA, 2000, for the complete proposed strategy).

Virus Transport Modeling

One method of addressing regulations associated with virus exposure, such as ground-water disinfection, the application of liquid and solid waste to the land, and wellhead protection zones, is the application of predictive virus transport models.

The states may choose to employ fate-and-transport models as screening tools to identify hydrogeologic barriers for a particular water supply aquifer. (U.S. EPA defines a hydrogeologic barrier as the physical, biological, and chemical factors, singularly or in combination, that prevent the movement of viable pathogens from a contaminated source to a public supply well.) To this end, the subject of modeling will become pertinent and will be discussed herein. Like most predictive modeling efforts, the results depend on the conceptual basis of the model as well as the quality and availability of input data (Corapcioglu and Haridas, 1984). Clearly, a thorough understanding of the processes and parameters associated with virus transport are essential elements in their application.

As shown in considerable detail in Table 3, some of the more important subsurface virus transport factors include soil water content and temperature, sorption and desorption, pH, salt content, organic content of the soil and ground-water matrix, virus type and activity, and hydraulic stresses. Berger (1994) indicated that the inactivation rate of viruses is probably the single most important parameter governing virus fate and transport in ground water.

Some of the existing models require only a few of these parameters which limit their use to screening level activities, while others require input information which is rarely available at field scale and is usually applied in a research setting. One limitation of most models is that they have been developed for use in the saturated zone. It has been shown, however, that the potential for virus removal is greater in the unsaturated zone than in ground water (Gelhar, 1992).

Despite the number of models developed at present, tests of the models against field data are not abundant. Simulation results of the developed models were either compared to the analytical solutions or fitted to data obtained for laboratory experiments. Even though some models were developed to handle the complex processes involved in virus transport, only simplified simulation results were compared against ideally controlled experimental conditions (Vilker and Burge, 1980; Matthess and Pekdeger, 1981; Tim and Mostaghimi, 1991; Teutsch et al., 1991).

The existing codes for virus transport can be placed into two categories. As shown in Table 5, the first group contains computer codes which are readily available to the public and which have user's manuals. The second group, shown in Table 6, contain computer codes which were developed for research purposes. Better understanding of virus transport mechanisms was the main motivation in developing these codes, rather than public dissemination. As a result, further discussions herein will be limited to the models in Table 5.

VIRALT, developed for EPA's Office of Drinking Water, is a modular, semi-analytical and numerical code that simulates the transport and fate of viruses in ground water. The code computes viral concentrations in extracted water describing both steady-state and transient transport including advection and dispersion in the vertical direction in the unsaturated zone. Along ground-water flow lines in the saturated zone it handles adsorption and inactivation.

CANVAS was developed in order to improve on its predecessor, VIRALT. The major enhancements implemented in CANVAS are:

- CANVAS can simulate multiple contaminant sources in the unsaturated or saturated zones whereas VIRALT is limited to a single source;
- transverse, as well as longitudinal and horizontal dispersion in the saturated zone is simulated by CANVAS whereas VIRALT is limited to longitudinal dispersion;
- a colloidal filtration term is designed to simulate the facilitated transport of viral particles through the unsaturated and saturated zones; and

Table 5. Publicly Available Virus Transport Codes: Group I

Program Name	Year	Authors	Description	Remarks
VIRULO v. 1.0	2002	Faulkner et al. @ U.S. EPA-ORD	A Monte Carlo-based screening model for predicting total virus mass attenuation in the unsaturated zone. Processes Considered: advection, dispersion, sorption, inactivation, and uncertainty.	Developed by EPA-ORD
VIRALT v. 3.0	1994	Park et al. @ Hydro-Geologic	A modular semi-analytic and numerical code for transport and fate of viruses in the unsaturated zones. Processes Considered: advection, dispersion, sorption, and inactivation.	Developed for EPA
CANVAS v. 2.0	1994	Park et al. @ Hydro-Geologic	A modular semi-analytical and numerical code for transport and fate of viruses in the unsaturated and saturated zones. Processes Considered: advection, dispersion, sorption, inactivation, and colloidal filtration.	Descendant of VIRALT
VIRTUS v. 1.0	1991	Yates et al. @ U.S. Salinity Laboratory	A numerical code for transport and fate of viruses in the unsaturated zone. The virus transport is coupled with the flow of water and heat through soil. Processes Considered: advection, dispersion, sorption, and inactivation.	Research-oriented code

Table 6. Other Virus Transport Codes (Developed for Research Purposes): Group II

Authors	Title of Research Paper	Journal	Solution Method	Processes Considered	Medium
Chu et al., 2001	Mechanisms of virus removal during transport in unsaturated porous media	WRR 37(2)	FDM	Advection, dispersion, mass-transfer, adsorption, and blocking	Un-saturated 1-D
Sim & Chrysikopoulos, 2000.	Virus transport in unsaturated porous media	WRR 36(1)	FDM	Advection, dispersion, adsorption, and mass-transfer	Un-saturated 1-D
Lindqvist et al., 1994	A kinetic model for cell density dependent bacterial transport in porous media.	WRR 30(12)	FDM & ANAL	Advection, dispersion, and non-equilibrium sorption	Saturated 1-D
Tan et al., 1994	Transport of bacteria in an aquifer sand: Experiments and model simulations.	WRR 30(2)	FDM	Advection, dispersion, and sorption (max retention capacity included)	Saturated 1-D
Hornberger et al., 1992	Bacterial transport in porous media: Evaluation of a model using laboratory observations.	WWR 28(3)	ANAL	Advection, dispersion, and clogging/declogging	Saturated 1-D
Tan et al., 1992	Transport of bacteria during unsaturated soil water flow.	SSSAJ 56(5)	Quasi-ANAL	Dispersion and sorption	Un-saturated 1-D
Harvey & Garabedian, 1991	Use of colloid filtration theory in modeling the movement of bacteria through a contaminated sandy aquifer.	ES&T 25 (1)	ANAL	Advection, dispersion, sorption, and filtration	Saturated 1-D
Lindqvist & Bengtsson, 1991	Dispersal dynamics of ground-water bacteria.	ME 21(1)	ANAL	Advection, dispersion, non-equilibrium sorption, and decay	Saturated Sand Column
Tim & Mostaghimi, 1991	Model for predicting virus movement through soils.	Ground Water 29(2)	FEM	Advection, dispersion, linear equilibrium, sorption, and first order decay. Program Name: VIROTRANS	Un-saturated Soil
Taylor & Jaffe, 1990	Saturated and biomass transport in a porous medium.	WRR 26(9)	FEM	Advection, dispersion, sorption, growth/decay, and shear/filtration. The change in parameter values due to biofilm clogging was included.	Saturated Column
Matthess et al., 1988	Persistence and transport of bacteria and virus in ground water - A conceptual evaluation.	JCH 2(2)	ANAL	Advection, dispersion, sorption, and filtration.	Saturated 1-D
Corapcioglu & Haridas, 1985	Microbial transport in soils and ground water: A numerical model.	AWR 8(188)	ANAL, FEM	Advection, dispersion, sorption, decay/growth, and clogging/declogging. Transport equation is coupled with nutrient concentration.	Saturated 1-D & 2-D
Matthess & Pekdeger, 1981	Concepts of a survival and transport model of pathogenic bacteria and viruses in ground water.	STE 21(149)	Not Clear	Discussion on controlling factors for bacteria/virus transport.	Saturated medium
Vilker & Burge 1980	Adsorption mass transfer model for virus transport in soils.	WR 14(783)	ANAL	Adsorption mass transfer model.	Batch & Column
Vilker et al., 1978	Application of ion exchange/adsorption models to virus transport in percolating beds.	AIChE 178(84)	ANAL	Ion exchange/adsorption	Saturated Column

WRR: Water Resources Research
ME: Microbial Ecology
JCH: Jour. of Contaminant Hydrol.
ES&T: Environ. Sci. & Tech.
SSSJ: Soil Sciences Society of America Jour.
WR: Water Research
AWR: Advances in Water Resources
STE: Science of the Total Environment
ANAL: Analytical
FEM: Finite Element Method
FDM: Finite Difference Method
AIChE: Am. Inst. for Chem. Eng. Symp. Ser.

- allows the virus inactivation coefficient to be either temperature-dependent or given as a user-specific value.

VIRTUS is a finite difference model for virus fate and transport in unsaturated soil. The model allows the virus inactivation rate to vary based on soil temperature. It supports unsteady flow, transport in layered soils, different inactivation rates for adsorbed versus freely suspended viral particles, and the flow of heat through soil. It assumes that viruses are introduced at the soil surface. VIRTUS is based on mass conservation of a contaminant in porous media and couples the flow of water, viruses, and heat through the soil. The model can be used to estimate the number of viruses that reach ground water after traveling through soil from a contamination source.

VIRULO was developed to fill the need for a predictive screening model. It uses the assumption of gravity flow infiltration and time averaging to solve governing equations for advection, dispersion, adsorption, and mass-transfer developed by Sim and Chrysikopoulos (2000). This model was produced with the idea that in many cases very little information may be known about a particular site. It supplies a small database of default parameters for water flow and virus transport. At a minimum, the user needs only to select one of the twelve USDA soil categories, a virus whose attenuation will be predicted, and a thickness of a soil bed of interest. The Monte Carlo method is employed with known or assumed distribution functions for the input parameters. Random number generators are used internally, and a graphical display of the histogram of predicted attenuations is produced, along with the number of times a user-specified level of attenuation was not achieved in a given number of simulations (1,000,000 by default).

Virus transport modeling is inherently fraught with uncertainty. It has been suggested that models have a tendency to under-predict virus transport, and hence their use as a sole criterion for purposes of determining regulatory compliance is questionable (Yates and Jury, 1995). Current rule-making considerations for the forthcoming Ground Water Rule consider modeling as a potential tool which can be useful, but a sampling program will always be a requirement.

Example Application of the *VIRULO* Screening Model

The use of municipal sewage effluent for irrigation is a growing trend in urban and suburban areas. It represents an attractive means by which water that has undergone treatment, but not to a level making it suitable for open distribution, can fulfill a need at greatly reduced costs. This is because the soil above the ground-water table through which irrigation water percolates can be viewed as a natural means of filtration. In a completely engineered system, the final stages of treatment to a level suitable for human consumption are very costly. Depending on the degree of disinfection applied to the effluent, viruses may have undergone little or no attenuation prior to irrigation. Therefore, there is a potential for viral contamination to underlying aquifers if natural filtration above the ground-water table is not sufficient to remove viable viruses. U.S. EPA Region VI recently conducted pilot Comprehensive Performance Evaluations for ground water for selected water supply systems in Texas and New Mexico (e.g., U.S. EPA, 2000). In these evaluations, the ground-water system itself is considered as a component of the overall water supply system performance. The evaluations frequently cite a goal of achieving “99.99% virus inactivation,” in order for the ground-water system to be considered acceptable.

These notions have implications for planning. Parks and golf courses are the most common sites of irrigation with municipal sewage effluent. In a gross sense, information from soil surveys, along with a screening model, can provide an indication of the level of risk of viral contamination (to the ground-water table) a particular site may have. Figure 4 shows a map of a portion of Wake County, North Carolina, which contains the city of Raleigh and its suburbs. The areas shown outlined in black represent locations of park lands and open space which might be suitable for irrigation with municipal sewage effluent.

The *VIRULO* screening model treats the total cumulative mass attenuation of viruses probabilistically. It contains a small database of input parameters describing soil properties that control rate of water percolation (Figure 5) and virus properties that control sorption, equilibrium partitioning between suspension and adsorption to soil particles, and inactivation rates (Figure 6). As shown in Figures 5 and 6, the parameters are grouped by default according to USDA soil type and virus of interest. The database was built from information in the USDA’s *UNSODA* database, managed by the U.S. Agricultural Research Service, and from an extensive literature search of experiments for virus behavior in soils. All parameters were assumed to be either normally or log-normally distributed, as determined by examination of histograms of experimental outcomes. VIRULO uses the Monte Carlo method with computer generated random numbers conditioned on the parameters to produce outputs of total time-integrated mass attenuation, defined as the average total amount of viable viruses leaving the bottom of a soil bed divided by the total amount arriving at the top of the soil bed (Figure 7). VIRULO presents the outcomes in a histogram of values of minus the base-10 logarithm of attenuation (Figure 8).

The soil survey for Wake County includes listings of the seasonal high water table, which depends on soil type as well as geographic location (proximity to streams and topography). The soil survey data can be used as input to the VIRULO model with the default parameters to get a spatially explicit representation of the possible level of risk associated with irrigating in a particular park or open-space area, as shown in Figure 9. The information produced is far from being definitive because of seasonal variability and soil in-homogeneity, but it can serve as a guide to highlight areas of concern. The assumptions used in VIRULO and the conditions under which large error may be incurred from employing them are listed in Table 7.

One reason predictive modeling of viral fate and transport is especially difficult is the fact that only one or two virus particles can infect any human who ingests them. Thus the margin for error is very small, and even very low probabilities of virus persistence may still represent cause for concern.

Setback Distances

Traditionally, state and county regulators have established fixed setback distances for all geologic settings in their jurisdictions. For example, the distance between a septic tank and a private well would, in many instances, be as little as 50 feet and would apply for tight clays as well as fractured rock. It would apply to areas where the water table was near the surface as well as at considerable depth. As discussed in this document, the travel time or transport distance of viral particles depends on a number of factors including moisture content, geological setting, type and depth of the soil overburden, and source loading, only to name a few.

Frequently, guidelines established as minimum distances became so standard that a well was often positioned precisely 50 feet from the septic tank. In the survey conducted as part of the proposed Ground-Water Rule, setback distances were found to be quite variable (U.S. EPA, 2000). Some of the distances were presumably based on scientific principles, while others were holdovers from past practices.

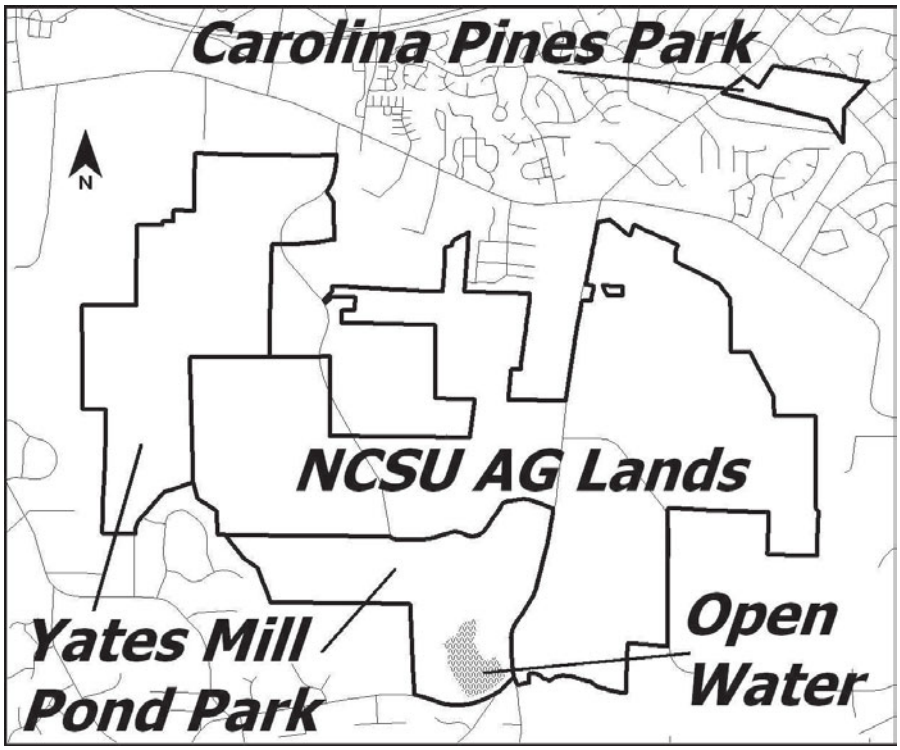

Figure 4. Map of portion of Wake County, North Carolina.

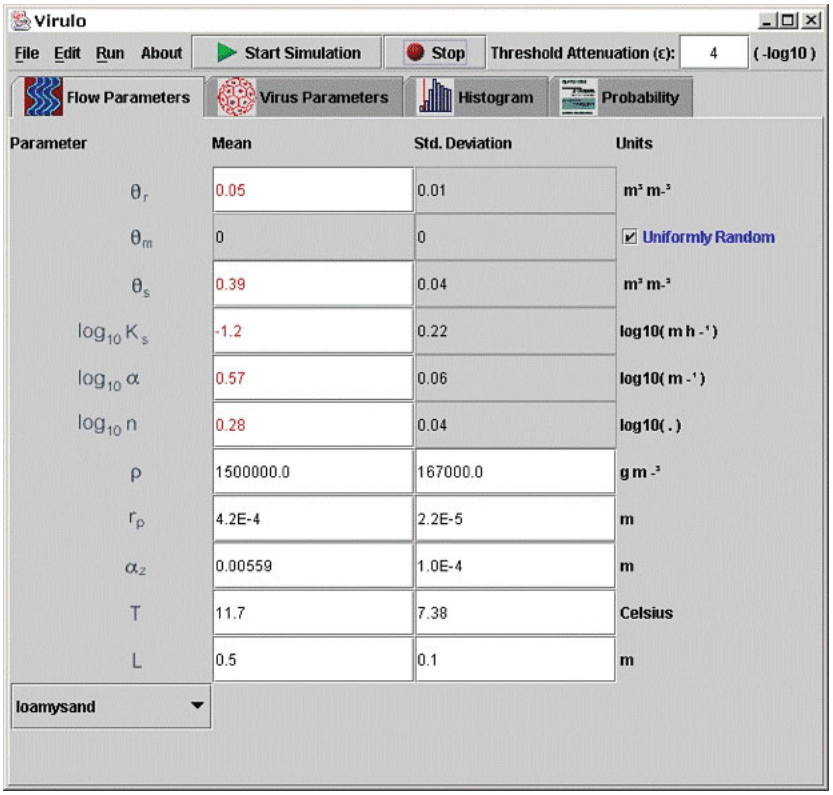

Figure 5. Flow parameters input panel in *VIRULO.*

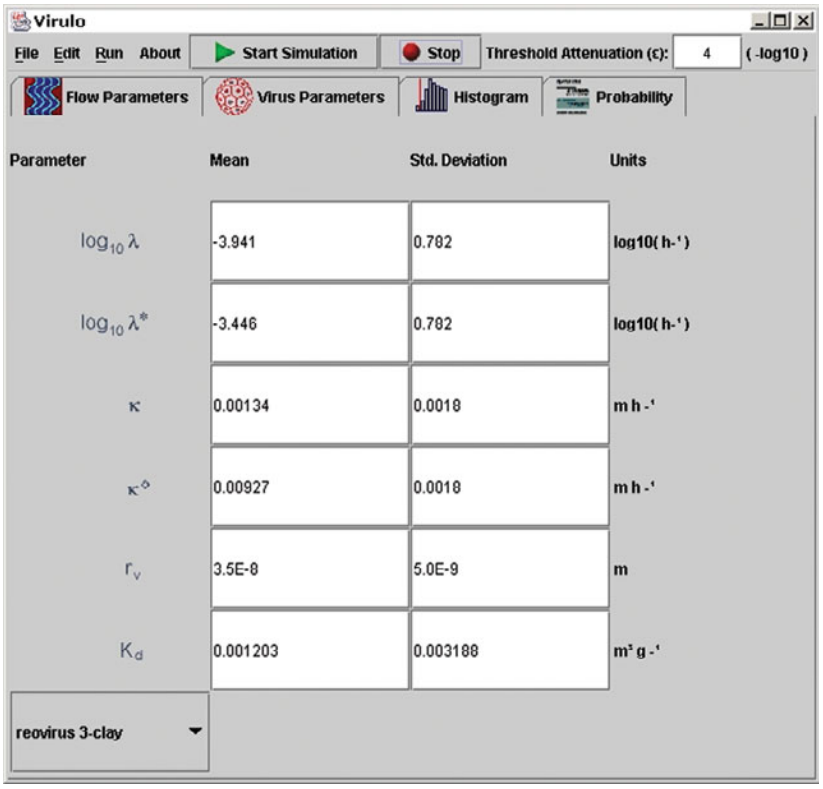

Figure 6. Virus parameters input panel in *VIRULO*.

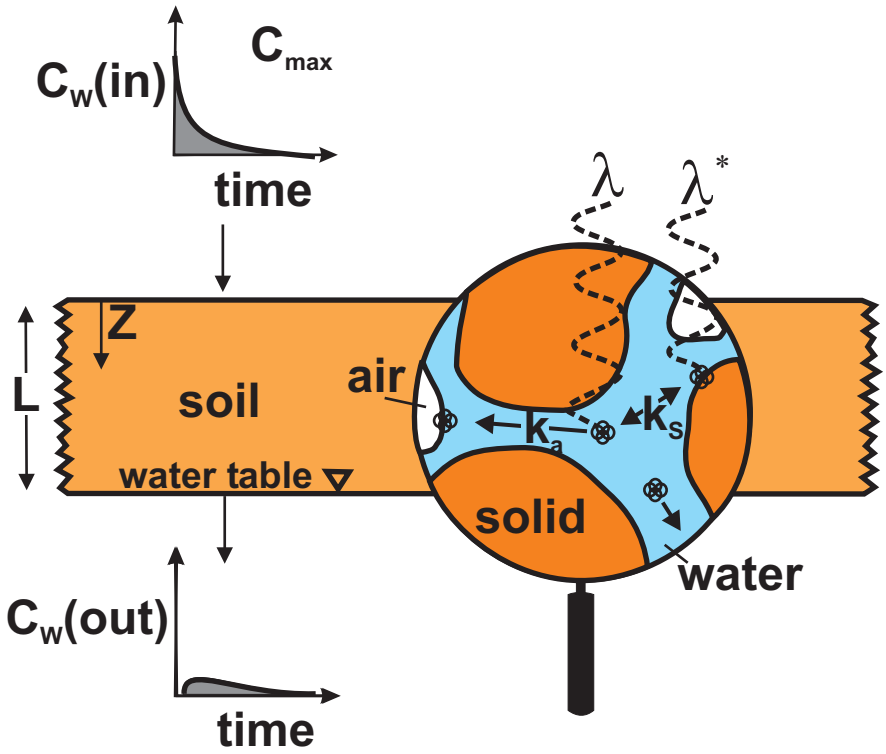

Figure 7. Conceptual depiction of soil bed in *VIRULO*.

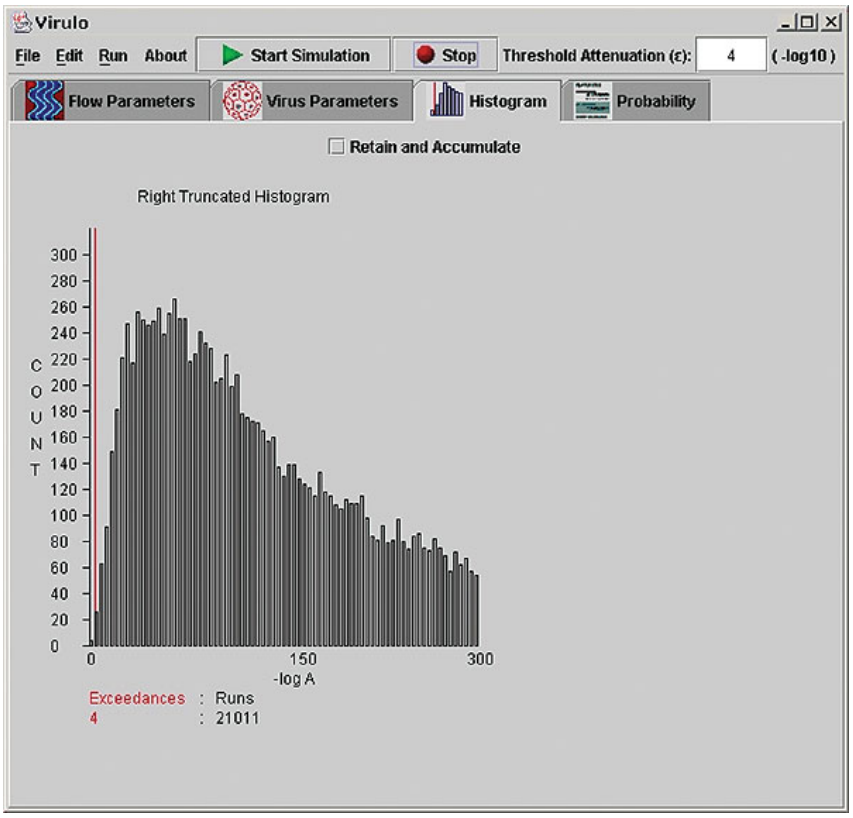

Figure 8. Histogram output panel in *VIRULO*.

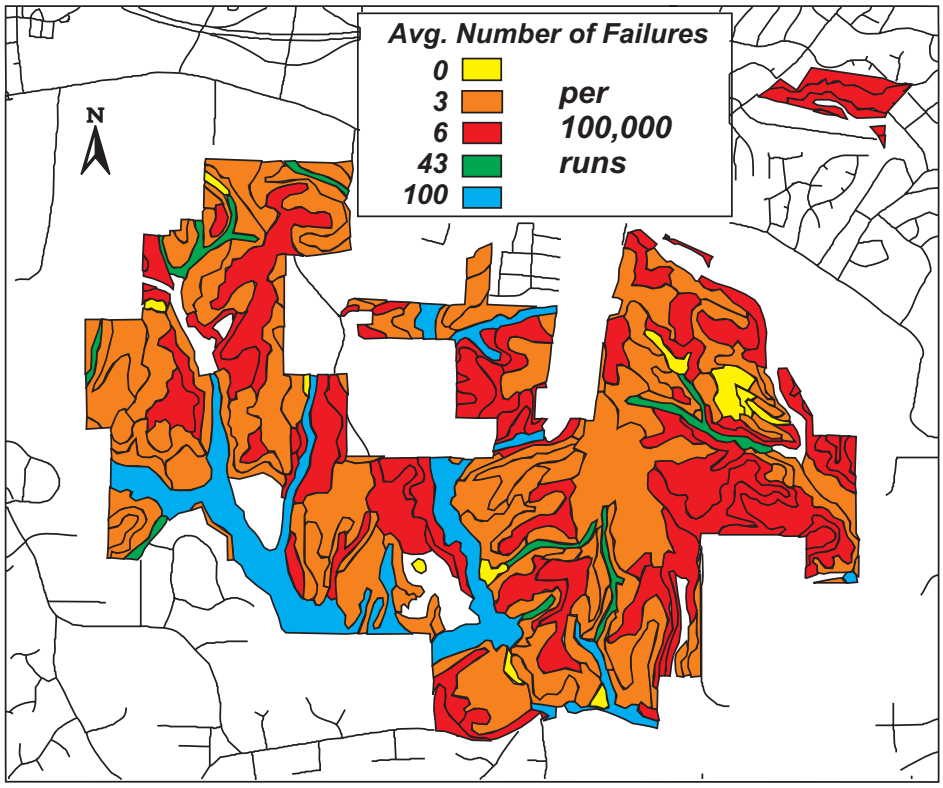

Figure 9. Map of average number of failures per 100,000 Monte Carlo runs based on soil type and seasonal high water table.

Table 7. Assumptions Made in *VIRULO* and Possible Consequences

Assumption	Potential Sources of Large Error in Assumption
Soil is homogeneous	Macropores, aggregates, not considered, though they may greatly affect hydraulic conductivity if present
Soil does not induce preferential flow	Roots, dessication cracks, worm holes and burrows may be present, providing a ready conduit for preferential flow to the water table
Soil and soil water are free of dramatic hydrophobic effects	Organic matter, recent fires can render predicted sorption, mass transfer, completely erroneous
Water percolation is due to gravity only	Abrupt rainfalls, floods, or periods of extended dryness can produce hydraulic pressure gradients in soils not considered in *VIRULO*'s hydraulic conductivity model
Soil is geochemically homogeneous	Microbial activity, decomposition, and mineral weathering can produce conditions that affect virus longevity
Microbial predation not considered	Protozoans present in near-surface can prey on viruses
Temperature not considered	Temperature can influence viral survivability and longevity
Irrigation water contains no surfactants	Municipal effluent that has undergone little treatment may contain surfactants, such as detergent residues, that can affect both viral sorption and hydraulic conductivity

One approach in determining setback distances for septic tanks in wellhead protection areas and bank filtration sites is to determine travel times using ground-water flow characteristics (Yates and Yates, 1987). This approach has been implemented in the Federal Republic of Germany, for example, where three concentric zones protect each drinking-water well. The zone immediately surrounding the well is faced with the most restrictive regulations which are founded on the belief that a 50-day residence time is adequate for inactivation of any pathogen present in contaminated water (Dizer et al., 1984). However, a comprehensive study by Matthess et al. (1988) involving the evaluation of "50 days zone" concluded that the reduction of viruses by 7 log units (current regulations) requires a much longer residence time. Matthess et al., indicated that a reduction of 7 log units occurred in "about 270 days (Haltern and Segeberger Forest) in one study, and about 160-170 days (Dornach)" would be required according to another study.

Another approach to this important issue is to consider the vulnerability to virus transport in the subsurface for portions of a state or county or for individual aquifers. Although there are a number of approaches to rank vulnerability, DRASTIC is one assessment methodology that utilizes hydrogeologic setting descriptions and a numerical ranking system to evaluate the ground-water pollution potential (Aller et. al., 1987). DRASTIC assumes that a potential contaminant will be introduced at the ground surface, have the mobility of water and be flushed toward the aquifer by infiltration. Utilizing existing information at variable scales, the methodology was designed to evaluate areas of 100 acres or larger.

DRASTIC is an acronym representing seven reasonably-available factors that are used to develop a numerical score. They are: **D**epth to water, net **R**echarge, **A**quifer media, **S**oil, **T**opography (slope), **I**mpact of the vadose zone media, and hydraulic **C**onductivity of the aquifer. DRASTIC uses a weighting system to create a relative pollution potential index that varies between 65 and 223 with the higher numbers expressing greater vulnerability.

Although DRASTIC was not designed specifically to evaluate the movement of viruses in the subsurface, the major transport mechanisms and flow paths for viral transport are considered and the flexibility of the systems' rating scheme allows many of these factors to be taken into account. For example, Depth to Water addresses saturated versus unsaturated flow conditions and their importance. Aquifer Media, Soil, and Impact of the Vadose Zone Media all are based on descriptive soil and rock terms that allow for variation due to fracturing, grain size, attenuation mechanisms and overall characteristics that affect flow. Topography addresses the tendency of viruses to be introduced into the subsurface or to be carried away by runoff. Hydraulic Conductivity addresses the relative ease of a contaminant to move with the velocity of water through the aquifer.

Clearly, meaningful setback distances can only be developed by using scientific principles that allow for the use of available knowledge. The establishment of setback distances from sources of viral contamination to points of extraction (wells) can be established using DRASTIC if both the hydrogeologic setting and sensitivity rankings are considered. For example, high pollution potential indices signal the need for greater setback distances. However, the hydrogeologic factors that control viral movement must be evaluated within this context in order to establish reasonable numbers for setback distances. A matrix that incorporates the important DRASTIC factors can be utilized to establish setback distances that include the vulnerability concept. Setback distances must incorporate the knowledge of saturated flow, transport pathway length, transport velocities, media interaction and potential attenuation mechanisms. These setback distances can be used on a regional scale, but can be modified if site-specific information is available. The beauty of DRASTIC is that its rationale and sensitivity factors are easily displayed, so that modification can be readily made.

Summary

Existing legislation addresses the protection of ground-water sources of drinking water with respect to pathogenic

microorganisms. It is a particularly salient issue since about half of the drinking water supplies in this country are obtained from aquifers, and between 1989 and 1990 in the United States 13 of 26 drinking water outbreaks were attributed to contaminated ground water with viruses being the main etiologic agents (CDC, 1991). Therefore, the transmission and survival of human pathogens, particularly human enteric viruses, through the soil to underground sources of drinking water are a serious risk to public health. Among the diverse sources of ground-water contamination, septic tank effluents, sludge disposal, and the application of waste water to the land are most pervasive.

The transport of pathogens in the subsurface depends on their retention to soil and aquifer materials and their survival. Some of the more important factors affecting virus transport include soil water content, temperature, sorption and desorption, pH, salt content, type of virus, and hydraulic stresses. There are indications that the inactivation rate of viruses is the single most important factor governing virus transport and fate in the subsurface.

There continues to be considerable controversy over the use of appropriate indicators for sanitary and biological states in environmental investigations. Since adsorption and inactivation are strongly virus dependent, it is important to realize that there is no single virus for which its transport characteristics can be used as a model to adequately describe the transport of all enteroviruses. One solution may be to use a cocktail of viruses with a range of soil passage characteristics. The selection of indicators is often influenced by the cost and time required for analyses as well as the efficiency of the assay method selected.

The concentration and loading of viruses and the hydrogeologic setting through which they move are major factors influencing their transport. Included among the most important hydrogeological factors that can be used to evaluate viral transport are the flux of moisture in the unsaturated and saturated zones, the media through which the particles travel, length of flow path, and time of travel. One tool which can be used to evaluate virus exposure is the application of predictive virus transport models (Hurst, 1997). Like most predictive modeling, the results depend on the conceptual basis of the model as well as the quality and availability of input data. Clearly, the success of predictive modeling depends on a thorough understanding of the processes and parameters involved in viral transport.

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Quality Assurance Statement

All research projects making conclusions or recommendations based on environmental data and funded by the U.S. Environmental Protection Agency are required to participate in the Agency Quality Assurance Program. This project did not involve the collection or use of environmental data and, as such, did not require a Quality Assurance Project Plan.

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