Final Report

Determination of an Appropriate Onsite Sewage System Setback Distance to Seasonally Inundated Areas

Presented to

Florida Department of Health
Tallahassee, Florida

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20 August 2001

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INTRODUCTION

Septic tank systems are the most frequently reported source of groundwater contamination in the United States (Yates, 1985). On site sewage treatment and disposal systems (OSTDS), such as septic tanks, are a common means of wastewater disposal in the State of Florida. Currently, Florida discharges 800 million gallons (MG) of wastewater into the ground per day. Thirty percent of Florida's population rely on 1.8 million OSTDS. Discharge from OSTDS in Florida was estimated at 450 MG per day in 1990 (DOH, 1999).

Water Quality Effects of OSTDS

In studies of OSTDS in the Indian Lagoon Basin of Florida, McNeillie, et al. (1994) found that while unsaturated soils contributed significantly to the renovation of septic tank effluent, by various processes, the ability of the soil to treat effluent may be affected by soil saturation. They found significant concentrations of effluent pollutants in ground waters directly below an OSTDS where saturated conditions existed within 30 to 60 cm below the drainfield. However, they could not find concentrations of key pollutants that were distinguishable from background levels within 12 meters (about 40 ft) downgradient of the system.

Microbiological Effects of OSTDS

Previous virus tracer studies and surveys have demonstrated the migration of viruses from septic tanks to coastal surface water in the Florida Keys (Paul, et al., 1995), Charlotte Harbor (Lipp, et al., 1999) and in Phillippi Creek, Sarasota (Rose and Zhou, 1995). The Florida Keys study took place in Key Largo in the upper keys and focused on coastal-influenced streams and marine waters. Bacteriophage tracers were added to a domestic septic tank by flushing a concentrated solution down the toilet over a six hour time period. Both grab samples and concentrated samples of surface waters were collected and assayed for the tracer. The tracer appeared in the canal adjacent to the home in 11 hours and moved to nearshore marine waters in 23 hours (Paul, et al., 1995). To date, little work has been done focusing on microbiological impacts from septic tanks on freshwater systems or seasonally inundated areas.

Two studies were conducted in four different infiltration trenches at a lysimeter station in Tampa Florida using the PRD1 *Salmonella* bacteriophage as the tracer (Nicosia, 1998). Virus transport through two feet of unsaturated fine sand was assessed for high (1.5gpd/ft^2) and low (0.75 gpd/ft^2) hydraulic loading rates. Breakthrough was defined as the first detection of PRD1 and peak concentration refers to the highest concentration of PRD1 detected throughout the sampling time. Peak concentrations were detected within approximately four days for all studies. The peak concentration was always seen in the high load trench prior to the low load trenches.

The bacteriophage remained stable over an average of 67 days for both of the tracer studies. The detection of PRD1 over an average of 67 days at high concentrations has been observed in other studies where virus concentrations increased to an apparent steady-state condition, followed by a decline (Bales, et al., 1989; Powelson, et al., 1993). Similarly, Nicosia (1998) found the concentration of PRD1 leveled off and remained stable for an extended amount of time, suggesting an absorption/desorption. Bales, et al. (1989) observed a slow desorption of PRD1 which resulted in a long-term release of viruses in the groundwater.

Total removal of PRD1 ranged between 2.6 log_{10} and 1.5 log_{10} in all three trenches. The removal of PRD1 observed in the high load trench, was an initial and total removal of 99% and 97%, respectively. A total removal of 97.7% of PRD1 was observed in this study. Powelson, et al. (1993) also observed 99.7% removal of PRD1 in an aquifer recharge experiment, after seeded secondary effluent percolated through 14.1 ft (4.3 m) of sand and gravel.

In a study of the human enterovirus from onsite disposal systems in Florida, Anderson, et. al., (1991) found no detectable virus in groundwater samples taken down gradient from two subdivisions using on-site disposal systems, although they did find virus in groundwater in the immediate vicinity of OSTDS drainfields. They also found that viruses shed in feces are discharged from the septic tank to the drainfield from 30 to 60 days after the last detectable virus in stool samples from the home.

No data have yet to be reported on natural bacterial and viral indicators of fecal pollution in Florida groundwaters associated with septic tanks. It is unclear as to how much removal drain fields may provide and how much may be site specific. Clearly in developing regulatory approaches for protection of both ground and surface waters more information is needed on the extent of fecal contamination, the problem associated with virus migration and the mitigation that the soil may provide.

Tracer Studies

One of the most unequivocal ways to ascertain the rates and pathways through a hydrological system, the hydraulic properties of an aquifer, or to link specific sites of contamination to discharge points is via artificial tracers. Ideally, tracers should have predictable properties, both intrinsically and in their interaction with the system into which they are introduced. Tracers should be chemically stable, conservative, inexpensive, readily available, and easily detected. The large volume of most hydrological systems means that tracers need to be detectable at low concentrations and have low natural, background concentrations.

In this tracer study, the conservative groundwater tracer sulfur hexafluoride was employed as the primary tracer to evaluate subsurface flow direction and velocity. Sulfur hexafluoride is very unreactive and detectable at low concentrations, but has the potential for degassing in mounded septic systems and shallow water tables (Corbett et al., 2000). Fluorescent dyes were used as a secondary tracer in each experiment, fluorescein during the winter/spring and rhodamine in the fall study. Rhodamine was used as the secondary tracer due to the presence of fluorescein in the well fields at the start of the fall experiment. Fluorescent dyes need to be used with caution as they are known to adsorb to subsurface media (Kasnavia et al., 1999; Trudgill, 1987; Omoti and Wild, 1979; Smart and Laidlaw, 1977). A biological viral tracer was also used to model migration rates of human viruses. Characteristics of these tracers are as follows:

Sulfur Hexafluoride

Sulfur hexafluoride (SF_6) is a water-soluble gas, is biologically and chemically inert, has a low background concentration (10^{-15} mol/L), and can be detected at extremely low concentrations (10^{-16} mol; Wanninkhof, et al., 1985). The gas has little industrial use although some was used since the 1960's as a gaseous electrical insulator (Wanninkhof, et al., 1991). Due to it's per fluorinated structure, SF_6 is an electrophilic compound that reacts readily with free electrons, but virtually nothing else. Therefore, it can be measured at very low levels with a gas chromatograph equipped with an electron capture detector (GC-ECD). SF_6 has been used for gas exchange studies in rivers (Clark et al., 1994) and lakes (Wanninkhof, et al., 1987; MacIntyre, et al., 1995; Upstill-Goddard, et al., 1990) as well as applications in atmospheric and oceanic sciences (Ledwell, et al., 1993; Upstill-Goddard, et al., 1991; Watson et al., 1991). The strong potential of SF_6 as a geothermal and groundwater tracer has also been reported (Upstill-Goddard and Wilkins, 1995; Wilson and Mackay, 1993). In those studies, SF_6 compared favorably with fluorescein dye applied in a 7.5 x 10^{-7} mass ratio of SF_6 to sodium fluorescein.

Fluorescein

Sodium fluorescein ($C_{20}H_{10}O_5Na_2$), referred to as fluorescein dye or simply fluorescein, is an inexpensive highly water soluble fluorescent dye (Quinlan, 1989). Fluorescein is bright yellow-green to the eye and has a maximum excitation of 491 nm and maximum emission of 513 nm (Gaspar, 1987). Many groundwater tracing studies have employed this dye since it is inexpensive, easily detectable, non-toxic, and stable over time (Reich, 1993; Quinlan, 1989; Duley, 1987; Smart, 1984; Smart and Laidlaw, 1977). However, the dye will break down if exposed to direct sunlight.

Rhodamine WT

Rhodamine WT ($C_{29}H_{29}O_5N_2Na_2Cl$) is another fluorescent dye commonly used in ground water systems. Rhodamine WT is bright orange with an excitation wavelength of 555 nm and an emission wavelength of 580 nm and like Fluorescein; it is inexpensive, easily detectable, non-toxic and stable overtime (Smart and Laidlaw, 1977). The fluorescent signature of rhodamine is distinct from fluorescein, enabling detection of both dyes in the same system.

PRD-1 bacteriophage (virus)

The bacteriophage PRD-1 is a virus that infects the bacterium *Salmonella typhimurium* as its host. Several aspects of this organism make it useful as a virus transport model: its size and transport properties are similar compared to human enteric viruses, detection methodology is relatively inexpensive and easy to perform, it is not commonly found as a natural inhabitant of environmental waters, it is harmless to humans, animals or plants, and it is rather persistent once introduced to groundwater aquifers. PRD-1 has been successfully used as a groundwater tracer in the Florida Keys (Paul et al., 1995), among other instances.

Ground Water and SIA Hydrology Modeling

Seasonally inundated areas (SIAs) experience annual inundation. Specifically,

> "seasonally Inundated Areas (SIAs) shall mean: specific soil mapping units, of at least 0.025 acre, that are classified in the Soil Legend of the applicable USDA Natural Resource Conservation Service (NRCS) Florida county soil survey as frequently flooded, ponded, depressional or slough, that are described in the detailed Soil Map Units of the applicable NRCS Florida county soil survey as very poorly drained; or that are classified in the Soil Legend of the NRCS county soil survey for Taylor County as commonly flooded." (99-395 Laws of Florida)

SIAs are intermediate between terrestrial and aquatic environments both in their spatial location and in the amount of water to which they are accustomed. It is the hydrology of SIAs that creates the unique physical and chemical environment to which a relatively few of the earth's plant species are adapted. In fact, "Hydrology is probably the single most important determinant for the establishment and maintenance of specific types of wetlands and wetland processes" (Mitsch and Gosselink 1986).

Carter (1986) suggested that hydrology is the primary driving force influencing wetland ecology, development, and persistence. Depth of inundation and duration of flooding (sometimes called hydroperiod) are, when taken together, what is commonly meant by the term wetland hydrology. Wetland hydrology is a dynamic interplay of water inflows and outflows. Figure 1 is an illustration showing the inflows and outflows of water that drive the water cycle for a typical SIA. Some of the pathways, like rainfall for instance, are driven by outside variability. Some are dependent upon the structural characteristics of the wetland, like groundwater recharge. Outflows through the organic layers in most wetlands are dependent on water levels within the wetland and the water levels in the surrounding landscape. Overland flows into wetlands from the surrounding landscape are dependent not only on the intensity of a given rainfall event, but also on characteristics of the surrounding watershed, as well as the antecedent soil moisture conditions. Evapotranspiration is a function of water levels within the wetland, humidity, and vegetation. Vegetation removes large amounts of water through transpiration--often, during peak activity, removing quantities comparable to or greater than surface out-flow or deep seepage (Heikurainen, 1963).

Heimberg (1984), in one of the few studies of water budgets in Florida wetlands, studied two isolated cypress swamps in north Florida: a swamp receiving treated wastewater and a control swamp. In the control wetland 71% of rainfall entered as through fall, 47% as surface inflow (run-in) and 2% as stem flow (calculated as a percent of rainfall, thus they do not add to 100%). Outflows were: infiltration (45%) and evapotranspiration (75%). There was no surface outflow from the control wetland.

Landscape position is extremely important to wetland hydrology (Odum 1978; Brown and Sullivan 1987; Brown 1989). Wetlands found in headwaters areas of watersheds are most often isolated depressional wetlands whose water inflows are primarily from rainfall and runoff from a relatively small surrounding watershed, often not more than four times the size of the wetland (Sullivan, 1986).

Since many SIAs are either depressional wetlands with relatively minor edge slopes, or occur in extremely flat expanses of minor elevational change, small changes in water levels can cause large changes in the areal extent of inundation. In studies of central Florida wetlands, Brown and Tighe (1990), quantified structural features of different wetland types. Many features including edge slopes (the slope of the ground surface between the wetland and upland) were analyzed for different wetland community types. Edge slopes for isolated wetlands averaged 2.6% (or about 0.26 meters of drop for each 10 meters of horizontal distance). With such gentle slopes, a small increase in water depth, inundates a large surface area and often extends upland past the edge of the seasonally inundated area in response to large storm events, or during the wettest periods of the year. Figure 2 illustrates an SIA and its upland fringe showing the extent of flooding in the fringe with increases in water levels in the wetland.

It is this storm flood zone around SIAs which presents the potential for interaction of ground and surface waters and may threaten public health when ground waters containing human pathogens from OSTDSs can emerge as surface water. Where pathogens being carried by groundwaters emerge and mix with overlaying surface waters, the potential for their release and human contact increases.

Modeling of ground water flows and surface water in wetlands can be used to evaluate the transport of constituents in ground waters and the effects of changes in water levels on flooding regime. Combined, the two modeling approaches can be used to develop insight into OSTDS setback requirements from SIAs to protect health and welfare of the public.

Plan of Study

To address the potential public health implications of OSTDS in close proximity to seasonally inundated areas (SIAs) and to determine the appropriate setback necessary to protect the public health, a three pronged approach was implemented. First, data were collected from operating OSTDS in five different locations across the State that were in proximity to SIAs to determine fate and transport of constituents from OSTDS in ground water. Second, models of ground water flows and pollutant transport were calibrated with these data and employed to ascertain the potential for transport of constituents through surficial ground waters and into surface waters in adjacent SIAs. Finally, SIA hydrology models, calibrated with both field data from these sites and data from other wetlands were simulated to predict the extent of flooding in areas immediately surrounding SIAs. This flooded area increases the potential for interaction of ground waters with surface waters, and while not “seasonally” inundated, is flooded on a regular basis with larger storm events.

The results of the study are to be used to establish a setback distance for OSTDS from SIAs that will be applied throughout the State of Florida.

METHODS

Standard methods for field data collection, sample analysis, data analysis and modeling were employed. In the following sections details of the methods are given, first for site selection and well installation, then field methods, followed by laboratory analysis, data analysis, and modeling.

Site Selection & Well Installation

Five sites were selected in north, central and southern Florida to provide some variation in hydrogeological and SIA characteristics (see Figure 3). Two different types of wells were installed on the sites: slotted sampling (SS) wells and multi-level sampling (MLS) wells (Figures 4 – 8). Throughout this report, wells are designated by the county initial followed by an S or M (indicating slotted or mulit-level) and the well number. County designations are as follows: Duval north = Dn; Duval south = Ds; Alachua = A, Lake = L, and Charlotte = C. Maps of each site give locations of each numbered well and are designated by S# indicating slotted wells or M#, indicating multi-level sampling wells. When samples are taken from a particular depth in a MLS well, the designation also includes the depth (for example LM2-1.4 indicates Lake county multi-level well #2 at a depth of 1.4 meters)

Site Selection

Florida Department of Health (FDOH) contacted county health department staff to locate sites having OSTDS adjacent to SIAs. Site visits by team members were conducted to make final selection of the five sites (Table 1). The Department used the site selection criteria from the seasonally inundated area definition of Chapter 99-395 of the Laws of Florida. To meet the seasonally inundated area definition, the sites selected met the following criteria: They were specific soil mapping units, of at least 0.025 acre, that were classified in the Soil Legend of the applicable USDA Natural Resource Conservation Service (NRCS) Florida county soil survey as frequently flooded, ponded, depressional or slough, that were described in the Detailed Soil Map Units of the applicable NRCS Florida county soil survey as very poorly drained;or that were classified in the Soil Legend of the NRCS county soil survey for Taylor County as commonly flooded. Areas were not considered to be a seasonally inundated area if they were physically altered in a manner that prevented future seasonal inundation.

Four of the sites had single family residential wastewater inputs to the OSTDS while the OSTDS at one site (Lake County) had inputs from a day-care center. All sites had three to seven feet of fine to medium sand over a clay-rich layer, with at least one foot of ground water (typically two to four feet) above the clay. Water in this shallow saturated zone above the clay lens typically flowed toward and discharged into the adjacent SIA. The distance between drainfield and SIA ranged from 30 to 174 feet (Table 1). This saturated zone above the clay lens was the focus of ground water quality sampling, tracer tests and ground water modeling.

Table 1. Selected sites

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Site	Site ID	SIA Type	Distance to SIA
Duval Co. – North (Sampson Rd)	SAM	Hardwood	70
Duval Co. – South (Maxville)	MAX	Impacted Cypress	35
Alachua County (Brook Point)	ALA	Hardwood	45
Lake County	LAK	Cypress Dome	30
Charlotte County	CHA	Marsh	174

Site Descriptions

Duval North (Sampson Road): The system site is located 70 feet from an SIA mapped in the NRCS Duval County Soil Survey as #86 Yulee clay, depressional, 0 to 2 percent slopes, and described in the detailed Soil Map Units as having a natural drainage setting of "very poorly drained".

Duval County-south (Maxville): The system site is located 35 feet from an SIA mapped in the NRCS Duval County Soil Survey as #66 Surrency loamy fine sand, depressional, 0 to 2 percent slopes, and described in the detailed Soil Map Units as having a natural drainage setting of "very poorly drained".

Alachua County (Brook Point): The system site is located 45 feet from an SIA mapped in the NRCS Alachua County Soil Survey as #22 Floridana sand, depressional, and described in the detailed Soil Map Units as a "very poorly drained soil in seasonally ponded, depressional areas and swamps."

Lake County (Groveland): The system site is located 30 feet from an SIA mapped in the NRCS Lake County Soil Survey as PmA, Placid and Myakka sands, 0 to 2 percent slopes, and described as "very poorly drained" and "in low marshy depressions".

Charlotte County: The system site is located 174 feet from an SIA mapped in the NRCS Charlotte County Soil Survey as #53 Myakka fine sand, depressional, and described as "poorly drained. In most years, under natural conditions, the soil is ponded for about 3 to 6 months."

Slotted Sampling Well Installation

Each site was instrumented with about 10 wells to characterize ground water quality and measure water levels between the drainfield and the adjacent SIA. A typical well pattern included wells located near the toe of the drainfield mound and wells that fanned out in a wider array approaching the SIA (see well location maps, Figures 4 - 8). One well was located upgradient of the drain field to provide background ground water quality at each site. Wells were installed typically to a depth of 3 to 7 feet using a hand auger. PVC well screen was used within the saturated zone with a solid casing attached which extended from the top of water table to the ground surface. After the well was placed at the appropriate depth, a sand pack with a bentonite plug was placed in the upper portion of the well bore. Details on the slotted wells are given in Appendix A.

A preliminary "picket" array of well locations was laid out to characterize ground water quality between the drain field and the SIA. As each well was installed, commercially available Hach field test kits (Hach Corp) were used to measure ammonia and nitrate in ground water samples extracted immediately after installation. This information provided a basis for adjusting the array, and in some cases adding additional wells at the site. However, not all sites were Hach sampled for nitrate or ammonia as the methodology appeared to be unreliable when groundwaters were highly colored. Measurements of electrical conductivity proved to be the best indicator of the nutrient plume at each site. This was due apparently to higher ionic concentrations in the drain fields compared with background levels, perhaps related to water softening chemicals that were discharged into the septic tank systems. Electrical conductivity was in good agreement with on-site ammonia data and was used exclusively at the last two sites that were instrumented.

Multi-Level Sampler (MLS) Well Installation

Slotted wells tend to provide integrated samples that are a mixture of different zones within the screened interval (Pickens, et al., 1978). Nesting wells or piezometers with short screens can be used to obtain samples from different depths, however this approach requires many bore holes and additional expense. The construction of a multi-level sampler (Figure 9) allows for sampling of groundwater at closely spaced intervals in a vertical direction from a single bore hole. The MLS device used in this study is a slight modification of wells used in previous groundwater studies (LeBlanc et al., 1991; Boggs et al., 1988; Pickens et al., 1978).

MLS wells are constructed using 1.9 cm OD PVC pipe as the housing to which 0.6 cm OD polypropylene tubing is attached. For this study, 3-7 polypropylene tubes were attached to the outside of a 1.5 to 3 m section of PVC pipe by plastic cable ties. The ends of the sampling tubes attached to the pipe were wrapped twice with 202 mm Nytex mesh and spaced 40 cm apart. Enough tubing was left above the PVC pipe for easy access and sampling (~0.5 m). Upon installation of the well, the PVC pipe was filled with material removed from the borehole and then capped. Sample depths were identified at the top of each piece of tubing.

MLS sampling wells were installed using a hand auger with a 7.5-cm hollow barrel. To prevent the hole from back filling during construction, a 10-cm PVC casing (outer-casing) was inserted into the hole and moved downward as the hole was dug deeper. Once the desired depth was reached, the well was inserted into the hole, contained by the outer-casing. The outer-casing was then removed from the hole, allowing the aquifer materials to collapse around the sampler, isolating sampling points of the MLS at each level in the borehole. Additional soil material, originally removed from the hole, was back filled to complete the well as necessary. Wells were typically cut flush to the ground and covered with a removable 15 cm plastic cover.

Multi-level samplers (MLS) were installed at three sites to a depth of 1.5 to 3 meters. The MLS wells were arranged in three rows down gradient from the drain field injection point (see Figures 5, 6 and 7). In this way flow rates at several intervals can be calculated: (1) from the injection point to the first row on the mound, (2) from the first row to the second row at the toe of the mound, and (3) from the second to the third row, can be determined. Additional

MLS wells were installed in the event that the tracer-laden wastewater plume traveled in an unexpected direction. There were 26 wells installed at Duval County – South site, 20 wells installed at the Alachua County site, and 20 wells installed at the Lake County site.

Site Surveys

All sites were surveyed by a licensed surveyor (with the exception of Lake County, where the Surveyor did not record elevations of the wells.. Location of OSTDS drainfield, installed slotted and multi-level sampling wells, the SIA edge, and elevations were recorded. Well elevation data were used to evaluate the slope of the surficial ground water, and head produced by the OSTDS drainfield.

Field Sampling

Field data collection from the five sites consisted of water quality sampling, microbiological sampling for several fecal indicators and enteric viruses, and tracer studies to determine flow rates and vertical mixing of constituents from OSTDS in surficial ground waters. Table 2 lists each of the sites and the activities undertaken at each site.

Table 2. Activities at selected sites

Site	W.Q. Sampling	Microbiological Sampling	Tracer studies
Duval Co. – North (Sampson Rd)	XX	XX	
Duval Co. – South (Maxville)	XX	XX	XX
Alachua County (Brook Point)	XX	XX	XX
Lake County	XX	XX	XX
Charlotte County	XX	XX	

Water Quality Sampling

Two rounds of ground water samples were collected from the shallow monitoring wells at each site. First round samples were collected immediately following well installation. The second round of sampling occurred approximately 4 – 6 weeks after the first samples were taken at each site.

Wells were sampled using peristaltic pumps purging the well bore volume prior to collecting samples. On-site measurements of electrical conductivity, pH, and temperature were recorded and samples were collected for laboratory analysis of ammonia, nitrate, and soluble reactive phosphorous. Water quality samples were preserved with sulfuric acid as needed for sample stabilization and stored in a cooler with ice and delivered to a State certified laboratory in Gainesville, Florida within 24 hours.

Ground water levels were measured following all well installation and sampling activities. During the second round of sampling, water levels were measured prior to pumping activities.

Microbiological Indicator Sampling

Five sites, in Duval-north, Duval-south, (Maxville), Alachua, Charlotte and Lake Counties were sampled for a variety of fecal indicator microorganisms and three sites (Duval--north, Lake, and Charlotte counties) were sampled for enteric viruses during the winter/spring-2000 phase. Microbial indicators of fecal pollution included: fecal coliforms (standard bacterial indicator recommended by the State of Florida), enterococci (bacterial indicator currently recommended by the USEPA for recreational waters), coliphage (a bacterial virus, used as a viral indicator), and *Clostridium perfringens*. *C. perfringens* is an anaerobic bacterium that has been recommended as an indicator organism in Hawaiian recreational waters. The species *C. perfringens* is not a natural inhabitant of environmental waters or soils (although some other species of the genus *Clostridium* are) and has been tested and used as an indicator of fecal pollution (Fujioka, 1985; Morinigo, 1990; Payment, 1993; Sorensen, 1989). Also, *C. perfringens* is unable to grow or reproduce in the natural environment, thus making it a potentially superior indicator for tropical environments where other bacterial groups may regrow.

In addition to microbial indicators of fecal pollution, samples were collected for the direct detection of human enteroviruses. Enteroviruses are a group of enteric human viruses. They are characterized by a naked icosahedral capsid and genome made up of one strand of RNA. The group includes poliovirus, echovirus and coxsackie viruses. The historical interest in polioviruses makes this group one of the better-studied groups of viruses and culture methods have become standardized. The use of the enteroviruses has been suggested as an index for the presence of other enteric viruses.

Water samples for microbiological analysis were collected from slot wells and multi-level sampling wells using a low flow peristaltic pump. For microbial indicators, up to 2 L of water were collected from each well, depending upon availability. Between collections at different wells, a 10% chlorine bleach solution was flushed through the tubing for two minutes and allowed to sit for ten minutes for disinfection. Following chlorination, the tubing was flushed for two minutes with 1% sodium thiosulfate and allowed to sit for five minutes for dechlorination. The system was then flushed for two minutes with new sample water before collection. All samples were collected in sterile one or two liter polypropylene containers and were kept on ice until processing in the lab. Sampling of wells at each site for microbial indicators occurred in December, 1999.

Because human viral pathogens generally occur at a lower concentration in the environment than microbial indicators, and because of methodological limitations, larger sample volumes were collected for enterovirus detection. To concentrate the large volume, water was pumped through a positively charged pleated filter (1-MDS, Cuno) using a peristaltic pump. The negative charge on the virus coat results in an electrostatic attraction between the filter and the viral capsid. Briefly, Tygon tubing was fit to the inlet of a 10" cartridge housing (Filterite) containing a 1-MDS filter. The outflow was connected to a flow meter to gauge the total volume collected. The housing containing the filter was kept on ice for the collection period (~12 hours). The volumes sampled for each site were 10 to 50 L. Filters were sent to the laboratory of Dr. Sam Farrah at University of Florida for cell culture analysis to detect infectious enteroviruses.

During the fall, 2000 phase of the tracer study, 1 L grab samples of standing surface water were collected occasionally throughout the study period, water availability permitting. The grab samples were analyzed for the microbial indicators listed above (except for enteroviruses), as well as for presence of the biological tracer (PRD-1 bacteriophage). Grab samples were taken in sterile 1 L polyethylene bottles and transported to the laboratory on ice for analysis. For the Duval County-south site, grab samples were taken from the SIA area south of the septic drain field. Sampling was performed on 09/18/00, 10/05/00, 10/16/00, and 11/27/00. For most of the period between 10/16/00 and 11/27/00, there was no standing water at this site. For the Lake County site, samples were taken from the only standing water in the area, from a pit in a ditch past the eastern edge of the well field. This water was quite stagnant and a heavy layer of duff had to be moved in order to sample water. Samples were taken from this location on 10/23/00 and 10/30/00, after which time no standing water was accessible.

Biological Tracer Study

Biological tracers were employed at three sites in the winter-spring of 2000, and two sites in the fall of 2000 in order to evaluate movement rates of the virus model and any variation in rates from the dry to the rainy season. The sites used for biological tracer studies were in Lake, Alachua, and Duval-south counties for the winter and spring study, and in Lake and Duval-south counties for the fall study. The bacteriophage PRD-1, a virus which infects a *Salmonella* bacterial host, was used. The tracer was injected into the septic system drain field, and samples of groundwater were taken from wells throughout the site to ascertain the extent and speed of virus transport with groundwater through the subsurface. Prior to each injection, pre-seed samples were taken from all possible wells to ensure that no background PRD-1 were present.

Injection - Viral tracer was injected by gravity feed at the same location as the chemical tracers. In the winter, 2000 study for Lake County, the PRD-1 suspension was diluted into 5 L of dechlorinated tap water and fed into the drain field over approximately 1 hour. For the fall, 2000 phase, PRD-1 was injected simultaneously with the chemical tracer in a diluted mixture over a period of 2 hours at each site. The tracer amounts and injection dates are as follows:

-Lake County, Winter 2000, 3.4×10^{13} plaque forming units (PFU) of PRD-1 were injected on January 17, 2000 at 12:00 p.m.
-Alachua County, Winter 2000, injection was done on February 29, 2000 (~10^9PFU)
-Duval County-south, Spring, 2000, injection was done on April 02, 2000(~10^9PFU)
-Duval County-south, Fall 2000, 1.29×10^{14} PFU were injected September 19, 2000 at 11:20 a.m.
-Lake County, Fall 2000, 4.2×10^{13} PFU were injected October 4, 2000 at 11:00 a.m.

Sample Collection – Samples were collected from multi-level and slot wells using 60-ml polypropylene syringes affixed with silicone tubing. Sampling syringes and tubes were sterilized by autoclaving prior to each sampling and changed for each well. Samples were transported on ice to the laboratory facility for processing within 16 hours. For the winter study at the Lake county site, samples were collected at 1, 2, 4, 6, 10, 14, 17, 24, 31, 38, 52, 73, and 107 days post-seed from wells as listed in Appendix C (from the shallowest depth with water, 1.0 – 1.8 m). At the Alachua County site, sample points were at 1, 3, 7, 10, 16, 22, 28, 46, 72, 104, 108 and 149 days post-seed. For Duval County-south, samples were taken at 18 and 37 days from wells in well field and at 23 days directly from the injection box, due to the lack of tracer detection. For the fall phase, samples were taken from the Duval County-south site at 0, 1, 2, 3, 7, 16, 21, 27, 35, 44, 56, 69, and 79 days post-seed from wells and depths listed in the appendix. For Lake County, samples were taken at 1, 2, 4, 6, 12, 19, 26, 40, 54 and 65 days post-seed.

Chemical Tracer Study

Chemical tracer studies were conducted during the winter/spring, dry season at three sites: Duval County south; Lake County; Alachua County. Additional studies were conducted during the fall, at the Duval County south and Lake County sites. The Alachua County wells were removed by request of the landowner and were not available for a fall experiment. The tracers were injected into the drain field and their concentrations in the well field were measured over time. Both initial observance and peak concentrations of the tracer were used to calculate flow rates.

<u>Injection</u> The injection solution was added by gravity feed into a pipe which was down stream of the septic tank and upstream of the drain field. Prior to injection, background samples were collected from the MLS wells nearest the site of injection, generally from the wells of the first picket. Each injection during the winter/spring study consisted of 500 grams of fluorescein dye dissolved into approximately 160 L of tap water which was bubbled with 99.8% pure SF_6 (Scott Specialty Gases) for at least 40 minutes. An additional 20-30 L of tap water was used to rinse the container to assure that the injection solution was flushed into the drain field. These methods were repeated in the fall study, however 250 ml of rhodamine WT was added instead of fluorescein. In order to more closely mimic the dosing rate of a typical drain field, the injection time was increased from 30 minutes in the winter/spring experiment to two hours in the fall.

<u>Sampling Frequency</u> It is critical that the sampling frequency be greatest immediately following tracer injection for two reasons. First, immediately following injection, the tracer slug is most concentrated and it is possible to miss its passing through a sampling picket array entirely. As time passes, the tracer slug disperses and widens so this is less of a factor. Second, nearer the time of injection, each day not sampled creates greater uncertainty. For example, if the wells are sampled on the 1^{st} and 3^{rd} days after injection, and the peak actually passes on the 2^{nd} day, an uncertainty of 33% results. Thus, the uncertainty introduced by missing a single day of sampling at three days after injection is much larger than the uncertainty of skipping a day ten days after injection, when one day is only 10% of the elapsed time.

Sample Collection Water samples were collected directly from individual sample depths on each MLS well in 30-mL serum vials using a peristaltic pump. After purging the tubing, a sample was pumped into a serum vial and allowed to overflow for three bottle volumes. The vial was then sealed with a rubber septa and a crimp cap. To prevent loss of SF_6 through the septa, the samples were stored on their sides until the samples could be extracted and analyzed. Fluorescent dye samples were also collected with a peristaltic pump and stored in 100-mL amber polycarbonate containers.

Sample Analysis

Water Quality Samples

Water samples were analyzed by a State certified laboratory (ppb Environmental Laboratories, Inc.) for ammonia, nitrate, and soluble phosphorous using standard methods.

Microbiological Indicator Samples

Samples were processed in the laboratory of Dr. Joan Rose at the University of South Florida, Dept. of Marine Science in St. Petersburg, within 16 hours of collection. For the well samples taken from the five study sites in winter, 2000, a 25-ml portion of each sample was poured off and used to measure turbidity (in NTU). The remaining sample material was used in the detection of bacterial and viral indicators. Turbidity measurements were not performed for the surface water samples taken in the fall.

Membrane filtration was used to enumerate each group of bacteria. Briefly, at least two volumes of collected water (1, 5, 10 or 25 ml for winter study) were filtered through appropriate membranes (0.45µm pore size, 47mm diameter cellulose filters, Gelman Sciences) and placed on selective nutrient media (see following sections). All bacterial assays were run in duplicate. Values are reported as the number of colony forming units (CFU) per 100 ml. For the fall surface water samples, high levels of suspended solids limited the amount of water that could be filtered, and as a result varying volumes were analyzed. The volumes filtered are presented in the results and appendix sections.

Fecal Coliforms - Processed filters were placed on mFC medium (modified medium for fecal coliform bacteria; Difco Laboratories, Detroit, MI) and sealed in plastic bags within 30 min after filtration. The plates were incubated for 24 hours in a water bath at $44.5 \pm 0.2^{o}C$. The bacterial colonies of various shades of blue were counted as fecal coliform bacteria (Standard Methods for Examination of Water and Wastewater, APHA, 1992).

Enterococci - Water samples were filtered as described above. The filters were then placed on mEI medium (USEPA Method 1600, modified medium for detection of enterococci plus indoxyl β-D glucoside, Difco Laboratories, Detroit, MI) and incubated at $41 \pm 0.5^{o}C$. After a 24-hour incubation, any colony with a visible blue halo was counted as enterococci per EPA method 1600.

***Clostridium perfringens* -** Water samples were filtered as described above. The filters were placed on mCP (modified medium for *C. perfringens*; Acumedia Manufactures, Inc., Baltimore, Maryland) plates and sealed in containers along with anaerobic Gas Paks (BBL GasPak, Becton Dickinson). After 24-hour incubation at 37^{o}C, plates with yellow colonies were exposed to ammonium hydroxide fumes and the colonies that turned red or dark pink were counted as *C. perfringens* as described in Bisson and Cabelli, (1979).

Coliphage - Coliphage samples were analyzed by plaque assay using a soft agar overlay technique with *Escherichia coli* ATCC 15597 as a host. Two ml of sample were added to test tubes containing 3 ml of melted 1% TSB top agar (48^{o}C) and 1 ml of a 3 hour culture of *E. coli* and poured onto solid TSA (1.5% agar) plates. Five replicates of each sample were done for a total of 10 ml sample analyzed. The plates were incubated for 24 hours at 37^{o}C. Plaques, areas where the viruses had grown and lysed the bacterial lawn, were then enumerated. Plaque forming units (PFU) per 100 ml were calculated (Standard Methods for Examination of Water and Wastewater, APHA, 1992).

Enteroviruses - Concentrated water samples from absorption/elution using the 1 MDS filter were prefiltered through 0.2-μm filters (25mm,Corning) and stored at -70^{o}C (Standard Methods, 1989; Jiang et al., 1992). Within 4 days of collection, samples were quickly melted in a 37^{o}C water bath. One milliliter of sample was inoculated onto each of a total of twenty 25 cm^2 flasks with a Buffalo green monkey (BGM) kidney cell monolayer without cell culture media. After incubation with the cell side down at 37^{o}C for two hours, maintenance medium (E-MEM with 5% fetal calf serum) was added to each flask. Generally, viruses are not detected prior to the third week of incubation and may require even further incubation. At three weeks, both positive and negative samples were frozen at -70^{o}C and thawed at 37^{o}C before being transferred (1ml of each) to a 13 X 100mm tube with a new BGM monolayer (this is referred to as the 2nd passage). The tubes were incubated at 37^{o}C for an additional three weeks and examined for CPE each day (Standard Methods for Examination of Water and Wastewater, 1989).

Biological Tracer – A similar method to the coliphage assay was used for detection of the biological tracer, PRD-1, using the PRD-1-specific host (*Salmonella typhimurium* ATCC 19585) rather than *E. coli*. Additionally, for each sample 2-ml duplicates were assayed. When necessary, samples were serially diluted in sterile 1x PBS (phosphate buffered saline) to obtain a readable plate.

Chemical Tracer Sample Analysis

Sulfur hexafluoride samples were extracted in the lab by adding a small head space (typically 4 ml) of ultra-high purity nitrogen to the samples. Simultaneously, a volume of water from the sample had to be removed and discarded to allow room for the head space. The serum vials were slightly over-pressurized with 1 cc of nitrogen to allow several injection volumes (100 uL or less) for the gas chromatograph (GC) to be pulled from each sample.

This method extracted 95+% of the SF_6 from a water sample (Dillon, et al., 2000, 1999; Dillon, 1998). The method has the advantage of extracting a larger volume of water with a smaller gas volume, which concentrates the sample. This results in a lower limit of detection of 0.01 pM (10^{-14} moles/L). Although it is possible to reach sensitivities of 0.03 ppm (3×10^{-17} moles/L) by concentrating the SF_6 from a 500-mL sample onto a cold trap (Wanninkhof, et al., 1991), this procedure is very time intensive and is unrealistic for the large numbers of samples generated in this study.

Samples were analyzed with a Shimadzu model 8A gas chromatograph equipped with an electron capture detector. The volume injected was 100 μL. The gas chromatograph contained a stainless steel column (180 cm x 0.1 cm I.D.) packed with molecular sieve 5A (80/100 mesh). Ultra-high purity nitrogen was used as a carrier with a flow rate of 25 mL/min. Column and detector temperatures were set at 90°C and 220°C, respectively.

Head space concentrations, C, in ppmv (parts per million by volume, = μL/L) of SF_6 were determined by reference to a 1.04 ppm standard (Scott Specialty Gases). The standard was run at the beginning of each day, after every thirty sample injections, and at the end of the day. Head space concentrations were converted to dissolved concentrations in μM as shown below:

$$C (\mu M) = (\mu L/L)/ (R((Latm)/(mol K)) * T (K) * E$$

Where R is the gas constant from the ideal gas law (PV = nRT), and T is temperature in degrees K. The parameter E is the extraction efficiency, which is determined by repeated extraction of some of the water samples. All head space gas was purged between extractions. The repeated extractions were continued until 99% of the gas of interest was extracted. E was then calculated by:

E = Quantity of gas in first extraction/Quantity of gas in summed extractions

Extraction efficiency for SF_6 was always at least 95%. Dilutions of the standard showed a linear relationship between SF_6 concentration and response of the GC over a very wide range (Figure 10).

Replicates were collected for at least 10% of the samples. In addition, duplicate injections were run on the gas chromatograph every tenth injection.

The fluorescein and rhodamine samples were analyzed using a Turner Designs TD-700 Fluorometer, which provides exact concentrations after calibration. For fluorescein, the fluorometer used the 10-089 blue mercury vapor lamp, 10-105 excitation filter (486 nm), and the 10-109R-C emission filter (510-700 nm), as specified by manufacturer. During rhodamine analysis, the fluorometer used the 10-046 clear quartz lamp, 10-103 excitation filter (550 nm), and 10-052R emission filter (>570 nm), as specified by manufacturer. The fluorometer was initially calibrated using fluorescein and rhodamine standards made in the laboratory where analysis was conducted (Department of Oceanography at Florida State University).

Modeling

Hydrologic modeling consisted of two distinct parts: groundwater modeling and SIA hydrology modeling. Groundwater modeling used existing, well-documented models, while the SIA model was developed specifically for this study.

Contaminant Transport Modeling

Two numerical models were employed to simulate conditions at each site. MODFLOW, was used to model ground water flow and MT3D was used to model solute transport. Both of the models are part of the Groundwater Modeling System (GMS) developed by Department of Defense [DOD (1998)]. The first step in the modeling effort was to approximate ground water flow conditions at each site. MODFLOW, a 3D, cell-centered, finite difference, saturated flow model developed by the United States Geological Survey (McDonald and Harbaugh, 1988) was used. Site maps (Figures 4 – 8) were used to locate the drainfield and installed wells on a uniform grid system used by MODFLOW. A simple grid system was used at each site to cover the area of interest. An aquifer thickness was used that approximated that observed above the clay layer found at each site. The clay was assumed to be horizontal which was confirmed by well logs collected during site instrumentation. Ground water levels were measured in all wells and used to calibrate the model under steady state flow conditions. Calibration involved applying a steady uniform recharge over the site that was representative of "dry" conditions (0.0013 ft/day was used, this is approximately 25% of the estimated annual recharge). In addition, a waste discharge load was applied over the drain field area (0.02 ft/day, which represented approximately four people using the septic system. Steady state flow was simulated and the hydraulic conductivity was adjusted to match the observed water table. A constant head boundary was applied at the boundary. Obviously reality is much more complex than this assumption, however, we assumed the constant head boundary represented the average condition

After ground water flow conditions had been simulated, solute transport was modeled using MT3D, a three dimensional modular solute transport code (Zheng, 1990). Initial simulations focused on matching the electrical conductivity data observed at each site. In this context, conductivity is viewed as a surrogate measure of wastewater characteristics in the aquifer. Conductivity values near the drain field were typically 500 to 1000 _S, therefore input concentration of the drain field was assumed to be the same. Simulations were conducted for approximately a one-year run which was sufficient to approach steady state. Porosity was assumed to be 0.36, which is typical of sands (Freeze and Cherry, 1979). Dispersion was initially assumed to be 1 ft^2/day. All other solute transport parameters were assumed to be the same as those used for the MODFLOW simulations.

In an effort to model the observed data, two approaches were used. Solute transport was first simulated assuming a conservative constituent (i.e., no sorption to the media or degradation in transport). After observing the results of this simulation, first order degradation was assumed and the decay coefficient was adjusted to approximate the observed electrical conductivity data. This provides some measure of the decay process that might be active at the site and can be compared to decay rates available in the literature for constituents of interest including human viruses. The second approach used literature values for degradation and included this in the model after adjusting the dispersion coefficient to best fit the observed conductivity data. Some sensitivity to the degradation coefficient was evaluated.

SIA Hydrology Modeling

Given in Figure 11 is a systems diagram of the SIA hydrology model. For a complete description of the symbols and resulting mathematics see Odum (1983). The systems diagram is a method of writing differential equations since each symbol is rigorously defined with explicit mathematical meaning. Differential equations are written directly from the diagram and programmed as difference equations in EXCEL. The complete set of equations is given in Appendix B.

Storages of water include surface water, soil water (as the interstitial waters in organic soils of the SIA), and groundwater. Inputs to surface water include rainfall ($J_{2.1}$), runoff from surrounding lands (called runin [$J_{2.2}$]), and "exchange" with soil water ($J_{4.1}$). Surface outflow from the SIA ($J_{4.2}$) occurs when surface water elevation exceeds the elevation of the SIA's outer edge. Evapotranspiration (J_3) includes evaporation from surface water ($J_{3.2}$) and transpiration ($J_{3.1}$). Ground water exchange with soil water ($J_{5.1}$) is driven by ground water elevation, which results from exchange with ground waters outside the system boundary ($J_{5.2}$). Numbered pathways in the diagram refer to corresponding line items in Appendix Table B- 2.

The water balance equation for each water storage is as follows:

$$\text{Surface water} = J_{2.1} + J_{2.2} - J_{3.2} - J_{4.1} - J_{4.2} \quad (1)$$

$$\text{Soil water} = J_{4.1} - J_{3.1} - J_{5.1} \quad (2)$$

$$\text{Ground water} = J_{5.1} - J_{5.2} \quad (3)$$

Rainfall is programmed as daily events from any climate data set. Runoff from surrounding lands depends on slope and conditions of the watershed, and is programmed by adjusting rate coefficients. Water level within the SIA is controlled by inflows of rain and surface run-in, and outflows of transpiration (exchange with soil water), evaporation, and surface outflow. Since vegetation is rooted in soils, and transpired water is "extracted" from the soil (not the water column) a storage of soil water is included in the model. The amount of soil water is controlled by input from surface water and outflows via transpiration and seepage.

The outflow via seepage is controlled by rate constants indicative of type of SIA and soil conditions. Transpiration is calculated as the product of a growth coefficient and solar insolation adjusted for the growing season. Evaporation was found as difference between evapotranspiration and transpiration. Surface outflow occurs if water levels exceed maximum water level in the SIA. Surface outflow can be controlled so as to mimic an isolated SIA (no surface drainage features and therefore no surface discharge) or a flow through SIA having a discharge elevation. In these simulations, seasonally inundated areas were considered isolated, that is, not connected by a surface drainage feature to waters of the State. As a result, there was no surface discharge from the SIA.

Output from the model is displayed on the computer screen during each simulation run. The output shows a yearly hydrograph and also a maximum water level plotted against a section view through the SIA and adjacent upland. Output graphs can also be saved and printed.

Sensitivity analysis, calibration, and validation of the model were done using data from previously studied SIA systems (see Heimburg and Wang, 1976; and Heimburg, 1986) and survey data collected from field measurements at the Lake County site.

Sensitivity analysis - Sensitivity analysis was conducted by evaluating the effect on model output of varying input parameters and flow pathway coefficients. Results obtained when parameters were increased and decreased by as much as 100% from programmed values were compared with expected model behavior (ie if an increase in a parameter should cause an increase in a flow or storage, the resulting behavior was compared with the expected result).

Calibration - The model was calibrated against a data set for a cypress wetland in north central Florida (Heimberg and Wang, 1976). Total flows into and out of the simulated SIA were compared to measured parameters in the cypress wetland. Predicted water levels that were generated by the model were compared to measured water levels. Once calibrated for the north-central Florida cypress wetland, the model parameters were adjusted to conditions at the Lake County site and simulation results compared to hydrological evidence of inundation elevation. In the absence of long-term water level data for the Lake County site, the elevations of lichen lines and cypress knees were used as indicators of depth of inundation (Brown and Doherty, 2000).

Validation - Rate coefficients and input parameters were adjusted based on results of the sensitivity analysis during calibration until a good fit between measured values for the cypress wetland and the simulation model was obtained. Of primary concern was the total flows into and out of the surface water storage (rainfall, run-in, ET, and seepage). The goal of calibration was to obtain simulation results for total flows within 5% of the measured values.

Simulation Runs - Water levels in the SIA were simulated for the base condition using actual precipitation for an average rainfall year. The base condition was 0% impervious surface, 2.3% watershed slope, four to one watershed to SIA ratio (four hectares of watershed to one hectare of SIA), watershed soil hydrologic group “C”, SIA water depth of 0.53 meters (1.75 feet), and an average rainfall year.

The model was then simulated for varying conditions and rainfall events to evaluate the area of upland immediately adjacent to the SIA that would be inundated. First different storm events were simulated during the rainy season by introducing a five, 10, 25, 50, and 100 year storm event on the 190th day of the year. Second, the percent impervious surface was increased in 10% increments to 50% to simulate development of the watershed.

RESULTS

Water Quality Parameters

Water quality was assessed at each site by measuring electrical conductivity, pH, temperature, ammonia, nitrate, and soluble phosphorous. The results of the first round of ground water samples collected immediately following well installation are presented in Figures 12a --16a. The second round data are given in Figures 12b-16b and the third round data are shown in Figures 13c -15c. Data not plotted imply that nearly all samples were below limits of detection. The data can be found in Appendix A. In general, the data indicate that high concentrations can be found near the drain field and levels decrease with distance from the drainfield. This trend is evident at all sites in the plots of electrical conductivity. Conductivity measures appear to be a good indicator of ground water quality with highest values near the drain field and levels dropping to near background with distance from the drainfield.

As a simple measure of ground water quality, electrical conductivity can provide some measure of the extent of the plume from the drain field. Looking at each site and estimating the distance between the drainfield and the point at which conductivity is approximately 15% of background, as determined by up gradient wells, provides a plume range from 40 to 130 feet with an average of 77.5 feet (Table 3). The trends observed in other water quality parameters are not as well defined but in general support the observation that ground water quality is impacted in a zone ranging from 40 to 130 feet from the drain field.

Table 3. Extent of groundwater plume detected by electrical conductivity

Site	First round measurement (ft)	Second round measurement (ft)	Third round measurement (ft)	Average (ft)
Duval North	90	100	Not sampled	95
Duval South	55	60	45	53
Alachua	60	40	40	47
Lake	60	70	No trend*	65
Charlotte	130	110	Not sampled	120
Average	79	76		76

*No observable trend we present in the data to define the extent of the plume.

Microbiological Sampling

Microbiological Indicators in Groundwater, December 1999

Duval County - North

Seven wells were sampled at the Duval County – North site on 12/14/1999: slot wells DnS1, DnS2, DnS3, DnS4, DnS5, DnS7, & DnS8 (see Figure 4). The volumes filtered and replicate plate counts for each well in colony forming units (CFUs) can be found in Appendix C. Mean values are listed in Table 4. Analysis of samples yielded the following results:

Fecal Coliforms - Mean CFU values per 100 ml for wells DnS3, DnS7, & DnS8 were ≤2. The mean CFU values for wells DnS1, DnS2, DnS4, & DnS5 were 1774, 3700, 3696, and 868 respectively. These levels are well above the Florida State standard for Class III waters of 800 CFU/100ml in a single sample.

Enterococci - No enterococci were detected. The limit of detection was a mean of <2 CFU/100 ml for all wells.

***Clostridium perfringens* -** Mean CFU values per 100 ml for wells DnS1, DnS3, DnS4, DnS5, DnS7, & DnS8 were 5, <5, 15, 10, <5, and 15 respectively. Due to overgrowth, plates for well DnS2 were not readable and therefore are not reported. Levels were below the 50 CFU/100ml that has been suggested as a standard for recreational waters in Hawaii.

Coliphage - Plaque forming units (PFUs) per 100 ml were calculated for 10 replicates of each well. No coliphage were detected. All wells sampled had <10 PFUs.

Table 4. Duval County - North (all values are given as CFU or PFU per 100 ml)

Well #	Fecal Coliform Bacteria	Enterococci	*Clostridium perfringens*	Coliphage	Turbidity (NTU)
DnS1	**1774**	<2	**5**	<10	46
DnS2	**3700**	<2	NR[2]	<10	NR[3]
DnS3	<2 [1]	<2	<5	<10	11
DnS4	**3696**	<2	**15**	<10	41
DnS5	**868**	<2	**10**	<10	38
DnS7	**2**	<2	<5	<10	48
DnS8	<2	<2	**15**	<10	78

[1] Overgrown with mucoid colonies, expect inhibition.
[2] NR – not readable due to background growth
[3] Inaccurate reading of >50 NTU, this sample was highly turbid (almost opaque)

Lake County (Groveland)

Wells sampled for this site include multi-level well LM2 at 1.4m, 2.2m, 3.0m depths, located in the drain field and slotted wells LS5, LS6, LS7, LS8 & LS9 which were SIA sites (see Figure 7). Sampling was performed on 12/06/1999. Volumes filtered and replicate plate counts for each well in colony forming units (CFUs) can be found in the Appendix C. Mean values are listed in Table 5. Analysis of samples yielded the following results:

Fecal Coliforms - Mean CFU values per 100 ml for wells LM2-1.4, LM2-3.0, and slotted wells LS5, LS6, LS7, LS8 & LS9 were ≤2. The mean CFU value for LM2-2.2 was 200.

Enterococci - Mean CFU values for wells LM2-1.4, LM2-2.2, LM2-3.0, and slotted wells LS5, LS7, & LS9 were <2 per 100 ml. Wells LS6 and LS9 had CFU values of <5 as turbidity affected the volume that could be assayed.

***Clostridium perfringens* -** Mean CFU values for wells LM2-1.4, LM2- 2.2, and LM2 -3.0 and slotted wells LS5, LS7, LS8 & LS9 were <5 per 100 ml. Well LS6 had a mean CFU of 55.

Coliphage - Plaque forming units (PFUs) were calculated for 10 replicates of each well. All wells sampled had <10 PFUs, except for well LM2 3.0. LM2 3.0 had a mean PFU value of 40.

Table 5. Lake County (all values are given as CFU or PFU per 100 ml)

Well #	Fecal Coliform Bacteria	Enterococci	*Clostridium perfringens*	Coliphage
LM2 (1.4)*	<2	<2	<5	<10
LM2 (2.2)*	**200**	<2	<5	<10
LM2 (3.0)*	<2	<2	<5	**40**
LS5	2	<2	<5	<10
LS6	<2	<5	**55**	<10
LS7	<2	<2	<5	<10
LS8	<2	<2	<5	<10
LS9	<2	<5	<5	<10

* One well: LM2, three depths sampled.

Charlotte County

Wells sampled for this site were slot wells CS1, CS2, CS3, CS4, CS5, CS6, and CS7 (see Figure 8). Volumes filtered and replicate plate counts for each well in colony forming units (CFUs) can be found in the Appendix C. Mean values are listed in Table 6. Analysis of samples yielded the following results:

Fecal Coliforms - Mean CFU values per 100 ml for wells CS3, CS4, and CS5 were <1; for wellsCS1 and CS2 were <2; CS7 was <10; and CS6 was 2.

Enterococci - Mean CFU values for wells CS3, CS4, and CS5 were <1, for CS1, CS2, and CS6 <2, and for CS7 <10. There were no enterococci detected in any wells at this site.

***Clostridium perfringens* -** The mean CFU value for well CS3 was 5, and for the other wells was <5.

Coliphage - Plaque forming units (PFUs) were calculated for 10 replicates of each well. All wells sampled had <10 PFUs.

Table 6. Charlotte County (all values are given as CFU or PFU per 100 ml)

Well #	Fecal Coliform Bacteria	Enterococci	*Clostridium perfringens*	Coliphage
CS1	<2	<2	<5	<10
CS2	<2	<2	<5	<10
CS3	<1	<1	**5**	<10
CS4	<1	<1	<5	<10
CS5	<1	<1	<5	<10
CS6	**2**	<2	<5	<10
CS7	<10	<10	<5	<10

Alachua County

Wells sampled for this site included AS5, AS6, AS7, AS8, AS9 (see Figure 6). A negative pump control was also collected. Sampling was conducted on 12/20/1999. Volumes filtered and replicate plate counts for each well in colony forming units (CFUs) can be found in Appendix C. Mean values are listed in Table 7. Analysis of samples yielded the following results:

Fecal Coliforms - Mean CFU values per 100 ml for well AS5 was <5. For wells AS6, AS7, & AS8 CFU values were 10, <10, and <10 per 10 ml, respectively. Well AS9 had a mean CFU value of <50. The pump control was negative.

Enterococci - Mean CFU values for wells AS5 & AS6 were <2 per 100 ml. Well AS7 had CFU values of <5. Wells AS8 and AS9 had 10 and <10 mean CFU/100 ml. The pump control was negative.

***Clostridium perfringens* -** Mean CFU per 100 ml for well AS5 was <5. Well AS6 had a mean CFU/100 ml of 5. Wells AS7, AS8, & AS9 had mean CFU values of <10, 40, and <50 respectively. The pump control was negative.

Coliphage - Plaque forming units (PFUs) were calculated for 10 replicates of each well. All wells sampled had <10 PFU/100 ml, except for well AS9. Well AS9 had a mean PFU value per 100 ml of 10.

Table 7. Alachua County (all values are given as CFU or PFU per 100 ml)

Well #	Fecal Coliform Bacteria	Enterococci	*Clostridium perfringens*	Coliphage	Turbidity (NTU)
AS5	<5	<2	<5	<10	33
AS6	**10**	<2	**5**	<10	25
AS7	<10	<5	<10	<10	NR[1]
AS8	<10	**10**	**40**	<10	27
AS9	<50	<10	<50	**10**	NR[1]
Pump control	<5	<5	<5	<10	

[1] Inaccurate reading of >50 NTU, this sample was highly turbid (almost opaque)

Duval County – South (Maxville)
Eight wells were sampled at the Duval County – south site: slot wells DsS1, DsS2, DsS3, DsS4, DsS5, DsS6, DsS7, & DsS8 (see Figure 5). The volumes filtered and replicate plate counts for each well in colony forming units (CFUs) can be found in Appendix C. Mean values are listed in Table 8. Analysis of samples yielded the following results:

Fecal Coliforms - Mean CFU values per 100 ml for all wells were ≤2.

Enterococci - No enterococci were detected. The limit of detection was a mean of <2 CFU/100 ml for all wells.

Clostridium perfringens **-** Mean CFU values per 100 ml for all wells except DsS5 were <5. Well S5 had a mean CFU/100 ml of 5.

Coliphage - Plaque forming units (PFUs) per 100 ml were calculated for 10 replicates of each well. No coliphage were detected. All wells sampled had <10 PFUs.

Table 8. Duval County - South (all values are given as CFU or PFU per 100 ml)

Well #	Fecal Coliform Bacteria	Enterococci	*Clostridium perfringens*	Coliphage
DsS1	<2	<2	<5	<10
DsS2	<2	<2	<5	<10
DsS3	<2	<2	<5	<10
DsS4	<2	<2	<5	<10
DsS5	<2	<2	**5**	<10
DsS6	<2	<2	<5	<10
DSS7	<2	<2	<5	<10
DsS8	<2	<2	<5	<10

Surface Water Microbial Indicator Sampling, September to November, 2000

Lake County (Groveland)

Two samples of surface water from near the Lake County well field were taken and analyzed in late October. It should be noted that this surface water was buried beneath a layer of plant duff approximately 6" thick, which was partly moved before a sample could be taken. The water was in a pit in a drainage to the east of the well field that was approximately 4 feet deep. Due to the foul smell and dark-brown color, it appeared that the water was quite stagnant. Volumes analyzed and the plate counts are found in Appendix C. Table 9 contains the mean values per 100 ml.

Fecal coliforms and enterococci were both present on both sampling dates and in high numbers, while *C. perfringens* and coliphage were not detected in the water. In addition, no tracer was detected.

Table 9., Lake County (Groveland), Surface Water Microbial Indicators. All Values in #/100 ml

Date	**Fecal Coliform**	**Enterococci**	***Clostridium perfringens***	**Coliphage**	**PRD-1 (Tracer)**
10/23/00	**3400**	**3950**	<5	<10	<25
10/31/00	**10250**	**4325**	NR	<10	<25

NR = No result, assay did not work

Duval County – South (Maxville)

Samples from standing water in the SIA area to the south of the drain field were taken on four occasions throughout the fall study period. Sample collection dates were 09/18/00, 10/05/00, 10/16/00, and 11/27/00. The volumes analyzed and plate counts are listed in Appendix C. Table 10 contains mean concentrations for each of the samples. The "nearest well" describes the approximate location of each sampling point within the SIA area. In addition, on 11/27/00, a sample was taken from the ditch by the road nearest to the mound and from a standing water puddle near well DsM21. In some instances, no valid results were obtained due to failure of the positive controls to give a confirmation that the assay indeed was working.

Fecal coliforms were present in at least one of the samples on 3 of the 4 sample dates, and may have been on 10/16/00, but were not detected due to problems with the analysis or culture media. Enterococci were also present in the 3 sample periods which showed fecal coliform, but not on 10/16/00. *C. perfringens* were detected near wells S6 and S8 on 09/18/00, near wells S6 and S7 on 10/05/00, and in the ditch near the road on 11/27/00. Coliphage were absent from all surface water samples. Coliphage were detected at low concentrations in the ground water and with die-off and dilution it is likely that these viruses would remain undetectable if reaching the surface water. The tracer PRD-1 was added at this site in September, but this was after a rainfall event and no rain was experienced after the tracer was added. Again the viral movement, dilution and die-off all influenced the non-detects in the surface water.

Table 10. Duval County-South, Surface Water Microbial Indicators All Values in #/100 ml

Date	**Nearest Well**	**Fecal Coliform**	**Enterococci**	***Clostridium perfringens***	**Coliphage**	**PRD-1 (tracer)**
9/18/00	DsS6	**1680**	**31**	**3**	<10	<25
	DsS7	**4**	**134**	<1	<10	<25
	DsS8	<1	**4**	**1**	<10	<25
10/5/00	DsS6	**127**	<3.3	**23.3**	NR	<25
	DsS7	**30**	**20**	**6.7**	NR	<25
	DsS8	<3.3	**130**	<3.3	NR	<25
10/16/00	DsS8-A	NR	<2.5	NR	<10	<25
	DsS8-B	NR	<2.5	NR	<10	<25
11/27/00	Ditch*	**760**	**93**	**3.5**	<10	<25
	DsM21	**1620**	**30**	<2.2	<10	<25
	DsS9	**12000**	**10**	<1.4	<10	<25

NR = No result, assay did not work
* Ditch by road nearest mound, 39 m from injection point

Average indicator concentrations from groundwater and surface water sampling conducted at the Lake County and Duval County - South sites are presented in Table 11. From these data it is evident that fecal coliform and enterococci concentrations are much greater in the surface water samples than in groundwater samples. *C. perfirngens* numbers are generally low in all four environments and coliphage were found in only one sample between all the sites examined here.

Coliphage and *Clostridium* are not found in soil and on plants as are fecal coliform and enterococci bacteria. Coliphage also die out rapidly. These two indicators are definitive identification of fecal contamination. In addition, *C. perfringens* is found in much lower concentration in feces and much less often in animals particularly birds than fecal coliform bacteria and Enterococci bacteria, therefore it is expected that the numbers would be low.

Table 11. Average Indicator Concentrations at Lake County(Groveland) and Duval County-south(Maxville) Sites

Organism	Site	Average #/100 ml	% Positives	Det. Limit (100 ml)
Fecal Coliform	Groveland, groundwater	**26.75**	25.0%	2
	Groveland, surface	**6825**	100.0%	50
	Maxville, groundwater	<2	0.0%	2
	Maxville, surface	1802.8	77.7%	3.3
Enterococci	Groveland, groundwater	<2.75	0.0%	2, 5[1]
	Groveland, surface	**4137.5**	100.0%	50
	Maxville, groundwater	<2	0.0%	2
	Maxville, surface	**41.8**	72.7%	3.3
C. perfringens	Groveland, groundwater	**11.25**	12.5%	5
	Groveland, surface	<5	0.0%	5
	Maxville, groundwater	5	12.5%	5
	Maxville, surface	**5.04**	55.5%	1 - 3.3[1]
Coliphage	Groveland, groundwater	**13.75**	12.5%	10
	Groveland, surface	<10	0.0%	10
	Maxville, groundwater	<10	0.0%	10
	Maxville, surface	<10	0.0%	10

[1] range of detection limits reflects multiple volumes were sampled

Enterovirus Sampling Results

No enteroviruses were detected from any groundwaters at any of the sites. However, given the nature of the drought conditions further sampling should be attempted in the summer of 2001 with an increase in the water table.

Biological Tracer Study at Lake County, January, 2000

The viral tracer was successfully detected in a number of wells throughout the study period, which allowed for determination of its movement as a plume from the septic tank drain field. The sequence of wells in which the tracer was detected allowed for determination of movement rates through the subsurface. Results of PRD-1 analysis from well water samples are given in detail in Appendix C. To summarize the movement of phage throughout the well field, by two days (T=2d) after injection, on 01/19/00, the tracer was detected at well LM2, at a distance of 13.2 m from the injection point. It remained detectable only at LM2, although it slowly declined in concentration for the next four sampling periods up to T=14d. By T=17d, the tracer was still present at LM2 and had moved off the drain field mound to wells LM6 and LM11, and an isolated pfu was detected at LDP1. Well LM11 is 15 m from LM2, and LDP1 is 18.2 m from the injection point (not in line with LM2). On T=24d, tracer was still present at wells LM2, LM6 and LM11. It also had appeared at LDP2 and LM3 on the mound and at LM5 off the mound. Distances for these wells are 9.1 and 11.2 m from the injection point for LM3 and DP2 respectively, and 5 m from M2 to M5. It remained detectable in these wells the next sampling period, T=31d. By T=38d, tracer was no longer detectable at LM5, and LM3 was dry, but it remained at wells LM2, LDP2, LM6, and LM11. It also made a first appearance in wells LM4 and LS3 on this day. These wells are 3.8 and 6.3 m from LM2 respectively. The distance from LM3 to LM4 is 5 m. At 52 days after seeding, tracer was still detected at wells LM2, LM4, LS3, LM6 and LM11, and re-appeared at well LM5. On the next two sampling days, T=73d and T=107d, tracer was only in well LM11. Based on this movement pattern, it appears the virus moved quickly through the drain field to well LM2, then moved at a more moderate rate off the drain field mound to wells LM6 and LM11, proceeding essentially south towards the SIA area. Concurrently, a slower lateral (westward) spread occurred which led to its presence in wells LM5, LM4, and LS3, along with LM3 on the drainage mound.

Table 12 contains a summary of the first appearance of the tracer at each well in which it was detected.

Table 12. First Appearance of PRD-1 Tracer, January 2000, (Groveland)

Well	Date	Days after seed
LM2	1/19/00	2
LM6	2/3/00	17
LM11	2/3/00	17
LDP2	2/10/00	24
LM3	2/10/00	24
LM5	2/10/00	24
LM6	2/10/00	24
LS3	2/24/00	38
LM4	2/24/00	38

Movement of the tracer plume to several key wells allows for determination of movement rates. Table 13 summarizes these distances, times and rates. Rates of movement on the drain field mound are 6.6 md^{-1} from the injection point to LM2, and 0.467 md^{-1} and 0.379 md^{-1} to wells LDP2 and LM3. However, due to the disturbed soil that comprises the mound and the presence of drainpipes which create preferential flow, the tracer migration rates to wells off the mound are more important. The movement of the tracer plume to wells LM6 and LM11 occurred first, indicating the primary direction of groundwater flow out of the drainage mound. By T=17d, the tracer had moved to both wells LM6 and LM11. Thus, the best estimate for movement rate of the viral tracer outside the drain field is from well LM2 to LM11, a value of 0.467 md^{-1}. In addition, movement of the tracer plume off the mound in a secondary southwesterly direction can be seen by the rates of movement from wells LM2 to LM5, LM4, and LS3. These rates are shown in Table 13.

Table 13. Tracer Movement Rates for January 2000, Lake County

Reference Point	to Well…	Distance (m)	Days	Movement Rate (m/d)
Injection	LM2	13.2	2	6.6
	LM3	9.1	24	0.379
	LDP2	11.2	24	0.467
LM2	LM6	4.6	15	0.306
	LM11	7	15	0.467
	LM5	5	22	0.227
	LM4	3.8	36	0.106
	LS3	6.3	36	0.175
LM3	LM4	5	14	0.357

Biological Tracer Study at Alachua County, February, 2000

The Alachua county tracer study commenced on 02/29/00 and proceeded for a period of about 5 months. Samples were collected from the most shallow water depth in the multi-level wells (for most wells, the 1m depth contained water). Although some wells were dry at times, if the wells contained water, the shallowest point remained constant throughout the study. Appendix C contains the results of phage analysis for the PRD-1 tracer. Tracer was not detected at this site for an extended period. The first detection of tracer was in well AM5, a distance of 15.4 m from the injection point, at T=104 days. It was again detected in this well the following sampling period at T=108 days. For samples collected the next, and final, sampling period (T=149 days), an enrichment procedure was performed to detect low concentrations of PRD-1. Approximately 100 ml of water were taken from the wells after purging. If the sample was clear, it was adjusted to 3% Tryptic soy broth and *Salmonella typhimurium* bacteriophage host was added directly to the tube (turbid samples were filtered prior to addition of host and media). After 24 hours, 1 ml was taken, centrifuged, and the supernatant assayed using soft agar overlay procedure by spotting a sample in the middle of the plate. Positive samples were those displaying large plaques at the point of inoculation. Following the enrichment procedure, wells AM1, AM2, AM3, and AM5 were positive for the presence of tracer at T=149 days.

Using the initial detection of phage tracer at well AM5 (15.4 m) on T=104 days, the movement rate calculated for this site was 0.148 md^{-1}. This is not considered an accurate rate due to low concentrations of the tracer and the use of an insensitive method early in the study. Therefore it is likely that the tracer reached certain wells before the times noted but were below the detection limits of the methods that were being used.

Biological Tracer Study at Duval County - South (Maxville), April, 2000

PRD-1 tracer was not detected in any wells in the spring Duval County-south tracer study. Appendix C details the wells sampled for this study. The last sampling was performed on T=37 days, with negative results from wells DsM1 - DsM7, DsM9 - DsM14, DsM19 - DsM24, and DsS1 - DsS7.

Additional biological tracer studies were successfully performed in the Fall of 2000 at the Lake County and Duval County-South sites.

Biological Tracer Study at Duval County - South (Maxville), September, 2000

The detailed results of sample analysis from the Duval County-south tracer study are presented in Appendix C. A summary of samples that were positive for the presence of tracer is given in Table 14. To summarize the tracer plume's movements, tracer was first detected in wells DsM1, DsM2, DsM6, and DsM10 on 10/10/00 (T=21d). The injection point at this site is very near to the wells on the drainage mound. Therefore, all distances for rate calculations are to the injection point. Distances for the initial wells where tracer was detected are one meter to DsM2, three meters to DsM1, 5.7 meters to DsM6, and nine meters to DsM10. There had been no PRD-1 detected in any wells the prior sampling day (T=16d). After initial detection of the tracer in these wells on T=21d, the tracer was not found in any wells the following sample day (T=27d), but did re-appear, albeit in greatly reduced numbers from its initial concentrations, on T=35d at wells DsM1, DsM2, DsS1, and DsS4. Distances for DsS1 and DsS4 are 5.4 and 9.7 meters respectively. The following sample day, T=44d, tracer was detected in low levels at wells DsM9 (10 m), DsM11 (10 m), DsM16 (12.9 m), DsM17 (14.1 m), DsM18 (13.1 m), and DsS6 (12.9 m). These wells are fairly well grouped together at the edge of the SIA area. On day 56 after seeding, no tracer was detected. However, it was detected in single wells the following two sample periods at extremely low concentrations: in DsM17 at T=69d and in DsM15 (13.3 m) at T=79d. Table 15 lists the day of initial detection for the tracer at Duval County-south.

Table 14. Detection of PRD-1 Tracer at Duval County-South (Maxville)

Date	Days After Seeding	Well	Depth (m)	Avg. Phage Conc. (pfu/ml)
10/10/00	21	DsM1	1.0	403.6
		DsM1	1.4	177.3
		DsM2	1.4	1.25
		DsM6	0.6	34.75
		DsM10	0.6	15
10/16/00	27	Note: all samples negative		

10/24/00	35	DsM1	1.4	1.75
		DsM1	1.8	2.75
		DsM2	1.4	0
		DsM2	1.8	0.25
		DsM2	2.2	0
		DsS1	0.47	3.25
		DsS4	0.26	0.25
11/2/00	44	DsM9	1.0	25
		DsM11	1.0	0.5
		DsM16	0.6	2.5
		DsM17	0.6	9
		DsM18	1.0	0.5
		DsS6	1.03	7
11/14/00	56	Note: all samples negative		
11/27/00	69	DsM17	0.6	0.15
12/7/00	79	DsM15	0.6	0
		DsM15	1.0	0.25

Table 15. First Appearance of PRD-1 Tracer, September, 2000, Duval County-South (Maxville)

Well	Date	Days after seed
DsM1	10/10/00	21
DsM2	10/10/00	21
DsM6	10/10/00	21
DsM10	10/10/00	21
DsS1	10/24/00	35
DsS4	10/24/00	35
DsM9	11/2/00	44
DsM11	11/2/00	44
DsS6	11/2/00	44
DsM18	11/2/00	44
DsM17	11/2/00	44
DsM15	12/7/00	79

Sporadic detection of the tracer was found after Day 21, due to the pulses of tracer moving through the soil system at the limit of detection. The rate determination, therefore was based the occurrence of the first movement pulse for the tracer plume, in which it moved to wells DsM1, DsM2, DsM6, and DsM10 by day 21. The rate for movement to DsM10 by this day is 0.429 md^{-1}. Subsequent detection of the tracer (after 10/10/00) was at very low concentrations. However, movement rates were also calculated for detection of tracer in the more outlying wells. These rates were determined by using the farthest well in which the tracer made its first appearance on that sampling day. On T=35 days, the tracer was detected at well DsS4 for the first time (9.7 m), on T=44 days, the most distant positive well was DsM17 (14.1 m), and on T=79 days, tracer was detected in well DsM15 (13.3 m) for the first time. Rates calculated to these wells are given in Table 16. When rates for this site were averaged, the mean movement rate was 0.299 md^{-1}.

Table 16. Tracer Movement Rates for Duval County - South, September 2000

Reference Point	to Well…	Distance (m)	Days	Movement Rate (m/d)
Injection	DsM10	9	21	0.429
	DsS4	9.7	35	0.277
	DsM17	14.1	44	0.320
	DsM15	13.3	79	0.168

Biological Tracer Study at Lake County, October, 2000

Results of sample analysis for the viral tracer are detailed in Appendix C. The PRD-1 tracer quickly migrated on the drain field mound as in the winter study, but this time was first detected in well M1, on T=1d. From there, the tracer plume moved in much the same fashion as in the winter study. On T=12d, tracer was detected at wells LM2, LDP2, and LM6. By T=19d, it was also present at LM11. This primary flow direction was the same as for the winter study, being detected first at wells LM6 and LM11 off the drain field mound. In contrast to the winter though, tracer was not detected in any other wells besides LM1, LM2, LM6, LM11 and LDP2. It remained detectable in these wells for the duration of the sampling efforts, up to T=65d. Table 17 contains a summary showing initial appearances of the tracer at Lake County. Unlike most years, conditions in the fall 2000 were very dry. Florida was in one of its worst droughts in history and as a result the water table fell below many of the wells. Only a few samples were possible from slot wells as almost all were dry. Appendix C details wells that were not sampled due to lack of water.

Table 17. First Appearance of PRD-1 Tracer, October 2000l, Lake County (Groveland)

Well	Date	Days after seed

LM1	10/5/00	1
LM2	10/16/00	12
LDP2	10/16/00	12
LM6	10/16/00	12
LM11	10/23/00	19

In spite of the dry conditions, it was still possible to calculate rates of tracer movement off the mound. Table 18 summarizes these calculations. The key movement for determining flow rates for this phase of the study is the migration of the tracer from well LM6 to LM11. Movement of the tracer from LM1 to LM6 and LM1 to LM11 are also important to consider. These rates are 0.590 md^{-1} from LM1 to LM6, 0.467 md^{-1} from LM1 to LM11, and 0.314 md^{-1} from LM6 to LM11. It is not accurate to consider the rate from LM2 to LM11, since the tracer was already detected past LM2, in LM6, on its first appearance in LM2. The mean rate for movement of the plume in its primary direction off the mound, taken from the three rates above, is 0.457 md^{-1}. This value is very close to that determined from the winter study of 0.467 md^{-1} for movement of the plume in its primary direction.

Table 18. Tracer Movement Rates for October 2000l, Lake County

Reference Point	to Well…	Distance (m)	Days	Movement Rate (m/d)
Injection	LM1	13.7	1	13.7
	LM2	13.2	12	1.1
	LDP2	11.2	12	0.93
LM1	LM6	6.5	11	0.59
	LM11	8.4	18	0.467
LM2	LM11	7	7	1
LM6	LM11	2.2	7	0.314

Chemical Groundwater Tracer Results

Flow rates of septic effluent towards SIAs have been calculated at the Duval County-south, Alachua County, and Lake County sites using results obtained from both SF_6 and fluorescent dyes. Most tracer studies calculate rates from the time it takes a tracer to move from the point of injection to wells down gradient. Since the tracers were injected at the pipe where the septic effluent enters the drain field, rates calculated in this manner include the residence time of the effluent in the drain field. The first row of wells, at all three sites, is on the edge of the top of the drain mound closest to the SIA, yielding flow rates of the injection slug as it travels through the drain mound and providing a reference point for the effluent leaving the mound. The rate of transport within the mound, and also away from the mound, may be influenced by the fill materials of the mound and changes in soil characteristics made during drain field installation. The primary direction of groundwater flow is indicated by water depth in the slotted wells and location of wells with multiple observations of relatively high concentrations of tracer. Rates calculated between wells, which are off the mound and in the primary direction of groundwater movement, yield results most representative of ground water movement between the drain mound and SIA.

Several flow rates between the same two wells can be calculated depending on which time references are used. Rates can be calculated using various combinations of the initial observation and peak concentration of a tracer. Additionally, more than one peak can occur in a well providing further time reference points. Since a concentration peak represents the passage of the bulk of the injection slug, rates calculated using the time of peak concentrations in both wells are preferable to rates involving an initial occurrence in either well. By applying a series of consistent preferences to a set of data, the rates, which are most representative of the movement of groundwater, can be determined.

Rates were first grouped by location of the reference wells; in order of preference, both wells at grade, well on mound to well at grade, and injection point to wells. Within these groupings, rates calculated from peak to peak are preferred over rates calculated from initial observation to peak concentration, which are preferred over those calculated from initial observation to initial observation. In several wells the initial observation was also a peak concentration and was considered a peak. The most reliable rates are obtained from well with multiple observations of tracer.

Duval County south Winter/Spring Tracer Experiment

The drain field was injected with SF_6 and fluorescein on 12/3/99 and flow rates have been obtained using both tracers. SF_6 was detected within a day at the mound wells, DsM1, DsM2, and DsM3 (Figures 17a-c). At this site, the injection point was only 1.5 m away from DsM2 and 3 m from DsM1 and DsM3. The drain field extends away from the SIA. The dry conditions and low slope of the drain mound suggest a large portion of injection slug entered the water table close to the injection point. Some of the injection slug was observed pooling beneath the header pipe and these initial observations of SF_6 are most likely a result from a portion of the injection slug that did not enter the drain field distribution pipes, but moved out from the injection point immediately to the monitoring wells. The later peaks are from the injection slug that did enter the distribution pipes and drain field. The largest concentrations of SF_6 were found in DsM2, with a peak occurring 15 days after injection. Smaller concentrations were observed at DsM1 and DsM3, but the results indicate the majority of the effluent traveled through the center of the mound, past DsM2 as it left the drain field (Figures 17a-c).

In general, SF_6 was detected away from the mound in two main events. On 12/29/99, 26 days after injection, SF_6 was detected in the slotted wells, DsS1, DsS2, DsS3 and DsS4, with a smaller amount in DsM8. By 50 days after injection, the tracer appeared throughout the well array, except DsM17 and DsM26, which contained SF_6 on day 61. The tracer was not observed at the remaining outer wells, DsM25, DsS5, DsS6, DsS7, DsS8, and DsS9 during the experiment (Appendix D-1, Figures 17d-i).

The initial observations of SF_6 before the second day after injection are most probably from the portion of the injection slug that did not enter the distribution pipes. A small leak was observed as the injection slug entered the header or distribution pipe. The larger, second peak at DsM2, observed on 12/18/99 is more representative of the portion of the injection slug that entered the distribution pipes of the drain field and thus the septic effluent. Of the three wells on the mound, DsM2 was the only well that continuously contained SF_6 and the concentrations were significantly larger than other wells on or off the mound. This indicates the bulk of the tracer flowed past DsM2; therefore this well is used as the primary time and location reference well for rate calculations. Since the first peak also contained significant amounts of SF_6, rates using the first and second peak of DsM2 as the time reference are reported. In addition to rates between the mound well DsM2 and wells at grade, rates were also calculated between wells that are both at grade. A wide range of ground water flow rates, 0.04 to 2.45 m/d, is calculated from the SF_6 data for the Duval County-south site (Appendix D-2.)

Besides DsM2 and the wells at the toe of the mound (DsM4-7), the MLS wells with the highest concentrations were DsM10 and DsM16, directly in line with DsM2 and the SIA, indicating the bulk of the flow traveled due south from the drain field. The data from the slotted wells is also consistent with this observation. Of the slotted wells, DsS1 and DsS3 had the highest concentrations and DsS6, also directly in line with DsM2, was the only outer slotted well in which SF_6 was observed (Appendix D-1). Rates derived from the above wells would be most representative of the movement of the bulk of the tracer and thus effluent. By choosing peak-to-peak calculations from the above subset of wells, a set of calculations can be determined that are most likely to represent the primary movement of groundwater. Since both the first and second peaks at DsM2 are relatively large, an argument can be made that both could be used as the time reference point. Rates of transport are reported for movement within the mound, from the mound, and away from the mound.

Table 19. Rates calculated from the Winter/Spring, Duval County-south SF_6 results. The drain field was injected on 12/3/99. The first peak at the mound wells occurred on the day of injection. The rates calculated between two wells at grade represent the best estimates for effluent movement towards the SIA. All rates are reported in units of meters/day.

Transport Rates Within the Mound		
	First Peak	**Second Peak**
Injection Point to DsM1		0.25
Injection Point to DsM2		0.15
Injection Point to DsM3		0.09
Average	0.16 ± 0.08	
Transport Rates Exiting the Mound		
Mound Well to Grade	Relative to First Peak	Relative to Second Peak
DsM2 to DsM5	0.08	0.10
DsM2 to DsM6	0.08	0.10
DsM2 to DsS1	0.15	0.24
Average	0.10 ± 0.04	0.15 ± 0.08
Average All Mound Well to Grade Well Rates	0.13 ± 0.06	

Injection Point to Grade	
Injection point to DsM5	0.11
Injection point to DsM6	0.11
Injection point to DsS1	0.21
Injection point to DsM10	0.16
Injection point to DsS3	0.31
Injection point to DsS6	0.26
Injection point to DsM16	0.25
Injection point to DsM17	0.23
Average	0.21 ± 0.07
Transport Rates at Grade	
DsS1 to DsM10	0.13
DsS1 to DsS6	0.32
DsS1 to DsM16	0.30
DsS1 to DsM17	0.26
DsS3 to DsM10	0.03
DsS3 to DsS6	0.20
DsS3 to DsM16	0.21
DsS3 to DsM17	0.18
Average	0.20 ± 0.09

The transport rate of 0.20 ± 0.09 m/d is the best estimate from the SF_6 data. Due to the close proximity of the injection point to the lip of the mound (<2m), the rate calculated using the point of injection as reference point, 0.21 ± 0.07 is consistent with the rates using another well as a reference point. This rate is probably a combination of a slower, background rate and a faster episodic rate resulting from rainfall or increased usage. The owner of the Duval County-south site reported having "laundry day" every few weeks. An increase in the water table height was observed on day 55, after a rainfall event on days 52. The increased water input into the system during either of these events would accelerate the transport of effluent out of the system.

The behavior of fluorescein in the well field did not mimic SF_6 as expected, but was retarded with respect to SF_6. Fluorescein was observed in only six wells, DsM1, DsM2, DsM3, DsM5, DsM6, and DsS1 and in each case lagged behind the SF_6 data (Figures 17j-m and Appendix D-3). Although used frequently as a tracer in septic systems, fluorescein is known to bind to organic matter in soils (Trudgill, 1987; Omoti and Wild, 1979; Smart and Laidlaw, 1977) and also alumina and carbonates (Kasnavia et al., 1998). The amount of separation between the SF_6 data and the fluorescein data increases with distance from the injection point, further supporting the hypothesis of its sorption to the soils. With respect to fluorescein, the soils in the well field are acting much as chromatography column. The rates calculated from the fluorescein data should be considered low for transport of the groundwater, but may be representative of some component of the effluent with similar adsorptive characteristics. Since fluorescein did not appear past the toe of the mound, only transport rates within the mound and exiting from the mound can be calculated.

Table 20. Rates calculated from the Winter/Spring, Duval County-south fluorescein results. The drain field was injected on 12/3/99. Flow rates from mound wells to grade wells are calculated using both peaks observed at DsM2 as the time reference. All rates are reported in meters/day.

	Transport Rates Within the Mound	
Injection Point to DsM1		0.03
Injection Point to DsM2		0.10
Injection Point to DsM3		0.03
Average		0.05 ± 0.04
	Transport Rates Exiting the Mound	
Injection Point to DsM5		0.05
Injection Point to DsM6		0.05
Injection Point to DsS1		0.05
Average		0.05 ± 0.00
	Relative to First Peak at DsM2	**Relative to Second Peak at DsM2**
DsM2 to DsM5	0.04	0.05
DsM2 to DsM6	0.04	0.04
DsM2 to DsS1	0.04	0.04
Average	0.04 ± 0.00	0.04 ± 0.01

Since the highest concentrations of fluorescein were observed at DsM2, the rate 0.10 m/d is the most representative of the transport of fluorescein within the mound. This is the same rate obtained from the SF_6 data. The transport rates of fluorescein out of the drain mound, 0.04 m/d, are approximately 50% of the rates obtained from the SF_6 data, 0.13 ± 0.6 meters/day.

Lake County Winter/Spring Tracer Experiment

The drain field was injected on 1/17/2000 and flow rates were obtained using both SF_6 and fluorescein. The SF_6 was observed within a day at all three wells on top of the drainage mound, LM1, LM2, and LM3 and also at LM4, LM5, and LM6, which were all at the toe of the mound (Appendix D-4). These initial observations are not thought to be representative of the normal flow of effluent through the drain field. The injection point at this site was on the side of the drain mound opposite the SIA with distribution pipes ending near the lip of the drain mound closest to the SIA (Fig 7). The size of the injection slug and flush, 190L, may have been too large to allow the slug to enter the drain field before the end of the distribution pipes. Some of the injection slug may have traveled to the end of the pipe, effectively delivering the slug immediately in the proximity of the wells. The bulk of the slug entered the drain field with the effluent and showed up in LM2, 10 days after injection (Figure 17n), indicating that the bulk of the tracer moved through the drain field at 1.3 m/day. After the first day, SF_6 was not found in LM1 or LM3, indicating the bulk of the effluent left the mound from the southeast corner and not in a due east or southerly direction. This is reasonable, as the lowest area in the well field is southeast of the mound. After 24th day, further SF_6 samples at LM1 and LM3 were not possible due to the low water table. The water table decreased over the course of the study and all slotted wells were dry on days 73 and 107, the last two sampling events (Appendix D-8). Off the mound, SF_6 was not observed until 2/17/2000, at well LS3. On 3/9/00, LM11 contained SF_6. On the next sample date, 3/30/00, SF_6 was observed in LM4, LM6, LM11, and LM12 (Appendix D-4)

The lack of more observations of SF_6 is most likely due to the very low water table during the study. Sampling for SF_6 requires a steady stream of water without air bubbles. In low water conditions, obtaining samples without air bubbles is very difficult. For example, LM1 yielded only two SF_6 samples, while numerous fluorescein samples where obtained for this well. Even with these limitations, rates of 0.06 to 0.30 m/d were calculated using the SF_6 data (Appendix D-5). The rates most representative of these rates are reported below.

Table 21. Rates calculated from the Winter/Spring, Lake County SF_6 results. The drain field was injected on 1/17/00. These rates, reported in meters/day, represent the best estimates for effluent movement towards the SIA.

Transport Rates Within the Mound	
Injection Point to LM2	1.32
Transport Rates Exciting the Mound	
LM2 to LM4	0.06
LM2 to LM6	0.10
LM2 to LS3	0.30
Average	0.15 ± 0.13
Transport Rates at Grade	
LM6 to LM11	0.06

The SF_6 concentrations indicate most of effluent flows towards LM2 and the best estimate for the flow rate in the drain field is 1.3 meters/day. The peak to peak rates from LM2 to grade, are the same or higher than the rate of 0.06 m/d, between LM6 and LM11. Since both of these wells are at grade, the rate of 0.6 m/d is the best estimate for ground water transport for this experiment. Both of these wells are at grade and the rate is calculated from peak to peak.

The fluorescein results from the three wells on the mound (LM1, LM2, LM3) present further evidence of fluorescein being retarded in the drain field with respect to SF_6 (Appendix D-4). Fluorescein was first observed two days after injection at well LM2. This initial observation was a concentration peak, and another peak was observed 30 days after injection. Both peaks lag behind the corresponding SF_6 peaks (Figure 17o). The separation of the two tracers is more evident at well LM3 where only one peak per tracer was observed (Figure 17p). Fluorescein was only observed once at well LM1, 17 days after injection. In contrast the one observation of SF_6 occurred on the first day after injection. Further samples at LM1 were not possible due to the low water table. These results provide further evidence that the bulk of effluent exits from the southeast corner of the mound.

The evidence for fluorescein being retarded in relation to SF_6 is less obvious off the mound due to the low water table and the difficulties obtaining samples. Since fluorescein samples are easier to obtain in low water conditions, there are many more observations of fluorescein. The first observation of fluorescein, off the mound, occurred at well LM11, 17 days after injection (Appendix D-4). Concentration peaks were first observed at LM5 and LM11, 24 days after injection. On day 52, concentration peaks were observed at wells LM4, LM6, LM11, and LS3 and initial concentrations were observed in LM16 and LM17. Peak concentrations occurred in both LM16 and LM10 on day 72 and in LM17 and LM9 on day 107 (Figures 17 q-r, Appendix D-4).

The larger data set allows for many more calculations of transport rates from wells at grade than the SF_6 data. (Appendix D-6). Well LM5, at the toe of the mound directly in front of LM2, contained the highest concentrations of fluorescein off the mound, indicating the bulk of the effluent flowed past LM5 towards the SIA. Rates calculated between LM5 and wells further from the mound, yield rates most representative of the bulk of effluent flow. LS3 is also directly in line with LM2 and the SIA, but the rates are less certain due to only one observation of fluorescein. After this initial observation, further samples were not possible due to the low water table.

Table 22. Rates calculated from the Winter/Spring, Lake County fluorescein results. The drain field was injected on 1/17/00. These rates represent the best estimates for the transport of fluorescein towards the SIA. All rates are reported in meters/day.

Transport Rates Within the Mound		
Injection Point to LM2	0.44	
Transport Rates Exciting the Mound		
	Relative to First Peak at LM2	**Relative to Second Peak at LM2**
LM2 to LM4	0.05	0.06
LM2 to LM5	0.13	
LM2 to LM6	0.13	0.29
Average	0.10 ± 0.05	0.18 ± 0.16
Average all Mound to Grade	0.13 ± 0.10	
Transport Rates at Grade		
LM4 to LM9		0.12
LM5 to LS3		0.05
LM5 to LM10		0.08
LM5 to LM11		0.05
LM5 to LM9		0.06
Average		0.06 ± 0.01
LS3 to LM9		0.07
LS3 to LM10		0.13
Average		0.10 ± 0.04
Average of All Grade to Grade Rates		0.08 ± 0.03

The slower rate of transport within the mound of 0.44 m/d from the fluorescein data, compared to 1.3 m/d from the SF_6 data, indicate fluorescein is retarded with respect to SF_6 in the drain mound. The rates for wells at grade obtained from both the SF_6 and fluorescein data are very consistent, at 0.06 and 0.08 ± 0.03 m/d respectively. The lack of a complete data set for the SF_6 tracer may account for the lower than expected rate.

Alachua County Winter/Spring Tracer Experiment

The drain field at the Alachua county site was injected with SF_6 and fluorescein on 12/20/99. SF_6 was observed on the mound in wells AM1, AM2, AM3, AM4, three days after injection. Wells AM1 and AM2 contained relatively high concentrations of SF_6 throughout the study. In contrast, AM3 peaked 7 days after injection with few, small observations afterwards. Well AM4 had few observations of SF_6 after the initial peak on day 3, and well AM5 did not contain SF_6 until day 37, two days after a large rain event. Off the mound, SF_6 appeared in two distinct events. The first event occurred during the first 7 days after injection, with a SF_6 peak observed in several wells off the mound: AM7, AM8, AM9, AM10, AM11, AM12, AM13, AM14, AM15, AM16, AM17, AM18, and AM19. The second occurrence of SF_6 peaks through out the well field occurred after a large rain event on day 35 of the study. On day 37, a peak was observed at AM10, AM12, AM14, AM15, AM17, AM18, AM21, AS1, AS3 and AS4. Peaks were observed at AM6, AM7, AM8, AM9, AM11, AM13, AM16, AM19, AM20, and AS2, on the next sampling event on day 43. Relatively large peaks were also observed at AM1 on day 43 and AM2 on day 66 (Figures 17s-u, Appendix D-7). The tracer was not observed in the slotted wells AS5, AS6, AS7, AS8, and AS9.

The injection slug was apparently more water than the drain field was used to seeing during the dry period, so some of the tracer fluid may have been stored in the lower part of the drain field prior to the rain event. It is hypothesized that during the dry period the tracer was not mobilized because the lower part of the drain field was not utilized by the small amount of water discarded by the household. Following the rain event, the lower part of the drain field flooded, freeing and mobilizing the tracer. Transport rates were calculated for both peaks.

Table 23. Rates calculated from the Winter/Spring Alachua, County SF_6 results. These rates represent the best estimates for effluent movement towards the SIA. Rates are calculated from the injection point and time to the tracer peaks of the wells off the mound. The second peak occurred after a rain event on day 35 of the experiment. A pre rain event peak was not observed in all wells. All rates are reported in meters/day.

Transport Rates Relative to Injection Point		
	First Peak	**Second Peak**
AM1	0.15	0.06
AM2	0.50	0.08
AM3	1.16	
	0.83 ± 0.47	0.07 ± 0.01
Injection Point to Grade	**First Peak**	**Second Peak**
AM6		0.46
AM7	2.23	0.35

AM8	1.87	0.30
AM9	3.75	0.24
AM10	1.53	0.29
AM11	3.39	0.54
AM12	7.48	0.57
AM13	2.77	0.45
AM14	6.25	0.48
AM15	2.38	0.45
AM16	5.34	0.35
AM17	4.72	0.36
AM18	2.19	0.40
AM19		0.46
AM20		0.54
AM21		0.38
AS1		0.31
AS2		0.31
AS3		0.50
AS4		0.62
Average	3.66 ± 1.90	0.42 ± 0.10

The higher initial rates during the dry period averaged 3.66 ± 1.90 m/d (n = 12). These rates were probably driven by the height of the mound and water running downhill. The slower rates averaged 0.42 ± 0.10 m/d (n=20). These rates represent the long distance rate of transport at the site. Note that well AM20, which was in the SIA, had a rate of 0.54 m/d and was a considerable distance (23m) from the injection point (Figure 6). The rate of 0.42 ± 0.10, calculated from the second peaks in the wells to the injection point and time, is a good estimate for the rate of transport out of the mound. Due to the differences in elevation between the top of the mound and grade, this rate is probably higher than if transport rates were obtained from two wells at grade.

As with the other two sites, fluorescein was retarded with respect to SF_6 by materials in the drain mound and/or well field. The lack of fluorescein observations, off the mound except in well AS2, supports this contention. The separation is not as obvious in comparing the concentration data in the mound wells as it was in the other two sites. This may be due to the fact that most of the distances traveled on top of the mound at the Alachua site occurred within the distribution pipes and not through the drain field fill material. Once the tracers entered the water table, retardation of fluorescein with respect to SF_6 is indicated by the lack of further fluorescein observations.

Table 24. Rates calculated from the Alachua County fluorescein results. AS2 was the only well off the mound where fluorescein was observed. All rates are reported in meters/day.

Transport Rates Mound to Grade	
Relative to Injection point	
AS2 Peak	0.20

Relative to Mound Wells	
AM1 to AS2	0.25
AM2 to AS2	0.21
AM3 to AS2	0.22
Average	0.23 ± 0.03

The rate calculated from the injection point to AS2, 0.20 m/d is very consistent with rates obtained between the mound wells, AM1, AM2 and AM3, and AS2, 0.23 ± 0.03 m/day. This observation suggests calculating rates from the injection point at this site does not inflate the results.

Duval County south Fall Tracer Experiment

The drain field at the Duval County-south site was injected with a solution containing SF_6 and rhodamine WT on 9/19/00, however transport rates could only be calculated for the SF_6 data. The continuing presence of fluorescein in the well field necessitated the use of the rhodamine dye. The injection slug was added over a two hour period in contrast to the 30 minute injection time during the winter/spring experiment. By spreading the injection time over two hours, the initial pooling observed at the header pipe, which occurred in the first experiment, was avoided. The site was very wet on the day of injection, with standing water covering wells DsM4 and DsM19. A day after injection, SF_6 was observed in the mound wells, DsM1, DsM2, DsM3, and by the third day throughout the drain field (Appendix D-9).

Background samples prior to this injection contained SF_6, presumably from the winter/spring experiment. The rains prior to this injection could have mobilized a portion of the SF_6/fluorescein injection slug. Relatively high concentrations of SF_6 were observed in well DsM2 four months after injection, indicating SF_6 can reside in septic mounds for long periods of time (Figure 17b, Appendix D-1). SF_6 was observed 3 days after injection in all wells sampled, including the slotted wells in the SIA (DsS5, DsS6, DsS7, DsS8, and DsS9), lending further support to this hypothesis. Rates could not be calculated for the SF_6 observations during the first three days of the experiment due to the uncertainty in the initial time reference. After three days, concentrations of SF_6 concentrations generally decreased and later peaks were observed associated with a second rain event on days 9 and 10 of the experiment (Figures 17v-ai).

Several wells contained additional, usually larger, concentration peaks of SF_6, allowing for additional rate calculations. These peaks occurred after the rain event 9/29-30/00. On day 12, the wells DsM4, DsM19, and DsM20 were all under water. The wells directly in front of the drain mound, DsM5, DsM6, DsM10, DsS1, and DsS3 (Figures 17z, aa, d, g), all contained concentration peaks on day 21, providing time reference points to wells further away from the mound, DsM8, DsM9, DsM10, DsM11, DsM12, DsS6 (Figures 17ab-af, ai). Wells DsM4, DsM9 and DsM11 had multiple peaks of SF_6 (Figures 17y, ab, ac). The observations of SF_6 in wells DsM4, DsM8 and DsM9 on day 79 occurred after a rise in the water depth of the slotted wells observed on day 69. (Figure17aj). All rates calculated from the SF_6 data are given in Appendix D-10. The rates calculated, for all wells, from the injection point and time, 0.36 ± 0.16 m/d agree with the rates calculated using DsM2 as a reference, 0.35 ± 0.18 m/d (Appendix D-10).

Table 25. Rates calculated from the Fall, Duval County-south SF_6 results. These rates represent the best estimates for effluent movement towards the SIA. All rate are reported in meters/day. Peaks in wells off the mound all occurred after the rain event on 9/29-9/30/00. All rates are report in meters/day.

	Transport Rates Within the Mound.		
	First Peak	**Second Peak**	
Injection Point to DsM1	3.47	0.14	
Injection Point to DsM2	0.68	0.05	
Injection Point to DsM3	0.50	0.14	
	1.55 ± 1.67	0.11 ± 0.05	
	Transport Rates Exiting the Mound		
Mound to Grade	**Relative to First Peak at DsM2**		
DsM2 to DsM5	0.23		
DsM2 to DsM6	0.22		
DsS1	0.21		
Average	0.22 ± 0.01		
Injection Point to grade	**To First Peak**	**To Second Peak**	**To Third Peak**
Injection point to DsM4	0.31	0.11	
Injection point to DsM5	0.27		
Injection point to DsM6	0.27		
Injection point to DsM7	0.24		
Injection point to DsM8	0.16		
Injection point to DsM9	0.28	0.18	0.13
Injection point to DsM10	0.43		
Injection point to DsM11	0.82	0.28	
Injection point to DsM12	0.37		
Injection point to DsS1	0.26		
Injection point to DsS2	0.38		
Injection point to DsS3	0.39		
Injection point to DsS4	0.36		
Injection point to DsS6	0.48		
Average	0.36 ± 0.16	0.19 ± 0.09	0.13
Average of all Injection Point to Grade		0.32 ± 0.16	
	Transport Rates at Grade		
	Relative to First Peak at Reference Well		
Peak of Outer Well:	**First Peak**	**Second peak**	**Third peak**
DsS1 to DsM9	0.38	0.15	0.09
DsS1 to DsM11		0.38	
DsM5to DsM9	0.30	0.12	0.07
DsM5to DsM8	0.13		

DsM6to DsM11		0.31	
DsM6to DsM12	0.87		
DsM10 to DsS6	0.65		
Average	0.47 ± 0.29	0.24 ± 0.13	0.08 ± 0.01
Average of All Grade to Grade Rates		0.31 ± 0.25	

The occurrence of multiple peaks at DsM9 and DsM11 allow additional rate calculations for the same distance. These multiple peaks may have occurred due to episodic increases in usage, such as "laundry day," as well as the changes in water table height associated with rain fall. After the flooded conditions on day 12, the water table decreased over the course of the study to the point that conditions were drier during the end of the fall study than during the winter/spring experiment. (Appendix D-8). The higher rate of 0.47 ± 0.29 m/d compared to the spring study (0.20 ± 0.09) for wells at grade reflects the increased hydraulic gradient presented by the very wet conditions.

Rhodamine was only observed on the mound in well DsM2 and DsM3 during the experiment and its appearance lagged behind SF_6. These results indicate rhodamine also is inhibited with respect to SF_6 in this system and to a greater degree than fluorescein. A literature search supports this observation and fluorescent dyes should be used with caution due to their adsorptive characteristics (Kasnavia et al., 1998, Sabatini and Austin, 1991).

Lake County Fall Tracer Experiment

The drain field at the Lake County site was injected with a solution containing SF_6 and rhodamine WT on 10/4/00 in a similar manner as during the fall injection of the Duval County-south site. Unlike the Duval County-south site, the SIA at the Lake County site was dry and the water table low (Appendix D-8). On the day of tracer injection, the wells LS1, LS2, LS10, LM14 were dry. Improved sampling techniques allowed for more SF_6 samples than the previous experiment, although sampling for SF_6 was still difficult to obtain under the dry conditions.

Multiple SF_6 peaks were observed at wells on and off the drain mound. The largest peaks occurred at LM1 on days 7 and 19 (Figure 17ak). Although LM1 one had the highest concentrations, LM2 was the only mound well to contain water and SF_6 throughout the experiment. In the mound wells LM2 and LM3, and also wells LM4, LM5, LM6, and LM7 at the toe of the mound, peaks were observed 2 and 7 days after injection (Figures 17al-ap). Well LM7 also had a third peak on the 19th day. Day 7 also saw peaks in the wells away from the mound, LM9, LM10, LM11, LM12, LM13 and LS4. Well LM11 also had later peaks on days 26 and 40 (Figure 17aq), which was the only well not on or at the toe of the mound to have peaks after the 7th day. The presence of these later peaks, makes LM11 the preferred outer well to use in rate calculations. Well LM6 is most directly in line with LM11, the mound, and the SIA (Figure 7). Since the peaks at the toe of the mound all occurred on the same days, the higher rates calculated from wells LM5 and LM7, to either side of LM6, reflect the greater distance to LM11. All calculations for the SF_6 data are presented in Appendix D-11.

Table 26. Rates calculated from the Fall Lake County SF_6 results. These rates represent the best estimates for effluent movement towards the SIA. All ratse are reported in meters/day.

Transport Rates Within the Mound.			
	First Peak	**Second Peak**	
Injection Point to LM1	1.96	0.72	
Injection Point to LM2	6.61	1.89	
Injection Point to LM3	4.56	1.30	
	4.38 ± 2.33	1.30 ± 0.58	
Transport Rates Exiting the Mound			
Mound to Grade	**First Peak**	**Second Peak**	
LM2 to LM4	0.77		
LM2 to LM5	1.01		
LM2 to LM6	0.91		
LM2 (peak 1) to LM7	1.11	0.33	
LM2 (peak 2) to LM7		0.46	
	0.95 ± 0.15	0.40 ± 0.09	
Transport Rates at Grade			
Peaks of outer well:	**First Peak**	**Second peak**	**Third peak**
LM6 (first peak) to LM11	0.48	0.10	0.06
LM6 (second peak) to LM11		0.13	0.07
Average		0.12 ± 0.02	0.07 ± 0.01
Average of LM11 Second and Third Peak Rates		0.09 ± 0.03	

The rates from LM6 to the second and third peaks at LM11 could be considered most representative of the ground water transport; both are peak to peak calculations from grade wells. However, the higher rate of 0.48 m/d is calculated from the largest concentrations found in both LM6 and LM11 and should be considered.

Rhodamine was observed in the three mound wells and in wells LM11 and LM6 off the mound. The presence of rhodamine in LM6 and LM11 and the lack of observations elsewhere in the well field, indicate that a substantial amount of the tracer injection flowed past these wells, which validates choosing rates from these wells as the most representative of ground water transport. The appearance of rhodamine lagged behind that of SF_6 in all wells except LM1 (Figures 17 ar-au). Since this is also the well with the highest concentrations of SF_6, we hypothesize that most of the distance traveled by the tracer slug to LM1 occurred in the distribution pipes. The time difference between the SF_6 and rhodamine is greater at LM11 away from the mound than at LM2 on the mound, suggesting the soil acted as a chromatograph column. Although enough rhodamine data was collected to calculate transport rates, these results are influenced by the adsorptive characteristics of the dye. The results of the Duval County-south and Lake County fall experiments indicate rhodamine WT does not act as a conservative tracer in mounded septic systems adjacent to SIAs.

Table 27. Rates calculated from the Fall, Lake County rhodamine WT results. Rates involving LM1 and LM2 are calculated using the first peak of rhodamine observed. All rates are reported in meters/day.

Transport Rates Within the Mound.		
	First Peak	
Injection Point to LM1	1.96	
Injection Point to LM2	0.51	
Injection Point to LM3	0.14	
	0.87 ± 0.96	
Transport Rates Exiting the Mound		
Injection Point to Grade	**First Peak**	**Second Peak**
Injection Point to LM6	0.62	0.25
Mound to Grade	**First Peak (LM6)**	**Second Peak (LM6, LM11)**
LM1 to LM6	0.34	0.11
LM2 to LM11		0.17
		0.14 ± 0.04
Transport Rates at Grade		
LM6 (first peak) to LM11	0.17	

Groundwater and SIA Hydrology Modeling

Contaminant Transport Modeling

Each site was modeled using MODFLOW and MT3D. The models were first calibrated by adjusting the hydraulic conductivity in order to approximate the observed heads at each site. The results of this effort are shown in Figures 18 – 25. The hydraulic conductivities used were 7 feet/day at the sites with finer sand and 50 ft/day at sties with medium sands. These numbers are in agreement with values reported in the literature (Freeze and Cherry, 1979). Based on the gradients measured at the sites and the hydraulic conductivity used, estimates of the pore water velocity are approximately 0.5 feet/day. This value is comparable to the results of the tracer tests conducted.

After calibrating the model for flow conditions, a solute transport simulation was conducted. In these simulations, the input concentration was assumed to be either 500 or 1000 units to approximate the observed electrical conductivity at the sites. The model was then run for approximately a one-year period assuming no degradation of the solute of interest. During these simulations, dispersion was assumed to be 1 ft^2/day. These results are presented in Figures 18 - 25. The results of the modeling effort suggest that some degradation or decay of the solute is required. Without decay the plume traveled much farther than observed. Based on this, simulations were conducted introducing a first order decay term which was adjusted to approximate the observed electrical conductivity data. The decay coefficient that produced reasonable results when applied to all sites was 0.005/days. This provides some estimate of the rate of decay occurring at the sites. This approach assumes that the conductivity measurements reflect the behavior of degrading constituents.

Given that viral transport to SIAs is of major concern, some estimate of virus transport in these systems is of interest. The modeling exercise can be used to assess the likely transport of human viruses in these systems. Assuming that the advective and dispersive transport observed in the electrical conductivity data would appropriately describe viral transport, the only remaining process that may diminish viral numbers is die off rate or inactivation. A number of studies have investigated viral transport in porous media (Scandura and Sodsey 1998, DeBorde et al.,1998, Chendorain et al, 1998, Bales et al., 1995, Harvey and George 1989, Funderberg et al., 1981). The studies estimated retardation and die off rates for viruses. DeBorde et al. (1998) investigated die off rates of coliphage as an indicator virus. Converting their finding to an equivalent decay rate for the fate and transport model produced a value of 0.1/days. This decay rate is higher than the value used to approximate the conductivity data. This suggests that viruses introduced into the aquifer would travel somewhat shorter distances than the observed conductivity data. Retardation of viruses has been reported over significant ranges. The retardation is dependent on pH. Retardation was not included in the model but would also tend to reduce transport distances or concentrations. Without local information on retardation or die off rates, a conservative approach is to consider the water quality data as indicative of viral transport.

The second approach, in which the conductivity data was used to calibrate the model, was applied at the Duval South site. The model was calibrated using dispersion as the calibration parameter. The simulation appropriately captured the plume defined by the conductivity data using a dispersion coefficient of 2 ft^2/day. Degradation was then introduced into the model to assess the plume behavior. Figure 26 shows the extent of the plume for degrading solutes with decay coefficients ranging from zero to 0.1/day. The plots indicate that degrading solutes (or viruses) do not travel significantly with reasonable degradation coefficients assumed. In the case of viral transport however, the concern may be at much lower concentrations or fluxes than observable in Figure 26. Virus flux to the SIA was investigated for different decay rates applied. A viral load of 1,000,000 was assumed applied to the drianfield. The number of viruses reaching the SIA was plotted as a function of the decay coefficient assumed in Figure 27. Again, the conditions are for those at the Duval South site. This suggests that for a decay coefficient of 0.1/day approximately 10 virus reach the SIA. To look at this in terms of setbacks from SIAs, the viral flux (or number of viruses reaching the SIA) was plotted as a function of distance assuming a decay coefficient of 0.1/day (see Figure 28). For the analysis done here, this suggests that if virus numbers such as 10 or 100 are of concern, then setback distances of 55 to 75 feet would be appropriate.

SIA Hydrology Modeling

The SIA hydrology model (Figure 11) was simulated for two different conditions to estimate the area of upland surrounding a SIA that would be flooded and therefore pose a potential transport mechanism for groundwaters to enter surface waters. Such a mixing of surficial ground waters and surface waters poses a threat to human health and welfare as pathogens present in the upper layers of the ground water may easily pass to the overlying surface water. Figure 2 illustrates the situation where ground and surface waters mix as a result of flooding conditions. During periods of the year when soils are typically saturated and water levels in the SIA exceed the elevation of the SIA edge (this can result from storm events, or because impervious surface has increased in the watershed) ground water and surface water interact, depending on slope and hydraulic conductivity.

The graphs in Figures 29 and 30 are simulation results from the model shown in Figure 11. In all cases the graphs are pared. The left graph is a hydrograph that shows the water levels in the SIA over the period of one year beginning in January. The vertical axis shows water levels above the ground surface in the center of the SIA. The horizontal red line (at 0.53 meters (21") is the depth of the SIA. The right-hand graph shows a cross-section of the SIA where distance from the center of the SIA is on the horizontal axis, and elevation on the vertical axis. The outer edge of the SIA is indicated by the short vertical black line at 57 meters from the center of the SIA (57 meters is the radius of a 1 hectare SIA). The red horizontal line of these cross-sections indicated the maximum elevation of water level in the SIA (the highest point on the hydrograph). Where this horizontal line intersects the ground plane (brown line) is the extent of inundation by the maximum water level and is indicated by the dashed vertical red line.

In the first set of simulations (Figure 29) five different storm events were programmed to occur at day 190 (about 30 days into the "normal" wet season) during an average rain year. The rainfall events that were programmed were a 5, 10, 25, 50 and 100 year storm events of 5.5", 6.5", 7.5", 8.5", and 9.5", respectively, with a duration of 24 hours. The right graph shows the yearly hydrograph that results from the rainfall, and the left graph which is a cross-section through the SIA and adjacent area, shows the maximum elevation that surface water achieves during the year. The first graphs (a) in Figure 29 result from simulation of the model using average rainfall conditions. During the wet season in this average rainfall year (Figure 29a), the maximum depth of surface water in the SIA is 0.53 meters (or about 21"). Figures 29b and 26c show the hydrographs and maximum elevations when a 5 and 10 year storm event is introduced during the wet season. Distances upland of the SIA edge that experience flooding are 16 meters (52 feet) and 19 meters (62 feet) respectively. When the rainfall event is increased to a 25 year storm the upland area that is inundated is 22 meters (72 feet) landward of the SIA edge (Figure 29d). Flooding occurs up to 24 meters (79 feet) and 27 meters (89 feet) from the SIA edge for a 50 and 100 year storm, respectively.

Figures 30 a-f are simulation results for the model when rainfall is held constant (average year) and the percent impervious surface in the watershed is increased in 10% increments. As the area of impervious surface increases, the amount of runoff increases and thus the total volume of water entering the SIA is greater. The net result of increased runoff from the watershed is an increase in flooding in the area immediately adjacent to the SIA. With a 10% increase in impervious surface, flooding occurs up to 5 meters (16 feet) into the upland fringe (Figure 30b). When 50% of the watershed has impervious surface (Figure 30f), the distance is 21 meters (69 feet).

Simulation results shown in Figures 29 and 30 are for an average rainfall year. Therefore they do not represent a "worst case scenario" since flooding would be much greater if the storm events depicted in the simulations were to happen in a wetter than normal year. In like manner, if increases in impervious surface were combined with storm events (a situation that is probably quite realistic, as development occurs) the combined effect is to increase the distances shown in Figure 29 by about 5 percent for each 10% increase in impervious surface. This means that the distance upland that is flooded increases from 16 meters (52 feet) to about 18 meters (60 feet) with a 5 year storm event when the impervious surface increases from 0% to 20 percent and would be about 20 meters (66 feet) with 30% impervious surface in the watershed.

DISCUSSION

Summary

Water Quality Sampling

Electrical conductivity and measurements of constituent concentrations in ground waters suggest that there is a “plume” extending in a down slope direction from the drainfield of OSTDS. Data indicate that the plume may range from 40 to 130 feet.

Microbiological Sampling and Tracer Studies

Three out of the five sites showed evidence of bacterial fecal contamination in the groundwater. Two sites (Lake and Alachua Counties) showed evidence of viral fecal contamination (coliphage). Duval County - North had the highest evidence of contamination with six of seven wells sampled positive for one or more indicators (three wells had two indicators of fecal pollution detected). At the Lake County site, three of six wells sampled were positive and two of the three lower depths at multi-level well LM2 were positive. Results for the presence of infectious human enteroviruses at the Duval County - North site were negative. At the Alachua County site, three of five wells were positive for one or more indicators (well AS6 had two indicators). At the Duval - South (Maxville) site only one well was positive for any indicator (*C. perfringens*).

The occurrence of any one indicator seems to be distributed in a heterogeneous fashion and may be related to excretion rates in the household, removal in the tank itself, survival, and adsorption to soil, as well as dilution. The indicators were found in ground waters at varying distances from the drain fields. The furthest well that was positive at the Lake County site was well LS6 for *Clostridium* (45 feet from the drain field). At the Duval County - North site, wells DnS7 and DnS8 situated at the lawn’s edge (80 feet from the drain field) were positive for fecal coliform bacteria and *Clostridium*. At the Alachua County site, well AS8 (120+ feet from the drain field) was positive for *enterococci*, *Clostridium*, and coliphage. In all, low levels of fecal bacteria and viruses, indicators of fecal contamination, were detected in ground waters at distances of 45 feet to greater than 120 feet from monitored drain fields.

Since the fecal coliforms and enterococci numbers are so much greater at the surface than in groundwater, one might assume that the septic systems at these sites were not significantly impacting the occurrence of fecal indicators in surface waters and this contamination is a result of input from other sources such as animals and soils. One should be cautious however, as a time correlation between these two environments was not made. Surface water samples were only taken from Lake County and Duval County - South, and only in the fall portion of the study. Groundwater indicator samples were only taken in the spring study. An additional point of caution should be noted as well; it is possible that once released into the rich organic surface waters that fecal coliform bacteria and enterococci will regrow. *Clostridium* is more restrictive to feces and while it can survive and accumulate months to years, there is no regrowth. Likewise the coliphage will not regrow, however their survival is not as long in the environment as *Clostridium*. The data on the *Clostridium* at the Duval County-south site at least, suggest that the septic tanks via contaminated ground water may be contributing small concentrations over time to the surface waters. The lack of rainfall during the fall study likely prevented an accurate assessment of viral transport into surface waters, especially at the Lake county site. At that site, movement of the tracer was far below the surface as indicated by the dry state of the slot wells. However, in both the winter/spring study at Lake county and the fall study at Duval county - south, the PRD-1 tracer was detected in slot wells, indicating that if the water table had been at or above the surface at these locations when the tracer was found there, some degree of virus transport into surface water would be likely.

Risk Assessment

On average the rate of virus migration ranged from a high of 1.95 feet/day at Lake County in October to a low of 0.944 feet at Lake County in Jan./Feb. There was less rain in January study as compared to the October study, although the rainfall was well below average. Table 28 gives the average virus movement rates for the three tracer studies. Overall for all sites the average movement was 1.289 feet/day. Depending on the rate of movement, there will be a difference in the time spent in the environment. The faster the virus moves, the less time in the environment and less virus inactivation achieved.

Table 28. *Average Tracer Movement Rates for Lake County and Duval County-south*

Site	Date	Rate off of the drain field m/day	Feet/day (3.281ft/m)
Lake County	October 2000	0.593	1.95
Lake County	January 2000	0.27	0.944
Duval County-south	September. 2000	0.299	0.981
Average for all sites	Jan., Sept. & Oct. 2000	0.393	1.289

Averages were taken from Tables 13, 16, 18 without, LM1, LM2 and LDP2

Yates and Yates (1988) proposed a model to estimate inactivation of viruses in soils using the coliphage MS2 as a standard (Log_{10} inactivation = -0.181 + 0.0214 x temperature in Celsius). The observed groundwater temperature at the Lake County site averaged 22.0^0C in the winter study. Using observed groundwater temperature of 22.0^0C, the inactivation rate using the model is 0.289 log_{10}/day. However, Gerba and Bitton (1984) estimated a Polio 1 virus inactivation rate in Florida groundwater of 0.0456 log_{10}/day. In evaluating virus inactivation rates it seems more appropriate to use a human virus and one that has been evaluated under more field conditions here in Florida. Using these inactivation rates and the tracer migration rates, various distances can be calculated to achieve various levels of virus reductions and thus levels of safety.

The quantitative microbial risk assessment approach uses modeling to estimate the likelihood or probability of infection after exposure to a pathogen. In this case, viruses were used as the pathogen target. The complete risk approach used in this study is described in the Appendix. However the data and assumptions used can be summarized as follows:

1. Assumption an individual in the house hold will at some time be infected and excrete viruses.
2. The excretion rates of enteroviruses and rotaviruses in infected individuals have been shown to be 10^6 and 10^{10} virus/gram, respectively (Yates and Yates, 1988). Assuming loading of 100 grams from an infected individual and a 4-$\log_{10}$ reduction in viral numbers in the septic tank, then 10^4 and 10^8 enteroviruses and rotaviruses respectively per liter would enter an impacted drain field. However this reduction is not known and could be less.
3. The reduction of viruses to protect public health varies depending on the concentrations of viruses and the potential for exposure. Regarding exposure, a high risk is associated with contamination of drinking water, moderate risk is associated with contamination of and exposure to recreational waters and a low exposure risk is associated with surface contamination (eg. soil and grasses) and hand or body contact.
4. Concentrations of viruses under these various exposure scenarios are dependent on the amount of inactivation time in the environment. Given this, a high to moderate concentration risk (termed high environmental risk) would be found with only 99.99% reductions (4 $\log_{10}$), moderate to low risk (termed moderate environmental risk) would be found with 6 $\log_{10}$ reductions and an insignificant risk (termed low environmental risk) would be achieved with 9 $\log_{10}$ reductions. These levels of risk can be used with the known migration rates to examine the types of setback distances that would be capable of achieving various levels of safety.

Setback distances in feet were calculated for high environmental risk (4 $\log_{10}$), moderate environmental risk (6 $\log_{10}$) and low environmental risks (9 $\log_{10}$) for the poliovirus inactivation model described above. The virus movement rates found for the three tracer studies at the two sites as well as the overall average for all sites were used (Table 28). The risk that might be accrued is dependent on the virus and the travel time, thus a given setback (wet soil or grasses, standing water associated with contact, recreational waters or potable aquifer) can be categorized as low, moderate or high exposure risks. MS2 virus data demonstrate that some viruses may not be as stable in the environment, however this is a coliphage (bacterial virus) and the risk may be better determined using a human virus and Florida conditions as our current studies suggest that coliphage are less stable in warm waters compared to human viruses. The data represent virus transport during saturated conditions, however this may be more reflective of the dry season and does not represent the rainy season or even averages in Florida, when the ground water table would be higher thus increasing the hydraulic head and more areas in the soil would be under saturated conditions. The data developed in this study is definitely "best-case" conditions in that the soil was quite dry and there was little rain.

The setback distances for high risk using the poliovirus model (and the various transport rates) ranged from a low of 83 ft, average of 113 ft, to a high of 171 ft (Table 29). For a moderate risk level the distances were, 124 ft, 170 ft, and 257 ft for low, average and high setbacks. Finally for low risks setbacks ranged from a low of 186 ft, average of 254 ft to a high of 385 ft. Thus it is possible to assign specific risk to various sites based on knowledge of water temperature, movement rates and distances to areas accessible by the public where exposure could take place. This risk will be bracketed particularly by season and influenced dramatically according to the level of rainfall influencing soil moisture and saturation conditions.

Table 29. Setback Distances Needed in Florida to Achieve Various Levels of Environmental Safety based on Poliovirus survival

Site and rate (feet/day) See Table 28.	**High environmental risk 4 $\log_{10}$ (99.99%) reductions (87.7 days)**	**Moderate environmental risk 6 $\log_{10}$ (99.9999%) Reductions (131.6 days)**	**Low environmental risks 9 $\log_{10}$ (99.999999%) Reductions (197.4 days)**
	In Feet		
Lake County 1.95	171	257	385
Lake County 0.9444	83	124	186
Duval County south 0.981	86	129	194
Overall average 1.289	113	170	254

These setbacks are really only appropriate for the low exposure (soil and grass) scenario. Table 30 shows the risk estimates based on this analysis using a rotavirus risk model (See Appendix for full description). Risk estimates are much higher than acceptable levels for drinking water (EPA suggested level is 10^{-4} and the risk estimates here are most often above 10^{-2}). For recreational exposure, setbacks would have to be in the low environmental risk range at 186 to 385 feet to achieve the acceptable level for the range of virus concentrations that might be found. However, even with low environmental exposure, with a high environmental risk (only 99.99% reductions) the probability of infection could get as high as a _ chance if on any particular day, high concentrations of viruses were excreted by an individual in the household. However, for average virus excretion rates, the risk levels are below 10^{-3} at setback distances between 83 to 171 feet.

Risk assessment is a tool that can be used to take scientific data and to evaluate in a specific context where by comparisons can be made. It is the approach used by governments and industries in order to make scientific-based, risk-based decisions. This assessment here used data from the literature as well as data specific to this study. However there are a number of assumptions and clearly this does not describe the wide range of conditions and situations that might be encountered here in Florida. It can be seen that the excretion rate of a virus by an individual is one of the more significant inputs influencing the model and thus the risk. This can not be controlled nor measured with any great certainty. However, further investigation into removal of viruses by septic tanks, dilution factors and more survival data on virus inactivation in Florida waters would allow for more definitive assessment of these factors and the development of policies which protect public health in regard to septic tanks and protection of environmental and water quality.

***Table 30**. Ranges of Probability of Infection Risk Estimates based on ranges of Virus excretion, Environmental Risk Assignments and Levels of Exposure.*

Risk Assignment based on Setbacks	**Exposure**		
	Low [Wet Soil/grass]	**Moderate [Playing in water]**	**High [Drinking]**
Environmental Risks	**Probability of Infection**		
Low risk [99.9999999% reductions]	$5.8x10^{-5}$ to $5.8x10^{-9}$	$5.7x10^{-3}$ to $5.8x10^{-7}$	$9.1x10^{-2}$ to $1.2x10^{-5}$
Moderate risk [99.9999% reductions]	$5.1x10^{-2}$ to $5.8x10^{-6}$	$5.6x10^{-1}$ to $5.8x10^{-4}$	$8.0x10^{-1}$ to $1.1x10^{-2}$
High risk [99.99% reductions]	$5.6x10^{-1}$ to $5.8x10^{-4}$	$8.7x10^{-1}$ to $5.1x10^{-2}$	$9.4x10^{-1}$ to $3.6x10^{-1}$
		Suggested acceptable risk is ~10^{-3} per swim	Suggested acceptable risk is 10^{-4} yearly

Chemical Tracer Studies

Chemical tracer studies were conducted to determine the flow rates for the movement of ground water between the drain mounds and the SIAs . Chemical tracers were injected in the drain fields at Duval County - South, Alachua, and Lake County. Two different tracer studies were conducted, one set in the winter/spring 2000 and a second study in the fall 2000. The purpose of conducting the second, fall tracer experiment was to gather data during a wet season. Unfortunately, Florida has been in a prolonged drought and conditions during the fall 2000 were much drier than typical years (Appendix D-13). The Duval County-south site was very wet on the day of the fall injection and experienced a rain event during both experiments. Except immediately following these rain events, standing water was not observed in the SIA(Figure 17ai).

The Lake County site was very dry during both experiments. The water table was observed to be at its highest level during the first several days of the winter/spring experiment

(Appendix D-8). The site stayed dry throughout the course of both studies. This part of central Florida did not experience the rain events observed in north Florida (Appendix D-13).

The rates that most represent the groundwater flow towards the SIA are calculated from SF_6 concentration peaks in two wells, both at grade. The similar conditions of the Duval south and Lake County sites during the two experiments are reflected by agreement between the following estimates for groundwater flow: at Duval south, 0.20 ± 0.09 m/d n=8 and 0.31 ± 0.25 m/d n=11 and at Lake County, 0.06 m/d n=1 and 0.09 ± 0.03 m/d n=4, for the spring/winter and fall studies. At the Alachua County site, rates could not be calculated between two wells, which are both at grade. After grade to grade rates, mound to grade rates give the next best estimate of transport rates from the drain field to the SIA. For the Alachua site, the mound to grade rate of 0.40 ± 0.12 m/d n=20, is the best estimate for groundwater flow.

Table 31. Comparison of the best estimates of groundwater transport from the SF_6 data at the three sites studied. The rates from the Duval County-south and Lake County experiments are calculated using SF_6 concentration peaks between two wells, both at grade. The Alachua rate is calculated between the injection point and time and wells off the drain field mound. All rates are reported in meters/day.

	Meters/Day
Duval County-south (Maxville) Winter/Spring	0.20 ± 0.09 n=8
Duval County- south (Maxville) Fall	0.31 ± 0.25 n=11
Lake County (Groveland) Winter/Spring	0.06 n=1
Lake County (Groveland) Fall	0.09 ± 0.03 n=4
Alachua County Winter/Spring	0.40 ± .10 n=20

Both fluorescent dyes were retarded in the septic systems with respect to SF_6. The first indication of this is the observation that in each experiment the SF_6 traveled further out into the well field and was observed in many more wells than either dye. At the Duval south site, the fluorescein peak occurred 5 days after the SF_6 peak at mound well DsM2. Approximately 4 meters further from the injection at the toe of the mound, the observed separation of the two tracer peaks was 55 days at DsM5 and 60 days at DsM6. The SF_6 rate of 0.11 ± 0.03 m/d from these wells is almost 3 times that of the fluorescein rate 0.04 ± 0.00 m/d (Table 31). Rhodamine WT was held preferentially to SF_6 by the septic mound at Duval south to an even greater degree, with the only observation of the dye at well DsM2. These observations are consistent with results obtained by Sabatini and Austin, 1991. They observed rhodamine WT being adsorbed to a greater extent than fluorescein in alluvial aquifer sands.

The Alachua winter/spring data also presents further evidence fluorescein is retarded compared to SF_6. The rates from the fluorescein data are approximately 50% of the SF_6 rates (Table 32). Additionally, SF_6 was observed throughout the well field during the experiment, while fluorescein was only seen at AS2 and the five mound wells (Appendix 8).

The very dry conditions at Lake County during the winter/fall experiment presented problems sampling SF_6. The low water volumes in several wells were not sufficient for the bubble free stream of water required for the SF_6 samples. The dye and biological samples are not limited in this way.These difficulties, likely caused some SF_6 peaks to be missed, making the differences in the two tracers less obvious than at the other two sites. The mound wells LM2 and LM3, show the separation in tracer peaks observed at the other sites (Figures17o-p). Off the mound, the difficulty in obtaining SF_6 samples during this experiment obscured any separation of tracer peaks that may have occurred. Separation of SF_6 peaks and rhodamine peaks were obvious during the Lake County fall experiment, especially at wells LM3, LM6 and LM11. (Figures 17at-av) The peaks at well LM6 were separated by 24 days and further from the mound, at well LM11, 33 days.

Table 32. Comparison of SF_6, fluorescein and rhodamine WT rates for the five tracer experiments. All rates are reported in units of meters/day.

Grade to Grade	**SF_6**	**Fluorescein**	**Rhodamine**
Duval Co. south Winter/Spring	0.20 ± 0.09 n=8		
Duval Co. south Fall	0.31 ± 0.25 n=11		
Lake County Winter/Spring	0.06 n=1	0.08 ± 0.03 n=7	
Lake County Fall	0.09 ± 0.03 n=4		0.17 n=1
Alachua County Winter/Spring			
Mound Well to Grade	**SF_6**	**Fluorescein**	**Rhodamine**
Duval Co. south Winter/Spring	0.11 ± 0.03 n=5	0.04 ± 0.00 n=6	
Duval Co. south Fall	0.22 ± 0.01 n=3		
Lake County Winter/Spring	0.08 ± 0.02 n=2	0.13 ± 0.10 n=5	
Lake County Fall	0.40 ± 0.09 n=2		0.14 ± 0.04 n=2
Alachua County Winter/Spring		0.23 ± 0.03 n=3	
Injection Point to Grade	**SF_6**	**Fluorescein**	**Rhodamine**
Duval Co. south Winter/Spring	0.21 ± 0.09 n=8		
Duval Co. south Fall	0.32 ± 0.16 n=18		
Lake County Winter/Spring	0.19 ± 0.04 n=2	0.41 ± 0.17 n=3	
Lake County Fall	2.06 ± 0.28 n=4		0.62 n=1
Alachua County Winter/Spring	0.41 ± 0.11 n=20	0.20 n=1	
Injection Point to Mound Wells	**SF_6**	**Fluorescein**	**Rhodamine**
Duval Co. south Winter/Spring	0.12 ± 0.03 n=3	0.10 n=1	
Duval Co. south Fall	0.11 ± 0.05 n=3		
Lake County Winter/Spring	1.32 n=1	0.44 n=1	
Lake County Fall	1.30 ± 0.58 n=3		0.87 ± 0.96 n=3
Alachua County Winter/Spring	0.07 ± 0.01 n=2		

Due to the difficulties obtaining SF_6 samples and the very low concentrations of SF_6 found in the wells (near detection limits), the rates calculated from the SF_6 data for Lake County are suspected of being artificially low. Since fluorescein was found to be severely retarded in the systems at both the Duval County-south and Alachua County sites, the rates from the PRD-1 tracer are thought to be the most representative of the groundwater movement at the Lake County site. Table 33 summarizes estimates of groundwater transport rates for the Duval County – south, Alachua County and Lake County sites.

Table 33. *Summary of the best estimates of groundwater transport from the* SF_6 *and PRD-1 data at the three sites studied.*

	Tracer	Meters/Day	Feet/Day
Duval County-south (Maxville) Winter/Spring	SF_6	0.20 ± 0.09 n=8	0.66 ± 0.30
Duval County- south (Maxville) Fall	SF_6	0.31 ± 0.25 n=11	1.02 ± 0.82
	PRD-1	0.30 ± 0.11 n=4	0.99 ± .36
Lake County (Groveland) Winter/Spring	PRD-1	0.27 ± 0.13 n=6	0.89 ± 0.43
Lake County (Groveland) Fall	PRD-1	0.59 ± 0.29 n=4	1.94 ± 0.96
Alachua County Winter/Spring	SF_6	0.40 ± .10 n=20	1.32 ± .33

SIA Hydrology Modeling

Simulation modeling of SIA hydrology suggested that flooding occurs on a regular basis in the areas immediately upland of the edge of an SIA. By its designation as a seasonally inundated area, the term suggests that the SIA is inundated regularly during each year, and in fact inundation must occur for sufficient time for hydric conditions to be generated in soils, or for hydrophytic vegetation to dominate. Yet flooding can and does occur upland from this SIA edge. The extent and duration depend on the size of the storm event and the impervious area (development) of the watershed. The model showed that the extent of flooding that results from a 5-year storm event with a 30% impervious area (Figure 34b) was of sufficient duration (over 4 days) and expansion toward the drainfield (66 feet) to allow inundation adjacent and into the mounded drainfield. Such conditions could allow direct upward migration of contaminants and pathogens into surface waters.

Anecdotal observations from the Duval County-south site (Maxville) during sampling events in the fall and winter 2000 provides important evidence that flooding in areas surrounding SIAs is a regular occurrence. Standing water was observed in contact with the drain mound following three different rain events during the two chemical tracer experiments. At the time of the fall injection of chemical tracers (9/19/00), the site was wet from a tropical storm (see Appendix D-14 for a rainfall history of the area during these events). Water was observed pooled around the drain field mound covering most of the area between the drainfield and the SIA and inundating wells DsM4, DsM19, DsM20, DsM21 (well covers are more or less flush with the ground surface). Another rain event occurred on 9/29-30/00. On 10/1/00, standing water was again observed pooled around the drain mound, with wells DsM4, DsM19, DsM20 covered by standing water. The water depth was 3.5 cm above well DsS2, about 2 cm above DsS3, and about 9.5 cm above DsS4 . During December tracer experiment, approximately 1.5 inches of rain fell on 1/23-24/00 and three days later wells DsM19 and

DsM20 were observed covered by water. Additional rain falling on 1/28–30/00 caused the area of standing water to expand, inundating DsM4. These events occurred during drought conditions in Duval County. DOH records indicate this OSTDS system was installed and inspected to code and had a setback of 35 feet from the SIA to the septic tank mound.

Conclusions

Measurements of conductivity and ground water modeling of the "plume" created in the groundwater by OSTDS showed that the plume extended an average of 77.5 feet (range from 40 to 130 feet) down slope from the drain fields toward the lower elevation SIAs. Microbiological sampling showed pathogenic organisms may travel in ground water as much as 120 feet from the drain field. Tracer studies suggested that movement of contaminants in ground waters ranged from about 6 inches per day to about 20 inches per day. Simulation modeling of SIA hydrology showed that flooding in areas adjacent to SIAs can occur on a regular basis but varies depending on size of storm event and amounts of development in the watershed. Anecdotal evidence collected on three occasions during the course of this study suggests that flooding outside of SIAs is not an uncommon occurrence, but conversely, happens on a relatively frequent basis.

Risk assessment using accepted inactivation models determined that distances required for virus inactivation to "acceptable" numbers could be as high as 1610 feet (for an insignificant risk to public health) or as low as 85 feet (representing a high risk to public health). These distances depend on many factors, but illustrate the minimum and maximum distances under high and low risk assessments calculated using the transport rates found in this study.

Considering the possibility of pathogenic organisms in ground waters at distances up to 120 feet from OSTDS drain fields and the potential for their migration into surface waters, especially when these areas are flooded, a SIA setback is recommended to protect public health. If one were to evaluate flooding occurrences and the distances affected in light of the risk each presents, a 5-year design storm may represent significant risks. The flooding that occurs with a 5-year storm and 38% impervious surface in the watershed is about 21 meters (69 feet) distance upland from the SIA edge. A typical residential development with one-quarter acre lots has a 38% impervious area, as suggested by the United States Department of Agriculture's Soil Conservation Service (Technical Release 55). Flooding during storm events may compromise the effective functioning of the drain field and reduce treatment levels in the soil matrix immediately below the drain field. Since these flooded soils increase the likelihood of pathogens entering surface waters and thus posing a serious threat to human health, a setback seems warranted. Larger storm events, occurring less frequently, flood greater distances into the upland and thus demand a larger setback. The trade off here is the frequency of occurrence versus the likelihood of pathogens in lower concentrations migrating into the surface waters.

Regardless of what distance is finally agreed upon, or how setbacks might be determined in the future, one is clear...the methodology used to approve OSTDS sites has deficiencies. The current method does not account for changes in the water table elevation due to site changes resulting from development. To keep the base of systems from being saturated (as observed in at the Duval County-south site)) a setback must be imposed that places the system in an un-ponded area. It also must be located such that it preserves the 2 foot vertical separation distance between the bottom of the drainfield and the top of the water table.

In summary, considering the potential for public health problems that may result from pathogenic organisms migrating into surface waters and the reduced functioning of treatment systems that may result from flooded soil conditions, we recommend a minimum OSTDS setback distance from SIAs of between 69-100 feet to protect water quality and public health. The risk associated with a setback of this dimension is still significant but when coupled with the fact that inundation of areas surrounding an SIA may only occur several times per year, lasting several days each time, we feel the risk may be reduced to acceptable levels. Certainly the larger the setback, the lower the risk.

LITERATURE CITED

American Public Health Association, American Water Works Association, and the Water Environment Federation, 1992. Greenberg, A.E., Clesceri, L.S.,and Eaton, A.D. (ed.), Standard Methods, 18th Edition, for the Examination of Water and Wastewater. Baltimore, MD.

Anderson, D.L., A.L. Lewis, and K.M. Sherman, 1991. Human enterovirus monitoring at onsite sewage disposal systems in Florida. In On-Site Wastewater Treatment Vol. 6: proceedings of the 6th national symposium on individual and small community sewage systems. Am. Soc. Of Ag. Eng. 2930 Niles Rd. St. Joseph, Mich. 49085. USA

Asano, T., Leong, L.Y.C., Rigby, M.G., and R.H. Sakaji. 1992. Evaluation of the California Wastewater Reclamation Criteria using Enteric Virus Monitoring Data. Wat. Sci Tech. Vol. 26, (No. 7-8):1513-1524

Bales, R.C., Gerba C.P., Grondin, G.H., and S. L. Jensen. (1989). Bacteriophage Transport in Sandy Soil and Fractured Turf. *Appl. Environ. Microbiol*. 55(8), 2061-2067.

Bales, R.C., S. Li, K.M. Maguire, M.T. Yahya, C.P. Gerba, and R.W. Harvey. 1995. Virus and bacteria transport in a sandy aquifer, Cape Cod, MA. Ground Water, 33(4), pp. 653-661.

Bisson, J.W., and Cabelli, V.J. 1979. Membrane filter enumeration method for *Clostridium perfringens*. Appl. Environ. Microbiol. 37 (1): 55-66.

Boggs, J.M., S.C. Young, H.F. Hemond, L. Richardson, and M.E. Schaefer, 1988. Evaluation of tracer sampling devices for the macrodispersion experiment. Tennessee Valley Authority, EA-5816.

Brown, M. T., and J. M. Schaefer. 1987. *An Evaluation of the Applicability of Upland Buffers for the Wetlands of the Wekiva Basin*. Final Report to the St. Johns River Water Management District, Palatka, FL. Gainesville, FL: Center for Wetlands, Univ. of FL.

Brown, M.T. 1989. Forested wetlands in urbanizing landscapes. In D.D. Hook and Russ Lea (eds.), *Proceedings of Symposium: The Forested Wetlands of the Southern United States: 1988 July 12-14; Orlando, FL.*, pp. 19-26. General Technical Publications GTR-SE-50. Asheville, NC: U.S. Department of Agriculture, Forest Service Southeastern Forest Experiment Station.

Brown, M.T. and M.F. Sullivan. 1987. The value of wetlands in low relief landscapes. In D.D. Hook (ed.), *The Ecology & Management of Wetlands.* Beckenham, England: Croom Helm. pp. 133-45.

Brown, M.T. and R.E. Tighe. (eds.) 1990. *Techniques and Guidelines for Reclamation of Phosphate Mined Lands.* Final Report to the Florida Institute of Phosphate Research. Gainesville, FL: Center for Wetlands, Univ. of FL. 704 pp.

Brown, M.T., J. Schaefer, and K. Brandt. 1989. *Buffer Zones for Water, Wetlands, and Wildlife in the East Central Florida Region*. Prepared for the East Central Florida Regional Planning Council, Winter Park, FL. Gainesville, FL: Center for Wetlands, Univ. of FL.

Carter, L.J. 1974. *The Florida Experience: Land and Water Policy in a Growth State.* John Hopkins U. Press, Baltimore, Md. 355 pp.

Chendorain, M., M. Yates, and F. Villegas. 1998. The fate and transport of viruses through surface water constructed wetlands, J. Environ. Qual., 27, pp. 1451-1458.

Clark, J.F., R. Wanninkhof, P. Schlosser, and H.J. Simpson, 1994. Gas exchange rates in the tidal Hudson river using a dual tracer technique. Tellus, 46B pp. 274-285.

Corbett, D.R., K. Dillon, and W. Burnett, 2000. Tracing groundwater flow on a barrier island in the northeast Gulf of Mexico. Estuarine, Coastal, and Shelf Science, submitted.

DeBorde, D.C., W.W. Woessner, B. Lauerman, and P.N. Ball, 1998, Virus Occurrence and Transport in a School Septic System and Unconfined Aquifer, Ground Water, vol. 36, No. 5, pp. 825-834.

Department of Defense, Groundwater Modeling System (GMS 2.1), Brigham Young University – Engineering Computer Graphics Laboratory, 1998.

Dillon, K., 1998. The use of sulfur hexafluoride as a groundwater tracer in the Florida Keys. M.S. Thesis, Department of Oceanography, Florida State University, 123

Dillon, K., D.R. Corbett, J.P. Chanton, W.C. Burnett and D.J. Furbish, 1999. The use of sulfur hexafluoride as a groundwater tracer of septic tank effluent in the Florida Keys. J. Hydrol. 220, 129-140.

Dillon, K., D.R. Corbett, J.P. Chanton, W.C. Burnett and L. Kump, 2000. Rapid transport of a wastewater plume injected into saline groundwaters of the Florida Keys, USA. Submitted to Groundwater.

Duley, J.W., 1987. Water tracing using a scanning spectrofluorometer for detection of fluorescent dyes. In: Environmental problems in karst terrains and their solution. Proceedings, National Water Well Association, Dublin, Ohio, 389-405.

Feachem, R.G., Bradley, D.J., Garelick, H. Mara, D.D. 1983. *Sanitation and Disease Health aspects of Excreta and Wastewater Management*. World Bank, John Wiley and Sons, New York, NY.

Freeze, R.A. and J.A. Cherry, *Groundwater*, Prentice Hall, 1979.

Fujioka, R.S., and L.K. Shizumura. 1985. *Clostridium perfringens*, a reliable indicator of stream water quality. J. Water Pollut. Control Fed. 57:986-992.

Funderberg, S.W., B.E. Moore, B.P. Sagik, and C.A. Sorber. 1981. Viral transport through soil columns under conditions of saturated flow, Water Res., 15, pp. 703-711.

Gaspar, E., 1987. Modern trends in tracer hydrology, vols I and II. CRC Press, Inc., Boca Raton, Florida.

Haas, CH., Rose, JB., and Gerba, CP. (eds) 1999. *Quantitiative Microbial Risk Assessment.* John Wiley and Sons, NY, NY.

Harvey, R.W., and L.H. George. 1989. Transport of Microshperes and indigenous bacteria through a sandy aquifer: results of natural- and forced-gradient tracer experiments, ES&T, 23, pp. 51-56.

Heikurainen, L., 1963. On using groundwater fluctuations for measuring evapotranspiration. *Acta Forestalia Fennica* 76(5): 1-16.

Heimberg, K., 1984. Hydrology of north-central Florida cypress domes. *Cypress Swamps*. K.C. Ewel and H.T. Odum, editors. Gainesville, FL: University of Florida Presses. pp. 72-82.

Kasnavia, T., De Vu, D.A. Sabatini, 1998. Fluorescent Dye and Media Properties Affecting Sorption and Tracer Selection. Ground Water 37, 3: 376-381.

Keswick, B. H., Gerba, C.P., Secorr, S.L., and Ceech, I., 1982. Survival of enteric viruses and indicator bacteria in groundwater. J. Environ. Sci. Health. A17, pg 903.

LeBlanc, D.R., S.P. Garbedian, K.M. Hess, L.W. Gelhar, R.D. Quadri, K.G. Stollenwerk, and W.W. Wood, 1991. Large-scale natural gradient tracer test in sand and gravel, Cape Cod,

Massachusetts: Experimental design and observed tracer movement. Water Resour. Res. 27, 896-910.
Ledwell, J.R. A.J. Watson and C.S. Law, 1993. Evidence for slow mixing across the pycnocline from an open-ocean tracer-release experiment. Nature, 364, 701-703.
Lipp, E.K. and J.B. Rose, R. Vincent, R.C Kurz, and C. Rodriquez-Palacios. 1999. Assessment of the microbiological water quality of Charlotte Harbor, Florida. Report to the Florida Dept. of Health and Southwest Florida Water Management District.
MacIntyre, S., R. Wanninkhof, and J.P. Chanton, 1995. Trace gas exchange in freshwater and coastal marine systems: flux across the air water interface. In *Methods in Ecology: Trace Gases*, P. Matson and R. Harriss (eds.), Blackwell Scientific.
McDonald, M.G., and A.W. Harbaugh, 1988, A Modular Three-Dimensional Finite-Difference Ground-Water Flow Model, Techniques of Water Resource Investigation 06-A1, United States Geological Survey.
McNeillie, J.I., D.L. Anderson, and T.V. Belanger. 1994 Investigation of the surface water contamination potential from on-site wastewater treatment systems (OWTS) in the Indian River Lagoon Basin. In Collins E. (ed) Onsite Wastewater Treatment. Proceedings of the 7th International Symposium on Individual and Small Community Sewage Systems. Am. Soc. Of Ag. Eng. 2930 Niles Rd. St. Joseph, Mich. 49085. USA
Mitsch, W.J. and J.G. Gosselink. 1986. *Wetlands.* Van Nostrand Reinhold. New York.
Morinigo, M.A., R. Corax, M.A. Munoz, P. Romero, and J.J. Borrego. 1990. Relationships between *Salmonella* spp. and indicator microorganisms in polluted natural waters. Water Res. 24:117-120.
Nicosia, L.A. 1998. Assessment of Florida On-Site Sewage Disposal System Regulations for the Removal of Viruses. Master's Thesis, University of South Florida, Dept. of Marine Science.
Omoti, U. and A. Wild, 1979. Use of fluorescent dyes to mark the pathways of solute movement through soils under leaching conditions. 1. Laboratory experiment. Soil Science. 128, 1:28-33.
Paul, J.H., J.B. Rose, C. Kellogg, J. Brown, E.A. Shinn, and S. Miller. 1995. Viral tracer studies indicate contamination of marine waters by sewage disposal practices in Key Largo, Florida. Appl. Environ. Microbiol. 61:2230-2234.
Paul, J.H., J.B. Rose, S. Jiang, C. Kellogg, and E.S. Shinn. 1995a. Occurrence of fecal indicator bacteria in surface waters and the subsurface aquifer in Key Largo, Florida. Appl. Environ. Microbiol. 61: 2235-2241.
Payment, P. and E. Franco. 1993. *Clostridium perfringens* and somatic coliphages as indicators of the efficiency of drinking water treatment for viruses and protozoan cysts. Appl. Environ. Microbiol. 59:2418-2424.
Pickens, J.F., J.A. Cherry, G.E. Grisak, W.F. Merritt, and B.A. Risto, 1978. A multilevel device for groundwater sampling and piezometric monitoring. Groundwater, 16, 322-327.
Powelson, D.K., Gerba, C.P. and M.T. Yahya.1993. Virus Transport and Removal in Wastewater During Aquifer Recharge. Water Research. 27(4):583-590.
Quinlan, J.F., 1989. Groundwater monitoring in karst terrains: recommended protocols and implicit assumptions. U.S. Environmental Protection Agency, Las Vegas, EPA/600/x-89/050.

Reich, C.D., 1993. Groundwater flow at Long Key, Florida Keys: a dye tracer study. Ms. Sc. Thesis, University of South Florida.

Rose, J. B. and Zhou, X. 1995. Phillippi Creek Water Quality Report. Sarasota Bay National Estuary Program.

Rose, J.B. and E.K. Lipp, 1997. A study on the Presence of Human Viruses in Surface Waters of Sarasota County. Report to the Florida Department of Health, Department of Marine Sciences, University of South Florida, St. Petersburg, FL

Sabatini, D.A. and T.A. Austin, 1991. Characteristics of Rhodamine WT and Fluorescein as Adsorbing Ground-Water Tracers. Ground Water 29, 3: 341-349.

Scandura, J.E., M.D. Sodsey. 1998. Viral and bacterial contamination of groundwater from on-site sewage treatment systems, Wat. Sci. Tech., 35(11-12), pp. 141-146.

Smart, P.L. and I.M.S. Laidlaw, 1977. An evaluation of some fluorescent dyes for water tracing. Water Resour. Res. 13, 15-33.

Smart, P.L., 1984. A review of toxicity of twelve fluorescent dyes used for water tracing. NSS Bulletin, 46, 21-33. Reich, 1993.

Sorenson, D. L., S.G. Eberl, and R.A. Dicksa. 1989. *Clostridium perfringens* as a point source indicator in non-point polluted streams. Water Res. 23:191-197.

Sullivan, M.F. 1986. Organization of low-relief landscapes in north and central Florida. MS Thesis. Gainesville, FL: Univ. of FL. 100 pp

Trudgill, S.T., 1987. Soil water dye tracingwith special reference to the use of rhodamine WT, lissamine FF and amino G acid. Hydrological Processes. 1, 149-170.

Upstill-Goddard, R.C., A.J. Watson, J. Wood, and M.I. Liddicoat, 1991. SF6 and 3He as sea-water tracers: deployment techniques and continuous underway analysis for SF6. Anal. Chim. Acta 249, 555-562.

Upstill-Goddard, R.C., A.J. Watson, P.S. Liss, and M.I. Liddicoat, 1990. Gas transfer velocites in lakes measured with SF6. Tellus, 42B, 364-377.

Upstill-Goddard, R.C., and C.S. Wilking, 1995. The potential of SF6 as a geothermal tracer. Water Res. 29, 1065-1068.

Wanninkhof, R., J.R. Ledwell, and A.J. Watson. 1991. Analysis of sulfur hexafluoride in seawater. J. of Geophysical Research. 96:C5, 8733-8740.

Wanninkhof, R., J.R. Ledwell, and W.S. Broecker, 1985. Gas exchange - wind speed relationship measured with sulfur hexafluoride on a lake. Science, 227, 1224-1226.

Wanninkhof, R., J.R. Ledwell, W.S. Broecker, and M. Hamilton, 1987. Gas exchange on Mono Lake and Crowley Lake, California. Jour. Geophys. Res., 92, 14567-14580.

Watson A.J., R.C. Upstill-Goddard, and P.S. Liss, 1991. Gas exchange in rough and stormy seas measured with a dual tracer technique. Nature, 349, 145-147.

Wilson R.D. and D.M. Mackay, 1993. The use of SF6 as a conservative tracer in a saturated sandy media. Groundwater, 31, 719-724.

Yates, M.V. 1985. Septic tank density and ground water contamination. Ground Water. 23: 585-591.

Yates, M. V. and S. R. Yates. 1987. Modeling microbial fate in the subsurface environment. CRC Critical Reviews in Environmental Control. 17(4): 307-344.

Zheng, C., 1990, "MT3D: A modular Three-Dimensional Transport Model for Simulation of Advection, Dispersion and Chemical Reactions of Contaminants in Groundwater Systems." S.S. Papadopulus & Associates, Inc.

FIGURES