A Case for the Safety & Sustainability of Class B Biosolids Land Application— Results of Microconstituent & Pathogen Research in Gainesville

Paul B. Davis, Ronald G. Herget, Richard H. Hutton, George Lukasik, Patricia V. Cline, Timothy M. Ptak, Jason Mau, and Allan H. Biddlecomb

Paul B. Davis, P.E., is a water/wastewater engineer with Gainesville Regional Utilities (GRU); Ronald G. Herget, P.E., is GRU's director of water and wastewater engineering; Richard H. Hutton, P.E., is a supervising utility engineer with GRU. George Lukasik, Ph.D, is executive director of BCS Laboratories in Gainesville. Patricia V. Cline, Ph.D, is a senior scientist with the engineering firm CH2M Hill in Gainesville; Timothy M. Ptak, P.E., is a client service manager in the firm's Gainesville office; Jason Mau, P.E., is a senior project engineer in the firm's Gainesville office. Allan H. Biddlecomb, P.G., is a senior hydrogeologist with the engineering/architectural/scientific firm Jones Edmunds & Associates in Gainesville. *Reprinted with permission from Proceedings of the Residuals and Biosolids 2010 Specialty Conference, May 23-26, 2010, Savannah, Georgia,* *Alexandria, Virginia. This article was presented as a technical paper at the 2010 Florida Water Resources Conference in May.*

The city of Gainesville, doing business as Gainesville Regional Utilities (GRU), owns and operates two water reclamation facilities that serve over 160,000 customers within Gainesville and the unincorporated area of Alachua County. As part of the water reclamation process, biosolids are generated and have been beneficially recycled through sustainable land application practices for over 27 years at Whistling Pines Ranch (WPR), a 1,100-acre farm west of the city of Archer in the southwest part of the county (see Figure 1, page 6). The biosolids replace or supplement inorganic fertilizer used to grow a variety of forage and row crops, and they also act as a soil amendment to the typically sandy soils at the site.

GRU's biosolids land application program is permitted by the U.S. Environmental Protection Agency (EPA) and the Florida Department of Environmental Protection (FDEP) through Section 503 of Title 40 Code of Federal Regulations and Chapter 62-640, Florida Administrative Code (F.A.C.). The biosolids program has been recognized for its exemplary performance, and GRU was awarded the EPA's Most Outstanding Biosolids Operation in the Southeast United States in 2004. Also, the Kanapaha Water Reclamation Facility was awarded the FDEP 2009 Plant Operations Excellence Award, which included a section on biosolids program operations.

In addition to biosolids treatment for its two water reclamation facilities, GRU provides biosolids treatment and recycling services to the University of Florida and the communities of Hawthorne, High Springs, and Waldo. In order to ensure the long-term capabilities of providing these services, the Gainesville City Commission approved the purchase of a portion of WPR with the condition of obtaining necessary permits. As a result of changes in land use regulations, GRU requested a special exception to the county's Unified Land Development Code to continue the beneficial use of biosolids.

As a condition of the special exception application process, GRU evaluated the potential exposure to microconstituents in biosolids applied at WPR. Microconstituents, as termed in this article, include a number of trace organic compounds of interest. Among them are those identified as "endocrine disruptors," pharmaceuticals, or personal care products.

The work to sample and evaluate microconstituents was termed Phase 1 and was developed in conjunction with the Alachua County Environmental Protection Department and the Florida Department of Health (FDOH)/Alachua County. Phase 1 sampling and analysis for microconstituents was performed on the biosolids, biosolids and soil mixture, and groundwater.

Phase 2 of the study evaluated exposure pathways from WPR with regard to pathogens and was developed in conjunction with the County Environmental Protection Department. Phase 2 consisted of sampling and analysis of (1) bioaerosols for pathogens, (2) transmission of soil metals and radionuclides to groundwater, and (3) vectors (flies, mosquitoes, etc.).

Figure 1 is an aerial photograph of the farm with field delineation. Local stakeholders typically live in areas to the west of the WPR property.

The EPA has established strict rules for the treatment of biosolids, sampling, analysis, and disposal in 40 CFR Part 503 (http://yosemite.epa.gov/r10/water.nsf/NPDES+Permits/Sewage+S825/$FILE/503-032007.pdf). Guidance documents have been added to simplify and aid in the compliance with the rule (EPA/625/R-92/013).

The guidance documents outline methodologies for the appropriate analysis and testing of biosolids to ensure that adequate treatment has been performed. The EPA is a significant proponent of the beneficial use of biosolids and has designed the current standards exclusively to maintain the significant benefits offered by biosolids reuse, while continuing its commitment to protecting public and environmental health.

The 40 CFR Part 503 rule was based on the results of extensive research, sampling, and analysis, as well as multiple extensive risk assessment studies. In these studies, the traditional risk assessment framework was modified in order to include state-of-the-art technology to evaluate the possible negative health impacts of land-applied biosolids.

Research is ongoing, but to date no conclusive evidence has been provided that indicates the 503 rule fails to protect public health as it is currently written and enforced. Subpart D of the 503 rule outlines the specific requirements for the reduction of pathogens within biosolids, as well as their attractiveness to vectors that may harbor additional pathogens.

Essentially the EPA essentially named two classes of biosolids: Class A biosolids are not considered to pose a risk to the general public

Continued on page 6

Continued from page 4

through direct contact, while Class B biosolids are treated to reduce pathogen levels significantly but are not free of pathogens. To be classified as either Class A or Class B, sludges must receive appropriate treatment to meet the specific requirements for reduction of pathogens.

For Class B biosolids application, natural processes occurring in the soil (desiccation, predation, ultraviolet radiation, etc.) are utilized to eliminate pathogens. In order to protect public health and ensure natural die-off of pathogens, EPA regulations restrict exposure to Class B biosolids for prescribed waiting periods.

The Kanapaha and Main Street Water Reclamation Facilities utilize aerobic digestion to convert raw waste activated sludge into a beneficially useable Class B biosolids product.

Methodology

Phase 1 - Microconstituents

The microconstituent sampling and analysis plan was developed in fall 2007 to address questions raised during public meetings regarding potential exposure to these compounds. Domestic wells present in the vicinity of the WPR site are used for drinking water, so impacts to groundwater were a priority of this study.

At that time, EPA Methods 1694 and 1698 had not been finalized, and most commercial laboratories had not validated these methods. Columbia Analytical Services in Kelso, Washington, was the laboratory identified as having validated these protocols for aqueous samples; no commercial laboratory was identified with validated methods for solid samples at that time.

The analysis of onsite groundwater samples provides information on current conditions resulting from over 27 years of biosolids land application practices at WPR. Future migration potential of microconstituents in surface soil was evaluated using standard leach tests, and the aqueous phase of the biosolids was collected to identify a worst-case source term for the soil-to-groundwater migration pathway.

Sample Location Selection & Protocol—Groundwater, soil, and biosolids samples were collected on January 31, 2008, and February 4, 2008, and analyzed for the microconstituents. The Alachua County Environmental Protection Department observed the sampling process.

Groundwater samples were collected from four onsite wells, as shown on Figure 2. These include three of the eight irrigation wells used for the farming operations and from the silo well, which is used to mix water with the biosolids before application. Wells were selected based on depth because microconstituent detection would be more likely from the infiltrating water through sandy and clayey-sandy soils. Table 1, see page 8, summarizes the wells sampled that pump from the uppermost portion of the Floridan Aquifer. The wells sampled were chosen based on casing and total depth construction characteristics.

Soil samples were collected from the fields surrounding each well sampled. Soils were collected from areas where the biosolids have been applied and disked into the soil for over 27 years.

Biosolids samples were collected from the GRU Main Street Water Reclamation Facility and the Kanapaha Water Reclamation Facility and were composited into one sample.

Sample Analysis—All samples were analyzed by Columbia Analytical Services. The microconstituents analyzed for this study include:

- Estrogenic hormones and their metabolites

Continued on page 8

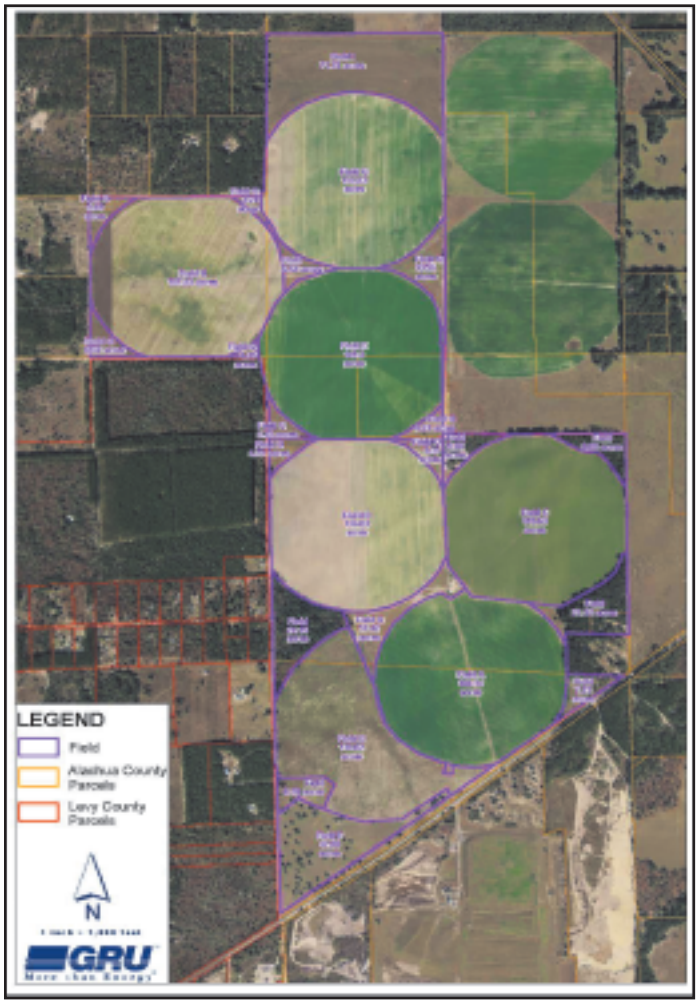

FIGURE 1: Whistling Pines Ranch Biosolids Land Application Site

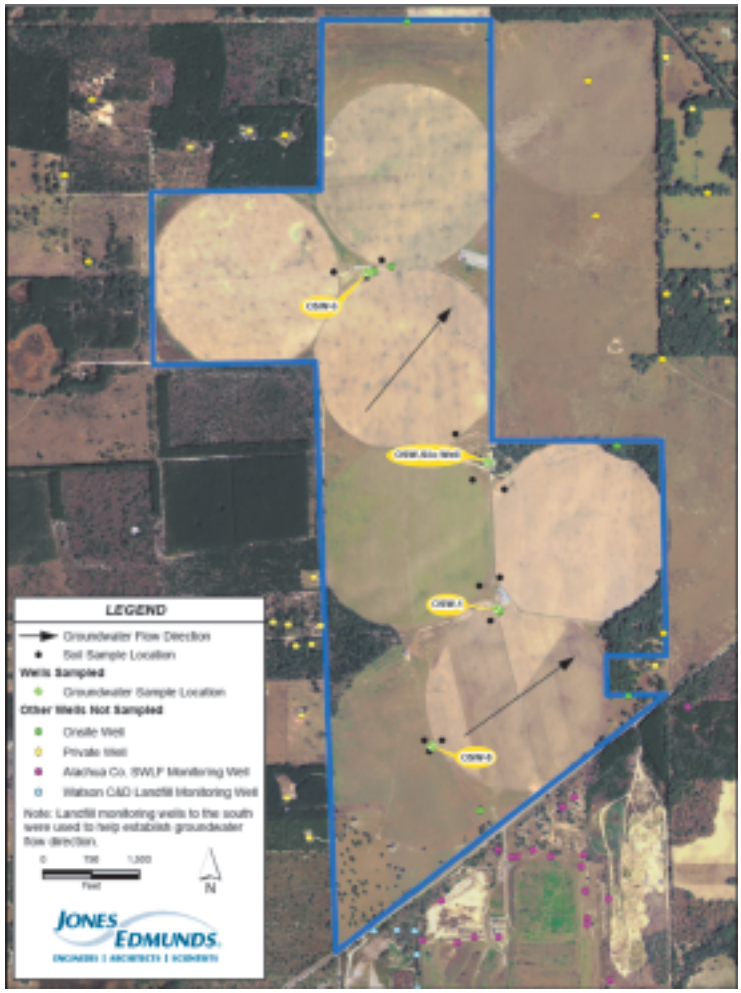

FIGURE 2: Whistling Pines Ranch Sample Locations

Continued from page 6

(17-a-ethynylestradiol, 17-a-estradiol, 17-ß-estradiol, Estriol, and Estrone)

- Pharmaceutical-related compounds (fluoxetine and Iopromide)
- Industrial/home products that potentially can be estrogenic (Bisphenol A) and detergent/surfactant compounds and their metabolites (4-tert-Octylphenol, Nonylphenol, Nonylphenol monoethoxylate, and Nonylphenol diethoxylate)

The detergent surfactant compounds and metabolites were analyzed by gas chromatography/mass spectroscopy/selected ion monitoring (GC/MS/SIM), while the remaining microconstituents were analyzed by liquid chromatography/mass spectroscopy/mass spectroscopy (LC/MS/MS).

Aqueous phase samples were obtained from soils and biosolids for microconstituent analysis. The soil samples were extracted using the Synthetic Precipitation Leaching Procedure (EPA Method 1312). The biosolids sample was centrifuged to separate the solid and aqueous phases.

Sampling Procedures—All samples were collected by a trained environmental technician experienced with environmental media sampling. Groundwater and soil sampling were performed in accordance with FDEP Standard Operating Procedure DEP-SOP-00/01 as required by FS 2200 and FS 3000, respectively. Groundwater samples were collected in four 1-liter amber glass jars. Two jars were unpreserved and two jars were preserved with sulfuric acid (H_2S0_4) to a pH <2.

Groundwater quality assurance/quality control (QA/QC) samples included a trip blank, a duplicate collected from OSW-Silo, a bottle blank, and a bottle of High Performance Liquid Chromatography (HPLC) water. The bottle blank was used in place of an equipment blank because the water samples were collected directly from the well taps without tubing or sampling equipment. For the bottle blank HPLC-grade sample, water was poured directly into the sample bottles.

Well (feet)	Total Depth (feet)	Casing Depth (feet)
OSW-1	180	70-90
OSW-6	190	60-90
OSW-8	150	100
OSW-Silo	90	60

TABLE 1: Well characteristics.

The purpose of the bottle blank was to determine if contaminants were transferred from the laboratory to the sample container before the samples were collected. An unopened bottle of the same HPLC-grade water was sent for analysis to ensure that no contaminants were present in this water. The trip blank was included in the sample kits and was returned with the samples. The samples were stored immediately in ice following sampling and were shipped to the laboratory by overnight delivery.

Four composite soil samples were collected from the fields surrounding the wells sampled. The sample locations are shown on Figure 2. Each soil composite consisted of three samples taken near the respective sampled well. The locations corresponded to areas of biosolids application.

Samples were collected approximately 0.5 to 1 foot below land surface–the depth of disking of the soil. A shovel was used to excavate a hole approximately one foot deep, and samples were collected from the side wall of the hole that had not contacted the shovel. The soil samples were stored immediately in ice. The individual soil samples were sent to the laboratory to be composited in equal proportions.

Soil QA/QC samples included a trip blank and a jar blank. The jar blank was prepared by pouring HPLC-grade water into extra soil jars and emptying these into the one-liter bottles sent to the laboratory.

One composite biosolids sample was collected consisting of one sample from the Main Street Water Reclamation Facility and one sample from the Kanapaha Water Reclamation Facility. These samples were collected from the gravity belt thickener conveyor belt using glass sample jars.

Four 32-ounce wide-mouth jars (two unpreserved and two preserved) were filled at each facility. The samples were sent to the laboratory to be composited as one-third from the Main Street Water Reclamation Facility and two-thirds from the Kanapaha Water Reclamation Facility (proportional the ratio sent to and applied at the site). Biosolids QA/QC samples included a trip blank and a jar blank collected using the same procedure as the soil QA/QC samples.

Phase 2 – Pathogens

Exposure to pathogens through direct or indirect contact with biosolids was assessed based on previously published scientific studies and the analysis of GRU biosolids, biosolids amended soils, and the dust/bioaerosols generated during land application. The methods for sampling and analysis of pathogens and interpretation of these results were developed based on an extensive review of the literature. The methods were selected and performed to evaluate health risks from exposure to biosolids.

Health Risks Associated with Direct Contact with Biosolids or Biosolids-Amended Soils—For as long as the practice of land application of biosolids has existed, there have been concerns regarding safety the and protection of public health. Class B biosolids typically contain a certain level of pathogens, but upon their application into the soil, factors such as desiccation, predation, ultraviolet inactivation, and additional environmental stressors result in a significant decrease in pathogen and indicator levels.

Numerous scientific studies have been performed and published in peer-reviewed literature to address the safety and sustainability of the land application of biosolids (Brooks et al., 2005a; Brooks et al., 2005b; Gerba et al., 2008; Gerba and Smith, 2005; Pepper et al., 2008; Tanner et al., 2008; and Zerzghi et al., 2009). The overall consensus within the scientific literature is that the current guidelines and practices for the treatment and use of wastewater biosolids are adequate in their protection of human and environmental health. The EPA also has accepted this conclusion.

Continued on page 10

Continued from page 8

Brooks et al. (2005b) assessed the risk of aerosols on communities adjacent to sites where biosolids were being land-applied. In the study, aerosols of indicator microorganisms (bacteria and viruses) were generated, and their survival and travel through the air was monitored at different conditions and distances.

The study concluded that the conservative estimated distance of 30.5 meters (assumed to be nearest adjacent residences) downwind from the application site, resulted in risks of infection of 1:100,000 to the more realistic 1:10,000,000 per exposure. Conservative annual risks were calculated to be no more than 7:100,000, whereas a more realistic risk was no greater than 7:10,000,000. Overall, the viral risk to residences adjacent to land application sites appears to be considerably low, both for one-time and annual probabilities of infection.

Few of the studies to date have looked at the potential risks of the land application of aerobically digested biosolids (the type of biosolids that GRU produces) compared to anaerobically digested biosolids (Brooks et al., 2005a; Brooks et al., 2005b; Gerba et. al., 2008; Gerba and Smith, 2005; Pepper et al., 2008; Tanner et al., 2008; and Zerzghi et al.; 2009). Aerobically digested biosolids generally contain, on an average, fewer pathogens and thus pose an even lower risk than those of anaerobically treated ones (Farrah and Bitton, 1983).

To better determine risks associated with the land application of biosolids in any one particular environment, data are needed on the concentrations of pathogens and indicators in wastes, the effectiveness of treatment processes, the dilution and survival following land application, the standardization of detection methodology, and better quantification of exposure.

Health Risk from Direct Contact & Ingestion of Biosolids—Models for the assessment of risks of infection from Salmonella have been developed by Haas et al. (1999) and Gerba et al. (2008). It is assumed that ingestion of biosolids-amended soil occurs via contamination of the hand, followed by ingestion and/or inhalation, followed by swallowing. Gerba et. al. (2008) estimated that it is safe to ingest anywhere between 50 and 480 milligrams (mg) of biosolids-amended soil during an eight-hour period, but conditions such as the individual's age, health, and type of activities he or she engages in ultimately dictate this risk.

Many scientists have utilized quantitative microbial risk assessment to calculate the risk of exposure to potentially pathogenic microorganisms and hazardous scenarios. This assessment is based on a specific framework developed by the National Research Council and refined by Haas et al. (1999). In general, risk assessment is based on 1) hazard identification, 2) exposure assessment, 3) dose-response assessment, and 4) risk characterization.

Quantitative microbial risk assessments were not performed using the data collected in this study, but the potential risk of exposure and infection for the purposes of this study is based on the reported infectious dose of the pathogens tested for (Salmonella and enteric viruses) and the levels that were detected or were absent in the biosolids, the biosolids-amended soils, and the bioaerosol sampling conducted in this study.

Health Risk from Inhalation of Bioaerosols—In order for a risk to be estimated for bioaerosols, it must be assumed that the bacteria are being aerosolized and transported. The results of the current study, as well as others, indicate that most pathogens either are not aerosolized at all or are inactivated during aerosolization and transport through the air; therefore, only extremely conservative estimates can be made with regard to the associated risks when bioaerosol data are used for exposure assessment.

The most stringent estimates of risk must assume 100 percent aerosolization and 100 percent survival. In order to stay consistent with previous risk assessment studies (Brooks et al, 2005a and 2005b) based on aerosolization and pathogen transport, a setback distance of 33.5 meters (100 feet) downwind from the site of biosolids application was used for this study's air sample collection.

Air Sampling—Air samples were collected during a wind event using BioSampler® (SKC, PA) liquid impinger air samplers. The air samples were concentrated in a liquid medium and analyzed for bacterial indicators and pathogens. These samplers are highly efficient in bioaerosol and bio-particle capture. They have been used in many previous studies (Baertsch et al., 2007 and Paez-Rubio et al., 2005) and were specifically acquired for this project to duplicate previous study efforts.

Fecal Contamination Indicators—The objective of this study was to evaluate the prevalence of pathogens in the soils and bioaerosols generated from the land application of biosolids. In addition to pathogen monitoring, it is usually necessary to sample for fecal indicator organisms to provide a thorough assessment of the microbiological quality of an environmental sample. Indicators are not pathogenic, but they are found in the same environments as pathogens.

Fecal indicator organisms are present in high numbers in fecal material and are easy to detect; their presence can sometimes be an indication of more-difficult-to-detect pathogens that are present in much lower numbers. This type of monitoring is a low-cost, conservative method for predicting the presence of pathogens and protecting public health; however, it does not always correlate to presence of the pathogens.

In contrast to fecal indicator organisms, vast numbers of bacteria are present naturally in the environment that are necessary for a variety of reasons, including nutrient cycling and bioremediation, etc. The presence of these microorganisms is essential to life on earth, as they are essentially the bottom link in the food chain. Without indigenous environmental bacterial flora, the natural decay process would not occur, many plants would be unable to take up nutrients such as nitrogen from their environment and would thus not survive, and many drugs (including many antibiotics) would not exist.

In the laboratory, this large group of organisms is referred to as "heterotrophic plate count" bacteria, or heterotrophic plate counts (HPCs). HPCs are bacteria that need an outside organic carbon source in order to grow, as opposed to photosynthetic organisms that make their own high energy carbon molecules using inorganic carbon dioxide (CO_2) and the energy of the sun. The HPCs are made up of thousands of species of bacteria that grow on standard microbiological media at standard biological conditions.

Usually absolute numbers of HPC bacteria are not indicative of human health risk. In fact, high HPC counts are likely the result of an actively metabolizing environment that is thriving and thus providing a strong founda-

Continued on page 12

Continued from page 10
tion for plant and animal life.

In this study, HPCs were monitored as an internal recovery control mechanism because of the fact that bioaerosol sampling inherently is insensitive. By monitoring for HPCs, it was possible to make the observation that organisms aerosolized in dust indeed were being captured and detected. This provides stronger evidence that negative (pathogen) results obtained in the bioaerosol samples were most likely due to the absence of these organisms and not an inability to detect them.

In contrast to HPCs, Escherichia coli (E. coli) are a fecal indicator organism that makes up approximately 90 percent of the fecal coliform population in a wastewater or fecal sample. E. coli are used as fecal indicator organisms because they inhabit the intestines of warm-blooded animals, therefore making them an indicator of fecal pollution.

Bacterial Enumeration—Indicator bacteria in liquid from the air samplers were quantified by EPA-approved Standard Methods using membrane filtration employing 47-millimeter (mm) cellulose acetate filters with a nominal pore size of 0.45 micron (µm). Total HPC bacteria were enumerated on nutrient agar plates (Becton Dickinson, Sparks, MD) at 25 degrees Celsius (°C) for 96 hours. Fecal coliform bacteria were enumerated on mFC agar for 24 hours at 44.5°C in a water bath.

Blue colonies were enumerated as fecal coliforms. E. coli (ATCC 9637) were used as the positive control for all coliform measurements. E. coli were cultured on modified mTEC agar. Plates were incubated at 35°C for 3 hours followed by 21 hours at 44.5°C.

Magenta colored colonies were enumerated as E. coli. Presumptive Clostridium perfringens were isolated on mCP agar (Acumedia Manufacturers, Inc.). Plates were transferred to gas pack bags (BBL GasPak; Becton Dickinson) and sealed.

After 24 hours of anaerobic incubation at 45°C, colonies were exposed to ammonium hydroxide fumes. All of the yellow/straw-colored colonies that turned pink/magenta were counted. Clostridium perfringens (ATCC 13124) were used as a positive control.

Biosolids and soil bacteriological analysis were conducted by ABC Research Laboratories in Gainesville. Fecal coliforms were enumerated by Standard Method 9221E, E. coli by AOAC 966.24, and Clostridium perfringens by AOAC 976.30. Salmonella spp. was analyzed according to the Bacteriological Analytical Manual for Food. The bacteria were reported as most probable number (MPN) per gram of dry weight.

Enteric Viruses—Liquid waste activated sludge, biosolids, and soil samples were processed according to American Society for Testing Materials (ASTM) methodology (ASTM 4994) and EPA/600/R-95/178. Samples processed were examined for cytopathic effect development on mammalian tissue cell culture as per EPA methodology. MPN determinations were performed using EPA-released software (Most Probable Number Calculator version 4.04; http://www.epa.gov/microbes/other.htm).

Helminth Ova—In the current study, the analysis for helminth ova was not requested by the agencies overseeing the study because of the acknowledgement that their large size renders them unlikely to be aerosolized and thus transported offsite. Even though the analysis for helminth ova was not recommended, BCS laboratories did analyze the biosolids from the water reclamation facilities for their presence. No viable helminth ova were detected in any of the biosolids samples collected (data not presented).

Sample Location Selection & Protocol—This study evaluated the presence of pathogens and indicator organisms related to GRU's biosolids land application program at the WPR farm. The study included analysis of untreated waste activated sludge and biosolids (digested waste activated sludge) from the Main Street Water Reclamation Facility and the Kanapaha Water Reclamation Facility. Also, analyses were performed on biosolids-amended soils and air samples taken during the land application and soil incorporation (via disking) of the biosolids.

Two sets of biosolids-amended soil samples were collected: one that had received biosolids within approximately one hour, and one that had received biosolids three months earlier. Air samples were collected at 100 feet downwind from the tractor during biosolids application and disking operations. These samples are intended to represent a reasonable worst-case exposure of what a neighbor of the site would experience.

Also, to test extreme conditions, air samples were collected 65 feet downwind during biosolids surface application and 42 feet downwind from the disking operation. At that distance, during the disking operation the sampling equipment and personnel were engulfed in a dust cloud as the tractor passed by. Background air samples (negative controls) were collected a significant distance away from and upwind of the biosolids operations.

Other Testing

Additional investigations and data gathering were undertaken to address questions raised by area residents, including the following.

Soil Metals—Soil metals sampling was performed by the FDOH/Alachua County at 18 locations around the parameter areas of the site on the surface soil to gather data on potential wind event dust components. Soil metal concentrations were compared to residential FDEP Soil Cleanup Target Levels. Also, soil evaluations to a depth of seven inches were performed to confirm soil series, soil texture, and "estimated season high water table."

Biosolids Testing for Radionuclides—Biosolids were sampled, sent to the FDOH, and tested for radionuclides; a comparison was made to site exposures from usual natural and manmade exposures. The FDOH radiological sample results are the detectable radionuclides in biosolids. These data were evaluated using the protocols presented in Interagency Steering Committee on Radiation Standards (ISCORS) Technical Report 2004-04: *ISCORS Assessment of Radioactivity in Sewage Sludge: Recommendations on Management of Radioactive Materials in Sewage Sludge and Ash at Publicly Owned Treatment Works* (ISCORS, 2004).

Entomologist Inspection—A University

of Florida entomology professor and graduate students inspected the site for flies/vectors.

Offsite Groundwater/FDOH—Offsite groundwater testing by the FDOH included some Primary Drinking Water Standard scans, microbiological indicators, and nitrate levels.

Additional Data—Testing of dioxin, uranium, and aluminum was also investigated.

Results & Discussion

Phase 1 - Microconstituents

Whistling Pines Ranch Soil & Groundwater—None of the microconstituents of interest were detected in any of the groundwater or soil leachate samples collected from the WPR site. Based on the QA/QC results, analyses demonstrate that these microconstituents were not present in the samples at concentrations above the method detection limit.

Biosolids—Estrogenic hormones, plasticizers, and surfactants were not detected in the GRU biosolids sample. Only one of the 12 microconstituents analyzed, fluoxetine (Prozac), was detected. The concentration of fluoxetine detected was 110 nanograms per liter (ng/L).

Note that there was some interference in the analysis of this source sample, potentially from the presence of some particulates or other dissolved materials in this matrix. Based on the QC review, this concentration may overestimate the dissolved concentration of this compound.

The lowest therapeutic dose for fluoxetine is 20 milligrams per day (mg/day), or 20,000,000 ng/day, for adults. One would need to consume over 48,000 gallons of this biosolid liquid to intake the equivalent of one pill.

This therapeutic dose also is used to estimate a "predicted no-effects concentration" (Schwab et al., 2005) by adding safety factors that consider sensitive receptors and a concentration with no observable adverse effects. For drinking water, this concentration is 42,000 ng/L. The liquid phase of the GRU biosolids tested is over 350 times lower than the predicted no effects concentration.

The evaluation of the concentration of microconstituents in water does not explicitly address potential exposures from dust; however, laboratory data from the liquid phase testing can be used to estimate dust concentrations. Using the liquid phase fluoxetine concentration, the solid phase concentration was estimated conservatively at 8.8 micrograms per kilogram (ug/kg) (dry weight).

Note that the material safety data sheet from Eli Lilly indicates the Log Kow for estimating partitioning is pH dependent (Log Kow: 1.0, 1.8, 2.6 [pH 5, 7, 9]). To roughly estimate a solids concentration, the following assumptions were used: Log Koc of 2; 2.5 percent solids, fraction of organic carbon (foc) of 0.8).

The 8.8 ug/kg (dry weight) is lower than the maximum concentration of 1,500 ug/kg reported in the U.S. Geological Survey biosolids study (Kinney et. al., 2006). At the estimated concentration of 8.8 ug/kg, a person would have to consume over 5,000 pounds of dry biosolids to ingest a 20-mg dose of fluoxetine.

Dust exposure is intermittent and typically represents only a small amount of material. A soil screening value for fluoxetine of 227 milligrams per kilogram (mg/kg) was estimated using the residential soil land use equation for incidental ingestion of soil for noncarcinogens (EPA, 2009) using the ADI of 0.0029 mg/kg/day (Schwab, 2005). This equation assumes incidental ingestion of 200 mg/day of soil by a child living on a residential property.

The 8.8 ug/kg (0.0088 mg/kg) estimated biosolids concentration is far below the soil screening value for residential property, and adjacent residents would not be exposed at levels assumed for an onsite child resident. Also, fluoxetine was not detected in the soils at the WPR site.

The use of comparisons of biosolids liquid and solid phases with drinking water cri-

Continued on page 14

Continued from page 13

teria or residential soil criteria illustrate extremely conservative safety factors for handling biosolids. Much lower concentrations would be present in WPR soils, dust, and/or groundwater from mixing and other attenuation mechanisms. This is supported by the results of testing completed on the WPR site soil and groundwater.

Phase 2 – Pathogens

As shown in the resulting data summarized in Table 2, see page 14,, which provides a comparison of the waste activated sludge results with the biosolids results, the pathogens and indicator bacteria present in biosolids are reduced significantly by the process of aerobic digestion at the GRU water reclamation facilities. With the exception of Clostridium perfringes, the inactivation was >90 percent.

Clostridium perfringes is ubiquitous in the environment and is a poor indicator of fecal pollution and the treatment process. The EPA does not utilize Clostridium perfringes as an indicator for the monitoring of water, wastewater, or biosolids quality. This microorganism was included in this study under the direction of the Alachua County Environmental Protection Department and is used as an internal recovery control as described previously.

Also, soil samples collected from a remote area not receiving biosolids contained approximately 10^2 colony forming unit per gram (cfu/gram) of Clostridium perfringes (data not shown). The aerobic digestion process used at GRU's water reclamation facilities results in biosolids that meet Class B EPA and the FDEP regulatory criteria for land application.

Two sets of biosolids-amended soil samples were collected, one that had received biosolids within approximately one hour, and one that had received biosolids three months earlier. As indicated in the biosolids-amended soil samples, once the biosolids from the GRU water reclamation facilities are land applied as a soil amendment at the Whistling Pines Ranch farm, the numbers of recovered pathogens and indicators are further substantially reduced.

Also, three months following land application, the pathogens are further reduced due to a variety of factors that include heat inactivation, desiccation, predation, ultraviolet inactivation, oxidation, etc. Note in Table 2 that the one-hour and month-month biosolids-amended soil sample results show that these samples meet the microbial criteria of Class A biosolids (biosolids of exceptional microbial quality).

As shown in Table 2, analysis of air samples downwind from sites of soil amendment with biosolids and/or disking activities indicated the absence of all pathogens and fecal indicators. The study was conducted on a dry day, with steady winds (average wind speed of 400 feet per minute [ft/min] or approximately 4.5 miles per hour [mph]).

Both the 100-foot-setback air samples and the close-proximity air samples did not recover any of the pathogens or indicator bacteria present at low levels in the biosolids prior to land application. The close-proximity sample results are presented in Tables 3 and 4.

The only organism groups detected in both the background air sampling and the downwind aerosolized particulate air sam-

TABLE 2: Average concentration of enteric pathogens and bacterial indicators in waste activated sludge, biosolids, biosolids-amended soils, and air samples following the application of biosolids to the soil.

Type of Sample	Enteric Viruses (MPN/4 dry g or vol. of air)	Fecal Coliforms [2] (CFU/ dry g or vol. of air)	*E. Coli* [2] (CFU/ dry g or vol. of air)	HPC [3] (CFU/ vol. of air)	*Clostridium Perfringens*[¥] (CFU/ dry g or vol. of air)	*Salmonella* (CFU/dry 4g or vol. of air)
Waste Activated Sludge	61.5	8.6×10^6	2.9×10^5	Not Done	7.1×10^6	154.8
Biosolids	4.9	3.9×10^5	4.5×10^4	Not Done	7.3×10^6	Non-Detect *
Biosolids Amended Soil (1 hour)	0.13 *	598 *	6.0×10^2	Not Done	1.2×10^4	0.2 *
Biosolids Amended Soil (3 months)	<0.03 *	18 *	18	Not Done	Non-Detect	Non-Detect *
Air Samples [†] (100 ft set back)	Non-Detect / 64.8 Liters	Non-Detect / 5.4 Liters	Non-Detect / 5.4 Liters	200 / 1.8 Liters	1.3 / 1.8 Liters	Non-Detect / 5.4 Liters
Air Samples [†] (Background)	Non-Detect / 172.5 Liters	Non-Detect / 19.2 Liters	Non- Detect / 19.2 Liters	101 / 1.8 Liters	0.33 / 1.8 Liters	Non-Detect / 19.2 Liters

[¥]- No value has been established for the maximum limit of Clostridia perfringens in biosolids; this microorganism is not recognized by the EPA as an indicator of fecal pollution or water quality, is ubiquitous in the environment and is a spore former, thus, it survives for prolonged periods of time in very harsh environments and is resistant to most conventional wastewater treatment. The presence of Clostridia perfringens at $<10^8$ / gram is typical for subtropical soils. The detected numbers per gram of dry weight are below the infectious dose of 10^8 cfu of bacteria. A search of the literature indicated that acceptable levels are around $10^3 - 10^4$ CFU/gram of food.

- 100,000,000 liters of air at a 100-foot setback from disking operations would have been inhaled and all the dust swallowed at any one time to be exposed to the potentially infectious dose.
- 1,000,000,000 liters of air at background conditions would have to be inhaled and all the dust swallowed at one time to be exposed to the potentially infectious dose.
- A typical human consumes about 7,200 liters of air per day; therefore, a human would have to consume a volume of about 3.8 years of air at once from the 100 ft setback to be exposed to a potentially infectious dose

* These samples meet the microbial criteria of Class A Biosolids (Class A Biosolids are of exceptional quality and safe for human contact as determined by the EPA).

[†] The air quality based on the microbiological data collected indicate that air collected from an area 100 feet downwind from a biosolids application site is statistically the same as air from an area not impacted by biosolids application or disking. Background air samples were collected in the northeast corner of the property along adjacent properties.

[2] Infectious doses for E. coli (main constituent of biosolids associated fecal coliforms) based on published literature are usually 10^8-10^{10}.

[3] Heterotrophic plate count (HPC) was included as a control for the air sampling study to ensure that recoveries are within range and methods are being performed appropriately. These organisms are very ubiquitous and generally harmless/beneficial microorganisms that are part of every living eco-system. As expected for the air samples, the values are increased with the higher dust content of samples taken adjacent to disking operations. The absence of this species would be indicative of the sterility of the environment, which is not a plausible scenario.

pling were HPC and Clostridium perfringens. Clostridia perfringens are ubiquitous in the environment and are very common in soils; and as indicated previously, are not recognized by the EPA as an indicator organism.

Also, HPC bacteria were detected in all the samples tested as expected. This microbial group was incorporated as an indication of the suitability and appropriateness of the methods selected for sampling and analysis. HPC are indigenous bacteria that are present in high numbers on all living and environmental surfaces. They are generally harmless and are often considered beneficial microorganisms that are part of every living ecosystem.

As expected for the air samples, the values are increased with the higher dust content of samples adjacent to disking operations. The absence of this species would be indicative of the sterility of the environment, which is not a plausible scenario, or the failure of the sample collection and analysis procedures.

In order to provide additional perspective on the level of potential hazard associated with biosolids handling and land application, the amount of material that would need to be con-

Continued on page 16

TABLE 3: Analysis of air samples for the presence of infectious enteric viruses in airborne particles/aerosols at setback distance much closer than the 100-foot setback distance. Multiple tractor passes during biosolids application and disking, and at higher speeds than usual during disking operations, were conducted to simulate extreme-case scenarios (8/6/2008).

Sample Site BCS ID	Air Sample Site Description	Total Air Volume Analyzed	Enteric Viruses (MPN)
GRU AIR Sample-1 BCS#:0808015	Two tractor pass: First sample 100 ft downwind during biosolids surface application; second at 65 feet (approximately 25 feet lateral distance from application path) **Note: This is an extreme-case scenario of exposure**	64.7 L	Non-Detect
GRU AIR Sample-2 BCS#:0808016		64.7 L	Non-Detect
GRU AIR Sample-3 BCS#:0808017	Two tractor pass: First sample 100 ft downwind from tractor **disking** surface applied biosolids (1hr); second tractor pass at 42 feet. (approximately 15 feet lateral distance from application path) Both equipment and personnel were engulfed in dust cloud. **Note: This is an extreme-case scenario of exposure**	57.5 L	Non-Detect
GRU AIR Sample- 4 BCS#:0808018		57.5 L	Non-Detect
GRU AIR Sample-11 BCS#:0808025	**Negative Control Sample:** Sample collected at NE Corner of Property (Background No Biosolids Application)	57.5 L	Non-Detect
GRU AIR Sample-12 BCS#:0808025		57.5 L	Non-Detect
GRU AIR Sample-13 BCS#:0808025		57.5 L	Non-Detect

TABLE 4: Analysis of air samples for the presence of cultivable fecal indicator organisms and bacterial pathogens in captured airborne particles and bioaerosols at setback distance much closer than the 100 ft setback distance. Multiple tractor passes during biosolids application and disking, and at higher speeds than usual during disking operations, were conducted to simulate extreme-case scenarios (8/6/2008).

Sample Site BCS ID	Air Sample Site Description	Air Volume Analyzed	Heterotrophic Plate Count (CFU)	Fecal coliform (CFU)	*E. coli* and *Salmonella* (CFU)	*Clostridium perfringens* (CFU)
GRU AIR Sample-1 BCS#:0808015	Two tractor pass: First sample 100 feet downwind during biosolids surface application; second at 65 feet (approximately 25 feet lateral distance from application path) **Note: This is an extreme-case scenario of exposure**	5.4 L	280	Non-Detect	Non-Detect	3 *
GRU AIR Sample-2 BCS#:0808016		5.4 L	360	Non-Detect	Non-Detect	8 *
GRU AIR Sample-3 BCS#:0808017	Two tractor pass: First sample 100 feet downwind from tractor **disking** surface applied biosolids (1hr); second tractor pass at 42 feet. (approximately 15 feet lateral distance from application path) Both equipment and personnel were engulfed in dust cloud. **Note: This is an extreme-case scenario of exposure**	4.8 L	430	Non-Detect	Non-Detect	18 *
GRU AIR Sample-4 BCS#:0808018		4.8 L	520	Non-Detect	Non-Detect	20 *
GRU AIR Sample-11 BCS#:0808025	**Negative Control Sample:** Sample collected at NE Corner of Property (Background Area - No Biosolids Application)	4.8 L	290	Non-Detect	Non-Detect	1
GRU AIR Sample-12 BCS#:0808025		4.8 L	200	Non-Detect	Non-Detect	Non-Detect
GRU AIR Sample-13 BCS#:0808025		4.8 L	320	Non-Detect	Non-Detect	Non-Detect

*Even though these samples demonstrated slightly higher counts than the other samples collected, the detected numbers per gram of dry weight are far below the infectious dose of 10^8 cfu of bacteria. A search of the literature indicated that acceptable levels are around $10^2 - 10^4$ cfu/gram of food

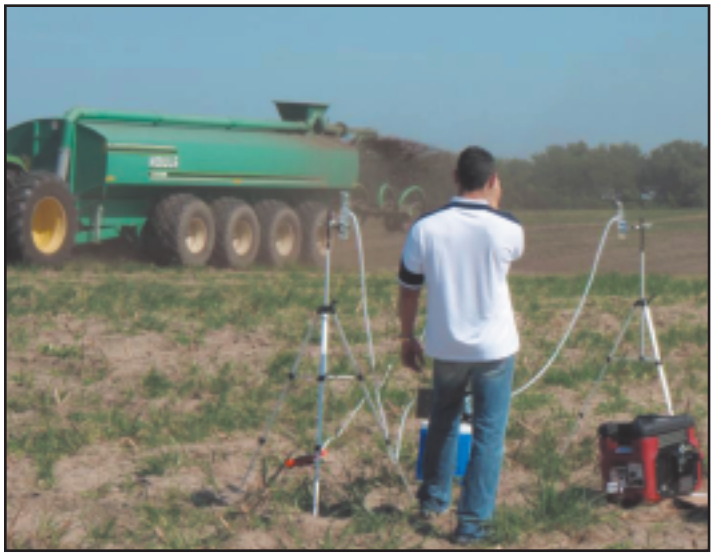

FIGURE 3: Bioaerosol Sampling during Surface Land Application of Biosolids {Field C}

FIGURE 4: Bioaerosol Sampling during Disking Operation (1-Day) [Northwest] {Field C}

Continued from page 15

sumed directly at one time by an individual to potentially cause illness was calculated. These were calculated based on the levels of enteric viruses detected in the samples and published values for infectious dose. Table 5 shows that a person would have to consume roughly 10 ounces of digested biosolids or 18.5 pounds of dried biosolids-amended soils (one-hour), or 45.4 pounds of dried biosolids-amended soils (three-month) at one time to potentially become ill.

No pathogens or fecal indicators were detected in the downwind aerosolized dust air sampling. The levels of the detected Clostridia numbers per gram of dry weight are below the infectious dose of 10^8 cfu of bacteria; therefore, even if the Clostridia perfringes levels were to be used as indicator of pathogens, 100,000,000 liters of air at a 100-foot setback from disking operations would have be inhaled at one time

and all the dust in the air swallowed to be exposed to the potentially infectious dose.

Also, a typical human consumes approximately 7,200 liters of air per day; therefore, a human would have to consume a volume of about 3.8 years of air at once from the 100-foot setback to be exposed to a potentially infectious dose. Based on the data, the exposure to a potentially infective dose could not be perceived, and thus dust inhalation could not be recognized as a realistic infectious pathway.

Figures 3 and 4 are photographs of bioaerosol sampling taken during biosolid application and disking operations, respectively.

Other Testing

Biosolids-Amended Soil Metals—Results showed all below residential Soil Cleanup Target Levels, as shown in Table 6.

Biosolids Radionuclides—Biosolids samples were collected and sent to the FDOH. Radiological sample results provided are the detectable radionuclides in biosolids. These data were evaluated using the protocols presented in ISCORS Technical Report 2004-04: *ISCORS Assessment of Radioactivity in Sewage Sludge: Recommendations on Management of Radioactive Materials in Sewage Sludge and Ash at Publicly Owned Treatment Works* (ISCORS, 2004).

For this evaluation, the reported concentrations are converted from picocuries per liter (pCi/L) to picocuries per gram (pCi/g) dry weight at approximately 1.5 percent solids. Estimated exposures in millirem per year (mrem/yr) for an onsite resident at an agricultural biosolids application site were calculated for each of the detected radionuclides.

These estimated exposures can then be compared to typical background exposures of about 300 mrem/yr natural and 70 mrem/yr man made. Natural potassium 40 and Tl-201 concentrations were below the screening concentration (1 mrem/yr for the most conservative exposure scenario of incineration).

For a resident at an agricultural site, these exposures were calculated to be about 0.00913 mrem/yr for potassium 40 and no additional radiation exposure for Tl-201. The contribution from I-131 for the land application scenario was 1.18E-15 mrem/yr.

As stated in the ISCORS document, the National Council of Radiation Protection and Measurements (NCRP, 1993) determined 1 mrem per year is a negligible individual dose. The estimated potential dose for biosolids application is orders of magnitude below this level.

University of Florida Entomologist Inspection—The report stated that flies were typical of farm and local area land uses, and from both onsite and offsite activities. Fly problems can be solved by best management practices.

TABLE 5: Calculated risk analysis conclusions for waste activated sludge, biosolids, biosolids-amended soil, and air sample.

Type of Sample	Calculated Sample Amount Consumed at a Single Time to Constitute a Potential Infectious Dose (Ingested or Inhaled) *
Waste Activated Sludge	0.6 – 3.7 fluid ounces
Biosolids (Kanapaha)	10.4 fluid ounces
Biosolids (Main Street)	No Enteric Viruses Were Detected
Biosolids Amended Soil (1 hour)	18.5 pounds
Biosolids Amended Soil (3 month)	>45.4 pounds
Air Sample/Aerosolized Dust (100 ft down wind tractor)	No Pathogens or Indicators Detected

* The calculations are based on the level of viable enteric viruses detected in the samples and the worst-case scenario assumption that utilizes rotavirus as a viral model. The assumption therefore assumes all viruses detected are the highly infectious rotavirus. Rotavirus has been shown to have one of the lowest infective doses among viruses (10 infectious units) therefore it is used in risk assessment studies to demonstrate worst-case scenarios of exposure and risk.

TABLE 6: Biosolids - Amended Soil Metals

Parameter	FDEP Soil Cleanup Target Levels*	FDOH Soil Sample Results**
Arsenic, mg/kg	2.1	0.2
Cadmium, mg/kg	82	0.2
Chromium, mg/kg	210	2.1
Copper, mg/kg***	150	3.9
Mercury	3	0.03
Nickel, mg/kg	340	1.3
Lead, mg/kg	400	2.2
Selenium, mg/kg	440	0.5
Zinc, mg/kg ***	26000	11.4

* Residential unrestricted use
** Based on average of 18 sample sites - Sampling and analysis and by Florida Department of Health
*** Considered micronutrients

Off-Site Groundwater Evaluation by FDOH—FDOH sampled over 30 area private wells for nitrate and pathogens. Nine wells were tested for all primary drinking water standards, which were met in all wells except two that exceeded the maximum contamination level for nitrate. Uranium was found in one well but was likely naturally occurring, according to the FDOH (see results below for uranium and aluminum).

Historically, GRU has sampled area residents' wells for nitrate and found that levels were very low. According to the University of Florida Institute for Food and Agricultural Sciences (IFAS), elevated nitrate is common in agricultural areas, and nitrates concentrations above 20 parts per million (ppm) have been found near farms in North Florida.

Additional Data

Dioxin—After a five-year EPA evaluation of dioxin in biosolids, they will not be regulated by the EPA as of a news release by the Agency on 10/17/2003, "EPA Makes Final Decision on Dioxin In Sewage Sludge used in Land Applications" (http://yosemite.epa.gov/opa/admpress.nsf/b1ab9f485b098972852562e7004dc686/209dab87e1b0a8b785256dc20050c977?OpenDocument).

Also, based on earlier testing from a Midwest Research Institute report, GRU dioxin test results Toxicity Equivalent (TEO) in parts per trillion (ppt) for the Kanapaha Water Reclama-

Continued on page 18

TABLE 7: Results of Uranium and Aluminum

	Uranium (mg/kg)	Aluminum (mg/kg)
GRU Biosolids	0.013	130
Natural Soil (Background)	0.3 – 1.4	800 – 3,300

Continued from page 17

tion Facility are a minimum of 1.96 and maximum of 8.78, and for the Main Street Water Reclamation Facility are a minimum of 22.2 and a maximum of 25.3—all well below the previously proposed regulatory limit of 300 ppt.

Uranium & Aluminum—Uranium and aluminum in GRU biosolids are well below background levels (Table 7).

Conclusions

Phase 1 - Microconstituents

Based on the analytical results, the following summary and conclusions are presented:

- The soil and groundwater data are most relevant for evaluating potential exposures to residents near the WPR.
  - None of these microconstituents were detected in the four groundwater samples tested at the WPR site.
  - None of the microconstituents were detected in the four composite soil leachate samples from the WPR site. Soils do not appear to be a potential future source of these microconstituents in groundwater.
- Biosolids were analyzed to provide information on potential source concentrations.
  - Eleven of the 12 microconstituents were not detected in the biosolids sample liquid phase.
  - One of the twelve parameters, fluoxetine (Prozac), was reported at a concentration of 110 ng/L in the biosolids liquid phase. Over 48,000 gallons of the biosolids liquid or over 5,000 pounds of dry biosolids would need to be consumed to equal one therapeutic dose of 20 mg.
- The results of this testing program show the GRU biosolids are not a significant source of microconstituents at the WPR site.

Phase 2 – Pathogens

This study provides the additional site-specific information requested by the residents and the Alachua County Environmental Protection Department, directly addressing the WPR land application of biosolids from the Main Street Water Reclamation Facility and the Kanapaha Water Reclamation Facility in Gainesville. The data indicate that pathogen and indicator organism levels in GRU biosolids are relatively low and that they pose minimal risk through direct exposure. Furthermore, based on the application of the biosolids to the soil at the sites tested during the observed operations, the risk to adjacent communities is considered extremely low.

Analysis of the current data set alongside existing risk assessment studies indicates a very low risk to human health from both direct contact and inhalation of bioaerosols generated from the application site. It is our conclusion that based on the current data, fields amended with GRU biosolids confer no greater risk of enteric pathogen disease to individuals in adjacent communities than to those living adjacent to fields that are not currently receiving biosolids.

Other Testing

- **Biosolids-Amended Soil Metals**—All soil metals are well below residential Soil Cleanup Target Levels.
- **Biosolids Radionuclides**—Site exposures were found to be orders of magnitude below usual natural and manmade exposures.
- **University of Florida Entomologist Inspection**—The report generally stated that the flies and vectors were typical of farm and local area land uses.
- **Off-site groundwater was tested by FDOH**—No pathogen or indicator organisms, and nitrate levels typical of groundwater on farms in North Florida.
- **Dioxin**—Dioxin will not be regulated by the EPA, and GRU biosolids dioxin test results were well below the regulatory levels proposed previously.
- **Uranium and Aluminum**—Uranium and aluminum in GRU biosolids are well below background levels.
- **Epidemiological Survey**—The FDOH performed an epidemiological survey of the area surrounding the biosolids land application site. No incidents of epidemiological anomalies were found.

The future of land application of Class B biosolids on the WPR is under review by local governmental authorities and regulatory agencies. The scientific evidence collected during this study indicates that the WPR land application process with Class B site restrictions is as safe as that of Class A processes from the standpoint of microconstituents and pathogens.

Acknowledgements

The authors would like to thanks the Kanapaha Water Reclamation Facility laboratory and operations staff, the Main Street Water Reclamation Facility operations staff, and all who participated in GRU's effort to continue the beneficial use of biosolids by utilizing scientific evidence to show the safety and sustainability of biosolids land application practices.

References

This paper was based primarily on the following documents that were generated to address the WPR area residents concerns:

- BCS Laboratories Inc. 2009. "Perceived and Calculated Human Health Risk Associated with Land Application of Aerobically Digested Biosolids." Prepared for Gainesville Regional Utilities.
- CH2M Hill. 2009. "Technical Memorandum, Whistling Pines Ranch - Microconstituent Sampling Results." Prepared for Gainesville Regional Utilities
- Jones Edmunds. 2008. Gainesville Regional Utilities Whistling Pines Ranch Microconstituent Sampling and Analysis. Jones Edmunds Project No.: 07125-047-01.

References used in the reports above and also in this paper are listed below.

Phase 1 - Microconstituents

- Florida Department of Environmental Protection (FDEP). 2005. Technical Report: Development of Cleanup Target Levels (SCTLs) for Chapter 62-777, Florida Administrative Code (F.A.C.).
- Kinney, C. A., E.T. Furlong, and S.D. Zaugg, , M.R. Burkhardt, S.L. Werner , J.D. Cahill, and G.R. Jorgensen. 2006. "Survey of organic wastewater contaminants in biosolids destined for land application." *Environmental Science & Technology*. 40: 7207-7215.
- Schwab, B. W., E. P. Hayes, J. M. Fiori, F. J. Mastrocco, N. M. Roden, D. Cragin, R. D. Meyerhoff, V. J. D'Aco, and P. D. Anderson. 2005. "Human Pharmaceuticals in US Surface Waters: A Human Health Risk Assessment." *Regulatory Toxicology and Pharmacology*. 42:296-312.
- U.S. Environmental Protection Agency (EPA). 2009. Regional Screening Levels for Chemical Contaminants at Superfund Sites User's Guide (December 2009) http://www.epa.gov/reg3hwmd/risk/human/rb-concentration_table/usersguide.htm. Accessed March 8, 2010.

Phase 2 – Pathogens

- Baertsch, C., T. Paez-Rubio, E. Viau, and J. Peccia. 2007. "Source tracking aerosols released from land-applied class B biosolids during high-wind events." *Applied Environ-*

Continued on page 20

Continued from page 18

mental Microbiology.;73(14):4522-31.

- Brooks, J.P., B.D. Tanner, K.L. Josephson, C.P. Gerba, C.N. Haas, and I.L. Pepper. 2005. "A national study on the residential impact of biological aerosols from the land application of biosolids." *Journal of Applied Microbiology*. 99(2):310-22.
- Brooks, J.P., B.D. Tanner, C.P. Gerba, C.N Haas, and I.L. Pepper. 2005. "Estimation of bioaerosol risk of infection to residents adjacent to a land applied biosolids site using an empirically derived transport model." *Journal of Applied Microbiology*. 98(2):397-405.
- Farrah, S.R., and G.Bitton. 1983. "Bacterial survival and association with sludge flocs during aerobic and anaerobic digestion of wastewater sludge under laboratory conditions." *Applied Environmental Microbiology*. 1983 Jan;45(1):174–181.
- Gerba, C.P., N. Castro-del Campo, J.P. Brooks, and I.L Pepper. 2008. "Exposure and risk assessment of Salmonella in recycled residuals." *Water Science Technology*. 57(7):1061-5
- Gerba, C.P. and J. E. Smith. 2005. "Sources of pathogenic microorganisms and their fate during land application of wastes." *Journal of Environmental Quality*. 34(1):42-8
- Haas, C.N. 1999. "On modeling correlated random variables in risk assessment." *Risk Analysis*. 1999 Dec;19(6):1205-14.
- Paez-Rubio T, E. Viau, S. Romero-Hernandez, and J.Peccia. 2005. "Source bioaerosol concentration and rRNA gene-based identification of microorganisms aerosolized at a flood irrigation wastewater reuse site." *Applied Environmental Microbiology*. 71(2):804-10.
- Pepper, I.L., H. Zerzghi, J.P. Brooks, and C.P. Gerba. 2008. "Sustainability of land application of class B biosolids." *Journal of Environmental Quality*. 2008 Sep-Oct;37(5 Suppl):S58-67.
- Tanner, B.D., J.P. Brooks, C.P. Gerba, C.N. Haas, K.L. Josephson, and I.L. Pepper. 2008. "Estimated occupational risk from bioaerosols generated during land application of class B biosolids." *Journal of Environmental Quality*. 37:2311-2321.
- Zerzghi, H., C.P. Gerba, J.P. Brooks, and I.L Pepper. 2009. "Long-term Effects of Land Application of Class B Biosolids on the Soil Microbial Populations, Pathogens, and Activity." *Journal of Environmental Quality*, Dec 30;39(1):402-8.

Other Testing

- Interagency Steering Committee on Radiation Standards (ISCORS). 2004. Technical Report 2004-04: *ISCORS Assessment of Radioactivity in Sewage Sludge: Recommendations on Management of Radioactive Materials in Sewage Sludge and Ash at Publicly Owned Treatment Works.* In EPA 832-R-03-002B, pages 6-8 to 6-10.