Microbial Pollution of Water: Human Health Risks

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The control of water pollution in the U.S. in the last two decades has focused on chemical risks and while great strides have been made, risks associated with microbial pollutants have seen a continuance, reemergence and escalation. As many as 900,000 cases and 900 deaths annually due to waterborne disease have been estimated in the U.S. by the Centers for Disease Control and Prevention. Outbreaks of disease from tap water is a national problem.The list of microorganisms that can be transmitted through contaminated water is long, and emerging pathogens of concern have now been documented as causing numerous outbreaks. There are over 100 different types of bacteria, protozoa and viruses which can be found in contaminated water. *E. coli* H0157 has been involved in serious illnesses and kidney failure, degenerative heart disease and insulin dependent diabetes are caused by coxsackievirus B infections, and peptic ulcers and stomach cancer are caused by the bacterium *Helicobacter pylori*. The protozoa, such as *Cryptosporidium* and *Cyclospora,* produce oocysts that are extremely resistant to disinfection. The recent outbreak of *Salmonella typhimurium* (a fecal-oral pathogenic bacterium), which caused illness in cattle and in humans, is alarming, as this bacterium carries with it multiple antibiotic resistance.   More recently, *Tropheryma whippelii*, an actinomycete, was detected in sewage, a cause of Whipple's disease, and nanobacteria, a cause of kidney stones; the potential for waterborne transmission is there.

Despite a growing uneasiness, most states have been unable to develop comprehensive microbial monitoring programs which will lead to protection of water resources for the future. Microbial pollution of water is a growing environmental health and public health crisis that has yet to be addressed using scientific risk assessment through a national program. While the coliform bacteria as indicators have served us well in assessing general fecal contamination, they have significant limitations in regard to emerging microbial contaminants and assessing public health risks. Greater emphasis has now been placed on evaluating specific water quality variables, sources, and loadings for the identification of watersheds at risk.   New tools and approaches are available for microbial contaminants, including immuno-based methods, PCR, source tracking, storm modeling and use of GIS. These can all be used for assisting with contaminant monitoring requirements. Better data bases on occurrences, along with watershed assessment, thus will ensure the protection of the nation's waters for the future.

A Historical Perspective of Detection Methods for *Giardia* Cysts and *Cryptosporidium* Oocysts in Water

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In the mid-twentieth century *Giardia* was classified as a nonpathogenic commensal organism and *Cryptosporidium* was not recognized yet. However, as early as 1946 a waterborne outbreak of giardiasis was suspected. From 1965 to 1979 it became clear that *Giardia lamblia* was indeed a human pathogen and was responsible for waterborne gastroenteritis. Unlike bacteria, there are no simple culture methods for enteric protozoan parasites, so detection and identification were limited to microscopic observations. Because the densities of cysts and oocysts in water often can be less than one per liter, volumes between 10 and 1,000 liters must be examined. The initial sampling approach of the Centers for Disease Control and Prevention was to use a sand filter on a flatbed truck, while that of the U.S. EPA was to use a fiber wound cartridge filter which became the standard. Buoyant density flotation protocols employing zinc sulfate, sucrose, Percoll, and Percoll-sucrose have been used to purify and further concentrate the cysts and oocysts. Percoll-sucrose eventually became the standard flotation medium.

Initial microscopic detection protocols utilized non-selective clinical stains such as Lugol's iodine and trichrome stains, but both the particulates and organisms in a water sample stained. To overcome this problem, Dr. Judy Sauch in 1984 developed a selective immunofluoescent antibody stain that was used in conjunction with phase contrast microscopy to detect and identify *Giardia* cysts. Although time consuming and skill dependent, this elegant method was quite successful in substantiating epidemiologic outbreaks of waterborne giardiasis.

In the 1980's *Cryptosporidium* was described not only as a human pathogen but as an agent of waterborne disease. *Cryptosporidium* became widely recognized as a result of the 1993 Milwaukee waterborne cryptosporidiosis outbreak, which holds the record of being the largest waterborne gastroenteritis outbreak ever caused by a microorganism. Following the lead of Dr. Sauch, others modified the existing *Giardia* method by adding an anti-*Cryptosporidium* fluorescent antibody to the staining solution. After substituting differential interference contrast for phase contrast microscopy, this methodology ultimately was applied in a monitoring study that was conducted under the Information Collection Rule (ICR). Numerous modifications were made to improve the recovery efficiency of the ICR method; however, they were of little benefit.   On average, multi-laboratory recoveries from seeded artificial water samples for *Giardia* ranged between 5-44%, and recoveries for *Cryptosporidium* oocysts ranged from 0-35%. In raw water samples, the average ICR method percent recovery for *Cryptosporidium* was 11%.

The U.S. EPA in 1996 initiated the development and validation of a new method to overcome the deficiencies of the ICR method. In method 1623, the volume sampled was reduced to 10 liters, buoyant density flotation was replaced by immunomagnetic separation, and parasites stained with fluorescent antibodies were counter stained with DAPI. Using this method, mean recoveries from seeded samples for both *Giardia* cysts and *Cryptosporidium* oocysts now are between 38 and 42%. Further research still is needed to improve percent recoveries.

Unregulated Contaminant Monitoring for Contaminant Candidate List Pathogens

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The 1996 amendments to the Safe Drinking Water Act require that selected unregulated contaminants be monitored in drinking water distribution systems. To meet this requirement, these contaminants will be monitored in some water distribution systems for one year under the Unregulated Contaminant Monitoring Regulation (UCMR). Data obtained from monitoring, along with information on exposure, health, treatment, and cost of monitoring will be used to make a determination of whether or not to regulate contaminants in drinking water. The contaminants to be monitored are selected from the drinking water Contaminant Candidate List (CCL), which is revised every 5 years. The current CCL has 10 microorganisms, 8 of which are occurrence priorities (63 FR10286,1998), meaning that they are priorities for monitoring, since more information is needed on their occurrence before a regulatory determination can be made.

Since analytical methods are in different states of availability for CCL contaminants, the contaminants are divided into different lists for monitoring purposes depending on the status of the methodology. The list that currently includes most of the CCL microorganisms is designed for contaminants that do not have well developed analytical methods. Surveys for these contaminants will be targeted at the most vulnerable water distribution systems and samples will be taken at vulnerable times of the year and locations within the systems. Sample size is flexible, but could be up to 200 systems. Cost of analyses would be a factor in determining how many samples were taken.

Before monitoring for the contaminants can begin, analytical methods will be needed. Microbiological analyses that demonstrate viability or infectivity are preferred since nonviable or noninfective organisms are not a threat to public health. The status of method availability for CCL microorganisms is variable. Only one CCL microorganism, *Aeromonas*, has a method that is suitable for UCMR monitoring; however, this method only identifies to the genus level, and does not indicate if colonies are potentially pathogenic strains of *Aeromonas*. Genetic analyses that could identify likely pathogenic aeromonads can be done on a small scale, but are too costly for UCMR monitoring. Analytical methods for the remaining CCL microorganisms are in various states of development. The methods that do exist for some of these microorganisms may not be satisfactory, and have not been standardized or validated. Therefore, more method development/evaluation is needed for the CCL microorganisms. Ideally, analytical methods to be used with the UCMR should be as inexpensive as possible. All methods will be validated.

To design UCMR surveys of CCL microorganisms at most vulnerable times and locations, information is needed on the occurrence of these microorganisms in environmental or distribution system waters. Some information is available on occurrence patterns for some CCL microorganisms such as *Aeromonas*, but less information is available for others, such as human microsporidia. The lack of occurrence information for several CCL microorganisms is a result of the inability to analyze for these microorganisms in environmental samples.

To assist UCMR surveys, analytical method development and more occurrence information are needed for CCL pathogens. Preliminary occurrence studies would greatly assist the implementation and design of the UCMR surveys. These surveys would not necessarily be restricted to analytical methods suitable for use the UCMR surveys.

Applying Geospatial Data to Health Surveillance

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USGS has long been active in global, national and regional geospatial database development. Some of these databases are used for health-related activities, although at times indirectly, e.g. studies of groundwater to guarantee safe drinking water, prediction of and reconstruction after natural hazards, and famine early warning. Datasets developed in these activities are archived and disseminated using Internet /clearinghouse technologies.

The challenge is to make new and creative use of existing archives for tackling health related problems. For example, the USGS/EROS Data Center (EDC) continually updates the data archive created for USAID's Famine Early Warning System (FEWS).

Data archived on an ongoing basis include Advanced Very High Resolution Radiometer-derived Normalized Difference Vegetation Index (NDVI) and Meteosat-derived rainfall estimates used to monitor vegetation condition and rainfall across sub-Saharan Africa. The database also includes national precipitation statistics and a wide range of biophysical and socioeconomic data such as infrastructure, landcover, elevation, soils, agro-climatic zones, etc.

The FEWS archive is now being applied to understanding health problems not specifically associated with famine or the FEWS project. As an example, the NDVI database is being used for the characterization of climatic conditions and landscape features that are required for outbreaks of chronic vector-borne diseases such as malaria, dengue fever, and Rift Valley fever in sub-Saharan Africa. Domestically, these techniques are being used to investigate the spread of hantavirus pulmonary syndrome, Lyme's disease, and encephalitis. Similar research into the climatic factors in water-borne diseases such as cholera (eg. Bay of Bengal, Peru) is being carried out by other institutions using geospatial data.

As our ability to manipulate data improves, our opportunities for new applications of these specific data do as well. Coupled with the rapidly growing datastream coming from new remote sensing technologies, it seems apparent we can tackle health and other problems for which many of these databases were never previously considered.

Drinking Water Microbial Regulations

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Regulations to prevent waterborne microbial disease are the cornerstone to federal drinking water standards. Monitoring requirements (Total Coliform Rule) and treatment requirements (Surface Water Treatment Rule) now in place were designed to bring consistency and proactively lower risks for both waterborne disease outbreaks and endemic illnesses across the country.

This process of protecting public water supplies has been iterative. As we continue to increase our understanding of waterborne microbial pathogens, their occurrence and effects, and as our monitoring and treatment capabilities improve, more-protective regulatory requirements are possible. Recent public discussions have led to the Interim Enhanced Surface Water Treatment Rule (IESWTR), which tightens filtration requirements for larger surface water systems. Similar requirements are soon to be proposed for smaller surface water systems (Longer-term 1 Enhanced Surface Water Treatment Rule, or LT1ESWTR). A recognition that groundwater-based public water supplies may be vulnerable to microbial contamination has led to proposed assessment and treatment requirements for such systems (Groundwater Rule).

This talk will cover the rationale and content of these new and proposed regulations. The IESWTR, promulgated in December 1998, will be enforced beginning December 2001. It will require surface water systems serving 10,000 or more people to meet more stringent filtration requirements and actively address *Cryptosporidium* contamination. Systems will also have to assess their current levels of disinfection. The LT1ESWTR, expected to be proposed in March 2000, promulgated in December 2000 and enforced by December 2003, would extend these requirements to surface water systems serving fewer than 10,000 people. The Groundwater Rule, expected to be proposed by May 2000 and promulgated early in 2001, would require systems using groundwater to assess the vulnerability of their sources, wells and distribution to microbial contamination. Identified vulnerabilities would have to be corrected.

The U.S. Environmental Protection Agency BEACH Program

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Background

A central goal of the Clean Water Act is to ensure that waters of the United States are "fishable and swimmable." Because of this, the U.S. Environmental Protection Agency (EPA) has actively supported efforts to protect and enhance the water quality of our nation's beaches and other recreational waters. Coastal and inland waters remain a rich heritage that is highly valued by the American people. The importance to the public is shown by that fact that recreational waters are important economically, generating billions of dollars in goods and services, including tourism.

EPA has increased its attention to beach water quality for several reasons. There are continuing public health concerns about the risk of infectious diseases resulting from exposure to pathogenic microbes in recreational waters. Epidemiological studies continue to show that people can become sick due to exposure to contaminated recreational waters. Beach advisories and closings by local health departments throughout the country indicate continuing problems. In addition, *E. coli* outbreaks in New York, Washington State, and elsewhere plus various food safety concerns have heightened general public awareness and fears about pollution and microbial pathogens.

EPA is also concerned about the institutional problems related to beaches and recreational waters. Currently there is no national consistency in water quality standards and the efforts devote to monitoring and public right-to-know notification vary widely. Most states rely on water quality standards that are not recommended by EPA (i.e., total coliforms or fecal coliforms). EPA's 1999 Beach Survey also confirmed that beach monitoring and notification throughout the U.S. are inconsistent.

EPA's BEACH Program

In response to these concerns, EPA Administrator Carol Browner formally announced the EPA BEACH program in May, 1997. To guide these efforts, in April 1999 we published our

Action Plan for Beaches and Recreational Waters (or "*Beach Action Plan*") which integrates EPA's beach programs and policy within the Office of Water (OW) with the Office of Research and Development's (ORD) research projects. Major provisions of the Beach Action Plan are to:

- Strengthen beach water quality programs nationwide.

  These efforts will strengthen state-adopted beach water quality standards; provide guidance to states and locals on how to improve monitoring and notification programs; and provide information for the public about beaches (such as: monitoring actions, advisories and postings, maps, standards, etc.) so they can make smart choices as they go to the beach.

•Improve ORD/OW science and research with focus on several areas:

> •better and faster water quality testing indicators and methods. These include better, faster indicators for gastroenteritis (GI) illnesses and non-GI illness; methods to distinguish human from non-human sources; new indicators for tropical waters; etc.

> •research will also focus on water quality models that can better predict when there will be public health problems; scientifically-sound approaches to beach monitoring (where, how often, etc.); and health effects research, including epidemiology studies.

Current activities within EPA's Office of Water focus on several areas.

<u>Water Quality Standards</u>. The Office of Water is developing policies and procedures to ensure that states make the transition to monitoring for *E. coli* and enterococci indicators, rather than total coliforms or fecal coliforms, no later than fiscal year 2003.

<u>Beach Survey and BEACH Watch Internet site.</u> Each year EPA conducts a national BEACH survey to assess beach advisories, closures, monitoring practices, etc. In 1999 almost 200 agencies participated and presented information on more than 1,000 marine beaches and selected freshwater beaches. Final preparation for the 2000 Survey is now underway. EPA's BEACH Watch website (www.epa.gov/OST/beaches) presents information on: water quality at specific beaches and on beach monitoring practices across the country from EPA's Beach Survey; current EPA policies and documents (testimony, relevant methods, studies and reports, etc.); as well as Internet links to other beach water quality and health information, such as state and local health agency web sites, environmental groups, public information, etc.

<u>BEACH Conferences.</u> In 1999 EPA completed two regional BEACH conferences to explain EPA's BEACH Action Plan; obtain feedback on major topic areas for EPA's subsequent document; and discuss local issues through case study presentations. The West Coast conference was held in San Diego CA. on August 31-Sept. 1, 1999 and the East Coast conference in Tampa Bay, Florida on Oct. 18-19, 1999. More than 200 people from wide range of organizations attended each conference. EPA is compiling results of both conferences into a Proceedings Document due out in spring 2000.

<u>Beach Monitoring Study.</u> The Office of Research and Development in conjunction with OW are completing the planning phase for a comprehensive, multi-environment beach monitoring study. Intensive sampling will be conducted during the summer of 2000 at five different beach environments.

<u>Guidance</u>. EPA is beginning work to develop detailed guidance to cover key aspects of beach monitoring and notification programs. It will be developed in consultation with EPA work group and assistance from state and local officials.

<u>EMPACT</u>. EPA's EMPACT program has funded several pilot projects for beaches. Several EPA regions are using EMPACT funds to conduct local, beach-specific projects that investigate biological indicators, monitoring methods, site-specific predictive tools, and make time-relevant

information available to the public. They are developing better means to record and display monitoring data, provide current beach advisory information, and explore other scientific advances such as bacterial genetic "fingerprinting."

Pending Federal Legislation

The U.S. House of Representatives passed H.R. 999, Beaches Environmental Awareness, Cleanup and Health Act, sponsored by Congressman Bilbray, on Earth Day (April 22, 1999). Main provisions of H.R. 999 are summarized here, but full text is available from Congress. *Jurisdiction:* The legislation would apply to coastal recreational waters and the Great Lakes. *WQS/Criteria:* States must adopt revised water quality standards consistent with EPA's 1986 ambient criteria for bacteria. (i.e., *E. coli* or enterococcus) within 31/2 years. *Program & Grants:* HR999 would allocate $30 million/year for EPA grants to states/local governments for coastal beach monitoring and notification programs. EPA would develop Performance Criteria for monitoring and notification programs. *Other:* EPA would also be required to conduct actual monitoring and notification at selected, high priority beaches, if not done by state/local governments. EPA would maintain a national public right-to-know database with information about exceedences of standards, information on beach advisories/postings & closures; "list of waters/beaches" covered by monitoring & notification actions.

The U.S. Senate held a hearing on 7/22/99 on two beach bills: Senator Lautenberg's bill (S. 522) and H.R. 999. Congressional consideration of the bills is continuing.

Detection of *Cryptosporidium* Oocysts in Stream Water Samples Using U.S. Environmental Protection Agency Method 1622

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Disease outbreaks from contaminated drinking water have been caused by the protozoan parasite *Cryptosporidium parvum* and oocysts of this parasite are found regularly in surface sources of drinking water. To improve surveillance for *Cryptosporidium* oocysts in water, the U.S. Environmental Protection Agency developed a new isolation and detection procedure now called Method 1622. Method 1622 is performance based and involves filtration, concentration, immunomagnetic separation, fluorescent antibody and DAPI counter staining, and microscopic evaluation. Currently, a capsule filter is described for use in Method 1622 and alternative filtration procedures are listed as acceptable; however, in a previous draft of the method, both the capsule filter and a membrane disk filter were described.

Capsule and membrane disk filters were compared in the analysis of 11 streamwater samples collected from diverse locations throughout the United States. Precision and recovery experiments from *Cryptosporidium*-oocyst-spiked 10-L volumes of reagent-grade water had averages of 39 percent (S.D.±13 percent) and 47 percent (S.D.±19 percent) with the membrane disk and capsule filters, respectively. Replicate 10-L streamwater samples, unspiked and laboratory spiked with approximately 100 to 250 oocysts, were tested to evaluate matrix effects from these environmental waters. Oocyst recoveries from the streamwater samples averaged 22 percent (S.D.±17 percent) and 12 percent (S.D.±6 percent) with the membrane disk and capsule filters, respectively. These results demonstrate that *Cryptosporidium* oocysts can be recovered from streamwater using Method 1622 but that recoveries are lower than from reagent-grade water.

Additionally, concentrations of *E. coli* and *Cl. perfringens* were evaluated in these streamwater samples by membrane filter methods. Average concentrations for *Cl. perfringens* ranged from 8 to 510 colony forming units per 100 mL, and *E. coli* concentrations ranged from 9 to 15,000 colony forming units per 100 mL. Because of the few oocyst-positive samples and the small sample size, relations between detections of oocysts and concentrations of *Cl. perfringens* or *E. coli* could not be determined.

Occurrence and Distribution of Viral Contamination in Shallow Ground Water in the Coastal Plain of Maryland

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In response to the 1996 Amendments to the Safe Drinking Water Act, the U.S. Environmental Protection Agency is developing the Ground Water Rule to protect users of public ground-water supplies from viral contamination. Following the custom of using total coliforms as an indicator of the risk of contamination from microbial pathogens, many ground-water suppliers use the absence of coliform as justification for not disinfecting source water. In addition, because of technical and fiscal restrictions, direct monitoring for viruses is seldom done in public water supplies. The Maryland Department of the Environment, in cooperation with the U.S. Geological Survey and the Wisconsin State Laboratory of Hygiene, has begun a study to characterize the occurrence and distribution of viral contamination in small (less than 10,000 gallons per day) public water-supply wells in the Coastal Plain and Piedmont Physiographic Provinces of Maryland.

In the Coastal Plain, the ground-water-flow and transport models MODFLOW and MODPATH were used to simulate two hydrogeomorphic regions and to determine likely flowpaths, recharge areas, and traveltimes, for a range of hydrogeologic characteristics known to exist in the shallow surficial aquifer. Assuming a 3-year travel time, simulation results showed that wells screened less than 50 ft below land surface are the most vulnerable to surface-derived contamination. Flow-model results also indicated that in some cases, recharge could be as far as 2,000 ft upgradient from the public water-supply well.

Sample sites were chosen by evaluating areal data (land use, natural soil group, and hydrogeomorphic region) and site data (well depth and age) with regard to susceptibility to viral contamination. Areal data within well-recharge areas were ranked according to likelihood of providing a source area for pathogens. Animal feeding and agricultural storage operations were considered among the most likely sources, and forested land ranked among the least likely. Soil groups were evaluated with regard to average depth to water table and percent sand. Sites with shallow depths to water or with very sandy soils were ranked as vulnerable to contamination. Wells drilled before 1960 also were considered vulnerable, because no installation or disinfection standards existed prior to that time.

Samples were collected by pumping 400 gallons of untreated well water through an electropositive filter. In addition to analysis for culturable viruses and virus groups through polymerase chain reaction (PCR)/gene-probe techniques, samples were analyzed for somatic and male-specific coliphage, *Clostridium perfringens*, Enterococci, *Escherichia coli*, fecal streptococcus, fecal coliform, total coliform, pH, specific conductance, temperature, dissolved oxygen, nitrate plus nitrite, dissolved organic carbon, ammonium, organic nitrogen, total phosphate, orthophosphate, and acid-neutralizing capacity.

Preliminary results indicate that more than 10 percent of samples analyzed have tested positive for either culturable or PCR/gene-probe viruses. About 19 percent of the samples analyzed have bacterial contamination. Although analyses are not yet complete, all sites exhibiting some form of viral contamination have also yielded positive results for coliform and one or more coliphages.

Monitoring of Fecal Contamination in the J.B. Converse Lake Watershed in Mobile County, Alabama

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J.B. Converse Lake is a 3,600-acre reservoir that serves as the primary drinking water supply for the city of Mobile, Alabama. The USGS-Alabama District has been working in cooperation with the Mobile Board of Water and Sewer Commissioners since 1990 to monitor and assess water-quality conditions in the watershed. Because of human health issues associated with the drinking water supply, the estimation of fecal indicator concentrations in the watershed is an important part of the monitoring effort. Many possible sources of fecal indicator bacteria exist in the watershed, such as cattle from dairy farms and pastures, humans from septic tank usage in residential areas, and wildlife from forested areas.

During 1990-1998, surface-water samples were collected from seven tributary and two lake sampling sites and assessed for fecal indicator bacteria. Fecal coliform and fecal streptococcus concentrations were assessed for the entire period; *Escherichia coli* concentrations, beginning in 1997. To further determine the presence of pathogens, samples from four lake sites were examined for the presence of *Giardia* cysts and *Cryptosporidium* oocysts. Elevated fecal bacterial concentrations were identified at several tributary sites. However, these sites received drainage from a variety of land uses, so the dominant source of fecal contamination could not be identified. The lake sites had much lower concentrations of fecal indicator bacteria (10 colonies per 100 milliliters or less); however, *Giardia* cysts and a *Cryptosporidium* oocyst were detected in the lake. Although infrequent, these detections indicate the periodic presence of pathogens in the lake.

In an attempt to differentiate between human and other possible sources of fecal contamination to the watershed, the sampling effort was expanded to seventeen sites that represented specific land-use activities. The first sampling event occurred in late August and early September 1999. The surface-water samples were collected and analyzed for fecal coliform and *Escherichia coli* concentrations. At nine sites where fecal contamination from septic tank usage was suspected, water samples also were analyzed for 47 wastewater indicator chemicals. These sites included 3 sites on Juniper Creek, above and below a dairy farm and above and below a residential area; 1 site on Collins Creek, below a residential area; 2 sites on Crooked Creek, above and below a residential area; and 3 sites on Hamilton Creek, above and below a high density residential area. Twelve different indicator compounds were detected among the sites. Caffeine, triclosan, cholesterol, and 3B-coprostanol were detected at Hamilton Creek at Snow Road and Collins Creek. Caffeine also was detected at another site on Hamilton Creek. *Giardia/Cryptosporidium* sampling was expanded to include sites on Juniper Creek, but no cysts or oocysts were found during the sampling run.

Microbial Indicators in Streams in the Chattahoochee River National Recreation Area Watershed, Metropolitan Atlanta, Georgia

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In the 48-mile reach of the Chattahoochee River downstream from Lake Sidney Lanier (Buford Dam) and within the Chattahoochee River National Recreation Area (CRNRA), fecal-coliform concentrations exceeding the Georgia Environmental Protection Division (GaEPD) standards are the principal reason for the Chattahoochee River and its tributaries not supporting or partially supporting designated uses of drinking water, recreation, and fishing. The extent of microbial contamination in the Chattahoochee River and tributary streams within the CRNRA is presented based on fecal-indicator bacteria data collected from March 1999 through February 2000 as part of a two-year U.S. Geological Survey (USGS) and National Park Service (NPS) project. Lake Sidney Lanier and this 48-mile reach of the Chattahoochee River are the principal drinking-water sources for Metropolitan Atlanta. The study area includes the entire CRNRA (an area of intense recreational use), six municipal water-supply intakes, and eight municipal wastewater-treatment-plant outfalls.

Fecal-coliform, enterococci, and *Escherichia coli (E. coli)*, concentrations were measured at three Chattahoochee River monitoring sites every five days during the summer-recreation season (May through October) and every eight days during the winter season (November through April). Synoptic surveys were conducted during summer wet-weather, summer baseflow, and winter baseflow conditions at four Chattahoochee River and eight tributary monitoring sites. Median fecal-indicator bacteria concentrations in the Chattahoochee River increased by approximately an order of magnitude from 9.5-13 colonies per 100 milliliters (colonies/ 100 mL) at the upstream site to 73-130 colonies/100 mL during the summer and 28-64 colonies/100 mL during the winter at the downstream site. In this highly regulated reach of the river, fecal-indicator bacteria concentrations poorly correlate with discharge and turbidity. Summer-recreation season fecal-coliform concentrations measured at the middle and downstream Chattahoochee River sites were significantly lower during the 1999 drought than those measured by GaEPD during the relatively wet summers of 1994 and 1995.

Results from the synoptic surveys indicate (1) much higher fecal-indicator bacteria concentrations during wet-weather conditions than during baseflow conditions, (2) generally higher concentrations during summer baseflow than during winter baseflow conditions, and (3) generally higher concentrations in tributary streams than in the Chattahoochee River. During the two baseflow synoptic surveys, fecal-indicator bacteria concentrations were lower in the Chattahoochee River downstream from the major wastewater outfalls than concentrations were 2 miles upstream, possibly the result of residual chlorine in the treated effluent. Concentrations of *Clostridium perfringens*, thought to be an indicator for microorganisms entering streams with chlorinated and untreated municipal wastewater, ranged from 32-350 colonies/100 mL during the wet-weather summer synoptic survey and, with one exception, were less than 42 colonies/100 mL during baseflow synoptic surveys.

Major sources and transport pathways of microbial contamination vary among and within tributary watersheds. In an attempt to identify potential differences in microbial sources among tributary sampling sites, a relatively new USGS organic sewage tracer laboratory procedure was used to analyze 47 constituents that may be useful as sewage tracers for samples collected during the summer synoptic surveys. Cholesterol and 3B-coprostanol are indicators of human fecal contamination and were 2 of 26 sewage tracers detected during wet-weather and 2 of 19 sewage tracers detected during baseflow synoptic surveys. Emphasis on evaluating wet-weather water quality in tributary watersheds and the development of improved methods for identification of major sources and transport pathways of microbial contamination are needed to aid in design of abatement programs. Project information and data are available on the project website http://ga.water.usgs.gov/ projects/chatm.

Microbiological Quality of Ground Water Used as a Source of Public Supply in the Ozark Plateaus Aquifer System, Missouri

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Missouri is widely dependent on ground water as a source of drinking water for public-water systems. Water from the deep bedrock aquifers in the Ozark Plateaus generally has been free from bacterial contamination. Little is known, however, about viral contamination and its relation to the bacterial characteristics of the ground water in the Ozark Plateaus. The Ozark Plateaus aquifer system is characterized as a carbonate system with numerous karst features throughout. In the Ozark Plateaus, the most important source of water for public supplies is the Ozark aquifer, both where it is unconfined and where it is confined by the Ozark confining unit in southwestern Missouri. The U.S. Geological Survey, in cooperation with the Missouri Department of Natural Resources, sampled 109 public-water-supply wells twice during 1997-98 to characterize the microbiological quality of ground water in the Ozark Plateaus aquifer system. Samples from each well were analyzed for the following microbiological organisms–total human enteric viruses, male-specific and somatic coliphage, and fecal indicator bacteria (fecal coliform, *Escherichia coli*, and fecal streptococcus).

The data indicate that microbiological contamination of public-water-supplies in the Ozark Plateaus is not widespread. A small but substantial percentage of the wells are contaminated by potentially pathogenic viruses or other pathogen indicator organisms. Of the 109 wells sampled in water year 1997, 94 (86 percent) showed no presence of microbiological contamination. Enteric viruses were present in a sample collected from one well at a density of 2.1 most probable number per 100 liters. Coliphage were present in samples collected from 11 wells at densities ranging from 38 to 2,600 plaque forming units per 100 liters, and fecal indicator bacteria were detected in three wells at a density of 1 colony per 100 milliliters. Enteric viruses, coliphage, and fecal indicator bacteria were not present in the same well. Of the 109 wells sampled in water year 1998, 98 (90 percent) showed no presence of microbiological contamination. Coliphage were present in three wells, including one that was fecal-indicator-bacteria positive in water year 1997, at densities ranging from 41 to 78 plaque forming units per 100 liters. Fecal indicator bacteria were present in eight wells at densities ranging from 15 to 50 colonies per 100 milliliters. Coliphage and fecal indicator bacteria were not detected in the same well.

Results varied considerably between the first and second rounds of sampling, and no apparent correlation exists between the presence of enteric viruses and coliphage or indicator bacteria. Most of the virus and coliphage detections were outside the area with the most mature karst features. The wells with detections generally were located where the Ozark aquifer is confined or where the Ozark aquifer is unconfined and karst features are not well developed. The locations generally correlated with the areas that have the most intense agricultural land use, have the largest population, or had a population increase of greater than 10 percent from 1990 to 1997.

Monitoring of Microbiological and Chemical Constituents in an Area of Induced Infiltration

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Recent regulations governing use of ground water under the direct influence of surface water necessitate programs to examine the factors that might affect transport of pathogens (such as *Giardia lamblia* or *Cryptosporidium parvum*) from surface water toward production wells. The City of Cincinnati, Ohio, currently maintains a well field in the permeable outwash deposits next to the Great Miami River and, with the U.S. Geological Survey, is examining hydrogeologic conditions that might affect the susceptibility of these wells to surface-water pathogens under varying conditions of surface-water infiltration.

Five monitoring wells were installed at each of two sites in the well field along potential flow-paths from the Great Miami River to the production wells. At each site, four vertical wells were completed at varying depths along a flowpath between the production well and the river. A directionally drilled flowpath well was installed approximately 15-20 ft under the riverbed at each site to monitor water quality directly beneath the river. Each well was instrumented with a dedicated pump for water-quality sampling, and a data sonde that monitors hourly specific conductance, pH, temperature, dissolved oxygen, and depth of water above the sonde. A turbidity probe was installed at the shallowest well at each site. One directionally drilled well also was instrumented with a chlorophyll probe. A surface-water gage was established to monitor stream stage, specific conductance, pH, temperature, dissolved oxygen, turbidity, and chlorophyll.

A weekly water-quality sampling program supplemented hourly data at the surface-water station and each of the monitoring wells. The analytes for this program included major cations and anions, total coliform, heterotrophic plate counts, plate counts, endospores, chlorophyll *a*, total organic carbon, ammonia, and nitrate. Additional analytes in the flowpath-study sampling included silica, THM formation potential, chlorine demand, and total organic carbon, and turbidity from all wells. Each month, a sample was collected from a selected well or surface-water site for microscopic particulate analysis and *Giardia lamblia* and *Cryptosporidium parvum* analyses. Relations between surface-water and ground-water constituents were examined under varying rates of infiltration, as inferred from river stage, ground-water levels, and pumping conditions at the production wells.

After a relation is developed for the occurrence and distribution of surface-water borne pathogens under varying rates of surface-water infiltration, event-oriented sampling will begin. Three intensive sampling events during high infiltration periods are planned to establish the extent that the river bed or aquifer material naturally attenuates surface-water pathogens.

Occurrence of Enteric Viruses in Surface Waters

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Human enteric viruses cause a number of diseases when individuals are exposed to contaminated drinking and recreational waters. Vaccination against poliovirus has virtually eliminated poliomyelitis from the planet. Other members of the enterovirus group to which poliovirus belongs, such as the coxsackieviruses and echoviruses that have been recently placed on EPA's Contaminant Candidate List, cause numerous diseases, including gastroenteritis, encephalitis, meningitis, myocarditis and perhaps diabetes and chronic fatigue syndrome. Hepatitis A and more recently hepatitis E have caused large waterborne hepatitis outbreaks. The second leading cause of illness in the United States is acute non-bacterial gastroenteritis. This disease results from infection of susceptible individuals with members of the Caliciviridae, Astroviridae, Reoviridae and Adenoviridae families.

The first step in establishing the risk of waterborne disease from these viruses is to determine their levels of occurrence in contaminated waters. Occurrence measurement requires methods to recover, identify and measure the concentration of viruses in affected waters. Viruses are recovered and concentrated from water by passage through a positively charged cartridge filter. Following virus elution from the cartridge filter with beef extract and concentration of the beef extract solution, viruses are usually assayed by cell culture. However, cultural methods are too time consuming and expensive for routine use, and many of the viruses that cause waterborne disease are either very difficult to culture or cannot be cultured.

To overcome these problems, rapid polymerase chain reaction (PCR) methods have been developed to measure virus levels in environmental waters. While PCR methods are rapid and can detect all the virus groups known to cause waterborne disease, the methods have several unique problems. One of the major problems is caused by the presence of potent environmental inhibitors of PCR that are co-concentrated along with viruses during sample processing. Because the number and types of these inhibitors vary among different water types and even within a single water type, PCR methods must be designed to remove as many inhibitors as possible from different water types without affecting virus recovery. This study was preformed to determine whether a multiplex PCR method developed to characterize the occurrence of enteroviruses, reoviruses, rotaviruses, hepatitis A virus and Norwalk virus in groundwater could be used with surface waters. The method was tested using surface waters from five USGS National Water Quality Assessment (NAWQA) Program sites selected to provide good geographic coverage of the United States and to span a range of hydroclimatic and land-use settings. All sites were found to be positive for two or more of the virus groups during the course of the study.

A second major problem with PCR methods is caused by the effectiveness with which PCR methods amplify nucleic acid target sequences. Aerosols containing amplified products can easily lead to false-positive results. The quality control measures needed to prevent this problem are much more complex than those used in traditional microbiology or chemistry laboratories.

A third major problem with PCR methods designed to detect waterborne viruses results from the fact that they target nucleic acid rather than infectious virus particles. Since only infectious viruses are of a public health concern, PCR methods potentially could lead to unnecessary regulatory action. The PCR findings from the NAWQA sites will be used to illustrate these problems and the proper interpretation of PCR-positive results.

Natural Filtration of Pathogens in Ground Water: What Can Surrogates Tell Us?

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Increasing concerns over deteriorating ground-water quality have stimulated new research which focuses specifically on the subsurface transport behavior of pathogens. Because of safety and ground-water quality issues, surrogates (well-characterized microbial-sized microspheres; non-pathogenic, fluorescently labeled bacteria and protozoa; and bacteriophage) are often used in lieu of microbial pathogens in flow-through column and field injection and recovery experiments. Controlled field and lab experiments were conducted to study the effect of trace organic contaminants (e.g., surfactants) and microbial properties (surface chemistry, size, and buoyant density) on the movement of surrogates through aquifer sediments. The results were applied to the predicted subsurface transport behavior of pathogens, assuming that the pathogens would be affected by these factors in a similar manner.

Differentiating Sources of Fecal Contamination

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Fecal contamination is a major concern in aquatic environments. This contamination can originate from human sources (HS) and nonhuman sources (NHS). Several attempts have been taken to develop methods to differentiate the sources of fecal contamination, such as the ratio of fecal coliform to fecal streptococci, species of *Streptococcus*, *Bacteroides fragilis*, and bacteriophages. However, these methods are not suitable for differentiating sources of fecal contamination. *Escherichia coli* is one of several fecal coliform bacteria that inhabit the intestines of many warm-blooded animals that sometimes contaminate water. Its presence does not specifically implicate human fecal input, therefore it is necessary to differentiate contamination sources to accurately assess health risks. *E. coli* were isolated from HS and NHS in the Apalachicola National Estuarine Research Reserve (ANERR) and analyzed for O-serogroups, multiple antibiotic resistance (MAR) and ribotype (RT) profiles. O-serogrouping showed less diversity for HS versus NHS isolates, and the predominant HS O-serogroups differed significantly ($P<0.01$) from those of NHS isolates. *E. coli* from HS showed significantly greater resistance ($P<0.05$) to antibiotics than isolates from NHS, except for penicillin G. HS and NHS isolates showed 41 and 61 RT profiles, respectively. At approximately 50% similarity index, HS and NHS isolates demonstrated four clusters. Isolates obtained directly from human and animal feces showed high homology with HS and NHS isolates, respectively. Discriminant analysis (DA) of MAR and RT profiles showed that 82% of HS and 97% of NHS isolates were correctly classified. DA of MAR profiles classified 77% and 93% of known and unknown HS isolates, respectively. DA of RT profiles were able to classify 100% and 92% of known and unknown NHS isolates. Application of these methods in the field studies showed that there was good correlation between MAR and RT profiles of *E. coli* and sources of fecal contamination. Therefore, O-serogroups, MAR and RT profiles of *E. coli* may offer useful methods to identify sources of fecal contamination within aquatic environments and to facilitate management practices.

Development of the RNA Oligonucleotide Hybridization Probing Technique to Determine the Source of Fecal Contamination

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Fecal contamination of surface and ground water remains a serious health concern in the U.S. and the world. Contamination problems cannot be adequately addressed until the sources of contamination are known and remediated. At present, there is no standard monitoring technique that can identify sources of the bacteria. The objective of a recent project by the U.S. Geological Survey has been to modify and apply the molecular technique known as "RNA oligonucleotide hybridization probes" to identify bacteria sources and adapt the method into a user-friendly kit. Fecal bacteria unique to a host species can be identified using this molecular technique. Tests have been conducted using RNA hybridization probes that target universal sequences (all bacteria), *E. faecalis* (warm blooded animals), *Lachnospira multiparus* (ruminants), *Fibrobacter succinogenes* (ruminants), *Fibrobacter intestinales* (ruminants), *Bacteroides distasonis* (humans), *Bacteroides vulgates* (primarily human), *Bacteroides fragilis* (human) and *Salmonella* sp. (poultry and human pathogen). The tests were conducted using a fluorometer method in addition to an epifluorescent microscope. The tests differentiated between human and other animals in blind tests with fecal-contaminated water. This success was tempered by the fact that the method required high numbers of bacteria (greater than 100,000 bacteria per 100 milliliters). Further modifications to the method were evaluated to determine if the sensitivity could be enhanced. These modifications focused on enhancing sensitivity of the detection signal. For example, different fluorescent tags were evaluated, enzyme-linked markers were used in place of fluorescent dyes, sample preparation methods were modified and polymerase chain reaction (PCR) was used. The preliminary results of the PCR experiments found that approximately 10 bacteria per liter are required for detection. This improved sensitivity came with the price of a more complicated procedure, which was counter to the original objective of developing a simple kit to track bacteria sources. The other method modifications had limited success at enhancing sensitivity. Despite the problem improving sensitivity, we had several successes in simplifying the RNA hybridization method. We successfully decreased the time required to run a test from 1-2 days to 4 hours by consolidating several steps. We have successfully identified bacteria without using an epifluorescent microscope. Based on preliminary field tests, a minimum of 2 bacteria species from a specific animal type were required to avoid false positives. This technique has potential to identify sources of fecal bacteria, but requires further development before it can be promoted as a user-friendly field kit.

Microbiological Quality of Selected Streams in the Puget Sound Basin Lowlands, and Identification of Contaminant Sources Using Microbial and Chemical Indicators

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In surface waters of the Puget Sound Basin in western Washington, state and local monitoring programs consistently identify the presence of fecal-indicator bacteria as an important water-quality issue. For the most part, data collections do not consist of multiple indicators of fecal contamination that could be used to describe the sanitary quality of water from other than a bacterial perspective or that might be used to infer the sources of contamination. This study was designed by the USGS Puget Sound Basin National Water Quality Assessment team to collect a set of different types of fecal-contamination indicators, including testing for the presence of coliphages, serotyping F^+RNA coliphage isolates to possibly identify the sources of fecal contamination, and employing a new analytical method for detecting a suite of chemicals known to occur in wastewaters.

Fecal coliforms, *Escherichia coli*, enterococci, and somatic coliphages were detected in all samples collected from 31 sites on streams draining urban and agricultural regions of the Puget Sound Basin Lowlands, Washington. F^+ coliphages were detected in samples from 15 of the 31 sites. During summer-time conditions, densities of *Escherichia coli* in 74 percent of the samples and of enterococci in 94 percent of the samples exceeded U.S. Environmental Protection Agency's freshwater recreation criteria; densities of bacteria in 81 percent of the samples exceeded Washington State fecal coliform standards. F^+RNA coliphages isolated from samples taken at South Fork Thornton and Longfellow Creeks were serotyped as Group II, implicating humans or raw sewage as potential contaminant sources. These two sites are located in densely populated, residential areas of metropolitan Seattle. F^+RNA coliphages in samples from 10 other sites, mostly in agricultural or low-density rural areas, were serotyped as Group I, implicating animals other than human as likely sources. Chemicals common to wastewater, including fecal sterols, were detected in samples from several urban streams and also implicate humans, at least in part, as possible sources of fecal bacteria and viruses to the streams. Because human sources of sewage or fecal contamination were implicated, water in Thornton, Longfellow, SF Thornton, Indian-Moxlie, and Wilkeson Creeks and perhaps Mission Creek might pose some level of risk for waterborne illness.

As an inexpensive method, serotyping appeared to function reasonably well as a screening tool to provide presumptive information about sources of contamination, particularly in the context of land use. However, this approach might also have been limited, in part, by the low numbers of F^+ coliphages in samples from most of the sites. Source differentiation by serotyping was not possible for several sites because the samples had few or no F^+RNA coliphage isolates for which serotyping could be performed.

Effects of Sampling Methods on *Escherichia coli* rep-PCR DNA Fingerprint Detection and Interpretation

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Bacterial DNA fingerprint methods may be used to (1) identify specific organisms for epidemiological purposes; (2) evaluate potential sources of bacterial pollution; and (3) develop improved models of delivery, transport and survival of contaminant bacteria in the environment. However, little information is available regarding the effects of sampling design on detection of specific fingerprints, or on the number of samples or isolates required to detect relations between bacteria taken from the environment and their sources. Our research addresses the environmental concentration and DNA-fingerprint diversity of the fecal indicator bacterium *Escherichia coli* in the Grand Traverse Bay Watershed (GTBW) in northern Michigan. Sampling was conducted at three sites (URBAN, FORESTED and ANIMAL-IMPACTED) during a period of base flow in June 1999. At each site, depth-and-width integrated (DWI) samples were taken at two stream transects, approximately 500 ft apart. In addition, grab samples were taken at up to three locations within the downstream transect for comparison with results from the DWI sample.

The largest numbers of *E. coli* per 100 mL occurred at the URBAN site, although at each site numbers of *E. coli* exceeding the Federal full body contact standard of 130 colonies/100 mL were detected. Estimates of *E. coli* numbers were generally lower for grab samples than for DWI samples. DNA fingerprints for 132 *E. coli* isolates (URBAN: 32; FORESTED: 52; ANIMAL-IMPACTED: 48) were determined using rep-PCR. Fingerprints at the 95% level of similarity, as determined by multivariate statistical methods, were considered identical. For the 32 URBAN isolates there were 26 different fingerprints. Only one identical fingerprint occurred within both the upstream and downstream transects. Likewise, at the FORESTED site there were 48 fingerprints for 52 isolates (2 identical upstream-downstream fingerprints) and at the ANIMAL-IMPACTED site there were 44 fingerprints for 48 isolates (2 upstream-downstream matches). When all isolate fingerprints were examined together, only one across-site (FORESTED and ANIMAL-IMPACTED) fingerprint identity occurred. *E. coli* from the URBAN site grouped together in a cluster. *E. coli* from the FORESTED and ANIMAL-IMPACTED sites grouped into two clusters—one that was similar to the URBAN isolates and one that was unique to these more rural sites. *E. coli* DNA fingerprints were highly diverse on spatial scales of a few hundred feet and sampling may affect ability to detect any specific isolate (as defined by its DNA fingerprint). Large numbers of *E. coli* isolates will be required to detect unique fingerprints and to relate fingerprints to sources.

Microbiological Monitoring in Surface Waters of the Santee River Basin and Coastal Drainages, North and South Carolina, 1995-97

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As part of the U.S. Geological Survey National Water-Quality Assessment Program, samples were collected and analyzed for selected water-quality constituents, including fecal-indicator bacteria, at 17 sites in North and South Carolina from October 1995 through September 1996. Fecal coliform and fecal streptococcus concentrations were measured monthly at these sites. Additionally, synoptic studies of two basins in the study area also were conducted. In September 1996, 16 sites were sampled in the Gills Creek Basin in South Carolina. Samples were analyzed for fecal coliforms and fecal streptococci. In October 1997, 20 sites were sampled in the South Fork Catawba River Basin in North Carolina. Samples were analyzed for fecal coliforms and *Escherichia coli* (*E. coli*).

Significant findings include:
- Samples collected at 11 of 17 sites had fecal coliform concentrations greater than the North and South Carolina guidance of 400 colonies per 100 milliliters (col/100 mL) of stream water.
- Of the 17 stations sampled, the highest fecal coliform concentrations were observed in samples from two predominantly agricultural basins, Indian Creek, North Carolina (21,600 col/100 mL), and Cow Castle Creek, South Carolina (12,000 col/100 mL).
- Samples with the highest fecal streptococcus concentrations were from the South Fork Catawba River, North Carolina (>20,000 col/100 mL), a site influenced by industrial wastewater discharge and agricultural runoff, and Cow Castle Creek, South Carolina (10,000 col/100 mL).
- Statistically significant correlations (at alpha = 0.05) were found between some fecal-indicator bacteria concentrations and streamflow, water temperature, and several nitrogen and phosphorus species.
- Correlations of water-quality constituents with fecal-indicator bacteria concentrations suggest that surface-water runoff is a significant source of contamination at Cow Castle Creek, South Carolina.
- Fecal coliform concentrations ranged from 37 to 1,700 col/100 mL in the Gills Creek Basin synoptic study area and were greater than 400 col/100 mL in 8 of the 16 synoptic sites.
- Concentrations of fecal coliform and *E. coli* in the South Fork Catawba River Basin synoptic study area were similar. Fecal coliform concentrations ranged from 22 to greater than 20,000 col/100 mL and *E. coli* concentrations ranged from 19 to greater than 20,000 col/100 mL. The highest concentration was observed at a site sampled immediately after a thunderstorm. Nine of the 20 synoptic sites had fecal coliform concentrations greater than 400 col/100 mL.

Bacteriological Quality of Ground Water Used for Household Supply, Lower Susquehanna River Basin, Pennsylvania and Maryland

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Ground water is an important source of household supply in the Lower Susquehanna River Basin. About 800,000 households and approximately 300,000 private wells are in the study area; approximately 40 percent of the households depend on ground water from private wells for water supply. In this study, we found that bacteriological quality of untreated ground water used for household water supply in the basin was related to land use and physiographic province. No significant relation existed between bacteria concentrations and selected water-quality constituents. Differences in bacteria concentrations among bedrock types were only statistically significant for *Escherichia coli (E. coli)*. Bacteria concentrations were not related to well characteristics such as total well depth or casing length, although further study is needed. Water samples collected from 146 household supply wells in the Lower Susquehanna River Basin were analyzed from 1993 to 1995 for fecal-indicator organisms including total coliform, fecal coliform, *E. coli*, and fecal streptococcus concentrations. Bacteria were not detected in water samples from 31 of the 146 wells sampled. Of the 146 water samples, 101 tested positive for total coliforms, 34 tested positive for fecal coliforms, and 92 tested positive for fecal streptococci. *E. coli* concentrations were determined in water from 88 of the 146 wells sampled. Of those 88 water samples, 26 tested positive for *E. coli.* Nearly one-third of the samples that contained total coliforms also contained fecal coliforms. Fecal streptococci were present in more than three quarters of the samples that tested positive for total coliforms and in all of the samples in which fecal coliforms were present. Land use was related to the presence of bacteria in water from household supply wells. Bacterial concentrations in agricultural and nonagricultural areas were compared. Concentrations of total coliform, fecal coliform, and fecal streptococcus were higher in agricultural areas than they were in nonagricultural areas; these differences in concentrations for different land uses were statistically significant. Differences in *E. coli* concentrations are not statistically significant among land-use categories.

Physiographic province was also related to the presence of bacteria in water from household supply wells. Bacterial concentrations in the Ridge and Valley and Piedmont Physiographic Provinces were compared. Water from wells sampled in the Ridge and Valley Physiographic Province was more likely to have bacteria than water from wells in the Piedmont Physiographic Province.

It is uncertain whether the aquifers sampled have widespread contamination or the bacteriological contamination is a site-specific occurrence. Other factors may exist, besides land use and physiographic province, that could affect bacteria concentrations. These factors include whether or not (1) the septic system is functioning, (2) manure has been applied to nearby fields, or (3) the well has been protected from surface contamination by grout and a sanitary seal. Hydrogeologic structures, such as sinkholes and fractures in the bedrock, transport the water rapidly from the land surface to the well and could affect bacteria concentrations. The large number of wells that did not have sanitary seals and were not grouted made it difficult to determine if bacterial contamination was a result of aquifer contamination or well characteristics. Further study with an assessment designed to compare different well-construction practices would provide the data needed to determine the relation between well construction and the bacteriological quality of well water.

Toward the Design of a National Microbiological Program: Results of Monitoring for Microbiological Indicators in Ground Water and Streamwater

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Data to characterize the microbiological quality of the Nation's fresh, marine, and estuarine waters are usually collected for local purposes, most often to judge compliance with standards for protection of public health in swimmable or drinkable waters. Methods and procedures vary with the objectives and practices of the parties collecting data and are continuously being developed or modified. Therefore, it is difficult to provide a nationally consistent picture of the microbiological quality of the Nation's waters.

A national microbiological monitoring program can be designed using the framework of the U.S. Geological Survey (USGS) National Water Quality Assessment (NAWQA). In this program, one could include monitoring for the three major groups of waterborne pathogens that affect the public health acceptability of waters in the United States—bacteria, protozoa, and viruses. Microbiological monitoring in NAWQA would assess the occurrence, distribution, and trends of pathogenic organisms and indicators in streamwater and ground water; relate the patterns discerned to factors that help explain them; and improve our understanding of the processes that control microbiological water quality.

In a pilot test of a NAWQA-based design, a total of 136 streamwater and 143 ground-water samples were collected in five major hydrologic systems of the United States and analyzed for microbiological indicators to test concepts for monitoring in a nationally consistent program. Total coliforms were found in 99 percent, *Escherichia coli* in 97 percent, and *Clostridium perfringens* in 73 percent of streamwater samples analyzed for each bacterium. Total coliforms were found in 20 percent*, Escherichia coli* in less than 1 percent, and *Clostridium perfringens* in none of the ground-water samples analyzed for each bacterium. Although coliphage analyses were done on many of the samples, contamination in the laboratory and problems discerning discrete plaques precluded quantification. Land use was found to have the most significant effect on concentrations of bacterial indicators in streamwater. Presence of septic systems on the property and well depth were found to be related to detections of total coliforms in ground water, although these relations were not statistically significant. A greater diversity of sites, more detailed information about some factors, and a larger data set in future studies may provide more insight into factors that affect microbiological indicators.

Pilot Study Results of a Same-Day *E. coli* Test for Application to Public Bathing Beaches

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The City of Milwaukee Health Department received an EPA EMPACT Metro grant in 1999 to (1) improve documentation and dissemination of environmental data specifically related to health risk associated with the recreational use of public beaches, (2) improve the type, quantity, and quality of environmental data collected at and around public beaches in Milwaukee in development of a public health risk model, (3) improve coordination and collaboration of environmental data collected between other agencies and organizations and standardize data collection, and (4) build community awareness around surface water pollution prevention. An important aspect of the grant project was to pilot and enzymatic rapid test for *E. coli* (Charm Sciences' LiteGuard™) . The claim of the company is that their enzyme-detection method requires only 6 hours of incubation time, allowing beach water quality results to be available by peak beach usage on the same day that beach samples are collected (7 hours later). The traditional Membrane Filtration method (MF) requires 24 hours of incubation time, within which water quality conditions can change dramatically. The City of Milwaukee Health Department Microbiology Laboratory found that the procedure for the enzymatic test involved long sample filtering times for turbid samples and a longer than expected total incubation (approx. 7.5 hrs). In a study performed using 35 known concentrations of *E. coli*, no relationship between Colony Forming Units (CFU) and the Relative Light Units (RLU) counted by the luminator was found. Forty water samples from each of The City of Milwaukee's three beaches were analyzed using both the traditional MF method and the enzymatic method resulting in correlation coefficient values between log CFU and log RLU of 0.51 (Bradford), 0.17 (McKinley), and 0.72 (South Shore). Additionally, blank determinations were performed along with the beach water analysis that resulted in RLU of at least 1481 and as high as 7701. Following the instruction of the company to average, then double these blank determinations for a control point (CP) that determines positive from negative, a value of 7298 RLU was achieved. A fourfold table analysis using the CP and the cutoff for *E. coli* of 235 CFU resulted in a high rate of false positives. Given the high false positive results obtained using the enzymatic test and the fact that the time required to perform the test does not offer a significant advantage over traditional methods (MF), the City of Milwaukee Health Department will likely discontinue its use and pursue other options to pilot during the second year of its EMPACT program.

Microbial Source Tracking Using Ribosomal RNA Typing in the Chattahoochee River National Recreation Area Watershed, Metropolitan Atlanta, Georgia—Study Design and Preliminary Results

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In Metropolitan Atlanta, fecal-coliform concentrations frequently exceed the Georgia Environmental Protection Division instream standards and are the principal reason that streams in the Chattahoochee River National Recreation Area (CRNRA) do not support or only partially support the designated uses. The CRNRA includes 48 miles of the Chattahoochee River downstream from Buford Dam to the mouth of Peachtree Creek and 14 park units adjacent to the river in northern Metropolitan Atlanta. Although fecal-coliform data provide information about the occurrence and distribution of microbial contamination, these data do not indicate the sources of contamination. Additional information is needed to determine the relative importance of various sources of microbial contamination in a watershed. In 1999, the U.S. Geological Survey (USGS), the National Park Service (NPS), and the University of Georgia began a study to analyze ribosomal RNA to statistically match *Escherichia coli (E. coli)* strains in water samples to *E. coli* strains in fecal samples from humans, domestic animals, and wildlife.

Ribotyping is a DNA fingerprinting technique utilizing highly conserved DNA sequences that ultimately determine the proteins that produce ribosomal RNA. The ribotyping technique consists of (1) positive identification of *E. coli* and pure culture growth, (2) DNA isolation and restriction enzyme digestion, (3) gel electrophoresis and southern hybridization, and (4) statistical analysis. Similar to forensic techniques, unknown ribotypes from water samples are matched to unique ribotypes in feces from individual warm-blooded species present in the watershed. These ribotypes from fecal-source samples will be added to a regional library of ribotypes so that future work may benefit from this study.

Prior to April 2000, 24 water samples are scheduled to be collected during low-flow and wet-weather synoptic surveys at four Chattahoochee River and eight tributary sites in Metropolitan Atlanta. In addition, 40 fecal-source samples are scheduled to be collected. Ribotypes from approximately 600 *E. coli* isolates from water samples and from approximately 1,000 *E. coli* isolates from fecal-source samples are scheduled to be generated. In order to determine the distribution of each source's contribution to contamination, the percent similarities among individual ribotypes will be matched statistically. The relative importance of human, domestic animals, and wildlife as sources of fecal contamination is expected to vary among watersheds and between low-flow and wet-weather conditions within watersheds. Data on the distribution of each source's contribution to contamination in the CRNRA should provide information to help identify principal sources of fecal contamination and more effectively design and implement nonpoint- and point-source-pollution controls.

Identification of Sources of Fecal Coliform Bacteria and Nutrient Contamination in the Shoal Creek Basin, Southwestern Missouri—A Watershed Approach

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Rapid growth in the livestock and poultry industries has caused concern about impacts on surface- and ground-water quality in southwestern Missouri. Shoal Creek drains an area of intense beef cattle and poultry production in Barry and adjacent counties, and more than 500 poultry houses are located within the upper 233 square miles of the basin. Between 1992 and 1999, densities of fecal coliform bacteria in water samples collected by the Missouri Department of Natural Resources (MDNR) from a site on the upper reach of Shoal Creek averaged more than 5,000 colonies per 100 mL (milliliters). These concentrations greatly exceed the Missouri limit of 200 colonies per 100 mL for whole-body contact recreation and have resulted in the upper Shoal Creek Basin being placed on the 303(d) list of impaired water bodies in Missouri. The U.S. Environmental Protection Agency, Region VII, MDNR, and U.S. Geological Survey initiated a cooperative study in 1999 to identify the sources of bacterial contamination in Shoal Creek.

This multi-discipline investigation combines standard water-quality assessment tools with emerging techniques, including microbial source tracking of *Escherichia coli* (*E. coli*) using ribotyping, and the determination of concentrations of optical brighteners, antibiotics, and hormones in water samples. A network of 13 stream and tributary sites is being monitored monthly for discharge, field water-quality parameters, distribution of indicator bacteria, nutrients, and optical brighteners. An expanded suite of analytes are being collected quarterly from 10 surface-water sites, 4 springs, and 3 stream sites during storm events. A geographic information system database is being used to normalize nutrient yields to basin parameters, including size, percent agricultural land use, and density of poultry operations, among others.

Preliminary results suggest that nutrient yields from the Shoal Creek Basin and its tributaries are among the largest in Missouri. The largest bacteria densities are from sites in agricultural areas and not associated with known sewage treatment plant effluents. A minimum of 16 isolates of *E. coli* from each site are being ribotyped by the University of Missouri, Department of Veterinary Pathobiology. Ribotype patterns from stream isolates and animal manure samples from the basin are being compared to determine the probable source of the *E. coli* in the stream samples. Serology also is being done on each isolate to test for the presence of the human pathogen *E. coli* O157:H7, which is responsible for recent outbreaks of waterborne illness across the country. This organism has not been associated with poultry litter and its presence may indicate impacts from cattle manure. During the October 1999 sampling event, 5 of 16 *E. coli* colonies isolated from a water sample collected at the MDNR sampling site were serologically identified as *E. coli* O157:H7. Previous isolates of *E. coli* O157:H7 have been identified in water samples from the basin.

Evidence of Trichloroethene Biodegradation at a Karst Site in Middle Tennessee

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Field and laboratory investigations examined chlorinated-ethene biodegradation in a karst aquifer contaminated with trichloroethylene (TCE). The study site, located in Middle Tennessee, was selected because of the presence of TCE degradation by-products in the karst aquifer, available site hydrologic information and chlorinated-ethene analytical data. The site is underlain by Ordovician limestone characterized by thin soil, vertical fractures, and horizontal bedding plane openings and dissolution openings. Additional chemical, biological and hydrological data were collected to evaluate if the occurrence of TCE degradation by-products in the karst aquifer was the result of biodegradation in the aquifer or simply transport into the karst aquifer from the soil zone. Geochemical analysis established that sulfate-reducing conditions, essential for reductive dechlorination of chlorinated solvents, existed in parts of the contaminated karst aquifer. Other areas of the aquifer fluctuated between anaerobic and aerobic conditions and contained compounds associated with cometabolism, such as ethane, methane, ammonia and dissolved oxygen. A large, diverse bacteria population inhabited the contaminated aquifer. Bacteria known to biodegrade TCE and other chlorinated ethenes, such as sulfate-reducers, methanotrophs, and ammonia-oxidizers, were identified from karst-aquifer water using the RNA-hybridization technique. Results from microcosms using raw karst-aquifer water showed that aerobic cometabolism and anaerobic-reductive dechlorination degradation processes were possible when appropriate conditions were established in the microcosms. These chemical and biological results provide circumstantial evidence that several biodegradation processes were active in the aquifer. Additional site hydrologic information was developed to determine if appropriate conditions persisted long enough in the karst aquifer for these biodegradation processes to be significant. Continuous monitoring devices placed in four wells during the spring of 1998 documented a dual-phase system within the karst aquifer characterized by dynamic areas where active flow occurs and stable areas isolated from active flow. The areas of active flow in the karst aquifer fluctuated between anaerobic and aerobic conditions in response to rain events. Bacterial communities and geochemical conditions conducive to cometabolism were associated with this dynamic environment. In contrast, the pH, specific conductance, dissolved oxygen concentrations (low) and oxidation-reduction potentials (low) changed very little in the stable areas isolated from active flow. These stable areas in the karst aquifer had geochemical conditions and bacterial communities conducive to reductive dechlorination of chlorinated ethenes. In summary, multiple lines of evidence developed from biological, chemical and hydrological data demonstrate that a variety of chlorinated-ethene biodegradation processes were active in this karst aquifer.

Methods in the Ohio District Microbiology Laboratory

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The Ohio District Microbiology Laboratory (ODML) is presented as an example of a well-equipped laboratory designed to support health-based microbiological monitoring of surface water and ground water. ODML personnel use traditional cultivation methods on various media for enumeration of bacterial indicators (total coliforms, fecal coliforms, *Escherichia coli*, *Clostridium perfringens*). Emerging national emphasis on viral and protozoan risks to public health has stimulated the ODML to establish an in-house testing program for coliphage and a cooperative association with the University of North Carolina for *Cryptosporidium* detection. Ongoing research with the U.S. Environmental Protection Agency has led the ODML to adopt a more sensitive method for coliphage detection (presence-absence enrichment test) and a direct detection method for potentially pathogenic enteric viruses (reverse transcriptase-polymerase chain reaction). The latter method involves concentration of the viruses in a water sample and chemiluminescent detection of PCR-amplified genetic material by hybridization with labeled probes. Details of current microbiological methods and their roles in microbiological monitoring will be discussed during the poster session.

Comparison of Methods for Determining *Escherichia coli* Concentrations in Recreational Waters

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Seventy water samples were collected from three Lake Erie beaches to compare recoveries of *Escherichia coli (E. coli)* using the USEPA-recommended method for recreational waters (mTEC) to recoveries using three alternative methods (MI, modified mTEC, and Colilert). Statistical tests showed no differences in recoveries of *E. coli* between MI and mTEC; however, statistically-significant differences were found between modified mTEC or Colilert and mTEC. The MI agar method provided the most similar assessment of recreational water quality to mTEC among the three alternative methods tested. The range of differences between Colilert and mTEC was widest among the three alternative methods. In a sample group with a range of values near the single-sample bathing-water standard, recoveries of *E. coli* were statistically lower using modified mTEC than mTEC; however, MI and Colilert compared well to mTEC in this range. Because samples were collected in a small geographic area, more work is necessary to test within-method variability of the modified mTEC, MI, and Colilert methods and to evaluate these methods as substitutes for the mTEC method in a variety of recreational waters.

Effects of Bacteria on Estuarine Waters in Hampton Harbor and Tributaries, New Hampshire

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Understanding bacteria levels and sources in estuarine waters is needed to improve the understanding of adverse effects on aquatic life and public health. Hampton Harbor and nearby tributaries comprise one of the most productive shellfish habitats in the State of New Hampshire. However, these waters have historically been closed to recreational and commercial harvesting due to elevated fecal coliform concentrations. An increase in fecal coliform concentrations has been observed following rainfall events, presumably as a result of contaminated storm water. The State of New Hampshire and local entities are interested in implementing improved storm-water management practices and developing improved water-quality forecasting techniques in order to maximize the periods of allowable shellfish harvesting. To assess variations in fecal coliform concentrations in shellfish harvesting areas, bacteriological data from several federal and state agencies will be analyzed to improve the understanding of coliform concentrations in relation to specific environmental, tidal, and rainfall conditions. As a result of this analysis, a fecal coliform monitoring program to complement on-going shellfish sanitation surveys will be implemented for purposes of developing additional non-point-source control strategies and improving a forecasting model for shellfish bed closures. This study will provide information and knowledge for making decisions on the harvesting of shellfish and designing future water-quality programs in estuarine waters.

Attenuation Rates for PRD-1 And MS2 During Artificial Recharge at a Constructed Research Basin in Los Angeles County

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The use of treated municipal wastewater effluent (recycled water) to replenish ground-water supplies through artificial recharge is increasing, especially in the arid southwestern United States. As a result, there is a need to predict the attenuation of viruses during recharge and subsurface transport to ensure that adequate soil-retention time requirements are met for removal of human viruses. Although mathematical models and laboratory column studies have been used as predictive tools in the past, field-scale experiments in the area of recharge also are needed. Field experiments conducted under actual recharge conditions with surrogates for human viruses will help provide the information to enable regulatory agencies to estimate soil-retention times and distances for virus attenuation necessary to protect human health.

The U.S. Geological Survey (USGS) and County Sanitation Districts of Los Angeles County (CSDLAC) have recently completed two such field-scale experiments. The experiments were conducted at a small research basin constructed adjacent to a large recharge facility (spreading grounds) located in the Montebello Forebay of Los Angeles County. The objective of the first experiment was to determine the feasibility of conducting field-scale recharge experiments using recycled water seeded with high concentrations of bacteriophage and KBr as tracers. On the basis of results of the first experiment, a second experiment was designed to determine attenuation rates of PRD-1 and MS2 used as surrogates for human viruses under actual recharge conditions.

Several conclusions were drawn from these two experiments: (1) Arrival times of the two bacteriophage and bromide were coincident at the 5-ft depth, and the bacteriophage arrived slightly ahead of the bromide at the 10-ft depth. (2) Bromide measurements indicate that little or no dilution occurred at depths less than 10 ft, and dilution was about 50% at deeper levels. (3) Bacteriophage was attenuated by removal and (or) inactivation. (4) Attenuation of PRD-1 was similar in both experiments and was greater than attenuation of MS2. (5) When breakthrough curves are constructed, both bromide and bacteriophage concentrations rise to a maximum and then decline slightly immediately after reaching their highest values, an unexplained observation also noted by others.

Ratios of bacteriophage to bromide were calculated for samples from the research basin and from depths of 1, 2, 3, 5, and 10 ft below the floor of the research basin. These ratios were calculated for 3, 6, 9, and 21 hours after the addition of bacteriophage and bromide. Regressions performed using data acquired at 9 hours, after steady-state conditions had been established at all depths, indicated an attenuation rate for MS2 of approximately 0.85 log units over 10 ft, and an attenuation rate for PRD-1 of approximately 1.5 log units over 10 ft. Extrapolation from these rates suggests that 7-log removal of bacteriophage, the amount required by regulators, should occur within less than 100 ft of travel through the subsurface.

Fecal-Coliform Concentrations in Streams in the Chattahoochee River National Recreation Area and Surrounding areas, Georgia, May-October 1994 and 1995

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The Chattahoochee River is the principal drinking-water source in Metropolitan Atlanta and water-based recreation constitutes 25-30 percent of the 3.5 million yearly visits to the Chattahoochee River National Recreation Area (CRNRA). The CRNRA includes 48 miles of the Chattahoochee River downstream from Buford Dam to the mouth of Peachtree Creek and 14 park units adjacent to the river in northern Metropolitan Atlanta. Within the Metropolitan Atlanta area, elevated fecal-coliform concentrations are the principal reason for streams not supporting or only partially supporting designated uses. Although, elevated concentrations of fecal-coliform bacteria do not necessarily pose a risk of illness, concentrations of these bacteria have traditionally been used to indicate that fecal contamination of the water has occurred, as well as the potential presence of pathogens.

In 1998, the U.S. Geological Survey (USGS) in cooperation with the National Park Service (NPS) began a study to evaluate the extent of microbial contamination in the CRNRA. As part of this study, the USGS retrospectively analyzed fecal-coliform data collected by the Georgia Environmental Protection Division (GaEPD) in the Chattahoochee River basin during the 1994 and 1995 summer recreation seasons. Fecal-coliform concentrations in many streams in the study area frequently exceeded GaEPD instream standards (based on the geometric mean of at least 4 samples collected within 30 days) and commonly exceeded maximum concentrations recommended for the designated uses of drinking water, recreation, and fishing. When four fecal-coliform samples are not collected at least 24-hours apart and within 30 days of one another, the U.S. Environmental Protection Agency (USEPA) recommends using a review criterion of 400 colonies per 100 milliliters (colonies/100 mL) rather than geometric-mean-based State standards. Fecal-coliform concentrations exceeded the USEPA review criteria in 1 to 89 percent of samples collected from 14 Chattahoochee River monitoring sites and in 42 to 100 percent of samples collected from 22 tributary monitoring sites.

During the 1994-95 summer recreation seasons, tributary streams having the lowest median fecal-coliform concentrations drained the least developed areas, generally upstream and downstream from Metropolitan Atlanta. Tributary streams having the highest median fecal-coliform concentrations drained densely developed urban and suburban areas. Fecal-coliform concentrations in streams increased during storm runoff in response to nonpoint-source contributions. High concentrations of fecal coliforms detected in a few urban streams during baseflow suggest that these tributaries may be affected by point-source contamination, such as leaking or overflowing sanitary sewers. In the 67-river-mile reach of the Chattahoochee River downstream from Buford Dam, median fecal-coliform concentrations increased from less than 20 colonies/100 mL to 790 colonies/100 mL downstream of Metropolitan Atlanta.

The GaEPD data set is the most extensive data set available on fecal contamination in Metropolitan Atlanta and retrospective analysis of these data provides the NPS a summary of the magnitude and scope of microbial contamination in the vicinity of its park units. By including these historical data from within and downstream of the CRNRA, spatial, temporal, and land-use coverages beyond the scope of the current USGS and NPS project were available to augment project design. These data indicate widespread and persistent exceedences of GaEPD standards and USEPA review criteria in Metropolitan Atlanta during the 1994-95 summer recreation seasons. Widespread exceedences further emphasize the need for improved methods for the identification of major sources and transport pathways of microbial contamination to aid in design of abatement programs.

Current and Future *E. coli* Monitoring Strategies at a Southern Lake Michigan Beach

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The problem of bacterial contamination along swimming beaches has been plaguing many of the nation's beaches. This problem is magnified by the ineffectiveness of the current monitoring programs for predicting real-time bacteria concentrations. The need for a rapid test is becoming more urgent, but the technology has not been adequate to address the interactions and obstacles associated with testing natural waters. Technology includes the use of galactosidase and glucoronidase detection, flow cytometry, and epifluorescence. We have been conducting research to assess the applicability and ease of these new technologies and to overcome the obstacles using a variety of techniques. Conclusions are still forthcoming, but the potential for success is promising. Another potential hazard to public health has been recognized in this ongoing *E. coli* research: bacteria in the sand. Our research has revealed a large population of interstitial *E. coli* harbored in the wetted beach sand. Sand along the shore is infiltrated by groundwater and lake water, and bacteria adsorbed to silt particles are being trapped in the sandy substrate. *E. coli* concentration in the sand is negatively correlated with pH, temperature, and specific conductance and positively correlated with ammonia concentration. There seems to be no relationship between *E. coli* concentration and distance from the shore. Furthermore, there seems to be some temporal relationship between concentrations of *E. coli* in groundwater, lake water, and sand, with concentrations highest in the sand. Given the inadequacy of current monitoring techniques and the numerous poorly understood contributing variables, the development of an effective monitoring approach is critical for these popular swimming areas.

A Bacterial Pathogen Reconnaissance of the Salton Sea

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The Salton Sea is an inland body of hypersaline water located in arid southern California. Created by accident in the early 1900's, the sea has become an important resource for wildlife, and recent mortality of fish and birds has prompted a high level of concern many resource agencies. A more intensive survey to document the distribution of known and potential microbial pathogens in the Salton Sea ecosystem was needed to provide baseline information for those tasked to evaluate proposed engineering projects at the Sea. This study was to determine the prevalence of *Pasteurella multocida* (avian cholera), *Salmonella* spp., *Aeromonas* spp., *Vibrio* spp., *Yersinia* spp., and *Erysipelothrix* spp. in the sediments and water of the Salton Sea ecosystem at various times of year. Fecal coliform determinations were done to provide a base-line to the study. Isolates were evaluated through molecular methods to determine potential epidemiological linkages and provide better insights into the their distributions.

Water and sediment samples were collected monthly at the Sea, frozen, and shipped for analysis. Samples were collected at the inflows to the Sea on a monthly basis and from six other sites at the Sea on a quarterly basis. Primary isolation of selected bacterial pathogens included both direct plating and selective enrichment culturing. Enrichment media for the enhanced recovery of *Pasteurella*, *Yersinia,* and *Salmonella* spp. and selective/differential media for the isolation of *Aeromonas, Vibrio* spp.,and *Erysipelothrix* spp. were used. All bacterial colonies were screened based on typical colony morphology and biochemical reactions on selective or differential media. Isolates were biochemically characterized and identified using an appropriate system (API or Vitek, bioMerieux, St. Louis, Missouri; Biolog, Hayward, California). Selected isolates were further analyzed by ribotyping (RiboPrinter™, Qualicon, Wilmington, DE via Cornell University, Laboratory of Molecular Typing, Ithaca, NY).

Results of this study will be used as part of the basis for ecosystem management actions which can be eventually directed toward the source of bacterial pathogens in an effort to mitigate their effects on both human and wildlife populations that use the Salton Sea. The molecular epidemiology of the isolates may be used to resolve sources of pollution and in potential future studies, application of this technology to the bacterial pathogenic isolates of the Salton Sea may help to identify the source of the isolates; human versus animal.

Toxicological Risk Assessment of Recreational Waters: The Use of Microscale Monitoring

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Waterborne microbial and chemical contaminants were assessed by a new tandem microscale monitoring approach that used Define Substrate Technology® (DST) to monitor coliform bacteria and microscale toxicity tests to detect chemical contaminants collected by a synthetic membrane device. Coliform bacteria are used globally as warning indicators of fecal contamination and as an index of general water quality and pathogenicity potential. This new approach uses the presence of characteristic enzymatic activity of indicator bacteria to differentiate fecal coliforms (b-glucu-ronidase) in the coliform group (b-galactosidase). DST (Colilert®) medium detects these specific enzymes. The test's endpoint—specific color or fluorescence—identifies and differentiates the enzymatic activity of coliforms and *E.coli* (i.e., a fecal coliform type) in water. Chemical contaminants were collected by a semipermeable membrane device (SPMD) that mimics the passive sorption of lipophilic contaminants through biological membranes and concentrates the pollutants in a thin film of lipid (triolein) enclosed in polyethylene tubing. SPMD-concentrated samples were diluted with organic solvents for toxicological analysis. This SPMD-extract of the sample is exposed to glowing luminescent bacteria in the Microtox Basic Test and the amount of light decrease from bacteria indicated the acute toxicity (EC50) of the sample. Alternatively, the test extract, with and without metabolic activation, is exposed to non-glowing luminescent bacteria (dark mutants) in the Mutatox Genotoxicity Test and the amount of light increase produced by bacteria indicated the genotoxicity (Yes-No) of the sample. Microbiological and toxicological evaluations of water samples from lentic and lotic systems were successfully made in < 24 h. These four procedures—DST, SPMDs, Microtox, and Mutatox—used in combination appeared to offer a rapid, sensitive, simple, and economical risk assessment of our Nation's recreational waters.

Factors Affecting *Escherichia coli* Concentrations at Three Lake Erie Public Bathing Beaches

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The environmental and water-quality factors that affect concentrations of *Escherichia coli* (*E. coli*) in water and sediment were investigated at three public bathing beaches—Edgewater Park, Villa Angela, and Sims Park—in the Cleveland, Ohio, metropolitan area. This study was done to aid in the determination of safe recreational use and to help water-resource managers assess more quickly and accurately the degradation of recreational water quality.

Water and lake-bottom sediments were collected and ancillary environmental data were compiled for 41 days from May through September 1997. Concentrations of *E. coli* in water were higher and more variable at Sims Park than at Villa Angela or Edgewater Park; concentrations were lowest at Edgewater Park. Short-term storage (less than one week) of *E. coli* in lake-bottom sediments may have occurred, although no evidence for long-term storage was found during the sampling period.

Turbidity, antecedent rainfall, volumes of wastewater-treatment plant overflows and metered outfalls, a sediment resuspension index, and wave heights were found to be statistically related to *E. coli* concentrations in water; however, wind speed, wind direction, water temperature, and the presence of swimmers were shown to be statistically unrelated. Multiple linear regression (MLR) was used to develop a model to predict *E. coli* concentrations, and thus the recreational water quality, at the three beaches. The chosen MLR model used weighted categorical rainfall, beach-specific turbidity, and wave height to predict *E. coli* concentrations. This model accounted for 58 percent of the variability in *E. coli* concentrations and for 1997, predicted the recreational water quality as well as, and in some cases better than, antecedent *E. coli* concentrations (the current method).

Effects of Hydrologic, Biological, and Environmental Processes on Sources and Concentrations of Fecal Bacteria in the Cuyahoga River, with Implications for Management of Recreational Waters in Summit and Cuyahoga Counties, Ohio

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Discharges of fecal bacteria (fecal coliform bacteria and *Escherichia coli*) to the middle main stem of the Cuyahoga River from storm water, combined sewers, and incompletely disinfected wastewater have resulted in frequent exceedences of bacteriological water-quality standards in a 23-mile reach of the river that flows through the Cuyahoga Valley National Recreation Area. Contamination of the middle main stem of the Cuyahoga River by bacteria of fecal origin and subsequent transport to downstream areas where water-contact recreation is an important use of the river are a concern because of the potential public-health risk from the presence of enteric pathogens.

Independent field investigations of bacterial decay, dilution, dispersion, transport, and sources, and bacterial contamination of streambed sediments, were completed in 1991-93 during periods of rainfall and runoff. The highest concentration of fecal coliform bacteria observed in the middle main stem during three transport studies exceeded the single-sample fecal coliform standard applicable to primary-contact recreation by a factor of approximately 1,300 and exceeded the *Escherichia coli* standard by a factor of approximately 8,000. The geometric-mean concentrations of fecal bacteria in the middle main stem were 6.7 to 12.3 times higher than geometric-mean concentrations in the monitored tributaries, and 1.8 to 7.0 times larger than the geometric-mean concentrations discharged from the Akron Water Pollution Control Station.

Decay rates of fecal bacteria measured in field studies in 1992 ranged from 0.0018 per hour to 0.0372 per hour for fecal coliform bacteria and from 0.0022 per hour to 0.0407 per hour for *Escherichia coli*. Most of the decay rates measured in June and August were significantly higher than decay rates measured in April and October. Results of field studies demonstrated that concentrations of fecal coliform bacteria were 1.2 to 58 times higher in streambed sediments than in the overlying water. Sediments are likely to be a relatively less important source of fecal bacteria during rainfall and runoff in the middle main stem relative to bacterial loading from point sources.

Numerical streamflow and transport simulation models were calibrated and verified with data collected during field studies. Of the constituents modeled, bacteria exhibited the poorest correspondence between observed and simulated values. The simulation results for a dye tracer indicated that the model reasonably reproduced the timing of dissolved constituents as well as dilution and dispersion effects. Calibrated and verified models for 1991 and 1992 data sets were used to simulate the improvements to bacteriological water quality that might result from reductions in concentrations of fecal bacteria discharged from two major sources.

The model simulation resulting in the greatest improvement in bacteriological water-quality was one in which concentrations of fecal coliform bacteria and *Escherichia coli* were reduced by 90 percent in the Cuyahoga River at the Old Portage gaging station, and to geometric-mean bathing-water standards in the effluent of the Akron Water Pollution Control Station (BWS/90 scenario).

Compared to the results of the base-simulation, when the BWS/90 scenario was applied in the 1991 model simulation, *Escherichia coli* concentrations were reduced 98.5 percent at Botzum, 97.5 percent at Jaite, and 91.1 percent at Independence. For 1992 model simulations, similar percent reductions in the concentrations of *Escherichia coli* were predicted at the three stream sites when the same reductions were applied to sources. None of the model simulations resulted in attainment of bacteriological water-quality standards.

Bacteria Source Tracking to Support Development of a Total Maximum Daily Load in Three Virginia Watersheds

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The concentration of fecal coliform bacteria in more than 160 stream segments in Virginia exceed the Commonwealth's standard of 1,000 colony-forming units per 100 milliliters (cfu/100 mL). The U.S. Geological Survey (USGS), in cooperation with the Virginia Department of Conservation and Recreation and Department of Environmental Quality, is utilizing bacteria source tracking to assist with the development of a total maximum daily load (TMDL) for fecal coliform bacteria in three of these impaired stream segments. The three sites, chosen to represent land uses commonly associated with impaired streams throughout the Commonwealth, are Accotink Creek in Fairfax County (urban), Christians Creek in Augusta County (agricultural), and Blacks Run in Rockingham County (combined urban-agricultural).

To identify the major sources of fecal bacteria in each watershed, a genetic fingerprinting method known as ribotyping will be used. The portion of DNA encoded for the production of ribosomal RNA is used to distinguish among the different strains of *Escherichia coli* (*E. coli*), the predominant form of fecal coliform bacteria. These *E. coli* strains are host specific, and as a result, *E. coli* in the stream can be linked to specific sources in the watershed. After identification of the dominant bacteria sources in each of the three watersheds, a watershed model (Hydrological Simulation Program – Fortran) will be used to simulate loading from these sources under current conditions and under selected load reduction strategies or best management practices. Incorporating data from the bacteria source tracking study will ensure that modeled scenarios for waste-load allocation and source-load reduction strategies accurately represent conditions in each of the three watersheds.

Total Maximum Daily Loads for Bacterial Indicators

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Introduction

Contamination by pathogens, primarily assessed using bacterial indicators, is among the leading causes of surface water impairment. One of the mechanisms for identifying these impaired waters is the Clean Water Act section 303(d) process. Section 303(d) requires the identification of waters needing Total Maximum Daily Loads (TMDLs) and the development of the TMDLs.

The *Protocol for Developing Pathogen TMDLs* (draft, January 2000) discusses the following steps in the TMDL development process.

Steps in the TMDL Process

Problem Identification

During problem identification available information is compiled, including water quality data, geographic setting and scale, and potential sources. An overall strategy is established to guide the TMDL development process.

Identification of Water Quality Indicators and Target Values

This component identifies the indicators and target values that can be used to evaluate attainment of water quality standards. Often the TMDL target will be the numeric water quality standard for the pollutant of concern. Implementation procedures associated with the water quality standards should also be noted, such as a seasonal primary recreation use.

For microbial pathogens, bacterial indicators are often used to indicate the likely presence of disease-causing organisms. Although many States, Territories and authorized Tribes use fecal coliform to protect recreation uses, EPA recommends *E. coli* or enterococci (*Ambient Water Quality Criteria for Bacteria – 1986*, EPA 440/5-84-002).

Source Assessment

During source assessment, a comprehensive list of potential sources is compiled and characterized by type, magnitude and location. Possible point sources include wastewater treatment plants, combined or separate sewer overflows, meat/poultry processing facilities and animal feeding operations. Potential nonpoint sources include wildlife, livestock, septic systems, land application of manure/biosolids, and domestic pets.

Linkage Between Water Quality Targets and Sources

This step establishes the cause-and-effect relationship between the pollutant sources and the in-stream pollutant response and allows for an estimation of the loading capacity. Seasonal variation in water quality must be addressed when discussing the linkages.

Water quality modeling is often used during this step. Numerous water quality models with a wide range of complexity are available for simulating bacterial indicators.

Allocations

Based on the established linkage, pollutant loadings that will lead to water quality standards attainment are determined and allocated among the significant sources. Wasteload allocations are the allowable loadings from existing or future point sources, while load allocations establish allowable loadings from natural background and nonpoint sources. The margin of safety is usually identified during this step to account for uncertainty in the analysis. The margin of safety is applied implicitly through conservative assumptions or explicitly by setting aside a portion of the loading.

Follow-up Monitoring and Evaluation

TMDL submittals should include a monitoring plan to determine whether the TMDL has resulted in attaining water quality standards and to support any necessary revisions to the TMDL.

Assembling the TMDL

In this component, those elements of a TMDL submittal required by statute or regulation are clearly identified and compiled, and supplemental information is provided to facilitate TMDL review.

Future Developments

On August 23, 1999, EPA published proposed changes to the TMDL rules at 40 CFR 130.2, 130.7 and 130.10. For more information, see http://www.epa.gov/owow/tmdl.

Methods for Determining Recreational Water Quality

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The goal of the Clean Water Act of 1972 was to restore and maintain the physical, chemical, and biological quality of the waters in the United States. Although great progress has been made in cleaning up our lakes, rivers, and coastal waters, many of them still do not meet water quality standards, and most of our beaches have been closed for at least one day because of high bacterial concentrations or other sources of contamination.   In 1986, EPA recommended the use of two new membrane filter methods, mE agar and mTEC agar, for monitoring recreational water for enterococci and *E. coli*, respectively, because the previous indicator organisms, total and fecal coliforms, were not specific for fecal contamination. The recommendation to use the new methods was based on epidemiological studies that showed that illness rates were directly related to enterococci and *E. coli* concentrations in the water, but not to the fecal coliform concentrations. Enterococci levels were correlated with illness rates in both fresh and marine recreational water, while *E. coli* concentrations were correlated in fresh water only.   Since then, two improved methods have been developed: the mEI agar method for enterococci and the modified mTEC agar method for *E. coli*. These methods allow faster and easier enumeration of the target microorganisms. The mEI method is able to recover the same number of enterococci in 24 hours that the mE method recovered in 48 hours, and the modified mTEC method has eliminated the filter transfer step to a second medium. However, results from both of these methods are not available until 24 hours after the samples are collected, and other enterococci methods cited in the literature may take up to 72 hours. This means that detection of unsafe levels of the indicator organisms in recreational water occurs after the exposure to the swimmers, bathers, and other users has taken place. Therefore, rapid methods, with results obtained the same day the sample is taken, preferably within hours, are needed to quickly assess the condition of the recreational water, so that the public can be warned of the risk of possible exposure to pathogens.