BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	
WATER QUALITY STANDARDS AND	)	
EFFLUENT LIMITATIONS FOR THE	)	R08-9
CHICAGO AREA WATERWAY SYSTEM	)	(Rulemaking - Water)
AND THE LOWER DES PLAINES RIVER:	)	
PROPOSED AMENDMENTS TO 35 Ill.	)	
Adm. Code Parts 301, 302, 303 and 304	)	

PRE-FILED TESTIMONY OF CHARLES P. GERBA

My name is Charles P. Gerba. I earned a Bachelor of Science degree from Arizona State University in 1969, and a Ph.D. from the University of Miami, Florida in 1973. Both of my degrees are in Microbiology. I was a postdoctoral research fellow and Assistant Professor of Environmental Virology at Baylor College of Medicine in the Department of Virology and Epidemiology from 1973 through 1981. I am currently Professor of Environmental Microbiology in the Departments of Microbiology and Immunology; Soil, Water, and Environmental Science; and Epidemiology and Biostatistics at the University of Arizona in Tucson, Arizona. I have authored more than 500 articles including several books in environmental microbiology and pollution science. I actively conduct research on the development of new disinfectants, new methods for the detection of enteric pathogens in the environment, occurrence and fate of pathogens in the environment, fate of pathogens during wastewater reuse and land application of biosolids, microbiology of domestic environments and microbial risk assessment.

For the last three years, I have participated in the District's Microbial Risk Assessment (MRA) Study as a member of the Geosyntec Team Senior Advisory Committee. In that role, I have worked closely with the project team providing direction and peer review in all aspects of the MRA Study, which evaluated the human health impacts of disinfection versus non disinfection at the District's three largest water reclamation plants all of which discharge into the

Chicago Area Waterway System (CAWS). In addition, at the onset of the study I provided on-site training to the District personnel on sample collection procedures.

The MRA study focused on microorganisms typically present in the feces of humans and other warm-blooded animals as indicators of fecal pollution. including the following *indicators and pathogens:*

- *Enteric viruses: i) total culturable viruses, (ii) viable adenovirus; and (iii) norovirus*
- *Infectious Cryptosporidium and viable Giardia lamblia*
- *Salmonella spp.*
- *Pseudomonas aeruginosa*
- *Fecal coliforms*
- *E. coli*
- *Enterococci*

This list was taken to be representatives of the likely universe of disease causing organisms and indicators that are used to assess fecal contamination. The indicators selected are those which have been traditionally used and those recommended by the United States Environmental Protection Agency and the World Health Organization for assessment of recreational water quality (NRC, 2004). *Salmonella* was also selected as it is one of the more hardy enteric bacterial pathogens and can always be found in wastewater and would be expected to be representative of the risks from other enteric bacterial pathogens. *Pseudomonas aeruginosa* was selected because it can be commonly isolated from sewage and causes recreationally associated eye, skin and ear infections (Hunter, 1997). Fecal coliforms, *E. coli*, enterococci were included in the list of organisms studied because of its use as an indicator recreational water

quality (NRC, 2004). The test did not detect pathogenic *E. coli*. Non-pathogenic forms of *E. coli* occur in much greater concentration than pathogenic forms in wastewater and their behavior would be expected to be similar to the pathogenic strains of *E. coli* (Nwachuku and Gerba, 2008). *Cryptosporidium* is the protozoan pathogen most commonly associated with recreational waterborne disease outbreaks in the United States today (Dziuban et al., 2006). *Giardia* is also associated with recreational water borne disease outbreaks (Dziuban et al., 2006). Total culturable virus assays have been used by the U. S. Environmental Protection Agency in the Information Collection to assess risks from enteric viruses in water and will largely detect the enteroviruses (Coxsackie, echo,), one is of the most common groups of enteric viruses found in wastewater. Norovirus and adenovirus are the viruses most commonly associated with recreational waterborne disease accounting for more than 90% of all reported outbreaks of viruses associated with recreational water. Norovirus is the most common cause of viral diarrhea in the United States. Adenoviruses are a cause of ear, nose throat and respiratory infections associated with recreational waters. They are also the second leading cause of viral diarrhea in children. Adenoviruses have been detected in greater concentration in wastewater than any other enteric virus, thus they may pose the greatest risk of infection in recreational waters of any of enteric pathogen (Gerba, 2008). Enteric viruses and the protozoan parasites were included in this study because they have a much lower infectious dose than the bacteria (i.e. takes fewer to cause infection) and they survive much longer in surface waters than the enteric bacteria pathogens.

I direct the operation of the Environmental Virology Laboratory, Department of Soil, Water and Environmental Science at the University of Arizona that performed the analysis of adenovirus and norovirus for this study using University of Arizona Standard Operating Procedures (SOPs). There are no U.S.EPA-approved methods for norovirus. The University of

Arizona method estimates the virus concentration, but does not determine or confirm viability or infectivity. Thus, this method is a conservative estimate of the number of infectious virus present in the water i.e. it detects both non-infectious (dead) and infectious viruses (live). Adenoviruses are believed to be more common in sewage than enteroviruses, and have been a cause of recreational waterborne illness (Gerba, 2007). There are no U.S.EPA-approved methods for adenovirus. A University of Arizona SOP was used for the analysis of adenovirus that includes cell culture and DNA confirmation.

The occurrence and concentration of protozoan parasites, total culturable viruses, adenoviruses and norovirus were generally equal to or lower than observed in other studies by me and others on wastewater discharges and surface waters in general during dry weather conditions (Gerba, 2008; Rodriquez et al., 2008; Rose et al., 1988, 1991,1996). These studies involved both disinfected and non-disinfected treated wastewater, and streams into which they were discharged. Some of these studies were conducted in Europe where disinfection of treated wastewater discharges is usually not practiced. The concentration of *Cryptosporidium* was lower than observed in studies in which I have been involved in previously and other studies reported in the scientific literature in which there where no known sewage discharges (Rose et al., 1988;1991) This is because cattle and other animals can be greater source of *Cryptosporidium* in surface waters than sewage discharges. The *Giardia* was also generally lower than that observed in several other sewage discharges from previous studies conducted by me and reported in the literature by others (Rose et al., 1996; Smith and Grimason, 2003). These studies were conducted in various locations across the United States. The total culturable viruses were also lower than observed in a study of a recreational stream in Arizona conducted by my laboratory in which bathers were the only source (Rose at al., 1987).

It is my expert opinion that decisions regarding the need for effluent disinfection must be made on a site-specific basis. Disinfection is warranted in situations where direct human contact in the immediate vicinity of an outfall is possible or where effluent is discharged to areas involving the production of human food. Disinfection is warranted in situations where its application leads to a reduction in the risk of disease transmission. As illustrated by post-disinfection regrowth of bacteria, relatively poor virucidal behavior, and generation of persistent disinfection by-products (DBPs), it is not clear that wastewater disinfection always yields improved effluent or receiving water quality.

There is a great variability in the performance and uncertainty in the efficacy of disinfection. There are many unanswered questions with respect to disinfection efficiency data for microbial indicators and pathogens. The available data for the evaluation of disinfection technologies are bench-scale or pilot-scale experiments and not full-scale operations. Therefore, it is uncertain if disinfection designed to remove indicators can be effective in the removal of pathogens and in the reduction of pathogen risks. In applying any disinfectant, it is important to strike a balance between risks associated with microbial pathogens and those associated with DBPs. DBPs are persistent chemicals, some of which have relevant toxicological characteristics. The inventory of DBPs that have the potential to cause adverse health effects is large and highly variable among Publicly Owned Treatment Works (POTW) effluents. The human health effects associated with chemical contaminants that are influenced or produced as a result of disinfection operations tend to be chronic in nature. Therefore, the development of a risk assessment for exposure to chemical constituents, including DBPs, is far more complex than the microbial risk assessment. Risk assessments of wastewater disinfection should consider microbial and chemical quality.

Respectfully submitted,

By: Charles P. Gerba

Testimony Attachments

1. Curriculum Vitae of Dr. Charles Gerba.
2. Dry and Wet Weather Risk Assessment of Human Health Impacts of Disinfection vs. No Disinfection of the Chicago Area Waterways System (CWA)

References

Dziuban et al. 2006. Surveillance of waterborne disease outbreaks associated with recreational water – United States, 2003-2004. MMWR 55(SS12):1-24.

Gerba, C. P. 2008. Virus occurrence and survival in the environmental waters. In: Human Viruses in Water. A. Bosch, ed. pp. 91-108. Elsevier, Amsterdam.

Hunter, P. H. 1997. Waterborne Disease. Wiley, New York.

NRC. National Research Council. 2004. Indicators and Waterborne Pathogens. National Academies Press. Washington, DC.

Nwachuku, N. and C. P. Gerba. 2008. Occurrence and persistence of *Escherichia coli* 0157:H7 in water. Rev. Environ. Sci. Biotechnol. 7:267-273.

Rodriquez, R. A., P. M. Gundy and C. P. Gerba. 2008. Comparison of BGM and PLC/PRC/5 cell lines for total culturable viral assay of treated sewage. Appl. Environ. Microbiol. 74:2583-2587.

Rose, J.B., R.L. Mullinax, S.N. Singh, M.V. Yates, and C. P. Gerba. 1987. Occurrence of rotaviruses and enteroviruses in recreational waters of Oak Creek, Arizona. Water Research 21:1375-1381.

Rose, J.B., C.P. Gerba and W. Jakubowski. 1991. Survey of potable water supplies for *Cryptosporidium* and *Giardia*. Environ. Sci. Technol. 25:1393-1400.

Rose, J. B., L. J. Dickson, S. R. Farrah and R. P. Carnahan. 1996.Removal of pathogenic and indicator microoganisms by full-scale water reclamation facility. Water Res. 30:2785-2797.

Smith, H. V. and A. M. Grimason. 2003. Giardia and Cryptosporidium. In: The Handbook of Water and Wastewater Microbiology. D. Mara and N. Horan. Pp. 695-756.Elaevier, London.

Attachment 1

CURRICULUM VITAE
of
CHARLES PETER GERBA

EDUCATION AND DEGREES

Arizona State University, Tempe, Arizona
B. S., Microbiology — June 1969
University of Miami, Coral Gables, Florida
Ph.D., Microbiology — January 1973

POSITIONS

Postdoctoral Fellow, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77030 — 1973

Assistant Professor of Environmental Virology, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77030

Adjunct Assistant Professor of Environmental Health, University of Texas School of Public Health, Houston, Texas 77030 — 1976-1983

Associate Professor and Professor, Department of Nutrition and Food Science and University Department of Microbiology and Immunology, University of Arizona, Tucson, Arizona 85721 — 1981-1990

Professor, Department of Soil, Water and Environmental Science
The University of Arizona, Tucson, Arizona
Phone (602) 621-6906 — 1990-

Adjunct Professor, Department of Nutritional Sciences
The University of Arizona, Tucson, Arizona — 1990-

Adjunct Professor, Department of Microbiology and Immunology, The University of Arizona, Tucson, Arizona — 1993-2005

Adjunct Professor, Department of Epidemiology and Biostatistics, The University of Arizona, Tucson, Arizona — 2000-

HONORS

Beta Beta Beta (biology scholastic honorary)	
Epsilon Tau Lambda (adult scholastic honorary: University of Miami)	1969-1972
National Institutes of Health Postdoctoral Traineeship	1973
Member, American Academy of Microbiology	1993
Waksman Lectureship Fellow, American Society for Microbiology	2005-2007

Listed in Who's Who in Technology Today, 1984, 1986, 1989, eds.
Listed in International Who's Who in American Education, 1992-1993, 1995, 1996-1997, eds.
Listed in Who's Who in the West, 1987-present
Listed in Who's Who in Emerging Leaders in America, 1989-1990, 1991-1992, eds.
Listed in Who's Who in the World, 1989-1995-present
Listed in American Men & Women of Science, 18th edition, 1992-1993, 1996-1997-present
Listed in Who's Who in Science and Engineering, 1992-1993, 1996-1997, eds.
Listed in Who's Who in America, 1994 - present
Listed in Who's Who in Medicine and Healthcare, 1997-1998-present

AWARDS

Outstanding Research Scientist Award, College of Agriculture, The University of Arizona	1984
Environmental Science and Engineering Fellow, American Association for the Advancement of Science	1984
Tribute of Appreciation, Criteria and Standards Division, Office of Drinking Water, U.S. Environmental Protection Agency	1984
Service Award for Public Health, Pima County Health Department	1984
Outstanding Team Research Award, College of Agriculture, The University of Arizona	1992
Co-Recipient of Mckee Award (for outstanding contribution to groundwater protection), Water Environmental Federation	1996
Recipient of the A.P. Black Research Award for outstanding contributions to Water Science, American Water Works Association	1997
Honorary Lifetime Membership Award for dedicated service to the water treatment industry, Water Quality Association	1998
Award of Excellence in Environmental Health for outstanding and innovative research program, The National Association of Country and City Health Officials	2000
Selected as one of the 21 most influential people in the water industry in the 21st century by Water Technology Magazine	2002
Best Paper Published in the Journal of the American Water Works Association, Water Resources Division	2005
Best Paper Published in the Journal of the American Water Works Association. Water Science and Research Division	2005
Shah Distinguished Lectureship in Risk Assessment, Stanford University	

Quentin Mees Research Award for outstanding research in applied water science
Arizona Water Pollution Control Association 2007

PROFESSIONAL ORGANIZATIONS

American Society for Microbiology
American Association for the Advancement of Science
Sigma Xi
International Water Association
American Water Works Association
Society for Applied Microbiology
Society for Risk Analysis
International Association for Food Protection

ELECTED POSITIONS IN PROFESSIONAL ORGANIZATIONS

Chairman-elect and Chairman, Applied and Environmental Division of the American Society for Microbiology 1982-1984
President-elect and President, Arizona Branch of the American Society for Microbiology 1983-1984
Councilor, Arizona Branch of the American Society for Microbiology 1985-1986
Chairman-elect and Chairman, Applied and Environmental Division of the American Society for Microbiology 1986-1988

EDITORIAL BOARD MEMBERSHIPS

Applied and Environmental Microbiology	1979-1985
CRC Critical Reviews in Environmental Control	1984-
Journal of Food Protection	1984-1990
Journal of Industrial Microbiology	1986-1989
Journal of Applied Microbiology	2000-2005
Letters in Applied Microbiology	2000-2005
Regional Editor – Journal of Water and Health	2002-
Reviews in Environmental Toxicology and Contamination	2006-

PROFESSIONALLY RELATED PUBLIC SERVICE

Member - U. S. Environmental Protection Agency Workshop on "Protocol Development: Criteria and Standards for Potable Reuse and Feasible Alternatives", Committee on Groundwater Criteria 1980
Member - U.S. Environmental Protection Agency Workshop on "Monitoring for Viruses in the Environment" 1980
Member - U.S. Environmental Protection Agency Workshop on "Microbial Contaminants in Drinking Water" 1981
Member - U.S. Environmental Protection Agency Workshop on "Land Application of Municipal Wastewater and Sludge", Denver 1983

Member - University Technical Advisory Committee to the Pima County Board of Health 1982-1984

Member - Demonstration Recharge Advisory Committee, Tucson Water, City of Tucson 1984-1987

Member - Technical Advisory Board to the Pima County Water Hyacinth Wastewater Treatment Project 1984

Member - U.S. Environmental Protection Agency, Office of Drinking Water, Workshop on Revised Drinking Water Regulations 1985

Member - Task Force for Microbiological Water Purifier Guide Standards and Testing, Office of Drinking Water, U.S. Environmental Protection Agency

Member - Pima County Board of Health 1986-1992

Member - Grant Review Panel, Office of Research and Development, U.S. Environmental Protection Agency 1986-1988

Member - Drinking Water Committee, Science Advisory Board, U.S. Environmental Protection Agency

Member - Task Group on Copper/Silver Ion Generators, National Sanitation Foundation 1989-1991

Member - Technical Advisory Committee, Soil Aquifer Treatment Facility, Santa Ana Watershed Project Authority 1990-1992

Member Ad Hoc - FIFRA Scientific Advisory Panel, Antimicrobial Test Methodology, Office of Pesticides, U.S. 1991-1997

Member - Task Group on Drinking Water Treatment Units for Bacteriostatic Testing and Cyst Reduction, National Sanitation Foundation 1991

Member - "Workshop on Drinking Water and Health in the Year 2000", U.S. Environmental Protection Agency and American Water Works Research Foundation 1991

Member - "Workshop on Virology for the Water Supply in the Nineties", National Institute of Public Health and Environmental Protection, The Netherlands 1991

Member - "Expert Panel on Hazards of Municipal Solid Waste Recycling," U.S. Environmental Protection Agency 1991-1992

Member - "Workshop of Research Needs in Drinking Water Microbiology", American Water Works Foundation 1992

Member - "Workshop on the Methodology for Deriving National Water Ambient Water Quality Criteria for the Protection of Human Health", U. S. Environmental Protection Agency, Office of Science and Technology 1992

Member - "Blue Ribbon Panel on Research needs for Ultraviolet Disinfection", State of California, Dept. of Health Services 1993

Member - "Blue Ribbon Panel on Assessment and Acceptability of Risk", State of Calif., Dept. of Health Services. 1993

Member - Workshop on "Research Needs in Microbial Risk Assessment", National Institute of Public Health and Environmental Protection, The Netherlands 1992

Chairman - Microbiology Working Group "Workshop for Revision of National Guidelines for Deriving Human Health Criteria for Surface Water", U.S. Environmental Protection Agency 1992

Member - Technical Advisory Committee, Water Campus Wastewater Reuse Project,

City of Scottsdale 1992-1994

Member - Science Advisory Board, Committee on Drinking Water and Committee on Research Strategies, U.S. Environmental Protection Agency 1994-1999

Member - Working Group on Microbial Risk Assessment, International Life Science Institute and U.S. Environmental Protection Agency 1995-

Member - Project Advisory Committee, UV Disinfection of Groundwater, American Water Works Research Foundation 1994-1996

Member - Project Advisory Committee
National Survey of Viruses in Groundwater,
American Water Works Association
Research Foundation 1995-1999

Member - Working Group on Microbial Risk
Assessment. International Life
Science Institute, and the United States
Environmental Protection Agency 1996-2000

Member - Workshop on Managing Microbial
Risks of Potable Water in Space NASA 1997

Member - Workshop on New Microbial Indicators for
Water, U.S. Environmental Protection Agency 1998

Member - Workshop on Water and Food Pathogen
Risk Assessment, U.S. Environmental
Protection Agency, and the International
Life Science Institute 1999

Member - Workshop on Groundwater
Indicator Evaluation, U.S.
Environmental Protection Agency 1999

Member - Early Warning Monitoring to Detect
Hazardous Events in Water Supply Systems
U.S. Geological Survey, U.S. Environmental
Protection Agency, Dept. of Defense 1999

Member – Susceptibility and Microbial Risk Assessment
Workshop G.W. University and the
Environmental Protection Agency 1999

Member - Mars Sample Handling Workshop, NASA 2000

Member – Research Needs for On-Site Wastewater Treatment Systems Workshop, U. S. Environmental Protection Agency 2001

Member – Recreational Water Quality Standards for Tropical Waters Workshop, U. S. Environmental Protection Agency 2001

Member – Research Needs for Biosolids and Animal Wastes, U.S. Department of Agriculture and U. S. Environmental Protection Agency 2001

Member –Workshop on Indicators for Pathogens in Wastewater, Biosolids and Stormwater, Water Environment Research Foundation 2003

Member – Expert Panel to Review Centers for Disease Control

Environmental Microbiology Program 2005
Member – Selecting Criteria for the Candidate Contaminate List,
U. S. Environmental Protection Agency 2006

SERVICE TO THE PROFESSION

Rapporteur - International Conference on Viruses in Water, Mexico City 1974
Vice-Chairman - Workshop on "Viral Pollution in the Environment", Fourth International Congress for Virology, The Hague, Netherlands
Member - American Society for Testing Material, subsection committee on standard methods for detecting virus on solids and soils
Member - American Society for Testing Material, subsection committee on standard methods for detecting viruses in fresh and marine waters 1978
Subcommittee on Virological Methods, "Examination of Seawater and Shellfish", published by the American Public Health Association
Chairman - Workshop on "Ecology of Viruses in Water", Second International Symposium on Microbial Ecology, University of Warwick, England
Session Chairman - "Distribution and Development of Pathogens", Second International Symposium on Microbial Ecology, University of Warwick, England
Session Chairman - International Conference on Viruses and Wastewater Treatment, University of Surrey, England 1980
Co-Chairman - Workshop on "Environmental Aspects of Viral Hepatitis Transmission", International Symposium on Viral Hepatitis, New York
Member - Session Committee, Institute of Food Technologists 1982
Member - Committee on Environmental Microbiology, American Society for Microbiology 1981-1983
Member - Microbial Problems in Drinking Water Committee, American Water Works Association
Co-Chairman - Round Table - Fate of Genetically Engineered Organisms in the Environment, American Society for Microbiology, Las Vegas
Senior Delegate - U.S. Committee of the International Association for Water Pollution

Control and Research for the American Society for Microbiology 1985-1991

Member - Planning Committee for Symposium on "Microbial Aspects of Surface Water Quality", Water Pollution Control Federation. 1988-1989

Co-Chairman - Organizing Committee of the 2nd International Symposium on Contamination of the Environment by Viruses and Methods of Control. Vienna, Austria 1987-1989

Chairman - Enteric Virus Committee, Joint Editorial Board, 17th edition supplement and 18th edition, Standard Methods for the Examination of Water and Wastewater 1989-1997

Member - Project Advisory Committee, National Groundwater Virus Survey, American Water Works Research Foundation 1991-2000

Member - Research Committee, American Water Works Association 1992-1994

Member - Workshop on "Microbial and Disinfection By-products Research Needs", American Water Works Research Foundation 1993

Member - International Scientific Committee, "Assessing and Managing Health Risks from Drinking Water Contamination: Approaches and Application". 1993-1994

Member - Organizing Committee, "Second International Symposium on Wastewater Reclamation and Reuse" 1993-1995

Member - Organizing Committee "Global Issues in Microbiological Water Quality for the next Century". Sponsored by UNESCO, U.S. Environmental Protection Agency, and the American Academy for Microbiology 1994-1995

Member - American Soc. for Microbiology delegate. United States National Committee of the International Water Quality Association (now International Water Association) 1992-1999

Member - Public and Scientific Committee of the American Soc. Microbiology 1996-2006

Member- Organizing committee for Workshop on Acceptable Microbial Risks in Water, American Academy for Microbiology 2006

Member – Workshop on Select Criteria for Drinking Water Candidate Contaminate List, Office of Water, United States Environmental Protection Agency 2006

Member – Scientific Review of the Proposed Risk Assessment Bulletin from the Office of Management and Budget, National Research Council 2006

DOCTORAL DISSERTATION

Gerba, C.P. 1973. Investigations into the effects of particulate matter on the survival of a virus in seawater, University of Miami.

PUBLICATIONS

BOOKS

Gerba, C.P., and S.M. Goyal (eds.). 1982. *Methods in Environmental Virology.* Marcel-Dekker, Inc., NY.

Bitton, G., and C.P. Gerba (eds.). 1984. *Groundwater Pollution Microbiology.* John Wiley and Sons, NY.

Goyal, S.M., C.P. Gerba, and G. Bitton. 1987. *Phage Ecology.* John Wiley and Sons, N.Y.

Pepper, I.L., C.P. Gerba, and J.W. Brendecke. 1995. *Environmental Microbiology - A Laboratory Manual.* Academic Press, NY.

Pepper, I.L., C.P. Gerba, M.L. Brusseau, and J.F. Brendecke (eds). 1996. *Pollution Science.* Academic Press, San Diego, CA.

Haas, C.N., J.B. Rose, and C.P. Gerba. 1999. *Quantitative Microbial Risk Assessment.* John Wiley, NY.

Maier, R.M., I.L. Pepper and C.P. Gerba. 2000. *Environmental Microbiology.* Academic Press, NY.

Pepper, I.L., C.P. Gerba, and J.W. Brendecke. 2004. *Environmental Microbiology - A Laboratory Manual.* Second Edition. Academic Press, San Diego.

Pepper, I. L., C. P. Gerba and M. L. Brusseau. 2006. *Environmental and Pollution Science*, Second Edition. Academic Press, San Diego.

PEER REVIEWED JOURNAL ARTICLES

Gerba, C.P., and G.E. Schaiberger. 1973. Biscayne Bay: bacteriological data interpretation. Flor. Sci. 36:104-109

Gerba, C.P., and G.E. Schaiberger. 1975. Effect of particulates on the survival of virus in seawater. J. Water Pollut. Contr. Fed. 47:93-103.

Gerba, C.P., and G.E. Schaiberger. 1975. Aggregation as a factor in loss of viral titer in seawater. Water Res. 9:567-571.

Gerba, C.P., C. Wallis, and J.L. Melnick. 1975. Microbial hazards of household toilets. Droplet production and the fate of residual organisms. Appl. Microbiol. 30:229-237.

Gerba, C.P., C. Wallis, and J.L. Melnick. 1975. The fate of wastewater bacteria and viruses in soil. Jr. Irrig. Drain. Div. ASCE 101:157-174.

Gerba, C.P., M.D. Sobsey, C. Wallis, and J.L. Melnick. 1975. Factors influencing the adsorption of poliovirus onto activated carbon in wastewater. Environ. Sci. Technol. 9:727-731.

Gerba, C.P., C. Wallis, and J.L. Melnick. 1975. Viruses in water: the problem, some solutions. Environ. Sci. Technol. 9:1122-1126.

Farrah, S.R., C.P. Gerba, C. Wallis, and J.L. Melnick. 1976. Concentration of viruses from large volumes of tap water using pleated membrane filters. Appl. Environ. Microbiol. 31:221-226.

Gilbert, R.G., R.C. Rice, H. Bouwer, C.P. Gerba, C. Wallis, and J.L. Melnick. 1976. Wastewater renovation and reuse: virus removal by soil filtration. Science 192:1004-1005.

Gerba, C.P., and J.S. McLeod. 1976. Effect of sediments on the survival of *Escherichia coli* in marine waters. Appl. Environ. Microbiol. 32:114-120.

Gilbert, R.G., C.P. Gerba, R.C. Rice, H. Bouwer, C. Wallis, and J.L. Melnick. 1976. Virus and bacteria removal from wastewater by land treatment. Appl. Environ. Microbiol. 32:333-338.

Farrah, S.R., S.M. Goyal, C.P. Gerba, C. Wallis, and P.T.B. Shaffer. 1976. Characteristics of humic acid and organic compounds concentrated from tapwater using the Aquella virus concentrator. Water Res. 10:897-901.

Payment, P., C.P. Gerba, C. Wallis, and J.L. Melnick. 1976. Methods for concentrating viruses from large volumes of estuarine water on pleated membrane filters. Water Res. 10:893-896.

Lance, J.C., C.P. Gerba, and J.L. Melnick. 1976. Virus movement in soil columns flooded with secondary sewage effluent. Appl. Environ. Microbiol. 32:520-526.

Lance, J.C., and C.P. Gerba. 1977. Nitrogen, phosphate and virus removal from sewage water during land filtration. Prog. Water Technol. 9:157-166.

Farrah, S.R., C.P. Gerba, S.M. Goyal, C. Wallis, and J.L. Melnick. 1977. Regeneration of pleated filters used to concentrate enteroviruses from large volumes of tap water. Appl. Environ. Microbiol. 33:308-311.

Gerba, C.P., C. Wallis, and J.L. Melnick. 1977. Disinfection of wastewater by photodynamic oxidation. J. Water Pollut. Contr. Fed. 49:575-583.

Hobbs, M.F., C.P. Gerba, C. Wallis, J.L. Melnick, and J.S. Lennon. 1977. Photodynamic inactivation of infectious agents. J. Environ. Eng. Div. ASCE 103:459-472.

Sobsey, M.D., C.P. Gerba, C. Wallis, and J.L. Melnick. 1977. Concentration of enteroviruses from large volumes of turbid estuary water. Can. J. Microbiol. 23:770-778.

Farrah, S.R., S.M. Goyal, C.P. Gerba, C. Wallis, and J.F. Melnick. 1977. Concentration of enteroviruses from estuarine water. Appl. Environ. Microbiol. 33:1192-1196.

Gerba, C.P., S.M. Goyal, E.M. Smith, and J.L. Melnick. 1977. Distribution of viral and bacterial pathogens in a coastal canal community. Marine Pollut. Bull. 8:279-282.

Gerba, C.P., E.M. Smith, and J.L. Melnick. 1977. Development of a quantitative method for detecting enteroviruses in estuarine sediments. Appl. Environ. Microbiol. 34:158-163.

Goyal, S.M., C.P. Gerba, and J.L. Melnick. 1977. Occurrence and distribution of bacterial indicators and pathogens in canal communities along the Texas coast. Appl. Environ. Microbiol. 34:139-149.

Gerba, C.P., C. Wallis, and J.L. Melnick. 1977. Application of photodynamic oxidation to the disinfection of tapwater, sea-water and sewage contaminated with poliovirus. Photochem. Photobiol. 26:499-504.

Stagg, C.H., and C.P. Gerba. 1977. Cyanophage as an indicator of animal viruses in wastewater. (Discussion). J. Water Pollut. Contr. Fed. 49:1915-1916.

Melnick, J.L., C.P. Gerba, and C. Wallis. 1977. Viruses in water: an increasing awareness of the problem and approaches to its solution. J. Viestnik AMN, USSR (J. Acad. Med. Sci., USSR) 6:70-75, (In Russian).

Farrah, S.R., S.M. Goyal, C.P. Gerba, R.H. Conklin, C. Wallis, J.L. Melnick, and H.L. Dupont. 1978. A simple method for concentration of enteroviruses and rotaviruses from cell culture harvests using membrane filters. Intervirology 9:56-59.

Gerba, C.P., S.R. Farrah, S.M. Goyal, C. Wallis, and J.L. Melnick. 1978. Concentration of enteroviruses from large volumes of tap water, treated sewage and seawater. Appl. Environ. Microbiol. 35:540-548.

Farrah, S.R., S.M. Goyal, C.P. Gerba, R.H. Conklin, and E.M. Smith. 1978. Comparison between adsorption of poliovirus and rotavirus by aluminum hydroxide and activated sludge flocs. Appl. Environ. Microbiol. 35:360-363.

Farrah, S.R., S.M. Goyal, C.P. Gerba, C.Wallis, and J.L. Melnick. 1978. Concentration of poliovirus from tapwater onto membrane filters with aluminum chloride at ambient pH levels. Appl. Environ. Microbiol. 35:624-626.

Smith, E.M., C.P. Gerba, and J.L. Melnick. 1978. Role of sediment in the persistence of enteroviruses in the estuarine environment. Appl. Environ. Microbiol. 35:685-689.

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Detection of rotavirus and hepatitis A in water, Environmental Protection Agency, Principal Investigator, 1982-1984, $117,000.

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Virus analysis of groundwater, Arizona Dept. of Health Services, Principal Investigator, 1984, $3,000.

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Studies on microbial contamination of groundwater, IBM Corporation, Principal Investigator, 1985-1986, $23,395.

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Water disinfection by material surface contact, U.S. Aid Program in Science and Technology, Principal Investigator, 1986-1989, $149,878.

Development of gene probes for rapid detection of enteric viruses in water and sewage, U.S. Aid Program in Science and Technology, Principal Investigator, 1986-1989, $149,651.

Salary support for Dr. Susan Stramer, Centers for Disease Control, Principal Investigator, 1985-1987, $31,958.

Detection and isolation of *Cryptosporidium*, *Giardia*, and *Entamoeba* from waters throughout the United States, U.S. Environmental Protection Agency, Co-Investigator, 1986-1989, $160,000.

Rotavirus survival and transport in the subsurface, U.S. Environmental Protection Agency, Principal Investigator, 1986-1988, $199,946.

Gene probes for enteric virus detection, The University of Arizona Biomedical Research Support Grant, Principal Investigator, 1986-1987, $6,995.

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Development of methodology for detection of enteric viruses in food and water, Dept. of Nutrition and Food Science, Hatch Project, Principal Investigator, 1988-1991, $41,929.

Rapid detection of enteric viruses in water using gene probes, Arizona Disease Control Research Commission, Co-Investigator, 1987-1988, $24,442.

The effect of liquid smoke on *Listeria monocytogenes*, Bar S Foods, Co-Principal Investigator, 1987-1988, $10,000.

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Effectiveness of hand washing for the removal of contaminating enteric viruses and *Giardia*, Dial Corp., Co-Investigator, 1987-1989, $19,285.

Ionic purification of water, Sigma Products, Inc., Principal Investigator, 1988-1989, $5,750.

Evaluation of a copper-silver electrolytic unit with chlorine under swimming pool conditions, U.S. Army, Principal Investigator, 1988, $9,950.

Evaluation of a thermal-activated carbon microbiological water purifier, Regal Ware. Principal Investigator, 1988, $7,200.

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Efficiency of reverse osmosis membranes in virus removal, Shaklee Corporation, Principal Investigator, 1988, $5,000.

Agricultural sludge reclamation, Pima County, Co-Investigator, 1988-1990, $23,000.

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Evaluation of gene probe technology for the detection of human immunodeficiency virus in hospital wastewater concentrates, National Science Foundation, Co-Investigator, 1989-1990, $29,994.

Gene Probe detection of pathogens in sludge-amended soils, U.S. Geological Survey, Co-Principal Investigator, 1989-1991, $174,693.

Demonstrations of nanofilter method for treating Colorado River water, Consolidated Utilities, Co-Investigator, 1989-1990, $33,575.

Microbiological evaluation of diapers, solid waste, and leachate from the Fresh Kill landfill, Proctor and Gamble Co., Co-Principal Investigator, 1989-1990, $85,268.

Determination of the inactivation kinetics of poliovirus after exposure to potassium permanganate, Carus Chemical Co., Principal Investigator, 1989-1990, $18,600.

Assessment of model equations for predicting survival and transport of microorganisms in groundwater in Arizona, Water Resources Center, Co-Principal Investigator, 1989-1990, $8,770.

Inactivation of MS-2 coliphage and *Legionella* by potassium permanganate, Carus Chemical Co., Principal Investigator, 1989, $10,950.

Virus removal by a wastewater treatment and recycling system, Thetford Systems, Inc., Principal Investigator, 1989, $15,000.

Microbiological characterization of hotel bathrooms, Brushguard, Inc., Principal Investigator, 1989, $6,950.

Microbiological evaluation of compost containing disposable diapers, Co-Principal Investigator, Proctor and Gamble Co., 1989-1990, $32,985.

Investigations into the invasive properties of *Campylobacter*, Arizona Disease Research Commission, Co-investigator, 1989-1991, $57,000.

Evaluation of the microbial efficacy of a porcelain cleaner, Musson Associates, Principal Investigator, 1989, $6,950.

The role of soil aquifer treatment in wastewater reclamation/reuse: hydrological, chemical and microbiological considerations, Salt River Project and Tucson Water, Co-Principal Investigator, 1990-1991, $283,665.

Subsurface transport of biocolloids, National Institute of Health, Principal Investigator, 1990-1992, $124,340.

Molecular methods for evaluation of microbial quality of groundwater, USDA Cooperative State Research Service, 1990-1992, $79,516.

Underground fate and transport of microorganisms, Water Resource Research Center. Co-Principal Investigator, 1990-1991, $33,637.

Human enteric viral contamination of groundwater, Dept. of Environmental Protection, State of New Jersey, Principal Investigator, 1990-1991, $48,325.

Health risks associated with bacterial and viral pathogens in groundwater, Arizona Disease Research Commission, Co-principal Investigator, 1990-1993, $85,600.

Research support for studies on solid waste, Procter and Gamble Co., Principal Investigator, 1990, $3,000.

Development of non-halogen disinfectants for swimming pools, 1990, Olin Corporation, Principal Investigator, 1990-1991, $43,650.

Underground fate and transport of microorganisms, Water Resources Research Center, Co-principal Investigator, 1991-1992, $40,531.

Evaluation of the hydraulic, chemical, and microbiological aspects of soil-aquifer treatment (SAT) during wastewater reclamation/reuse: laboratory and field studies, Tucson Water and the Salt River Project, Co-principal Investigator, 1992-1993, $137,951.

Detection of viable *Giardia* cysts in water by polymerase chain reaction, Metropolitan Water District of Southern California, Co-principal Investigator, 1991-1992, $52,809.

Transport of subsurface bacteria in porous media, Dept. of Energy, Co-Investigator, 1991-1993, $300,100.

Microbial contaminate removal/inactivation by Asian point-of-use treatment system, Amway Corporation, Principal Investigator, 1991-1992, $104,000.

Studies on viruses and parasites in reclaimed water, Microbial Analytical Laboratory, Principal Investigator, 1985-1993, $972,809.

Delineation of wellhead protection zones: considerations of virus transport, U.S. Environmental Protection Agency, Principal Investigator, 1991-1994, $200,000.

Determination of the inactivation kinetics of hepatitis A virus and *Giardia* cysts after exposure to potassium permanganate, Carus Chemical Company, Principal Investigator, 1992, $52,000.

Transport of biocolloids in the subsurface, National Institute of Environmental Health Science, Co-principal Investigator, 1992-1995, $425,000.

Agricultural sludge reclamation, Pima County Wastewater Division, Co-investigator, 1991-1992, $83,793.

Multi-laboratory evaluation of the guide standard and protocol for testing microbiological water purifiers, U. S. Environmental Protection Agency, Principal Investigator, 1992-1994, $164,964.

In use antibacterial dish detergent efficacy study, L and F Products, Principal Investigator, 1992-1993, $35,716.

Characterization of the microflora of households and estimation of the impact of disease transmission by surfaces, Co-Principal Investigator L and F Products, 1992-1993, $66,270.

Stability of HIV viral RNA under environmental conditions, Co-investigator, National Science Foundation, 1992-1993, $50,000.

Incidence of pathogens in Mamala Bay: molecular and risk assessment, Co-Principal Investigator, Mamala Bay Commission, 1993-1995, $350,000

Physical, chemical, and biological properties of the Schmutzdecke, Co-Investigator, U. S. Department of Agriculture, 1993-1996,
$180,000.

Microbial risk assessment for drinking water, Co-investigator, American Water Works Research Foundation, 1993-1995, $200,000.

Studies on the inactivation of *Giardia* by pH, pressure, and disinfection, Principal Investigator, CDM Engineering, 1993, $59,000.

Solas Water System Testing, Solas Corporation, Principal Investigator, 1993-1994, $12,000.

SC Johnson Wax R&D Fellowship Grant, Johnson Wax, Principal Investigator, 1993-1994, $20,000.

Biocolloid Transport in Groundwater. United States-Israel Binational Agricultural Research and Development Fund. Co-Principal Investigator. 1993-1995, $250,000.

Application of PCR Technologies for virus detection in groundwater. American Water Works Research Foundation. Co-Principal Investigator. 1993-1997, $400,000.

Soil treatability pilot studies to design and model a soil aquifer treatment system. American Water Works Research Foundation. Co-investigator. 1994-1995, $224,000.

Field Tracer Experiments at Oak Ridge National Laboratory. University of Tennessee. Co-investigator. 1994. $8,000.

Evaluation of a potable POU. Sweetwater Inc., Principal Investigator, 1994, $19,000.

Risk Assessment of a Distillation Water Treatment System, In-sink-erator, 1994, $57,000.

Microsporidium Reduction Testing. Amway Corp., Principal Investigator, 1995, $13,000.

Efficacy of chlorine bleach disinfection on surfaces against *Giardia*, Principal Investigator, Clorox Corp., 1995, $13,250.

Rapid PCR based monitoring of infectious enteroviruses in drinking water. Co-investigator, Amer. Water Works Research Foundation, 1995-1997, $191,896.

Comparison of POU devices for microbial removal, Principal Investigator, Sweetwater Inc., 1995, $53,000.

Evaluation of point-of-use water treatment devices for outdoor use, L.L. Bean, Inc. Principal Investigator, 1995, $5,000. Evaluation of tablet formulations for water disinfection, Principal Investigator, Sweetwater, Inc., 1995, $10,000.

Optimal Secondary Wastewater Reuse with Minimal Environmental Risks. United States-Israel Binational Agricultural Research Development Fund, Co-Principal Investigator, 1996-1999, $339,000.

Enter Pathogen Reduction by Artificial Wetlands. Wyoming Water Resources Research Center, Principal Investigator, 1996-1998, $126,350.

Quantitative microbial risk assessment of foods. Co-investigator. $23, 206. International Life Science Institute, 1997-1998.

Development of low cost indicators of viruses and parasites on foods, Principal Investigator, $18,500, USDA, 1996-1998.

Investigation of Soil Aquifer Treatment, Co-investigator, $400,000, Amer. Water Works Research Foundation, and cities of Phoenix and Tucson, 1997-1998.

Evaluation of the economics and public health benefits from water chlorination for cholera using risk assessment, Co-Principal Investigator, $90,000, Chlorine Chemistry Council, 1996-1997.

Inactivation efficiencies of emerging waterborne pathogens by chemical disinfection process. Co-investigator. Amer. Water Works Res. Foundation, 1998-2000, $250,000.

Investigation of soil aquifer treatment, Co-investigator, EPA, $1,500,000, 1998-2002.

Residential graywater systems, Co-investigator, CASA, $16,413, 1998-2000.

Molecular detection of pathogens in irrigation water and their significance, Principal investigator, USDA, $275,000, 1999-2001.

Impact of wildlife on enteric pathogens in a constructed wetland, Co-investigator, City of Phoenix, 2000-2001, $48,651.

Microbial risk analysis of iceberg lettuce due to manure application. Co-investigator. Arizona Iceberg Lettuce Research Council. $40,978. 2000-2002.

Effect of hetrotrophic plate count bacterial populations in drinking water. Co-investigator. NSF Water Quality Center. $50,000. 2000-2002.

Use of risk modeling to determine the benefit of topical antimicrobial products. Soap and Detergent Association. Co-investigator. $20,000. 2000-2002.

Virus transport through soil. U.S. Dept. of Interior. Co-investigator. $12,000. 2000-2002.

Measurement of Hormonal Activity and Volume Contribution of Treated Wastewater in Water from Wells along the Santa Cruz. U.S. Dept. of Interior. Co-investigator. $12,700. 2001-2002.

Microbial risk analysis of water in the production of produce in Arizona. Co-PI. USDA. $525,000. 2000-2003.

Assessment of bacterial contamination of oysters. Co-PI. USDA. $1,200,000. 2001-2004.

Role of irrigation methods on microbial food safety. Co-PI. FDA. $525,000. 2001-2004.

Giardia/Cryptosporidium transport and fate during subsurface infiltration: integrated laboratory and field study. Co-PI. EPA. $519,725. 2001-2004.

Bioaerosol generation from biosolids. Co-PI. NSF Water Quality Center. $60,000. 2001-2004.

Occurrence of emerging pathogens in the waters of Arizona. P.I. NSF Water Quality Center and State of Arizona. $200,000. 2003-2005

Survival of the SARS virus in water and wastewater. P.I. NSF Water Quality Center. $10,000. 2003-2004.

Development of an infectivity assay for norovirus in cells. Co-PI. American Water Works Research Foundation. $400,000. 2004-2007.

Occurrence of viruses on fomites in work environments. PI. Clorox Company. $52,000. 2004-2005.

Microbial quality in individual and small water systems in Arizona. PI. NSF Center for Water Quality and the State of Arizona. $200,000. 2004-2006.

Development of a Ct for chlorine for enteroviruses. PI. U.S. Environmental Protection Agency. $20,000. 2005.

Adenovirus and norovirus occurrence in sewage discharges. PI. Geosyntec. $38,000. 2005.

Virus removal from Combined Sewage overflows. PI. CH2M Hill. $20,000. 2005.

Occurrence of viruses on fomites in public facilities. PI. Clorox Company. $35,000. 2005.

Assessment of a thermal point of use device for microbial treatment of water. PI. Johnson Research. $28,000.

Center for Advancing Microbial Risk Assessment . Co-investigator. U. S. Environmental Protection Agency/Department of Homeland Security. $1,100,000. 2005-2010.

Disinfectants in disease reduction in public schools. P.I. Clorox Company. $224,000. 2005-2006.

Occurrence of bacteria in liquid soap. P.I. GOJO Industries. $28,000, 2006.

Control of *Naegleria fowleri* in ground water in Arizona. P.I. NSF Water Quality Center and the State of Arizona. $185,000. 2006-2008.

Development of a universal microbial concentrator. Co-PI. U. S. Environmental Protection Agency. STAR Grant Program. $450,000. 2006-2009.

Survival of prions in biosolids. PI. NSF Water Quality Center. $140,000. 2006-2009.

Development of an ozone/UV light disinfection system. P.I. NSF Water Quality Center/Vortex Technologies. $78,000. 2006-2007.

Development of new disinfectant technologies. P.I. The Clorox Company $84,000. 2006.

Microbiology of home vs. work offices. P.I. The Clorox Company $59,000. 2007-2008.

Evaluating proposed operational practices for control of *Naegleria fowleri* in Arizona's Public Drinking Water systems. Co-investigator. Arizona Water Institute/City of Peoria $58,028. 2008.

A new generation of anti-micriobial materials. Nexra. $20,000. 2007

Microbial contamination of hospital scrubs. P. I. Molnlycke Health Care, Inc. $9,000. 2007.

New Generation of water treatment for the developing world. P. I. Vestgaard. $104,000. 2007.

Assessment of Lumilife Systems. P. I. Lumilife. $8,000, 2007.

Evaluation of a foaming hand product formulation in preventing the transfer of rhinovirus. P. I. Procter and Gamble Company. $31,000. 2007.

Environmental microbial assessment of fomites. P.I. Microban. $14,000. 2007

Assessment of the microbial contamination of vacuum cleaners. Oreck. $14,500. 2007.

INSTRUCTION

FORMAL COURSES TAUGHT AT BAYLOR COLLEGE OF MEDICINE

Environmental Virology (3 units) (1978-1980)

FORMAL COURSES TAUGHT AT THE UNIVERSITY OF ARIZONA

Food Microbiology (3 units) (1981-1990) (100% effort)

Food Microbiology Laboratory (1 unit) (1981-1990) (100% effort)

Food Safety (2 units) (1981-1988) (10-50% effort)

Advanced Food Science (3 units) (1988-1991) (10% effort)

Groundwater Pollution Microbiology (3 units) (1982) (90% effort)

Introduction to Virology (3 units) (1986-1987) (10% effort)

Environmental Microbiology (3 units) (1992-1997) (15-20% effort)

Environmental Microbiology Laboratory (2 units) (1992-) (50% effort)

Environmental Biotechnology (2 units) (1993- 2001) (40% effort)

Pollution Science (3 units) (1994-) (40% effort)

Risk Assessment (3 units) (2005-) (25% effort)

SHORT COURSES TAUGHT AT OTHER UNIVERSITIES
(These courses are usually 1-2 weeks in length)

Virus and Parasite Detection in Reclaimed Water. Mexico City, 1988 (Sponsored by the World Bank and Pan American Health Association)

Methods for the Detection of Viruses in the Environment. Cochabamba, Bolivia, 1989. (Sponsored by the University of San Simon)

Application of Biotechnology to the Detection of Viruses, Parasites, and Bacteria in the Environment. Culiacan, Mexico, 1990. (Sponsored by the University of Sinaloa)

Detection of Parasites, Viruses and Bacteria in Water and Wastewater. Santiago, Chile, 1991. (Sponsored by the University of Chile and the American Society for Microbiology)

Applications of Biotechnology to the Detection of Enteric Microorganisms in the Environment. Panama City, Panama, 1992 (Sponsored by the University of Panama and the United States Agency for International Development)

Advances in the Detection of Enteric Bacteria, Viruses, and Parasites in Water and Wastewater, Maracaibo, Venezuela, Sept., 1992. (Sponsored by the University of Zulia).

Virus and Parasite Detection in water and Wastewater. Buenos Aires, Argentina, July, 1993 (Sponsored by the University of Buenos Aires and the International Life Sciences Institute).

Molecular methods for the Detection of Microorganisms in water, San Paulo, Brazil, August, 1994, University of San Paulo.

Detection of Microorganisms in Water and Food. University of Panama, Panama City, Panama, May, 1997. (Sponsored by the University of Panama)

Microbial Detection in Water and Environmental Microbiology. Univerdidad del Valle, Guatemala City, Guatemala. Feb. 25-28, 2000. (Sponsored by USDA, USAID, Merck, Procter and Gamble, and Universidad del Valle).

Environmental Microbiology. University of Panama. Panama City, Panama. February, 2005. (Sponsored by the American Society for Microbiology Latin American Lectureship Program).

Quantitative Microbial Risk Assessment. University of Sao Paulo, Brazil. June 14-16, 2005.

Transmission of Pathogens through the Environment. November 29-December 1, 2005. University of Sonora. Hermosillo, Mexico.

PREPARATION OF INSTRUCTIONAL MATERIALS

Prepared the first training manual in Spanish on methods for the detection of viruses in water "Manual de Vigilancia de Virus Entericos en el Agua" (R.C. DeLeon, C.P. Gerba and J.B. Rose) under the sponsorship of the World Bank and Pan American Health Association. This has since been used in numerous training courses in South America at various universities.

Preparation of a laboratory manual with I. Pepper entitled "Environmental Microbiology Laboratory". Published in 1995 by Academic Press.

Aided in preparation of manual for training course in "Water Microbiology for the 21st Century" which has been used in training courses at Macquarre University (Sydney, Australia, Sept., 1993), the University of Washington (Seattle, March, 1994) and the University of York (York, England, Sept., 1994).

Designed and prepared wall posters for laboratory training in Environmental Microbiology "Procedure for the Concentration and Detection of Enteric Viruses in Water", "Detection of Enteroviruses by the Polymerase Chain Reaction", and "Procedure for the Concentration and Detection of *Giardia* and *Cryptosporidium* Oocysts".

Preparation of textbook with I. Pepper, and M. Brusseau, entitled "Pollution Science". Published in 1996 by Academic Press. Preparation of textbook Environmental Microbiology with R. Mier and I.L. Pepper, published 2000 by Academic Press.

INSTRUCTIONAL VIDEOS

Prepared instructional video "Environmental Microbiology Laboratory" for training in methods for the detection of enteric viruses and parasites in water. 1991.

Participated in preparation of instructional video "Cleaning Products....In Our Homes, In Our Environment" under sponsorship of The Soap and Detergent Association and the University of Ohio. 1992.

Participated in preparation of instructional video "The World's Largest Landfill: A multidisciplinary Investigation". Sponsored by Proctor and Gamble and the Council for Solid Waste Solutions.

OTHER

NFS (Micr) 470, Food Microbiology selected by Arizona Ambassadors, a student volunteer organization that assists the Office of Admissions to provide prospective students a positive teaching experience. 1989.

Participated in College of Agriculture "Horizons Unlimited" Program 1989-1995. A one-week course to provide high school students with an introduction to college level instruction.

Participated 1989-present in the Undergraduate Biology Research Program, The University of Arizona. This program is designed to provide undergraduates with an interest in research to work in the laboratories of faculty.

LIST OF THESES AND DISSERTATIONS DIRECTED

1. LaBelle, Raymond. Ph.D., 1979. The role of sediment in the ecology of enteric viruses in the marine environment. Systems Analyst. Honeywell Corp., Houston, TX

2. Smith, Eric. Ph.D., 1980. Development of a method for detection of rotavirus in water. Professor of Microbiology, University of Texas Medical School at Galveston, TX.

3. Hurst, Christian. Ph.D., 1980. Viral detection and persistence during the land treatment of sludge and wastewater. Environmental Virologist, Risk Reduction Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH. Retired.

4. Zerda, Katherin S. Ph.D., 1982. Adsorption of viruses to charge-modified silica. University of Housston, Houston, TX.

5. Hurst, Pei-Fung Liew. Ph.D., 1982. Development and evaluation of an enzyme-linked immuno-sorbent assay for the detection of viruses from wastewater. Senior Group Leader, Dames and Moore, Environmental Consulting Engineers. Cincinnati, OH.

6. Soria, Gary A. Toranzos. M.S., 1983. Development of a microporous filter method for concentration of rotavirus from tap water. Professor, Dept. of Biology, University of Puerto Rico, Rio Piadras, PR.

7. Bassous, Marlene. M.S., 1983. Use of dyes and proteins as indicators of virus adsorption to soils. Clinical Laboratory Supervisor, VA.

8. Yates, Marylynn V. Ph.D., 1984. Virus persistence in groundwater. Professor and Chairperson, Department of Environmental Science, Univ. of Calif., Riverside, CA.

9. Toranzos, Gary A. Ph.D., 1985. Occurrence of enteric viruses in drinking water in South America. Professor, Dept. of Biology, University of Puerto Rico, Rio Piadras, PR.

10. Musial, Coral A. Ph.D., 1985. Development of a method for the detection of *Cryptosporidium* in water and selected studies on hepatitis A virus. Physician, Dept. of Infectious Disease, George Washington University, St. Louis.

11. Mullinax, Rebecca L. M.S., 1985. Isolation of enteric viruses from the recreational waters of Oak Creek. Research Associate, University of Calif. at Davis, CA.

12. Rose, Joan B. Ph.D., 1985. Virus removal during conventional drinking water treatment. Professor, Dept. Fisheries and Wildlife, Michigan State University, East Lansing

13. Payne, Holly. M.S., 1985. Development of methods for enteric virus detection in freshwater clams. Quality Control Supervisor, Soufer Foods, NC.

14. Margolin, Aaron B. Ph.D., 1986. Use of cDNA-blot hybridization techniques for detection of enteric viruses in water. Professor and Head, Dept. of Microbiology, University of New Hampshire, Durham, NH.

15. Badaway, Amin S. Ph.D. Survival and detection of enteric viruses on vegetables. Professor, Mosul, Iraq.

16. Thurman, Robert. Ph.D., 1987. Mechanisms of virus inactivation on modified soil surfaces. Associate Professor, Australian Catholic University Ballorat, Victoria.

17. Sun, Gwoshing. M.S., 1986. Gray water reuse for irrigation.

18. Madore, Mary. M.S., 1986. Resistance to *Cryptosporidium* to chlorine disinfection.

19. Grondin, Gerry. M.S., 1987. (Co-advisor). Modeling virus transport in ground water. National Groundwater Modeling Center, OH.

20. Kayed, Dima. M.S., 1986. Methods for the isolation of oocysts of *Cryptosporidium* from sludge and *Giardia* cysts from stool. Ph.D. Research Microbiologist. Phoenix, AZ.

21. Bradford, Alan. M.S., 1987. (Co-advisor) Transport of MS-2 virus through saturated soil columns. Working for a bioremediation company in Irvine, CA.

22. Messina, Maria Cipolla. M.S., 1989. The effect of liquid smoke on *Listeria Monocytogenes*. Working for a biotechnology company in New Jersey.

23. Landeen, Lee Kevin. M.S., 1989. Inactivation of *Legionella pneumophila* by copper, silver ions and free chlorine. Working for a biotechnology company in San Diego, CA.

24. Manthriratna, Gothami Anoma. M.S., 1989. Efficacy of handwashing as an aid in the control of rotavirus and *Giardia* transmission.

25. Kroeger, Thomas William. M.S., 1989. (Co-advisor) Hydrophobic partitioning of the bacteriophage MS-2. Dept. of Water Resources, State of Wisconsin.

26. Stocking, Kristin. M.S., 1989. (Co-advisor) Adsorption of MS-2 bacteriophage to silica. Hydrologist, City of Tucson, AZ.

27. Richardson, Kenneth James. Ph.D., 1989. Use of nucleic acid probes on a nonradioactive labeling system for the detection of enteroviruses in water. Lawyer.

28. DeLeon, Ricardo. Ph.D., 1989. Use of gene probes and an application method for the detection of rotaviruses in water. Head, Microbiology, Metropolitan Water District, LaVerne, CA.

29. Hinkle, Stephen. M.S., 1990. (Co-Advisor) Modeling colloid transport in saturated porous media: an assessment of the importance of pH and kinetics in virus transport.

30. Cassels, Jenna Marie. M.S., 1990. Inactivation of *Naegleria fowleri* amoebas by copper, silver and chlorine. Research Microbiologist, MBX Corporation, Tucson, AZ

31. Luedeman, Rene Annette. M.S., 1990. (Co-advisor) Development of *in vitro* primary cell cultures from the penaeid shrimp, *penaeus stylirostris* and *penaeus vannamei* and evaluation of a potential application. Research Associate, University. of Arizona, Tucson, AZ.

32. Soares, Ana Cristina Fermino. M.S., 1990. Occurrence of enteroviruses and *Giardia* cysts in sewage sludge before and after anaerobic digestion.

33. Zhou, Xia. M.S., 1991. Inactivation of *Escherichia coli* and coliphage MS-2 by chloramine and copper. Working for a biotechnology company in Los Angeles, CA.

34. Kinoshita, Takashi. M.S., 1991. Effects of pH and hydrophobicity on the transport of viruses and bacteria in saturated media. Consulting Engineering Firm in Japan.

35. Chiou, Ipeng. M.S., 1991. (Co-advisor) Inactivation of *Listeria monocytogenes* by copper, silver ions and free chlorine. Microbiologist, Hunts Foods, Los Angeles, CA.

36. Straub, Timothy Mark. M.S., 1991. Inactivation of bacteriophages MS-2 and PRD1 and poliovirus type 1 in Pima clay loam and Brazito sandy loam soils amended with anaerobically digested sewage sludge. Research Scientist, Battelle Labs Northwest. Richmond, WA.

37. Abbaszadegan, Morteza. Ph.D., 1991. Detection of *Giardia* cysts by cDNA probe and application to water samples. Professor, Dept. of Environmental Engineering, Arizona State University, Tempe, AZ.

38. Huber, Mary Susan. M.S., 1992. Occurrence of enteric viruses in disposable diapers from three landfills.

39. Lenczewski, Melisa E. M.S., 1993. Comparative transport of bacteriophage and microspheres in an aquifer under forced-gradient conditions. Associate Professor, Dept. of Geology, University of Northern Illinois.

40. Straub, Timothy Mark. Ph.D., 1993. (Co-advisor). Detection of enteroviruses and hepatitis A virus in sludge and sludge amended soil using the polymerase chain reaction. Research Scientist, Battelle Labs Northwest. Richmond, WA.

41. Hasan, M.N., M.S. 1994. Evaluation of a microbial water purifier for inactivation/removal of viruses, *Giardia lambla* cysts, and *Cryptosporidium* oocysts, Project Officer, American Water Works Research Foundation, Denver, CO.

42. Enriquez, Carlos. Ph.D., 1994. Detection and Survival of Selected Viruses in Water. Research Scientist, Clorox Company, Pleasanton, CA.

43. Ma, Ju-Fang. Ph.D., 1995. Development of methods for concentration and detection of enteroviruses in water. Physician, NY.

44. Reynolds, Kelly A. Ph.D., 1995. Detection of enteroviruses in marine waters using RT-PCR. Research Assistant Scientist, The University of Arizona, Dept. of Soil, Water and Environmental Science.

45. Meng. Q.S. 1995. Comparative inactivation of enteric adenovirus, poliovirus, and coliphages by ultraviolet irradiation.

46. Johnson, Dana C. Ph.D., 1996. The fate of *Giardia* and *Cryptosporidium* in marine water. Microbiologist. Austin, TX

47. Crabtree, Kristina D. Ph.D., 1996. Risk assessment of virus in water. Assistant Professor. College of Public Health, University of Texas, El Paso, TX.

48. Asthana, Seema, M.S. 1996. Influence of hydrocarbons on the virulence factors associated with *Pudomonas aeruginosa*, Senior Research Assistant, Gen-Probe, San Francisco, CA.

49. Carroll, Sean M. M.S., 1996. Evaluations of virus removal by sandy soils during soil-aquifer treatment using indigenous bacteriophage as indicator organisms. Consulting Engineering Firm, Boulder, CO.

50. Falabi, Jeanne A. M.S., 1996. Pathogen removal by duckweed (*Lemna gibba* L.) - covered pond.

51. Vinlvan, Edlin Artruz. M.S., 1996. Survival of microbial indicators in a constructed wetland.

52. Panelli, Manuela. M.S., 1996. Concentration and detection of *Septata intestinalis* in water. Instructor at a junior college, Dallas, TX.

53. Thurston, Jeanette Ann. M.S., 1997. Fate of pathogenic and indicator microorganisms in two subsurface multispecies constructed wetlands. Research Scientist, Agricultural Research Service, USDA, Lincoln, NE.

54. Kamper, Matthew Frederic. M.S., 1997. The occurrence of microsporidia in environmental waters.

55. Nokes, Rita Lynn. M.S., 1998. Reduction of enteric viruses in small scale, subsurface flow constructed wetlands. Graduate Student, Northwestern University, Chicago, IL.

56. Papp, Julie Dawn. M.S., 1998. The concentration of animal waste to the microbial load of municipal solid waste. Reserved Technician, City of Hope, Los Angeles, CA.

57. Sabalos, Constantine Marc. M.S., 1998. Detection of enteric viruses in treated wastewater sludge using cell culture and molecular methods. Microbiologist. Biotechnology Company, Tucson, AZ.

58. Manshadi, Faezeh Dehghan. M.S., 1998. Occurrence of indicator and pathogenic enteric microorganisms in natural wetlands. Research Associate, Arizona State University.

59. Watson, Suzanne Michelle. M.S., 1999. Bacterial Survival during laundering with and without disinfectants. Microbiologist East Bay Municipal District, Oakland, CA.

60. Mahalahabis, Madhumita. M.S., 1999. Detection of infectious poliovirus by multiple passage ICC-PCR and cell culture. Graduate student, University of Washington, Seattle, WA.

61. Chaidez, Quiroz Cristobal. Ph.D., 1999. Risk assessment of selected opportunistic pathogens in drinking water: Microbiologist, Centro de Investigacion en Alimentacion y Desarrollo, Culican, Mexico.

62. Quinonez-Diaz, Maria de J. Ph.D., 1999. Removal of pathogens and indicator organisms by natural wetlands. Microbiologist. Centro de Investigacion en Alimentacion y Desarrollo, Culiacan, Mexico.

63. Karim, Mohammad R. Ph.D., 1999. Survival of indicator bacteria and enteric pathogens in wetlands. Research Microbiologist, U. S. Environmental Protection Agency. Cincinnati, OH.

64. Watt, Pamela. M.S., 1999. Research Specialist, Agricultural Research Service, USDA, Salinity Labs, Riverside, CA.

65. Sanchez, Luis R., Ph.D. 1999. Pathogen removal in diary wastewater using a wastewater constructed treatment system with wetland cells. Quality Control Supervisor, produce company, Los Angeles, CA.

66. Gramos, Dawn M. M.S. 2000. Inactivation of selected enteric viruses using ultraviolet light. Hazardous Waste Risk Manager. U.S. Dept. of Defense. Kwajalein Atoll, Marshall Islands.

67. John, David. M.S. 2000. Inactivation of *Encephalitozoon intestinalis* by chlorine and ultraviolet light. Research Associate, University of South Florida.

68. Vladich, Frank, M.S. 2000. Development of a method for concentration of microsporidia from water. Research Specialist, University of Arizona, Tucson, AZ.

69. Vidales, Juan A. Ph.D. 2001. Removal of viruses and pollution indicators in constructed wetlands. Professor, Univ. of Monterrey, Mexico.

70. Alum, Absar, Ph.D. 2001. Control of viral contamination of reclaimed irrigated vegetables by drip irrigation. Research Associate, Arizona State University, Tempe, AZ.

71. Thurston, Jeanette A. Ph.D., 2001. Occurrence of human pathogens microsporidia in irrigation water and ultraviolet light and chlorine inactivation of enteric adenovirus type 40 and feline calicivirus. Research Scientist, Agricultural Research Service, Lincoln, NE.

72. Orosz-Coghlan, Patricia. A., M.S. 2001. Impact of wildlife on *Escherichia coli* in a constructed wetland. Senior Research Specialist, University of Arizona, Tucson, AZ.

73. Crenshaw, Tristen. N. M.S., 2002. Survival of enteric bacteria in chicken manure utilized as vegetable crop fertilizer. Research Specialist, University of Arizona.

74. Carreon, Joesph. D. M.S., 2003 Risk of infection by Campylobacter, Salmonella, and Norwalk virus in commercially grown oysters: a novel application of quantitative microbial risk assessment. Research Assistant, George Washington University, Washington.

75. Seidel, Georgetta Ph.D., 2003 Detection on non-CPE producing enteroviruses via ICC-PCR at wastetewater land application sites in Arizona and California; Endocrine disruption activity after wetland, pond, and soil aquifer treatment of wastewater. Senior Epidemiologist, Tucson Medical Center, Tucson, AZ.

76. Manshadi, Fashi. D. Ph.D., 2003. Occurrence of pathogenic and indicator microorganisms on produce irrigated with dairy wastewater. Research Assistant. Arizona State University. Phoenix, AZ.

77. Kayed, D. Ph.D. 2003. Microbial quality of irrigation water used in the production of fresh produce in Arizona. Microbiologist. Phoenix, AZ.

78. Bright, K. Ph.D. 2003. Reduction of pathogenic bacterial populations on stainless steel by silver and zinc ions: potential use in preventing cross contamination of environmental surfaces. Assistant Research Scientist, University of Arizona. Tucson, AZ.

79. Woo, H. M.S. 2004. Bacteriophage surrogate for human enteric viruses in the testing of point-of-use (POU) devices. Microbiologist. Analytical Associates, VT.

80. Balkhyour, M. Ph.D. 2004. Factors that affect respirator fit- testing programs. Professor, Saudi Arabia.

81. Tanner, B. D. Ph.D. 2004. Aerosolization of microorganisms and risk of infection from reuse of wastewater residuals. Research Scientist, Clorox Company. Pleasanton, CA.

82. Stine, S. W. Ph.D. 2004. Survival of enteric pathogens on the surface of fresh produce and intake of heterotrophic bacteria in the United States. U. S. Environmental Protection Agency. Dallas, TX.

83. Sutton, S. J. M.S. 2004. Inactivation of *Encephalitozoon intestinals* by chlorine dioxide. Microbiologist. Aerotech Labs,. Phoenix, AZ.

84. Law, B. F. Ph.D. 2005. Assessment of the pathogenicity of *Campylobacter* from broiler chickens. Research Scientist, University of Arizona.

85. Boone, S. 2005. Occurrence and persistence of viruses on fomites. Research Scientist, Agricultural Research Service, United States Department of Agriculture, New Orleans, LA..

86. Jones, E. L. Ph.D. 2006. Norovirus in recreational waters of Arizona. Microbiologist. Clorox Company. Pleasanton, CA.

87. Black, S. M.S. 2006. Determination of Ct values of resistant enterovirus by chlorine inactivation. Research Specialist, University of Arizona.

88. Bronson-Lowe, D. L. Ph.D. 2006. Impact of an environmental hygiene intervention on illness and microbial levels in child care centers. Epidemiologist. State of Arizona Department of Health. Phoenix, AZ.

89. Chattman, M. M.S. 2006. Bacterial contamination of liquid hand soaps. Research Technician. University of Arizona. Tucson, AZ.

90. Blair, Barbara. M.S. 2006. Occurrence of *Naegleria fowleri* in wells in Arizona. Ph.D. student at the University of Arizona.

91. Castro, Nehelia. 2007. Ph.D. Survival of enteric bacteria and viruses in biosolids.

92. Goartares-Moroyoqui, Pablo. 2007. Ph.D. Microbiological water quality in irrigation water, treated wastewater, and untreated wastewater and its impact on vegetables in Sonora, Mexico. Director of Biotechnology Programs, Sonora Institute of Technology. Obregon, Sonora.

93. Ruelas, Enue Erdemely Sicaros. 2007. Ph.D. The development of alternative methods of disinfection. Research scientist. The Clorox Company. Pleasanton, CA.

94. Marrero-Ortiz, Roberto. 2007. Ph.D. Assessment of the microbial and chemical water quality of individual and small water groundwater supplies in Arizona. Research Associate. WI.

95. Rodriguez, R. A. 2007. Ph.D.Occurrence of enteric viruses on combined sewer overflows. Postdoctoral Fellow. University of North Carolina. Chapel Hill, NC.

96. Oswald, A. 2007. Ph.D. Removal of indictors and pathogens by an artificial wetland used to treat secondary treated wastewater. Director of Research. Orange County Water District. Orlando, FL.

97. Carpenter, K. 2007. M. S. The ecology of bacterial pathogens and *Salmonella* in Irrigation canals. Laboratory Manager. The Clorox Company. Oakland, CA

98. Moghe, A. 2007. M.S. Persistence of bacteria on fomites.

POSTDOCTORAL FELLOWS

1. Dr. Samuel Farrah, Professor of Microbiology, Department of Microbiology and Cell Science, University of Florida, Gainesville, FL.

2. Dr. Pierre Payment, Professor of Microbiology, Institute Armand-Frappier, University of Quebec, 531, Blv. Des Prairies, Laval-des-Rapides, Quebec, Canada.

3. Dr. Sagar Goyal, Professor of Microbiology, Veterinary Diagnostic Laboratories, College of Veterinary Medicine, University of Minnesota, St. Paul, MN.

4. Dr. Bruce Keswick, Director, Global Microbiology, Proctor and Gamble Co., Cincinnati, OH.

5. Dr. Thomas Hejkal, Physician, Lincoln, NE.

6. Dr. De-Shin Wang, Compac Computers, Houston, TX.

7. Dr. Marylynn Yates, Professor and Chairperson, Department of Soil and Environmental Science, University of California, Irvine, CA.

8. Dr. Shri N. Singh, Professor, Director of Diagnostic Microbiology, University of Kentucky, Hopkinsville, KY.

9. Dr. Susan Stramer, Research Scientist, Centers Disease Control, Atlanta, GA.

10. Dr. Susan M. Bradford (Kutz), Private consultant to the water industry, Fountain Valley, CA.

11. Dr. Joan Rose, Professor, Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI

12. Dr. Aarron Margolin, Professor, Department of Microbiology, University of New Hampshire, Durham, NH.

13. Dr. Morteza Abbaszadegan, Professor, Department of Environmental Engineering, Arizona State University, Tempe, AZ.

14. Dr. David K. Powelson, Assistant Research Scientist, University of Florida, Gainesville.

15. Dr. Timothy Straub, Research Scientist, Battelle Labs Northwest. Richmond, WA.

16. Dr. Patricia Rusin, Associate Research Scientist, Dept. of Soil, Water and Environmental Science, The University of Arizona, Tucson, AZ.

17. Dr. Carlos Enriquez, Xavier College, CA

18. Dr. Dana Johnson, Microbiologist, San Jose, TX

19. Dr. Kelly Bright, Assistant Research Scientist, University of Arizona, Tucson, AZ.

20. Dr. Stephanie Boone, Research Scientist, Agricultural Research Service, New Orleans, LA.

21. Ryan Sinclair, current

Attachment 2

Prepared for

Metropolitan Water Reclamation District of Greater Chicago

DRY AND WET WEATHER RISK ASSESSMENT OF HUMAN HEALTH IMPACTS OF DISINFECTION VS. NO DISINFECTION OF THE CHICAGO AREA WATERWAYS SYSTEM (CWS)

Prepared by

Geosyntec consultants

engineers | scientists | innovators

55 West Wacker Drive, Suite 1100
Chicago, Illinois 60601

Project Number CHE8188

April 2008

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LIST OF ACRONYMS

ANOVA	Analysis of Variance
AWQM	Ambient Water Quality Monitoring
BEACH	Beaches Environmental Assessment and Coastal Health
BGMK	Blue Green Monkey Kidney
BR	Backflow Regulator
CCL	Contaminant Candidate List
CDC	Center for Disease Control
CEC	Clancy Environmental Consultants, Inc.
COD	Chemical Oxygen Demand
CLHA	Cecil Lue-Hing and Associates
CPE	Cytopathic Effects
CSC	Calumet-Sag Channel
CSO	Combined Sewer Overflow
CSSC	Chicago Sanitary and Ship Canal
CT	Contact Time
CDF	Cumulative Distribution Function
CWA	Clean Water Act
CWS	Chicago Area Waterway System
DAPI	4',6-diamidino-2-phenylindole
DBPs	Disinfection Byproducts
DIC	Differential Interference Contrast
DPR	Des Plaines River
DNS	Downstream
DQO	Data Quality Objective
E. coli	*Escherichia coli*
EPA	US Environmental Protection Agency
FA	Fluorescence Assay
FITC	Fading/Diffusion Of Fluorescent Isothiocyanate
FS	Flowing Stream
Geosyntec	Geosyntec Consultants
GPS	Global Positioning System
HAV	Hepatitis A Virus
HEV	Hepatitis E Virus
HML	Hoosier Microbiological Laboratory, Inc.
IDPH	Illinois Department of Public Health
IEPA	Illinois Environmental Protection Agency
IPCB	Illinois Pollution Control Board
LCR	Little Calumet River
LP&L	Lockport Powerhouse and Lock
MG	Million Gallons
MGD	Million Gallons per Day
MF	Membrane Filtration
MLE	Maximum Likelihood Estimation

LIST OF ACRONYMS (Continued)

MPN	Most Probable Number
MS	Matrix Spike
MWRDGC	Metropolitan Water Reclamation District of Greater Chicago
NAC	Negative Assay Control
NOM	Natural Organic Matter
NPDES	National Pollutant Discharge Elimination System
NSC	North Shore Channel
NTU	Nephelometric Turbidity Units
OPR	Ongoing Precision And Recovery
PAC	Positive Assay Control
PCR	Polymerase Chain Reaction
PDF	Probability Density Function
PMF	Probability Mass Function
PEC	Patterson Environmental Consultants
PFU	Plaque Forming Units
PR	Regulator Module
QA	Quality Assurance
QC	Quality Control
QAPP	Quality Assurance Project Plan
QMRA	Quantitative Microbial Risk Assessment
RFP	Request for Proposal
RT	Reverse Transcriptase
SAC	Senior Advisory Committee
SAP	Sampling and Analysis Plan
SC	Specific Conductance
SF	Swivel Female Insert
SOP	Standard Operating Procedure
SWW	Significant Wet Weather
UAA	Use Attainability Analysis
UV	Ultraviolet
UPS	Upstream
VIRADEL	Virus Adsorption-Elution
WCC	Waterway Control Center
WHO	World Health Organization
WRP	Water Reclamation Plant

EXECUTIVE SUMMARY

The Metropolitan Water Reclamation District of Greater Chicago (MWRDGC or District) has retained The Geosyntec Team, which includes Geosyntec Consultants (Geosyntec) and its subcontractors, Patterson Environmental Consultants (PEC); Cecil Lue-Hing & Associates (CLHA); Dr. Charles Gerba of the University of Arizona (UA); Hoosier Microbiological Laboratory, Inc. (HML); and Clancy Environmental Consultants, Inc. (CEC) to perform a Risk Assessment of Human Health Impacts of Disinfection Vs. No Disinfection of the Chicago Area Waterways System (CWS).

The CWS consists of 78 miles of canals, which serve the Chicago area for two principal purposes: (1) the drainage of urban storm water runoff and treated municipal wastewater effluents from the District's three major water reclamation plants (WRP) (North Side, Stickney and Calumet), and (2) the support of commercial navigation (See Figure ES-1). Approximately 75 percent of the length of the CWS includes manmade canals where no waterway existed previously, and the remainder includes natural streams that have been deepened, straightened and/or widened to such an extent that reversion to the natural state is not possible. About 70 percent of the annual flows in the CWS are from the discharge of treated municipal wastewater effluent from the District's WRPs (MWRDGC, 2004).

Over time, there have been major improvements in water quality, altered land use and additional public access along the CWS. Such improvements and conditions have produced both greater opportunity and heightened public interest in environmental and recreational uses within and along the waterways. Currently, the waterways are used for recreational boating, canoeing, fishing and other streamside recreational activities. These waterways also provide aquatic habitat for wildlife.

The Illinois Environmental Protection Agency (IEPA) has conducted a Use Attainability Analysis (UAA) of the CWS in accordance with 40 CFR 131.10(d). The IEPA and UAA stakeholders have agreed that swimming and other primary contact recreation should not be considered as a viable designated use of the CWS. The IEPA initially attempted to develop water quality standards for the CWS based on the *Ambient Water Quality Criteria for Bacteria*-1986 (EPA, 1986) and EPA guidance (EPA, 2003). In order to

assist IEPA in evaluating the proposed bacterial water quality standards, the District commissioned qualified consultants (research scientists and water quality experts) to conduct a peer review of the EPA's Water Quality Criteria for Bacteria – 1986, and the November 2003 draft implementation guidance document (EPA, 1986 and 2003). The findings of the expert review panel indicated that these EPA documents provide no scientific basis for developing protective bacteria standards for the designated CWS recreational uses. One of the recommendations from the expert review panel report was that more science is needed before bacteria criteria can be established for effluent dominated urban waterways. To address this recommendation, the District has conducted a microbial risk assessment study to determine health impacts of recreational use of the CWS.

Microbial Risk Assessment Objectives

The main objective of this risk assessment study was to evaluate the human health impact of continuing the current practice of not disinfecting the effluents from the District's Calumet, North Side, and Stickney WRPs versus initiating disinfection of the effluent at these three WRPs. The study includes dry and wet weather microbial sampling data. The dry weather risk assessment sampling was completed during the 2005 recreational season when the climatic conditions were not suitable for wet weather sampling. The wet weather sampling took place during the 2006 recreational season. Dry and wet weather microbial sampling results of the surface water in the CWS and the WRP effluents formed the basis for the risk assessment. The dry and wet weather microbial results were integrated to enable an evaluation of the potential impacts of disinfection on overall risks associated with the recreational use of the waterway.

This study focused on the detection of microorganisms typically present in the feces of humans and other warm-blooded animals as indicators of fecal pollution. Hence, a group of EPA-approved indicator microorganisms, such as *E. coli*, *enterococci*, and fecal coliform was selected for this study. In addition to the indicator microorganisms, pathogens representative of those present in the wastewater that are also of public health

concern were selected. The rationale for selecting the pathogens for this microbial risk assessment study included the following criteria:

- The pathogens selected are associated with documented outbreaks of disease, including gastrointestinal and respiratory diseases and infections
- There are EPA-approved methods or laboratory standard operating procedures (SOPs) available for the measurement of the selected pathogens.

Based on the rationale and selection criteria outlined above, the objective of the dry and wet weather microbial risk assessment sampling was to determine the concentrations of the following indicators and pathogens:

- Enteric viruses: i) total culturable viruses, (ii) viable adenovirus; and (iii) *Calicivirus*
- Infectious *Cryptosporidium parvum* and viable *Giardia lamblia*
- *Salmonella* spp.
- *Pseudomonas aeruginosa*
- Fecal coliforms
- *E. coli*
- *Enterococci*

Dry Weather Microbial Risk Assessment Objectives

During dry weather, the District's North Side, Stickney and Calumet WRPs contribute the majority of the flow in the CWS. The specific objectives of 2005 dry weather sampling were as follows:

1. Evaluate the impact of the treated effluent from the District's three major WRPs (North Side, Stickney, and Calumet) on the microbial quality of the CWS.

2. Estimate health risks to recreational users of the CWS due to incidental contact pathogen exposure under dry weather conditions.

3. Quantify any reduction of risk that would result from disinfection of WRP effluents during dry weather.

Wet Weather Microbial Risk Assessment Objectives

During wet weather, in addition to the WRP effluents, several sources contribute to the microbial load in the CWS, including: CSOs, discharges from storm drains, overland runoff, land-use activities such as agriculture and construction, erosion, and habitat destruction. The specific objectives of 2006 wet weather sampling were as follows:

1. Evaluate the impact of the WRP wet weather flow on the microbial quality of the WRP outfalls.
2. Evaluate the impact of combined sewer overflows (CSOs) on the microbial quality of the CWS.
3. Estimate health risks to recreational users of the CWS due to incidental contact pathogen exposure under wet weather conditions.
4. Quantify any reduction of risk that would result from disinfecting WRP effluents during wet weather.

Microbial Sampling and Analysis

Sampling and Analysis Plans (SAPs) and Quality Assurance Plans (QAPs) were developed that provided a detailed sampling strategy, including sampling locations, the number of samples and sampling frequency. A subset of the Ambient Water Quality Monitoring (AWQM) sampling stations employed by the MWRDGC along the 78 miles of the CWS, was used for this study. Figures ES-1 and ES-2 show the dry and wet weather sampling locations, respectively.

One of the components of the microbial risk assessment was to conduct water sampling and analysis of the CWS. Dry weather sampling was conducted between July and September 2005. Seventy five (75) dry weather water samples were collected at the North Side, Stickney and Calumet waterways, including upstream, downstream and outfall samples. Wet weather sampling was conducted between June and October 2006. Fifty (50) wet weather samples were collected at the North Side, Stickney and Calumet waterways, including upstream, downstream and outfall samples. The wet weather locations were spaced at significantly larger distances away from the WRPs compared to the dry weather locations to account for the contributions of storm water runoff, CSO outfalls, and pumping stations (see Figures ES-1 and ES-2). At the North Side, wet

weather samples were also collected near the North Branch Pumping Station (NBPS) and at Stickney, wet weather samples were collected near the Racine Avenue Pumping Station (RAPS). Overall, one hundred and twenty five (125) samples were collected and analyzed during the dry and wet weather events.

Sampling and analysis of microbial samples were conducted in accordance with the procedures described at http://epa.gov/microbes and in Standard Methods for the Examination of Water and Wastewater (Standard Methods, 1998). The samples were analyzed for three major groups of indicator and pathogenic microorganisms including bacteria, protozoa, and viruses. The microbial methods of analysis include the following:

- Enteric viruses: i) (total culturable viruses) using the methods described in the ICR Microbial Laboratory Manual, EPA 600/R-95/178 (EPA, 1996); ii) viable adenovirus; and iii) *Calicivirus*. The samples for total culturable viruses were analyzed by HML and the samples for adenovirus and *Calicivirus* were analyzed by the UA Laboratory using the UA SOPs. There are no EPA-approved methods for viable *Calicivirus*. The method used involves a Polymerase Chain Reaction (PCR) method that offers an estimate of the virus concentration, but does not determine or confirm viability. *Calicivirus* is a family of human and animal viruses. For this risk assessment study *Calicivirus* refers to human *Caliciviruses*, specifically the genus norovirus.
- Infectious *Cryptosporidium parvum* and viable *Giardia lamblia* were determined using EPA Method 1623 (EPA, 2001) in conjunction with cell culture infectivity for the *Cryptosporidium* and viability staining (DAPI-PI) for the *Giardia*. The samples for protozoa were analyzed by CEC.
- *Salmonella* spp. using Standard Method 9260D (Standard Methods, 1998)
- *Pseudomonas aeruginosa* using Standard Method 9213E (Standard Methods, 1998)
- Fecal coliforms using Standard Method 9222D (Standard Methods, 1998)
- *E. coli* using EPA Method 1103.1 (EPA, 2002)
- *Enterococci* using EPA Method 1106.2 (EPA, 2001a)

Microbial Results and Conclusions

The microbial analytical results generated during this study were evaluated and interpreted within the framework of dry and wet weather conditions. However, for the microbial risk assessment estimates, the dry and wet weather microbial results were integrated in a comprehensive dataset representative of all weather conditions in the waterway. The following sections discuss the dry and wet weather analytical results of bacteria, protozoa and viruses.

Bacteria Results

Bacteria were the most abundant microbial species detected in the waterway compared to viruses and protozoa during both dry and wet weather events. The results were analyzed and evaluated statistically using the Minitab computing software and the procedures in Helsel and Hirsch (2002) and Helsel (2005). Analysis of Variance (ANOVA) ANOVA tests were performed for the dry and wet weather bacteria results to determine differences of bacteria concentrations by site (i.e., North Side, Stickney, and Calumet), by location (i.e., upstream, downstream, and outfall), and by depth (for dry weather only) (i.e., surface and 1-m depth).

Also, the geometric mean values of the bacteria concentrations were calculated as a measure of the central tendency of the bacteria data sets under both dry and wet weather conditions. In addition, semi-log box plots, indicating the 25^{th}, 50^{th}, and 75^{th} percentile values of the data were created to graphically demonstrate the central tendencies and variability of the various bacteria datasets. For the dry weather results, the spatial (upstream, downstream, outfall) percentile box plots were created. An examination of the spatial variability of the wet weather data did not reveal any discernable trends. Therefore, for the wet weather results, the box plots were used to evaluate any temporal trends that may be attributable to the different weather conditions and the occurrence or non-occurrence of discharges from the pumping stations.

Dry Weather Bacteria Results

For dry weather, ANOVA analysis was only conducted on *E. coli*, fecal coliform, and *Enterococcus* data as these groups had the most statistically significant (by percent detect) datasets. *E. coli,* fecal coliform, and *Enterococcus* were detected at a frequency ranging from 99 to 100%, while *Pseudomonas aeruginosa* was detected in 75% of the samples and *Salmonella* spp. in only 13% of the samples.

The dry weather results are consistent for all bacteria groups in that there is a significant difference between concentrations by site (North Side, Stickney and Calumet), and by location (upstream and downstream). This finding is consistent with a physical understanding of the waterway system, that different sites have varying loading and dilution conditions which results in varying concentrations, and that bacteria concentrations will generally increase downstream of the WRP outfalls compared to the upstream locations. Dry weather downstream concentrations at North Side are generally greater than Stickney, which are greater than Calumet. Also, downstream concentrations are consistently greater than upstream. All bacteria groups in dry weather samples showed no statistically significant difference in concentration by depth.

The dry weather geometric mean results confirm that the dry weather microbial concentrations tend to increase immediately downstream of the WRPs. For dry weather results, the semilog box plots show concentrations increasing downstream, except for *P. aeruginosa* at Stickney and Calumet, and *Enterococcus* at Calumet. *P. aeruginosa* percentile results are highly influenced by non-detect results, therefore downstream increases can not be seen in these box plots. Geometric mean values (generated using the maximum likelihood method) are better indicators of this trend for significantly censored datasets. The fecal coliform dry weather concentrations upstream of the North Side and Stickney WRPs were greater than the IEPA proposed effluent limit of 400 colony forming units (CFU)/100 mL.

For dry weather results, the box plots demonstrate a modest spread of the concentration data around the median (around 1 log between the 1st and 3rd quartiles), as well as the occasionally significant skewedness (in log space) of these results. Moreover, all the box plots consistently show that downstream concentrations exhibit less variability than upstream concentrations.

Wet Weather Bacteria Results

The results of the wet weather data ANOVA analysis indicate that the wet weather *E. coli*, and *Enterococcus* data are significantly different by site (i.e. North Side, Stickney and Calumet waterway) only. Fecal coliform, *P. aeruginosa* and *Salmonella* spp. do not differ by site or any other factor.

The wet weather geometric means at each sampling location (upstream, downstream, outfall) at the North Side and Stickney WRPs indicate that most of the North Side and Stickney geometric mean bacteria concentrations upstream and downstream of the WRPs are higher than the outfall concentrations. Also, the wet weather upstream and downstream geometric mean concentrations at Stickney and North Side are greater than Calumet. Fecal coliform and *E. coli* wet weather concentrations are greater than the other bacteria geometric means at each sampling location at all WRPs. The results also indicate that the wet weather fecal coliform concentrations upstream of the North Side, Stickney and Calumet WRPs were above the IEPA proposed effluent limit of 400 CFU/100 mL.

The outfall samples show lower levels of *Pseudomonas aeruginosa* than the corresponding upstream and downstream wet weather samples. This suggests that the major inputs for *Pseudomonas aeruginosa* in the waterways are sources other than the WRP effluents.

The wet weather results indicate that the occurrence of pumping station discharges resulted in elevated concentrations of bacteria in the Stickney and Calumet waterways, except for *Salmonella* spp. The large variability of the North Side bacteria results is probably masking the effect of the NBPS discharge.

Comparison of Dry and Wet Weather Bacteria Results

The results of the dry and wet weather ANOVA analysis indicate that dry and wet weather combined bacteria data (*E. coli*, *Enterococcus*, *P. aeruginosa*) are significantly different by site (i.e. North Side, Stickney and Calumet waterway) and weather (dry and wet). Fecal coliform differs by weather only (not by site). The *Salmonella* spp. dry weather results had statistically insignificant detections and therefore an ANOVA analysis of both the dry and wet weather results was not performed.

The wet weather bacteria concentrations are significantly greater than the dry weather concentrations at each WRP waterway. The most significant differences are observed at the North Side and Stickney waterways. The geometric mean concentrations of *Salmonella* spp. were low in both dry and wet weather conditions. The *Salmonella* spp. concentrations in the upstream and downstream samples were similar during wet weather conditions at the North Side, Stickney, and Calumet segments of the waterway. The *enterococci* concentrations were lower than *E. coli* and fecal coliform concentrations under wet weather conditions. *Pseudomonas aeruginosa* wet weather concentrations were slightly higher than the dry weather levels. However, the effluent samples show lower levels of *Pseudomonas aeruginosa* than the corresponding upstream and downstream wet weather samples.

Cryptosporidium and *Giardia* Results

The following sections discuss the *Cryptosporidium* and *Giardia* results under dry and wet weather conditions.

Dry Weather *Cryptosporidium* and *Giardia* Results

At North Side, dry weather enumeration results indicate that *Giardia* cysts (cysts) were detected in all outfall samples and in all downstream samples except two (2). Cysts were also detected in four (4) of 10 upstream samples. *Cryptosporidium* oocysts (oocysts) were detected in three (3) of five (5) outfall samples, one (1) of 10 upstream samples and six (6) of 10 downstream samples.

At Stickney, dry weather results show *Giardia* cysts detected in all outfall samples. Cysts were detected in the upstream samples collected during the last four dry weather sampling events. Cysts were not detected in two (2) of 10 downstream samples analyzed. *Cryptosporidium* oocysts were detected in three (3) of five (5) outfall samples analyzed, in one (1) of 10 upstream samples, and in three (3) of 10 downstream samples.

At Calumet, dry weather *Giardia* cysts were detected in four (4) of five (5) outfall and in four (4) of 10 downstream samples. Cysts were not detected in any of the samples upstream of the Calumet WRP. *Cryptosporidium* oocysts were detected in one (1) of five (5) outfall and in four (4) of 10 downstream samples at the Calumet waterway. Only one upstream sample had detectable *Cryptosporidium* oocysts at the Calumet waterway.

For dry weather samples, no infectious *Cryptosporidium* oocysts were detected. Also, for dry weather, most *Giardia* cysts were non-viable. The average dry weather percentage of viable *Giardia* cysts found in each waterway segment, including outfall and in-stream concentrations, are provided below:

- Calumet: *Giardia* viability=10%
- Stickney: *Giardia* viability=21%
- North Side: *Giardia* viability=26%

Outfall samples at the North Side and Stickney WRPs contained higher levels of viable cysts compared to Calumet. The average dry weather percentage of viable *Giardia* cysts found in the outfall only of each WRP is provided below:

- Calumet Outfall: *Giardia* viability=10%
- Stickney Outfall: *Giardia* viability=47%
- North Side Outfall: *Giardia* viability=51%

Wet Weather *Cryptosporidium* and *Giardia* Results

Overall, the concentrations and frequency of detection of *Cryptosporidium* oocysts and *Giardia* cysts were greater during wet weather compared to dry weather sampling. Wet weather enumeration results from samples collected at the North Side designated locations indicate that *Cryptosporidium* oocysts were detected in one of three upstream samples, in 10 of 12 downstream samples, and in the one (1) outfall sample collected. *Giardia* cysts were detected in all samples analyzed at the North Side.

Wet weather enumeration results from samples collected at the Stickney designated locations indicate that four (4) of six (6) upstream samples, four (4) of six (6) downstream samples and two (2) of three (3) RAPS samples had detectable concentrations of *Cryptosporidium* oocysts. All Stickney samples, except one (1) upstream sample, had detectable concentrations of *Giardia* cysts.

Wet weather enumeration results from samples collected at the Calumet designated locations indicate that two (2) of the three (3) outfall samples had detectable concentrations of *Cryptosporidium* oocysts. None of the wet weather samples collected upstream of the Calumet WRP had detectable concentrations of *Cryptosporidium* oocysts and *Giardia* cysts. Two (2) of the three (3) Calumet outfall samples had detectable concentrations of *Cryptosporidium* oocysts. Seven (7) of 12 downstream samples had detectable concentrations of *Cryptosporidium* oocysts. All outfall samples at the Calumet WRP had *Giardia* cysts. However, only four (4) of 12 downstream samples had detectable *Giardia* cysts.

For wet weather samples, no infectious *Cryptosporidium* oocysts were detected with one exception. The average wet weather percentage of viable *Giardia* cysts found in each waterway segment, including outfall and in-stream concentrations, are provided below:

- Calumet: *Giardia* viability=10%
- Stickney: *Giardia* viability=47%
- North Side: *Giardia* viability=49%

The average wet weather percentage of viable *Giardia* cysts found in the outfall only of each WRP is provided below:

- Calumet Outfall: *Giardia* viability=10%
- Stickney Outfall: *Giardia* viability=50%
- North Side Outfall: *Giardia* viability=42%

Comparison of Dry and Wet Weather *Cryptosporidium* and *Giardia* Results

For dry weather samples, no infectious *Cryptosporidium* oocysts were detected. Similarly, for wet weather samples, no infectious *Cryptosporidium* oocysts were detected with one exception. Also, two (2) subsamples of the wet weather matrix spike sample of the North Side waterway had infectious foci. Overall, the combined wet and dry weather percentage of infectious foci is estimated to be approximately 2.4% (3 of 125 samples [75 dry weather and 50 wet weather samples]).

The Calumet waterway under both dry and wet weather contained the smallest percentage (10%) of viable *Giardia* cysts compared to Stickney and North Side. The viability of *Giardia* cysts increased at the Stickney and North Side waterways during wet weather. The WRP outfalls had similar *Giardia* viability under wet and dry weather conditions.

Virus Results

The following sections summarize the analytical results for enteric viruses, adenovirus and *Calicivirus* (norovirus) under dry and wet weather conditions.

Enteric Viruses

Dry Weather Enteric Virus Results

The dry weather results indicate that a relatively small number of samples (17 of 75 samples or 23%) had detectable concentrations of enteric viruses. Eight (8) of 25 dry weather samples (29%) upstream, downstream and at the outfall of the North Side WRP had detectable enteric virus concentrations. Six (6) of 25 dry weather samples (24%)

upstream and downstream of the Stickney WRP had detectable virus concentrations. There were no detectable enteric virus concentrations at the Stickney WRP outfall. Only three (3) of 25 dry weather samples (12%), one at each upstream, downstream and outfall location of the Calumet WRP had detectable concentrations of enteric viruses.

Wet Weather Enteric Virus Results

During the North Side wet weather sampling, 11 of 16 samples (69%) had detectable enteric virus concentrations. Only one (1) wet weather outfall sample was collected at the North Side WRP; that sample had a detectable enteric virus concentration. Due to safety concerns, the discharge of the NBPS was sampled at the nearest downstream location and only one (1) of the three (3) samples collected had detectable virus concentrations.

During the Stickney wet weather sampling, 14 of 16 samples (88%) had detectable enteric virus concentrations. Only one (1) wet weather outfall sample was collected at the Stickney WRP; that sample had a detectable enteric virus concentration. All three (3) RAPS samples had detectable concentrations of total enteric viruses

During the Calumet wet weather sampling, 14 of 18 samples (77%) had detectable enteric virus concentrations. Two (2) of the three (3) wet weather outfall samples collected at the Calumet WRP had detectable enteric virus concentrations.

Comparison of Dry and Wet Weather Enteric Virus Results

The percentage of enteric virus detections during wet weather were greater than the dry weather detections. The percentage of enteric virus detections at the North Side waterway segment increased from 29% during dry weather to 69% during wet weather. The percentage of virus detections at the Stickney waterway segment increased from 24% during dry weather to 88% during wet weather. The percentage of enteric virus detections at the Calumet waterway segment increased from 12% during dry weather to 77% during wet weather. In addition, the concentrations detected during wet weather sampling are generally greater than the dry weather concentrations.

Adenovirus

Dry Weather Adenovirus Results

Of 75 dry weather samples, 42 or 56% demonstrated the presence of detectable virus by assay in the PCL/PRF/5 cell line. Of 42 samples that were cell culture positive, adenoviruses were detected in 31 or about 74% of the samples by PCR. During the North Side dry weather sampling, 12 of 25 samples (48%) had detectable adenovirus virus concentrations. During the Stickney dry weather sampling, 13 of 25 samples (52%) had detectable adenovirus concentrations. During the Calumet dry weather sampling, six (6) of 25 samples (24%) had detectable adenovirus concentrations. There were no detectable concentrations upstream of the Calumet WRP during dry weather sampling.

Wet Weather Adenovirus Results

Of 50 wet weather samples, 42 or 84% demonstrated the presence of infectious virus by assay in the PCL/PRF/5 cell line and had adenoviruses detected by PCR. During the North Side wet weather sampling, 14 of 16 samples (88%) had detectable adenovirus concentrations. Several of the upstream and downstream locations had concentrations greater than the outfall. Due to safety concerns, the discharge of the NBPS was sampled at the nearest downstream location and all three (3) samples collected had detectable adenovirus concentrations.

During the Stickney wet weather sampling, 15 of 16 samples (94%) had detectable adenovirus concentrations. Only one (1) wet weather outfall sample was collected at the Stickney WRP; that sample had a detectable adenovirus concentration. All three (3) RAPS samples had detectable concentrations of adenovirus

During the Calumet wet weather sampling, 13 of 18 samples (72%) had detectable adenovirus concentrations. Only one (1) out of three (3) upstream samples at the Calumet WRP had detectable adenovirus concentrations. Nine (9) of the 12 downstream samples had detectable adenovirus concentrations. All three (3) wet weather outfall samples collected at the Calumet WRP had detectable adenovirus concentrations.

Comparison of Wet and Dry Weather Adenovirus Results

The percentage of adenovirus detections during wet weather were greater than the dry weather detections. The percentage of adenovirus detections at the North Side waterway segment increased from 48% during dry weather to 88% during wet weather. The percentage of adenovirus detections at the Stickney waterway segment increased from 52% during dry weather to 94% during wet weather. The percentage of adenovirus detections at the Calumet waterway segment increased from 24% during dry weather to 72% during wet weather. In addition, the concentrations detected during wet weather sampling are generally greater than the dry weather concentrations.

Calicivirus (Norovirus)

Dry Weather *Calicivirus* (Norovirus) Results

During dry weather, norovirus was only detected in five (5) samples or about 7% of the 75 samples. At North Side, only one (1) outfall sample (one [1] of 25 samples [4%]) had a detectable norovirus concentration. During the Stickney dry weather sampling, three (3) of 25 samples (12%) had detectable norovirus concentrations. During the dry weather sampling the Stickney WRP outfall did not have any detectable norovirus concentrations. During the Calumet wet weather sampling, only one (1) outfall sample (one [1] of 25 samples [4%]) had a detectable norovirus concentration. Norovirus infection is most common in the winter and that may explain the low concentration of norovirus observed in this study (Gerba, 2006).

Wet Weather *Calicivirus* (Norovirus) Results

During wet weather, *Calicivirus* or norovirus were only detected in 20 samples or 40% of the 50 samples. The greatest concentration of norovirus was observed at RAPS, which is located upstream of the Stickney WRP.

During the North Side wet weather sampling, seven (7) of 16 samples (44%) had detectable norovirus concentrations. There were no detectable concentrations of norovirus upstream of the North Side WRP. Only one (1) wet weather outfall sample

was collected at the North Side WRP and it did not have a detectable norovirus concentration. Due to safety concerns, the discharge of the NBPS was sampled at the nearest downstream location. One (1) of three (3) NBPS samples had detectable norovirus concentration.

During the Stickney wet weather sampling, 10 of 16 samples (63%) had detectable norovirus concentrations. Two (2) upstream and five (5) downstream samples had detectable norovirus concentrations. Only one (1) wet weather outfall sample was collected at the Stickney WRP; this sample had a detectable norovirus concentration. Two (2) of the three (3) RAPS samples had detectable concentrations of norovirus

During the Calumet wet weather sampling, three (3) of 18 samples (17%) had detectable norovirus concentrations. There were no detectable norovirus concentrations upstream of the Calumet WRP. There was only one (1) detectable concentration downstream of the Calumet WRP. Two (2) of the three (3) wet weather outfall samples collected at the Calumet WRP had detectable norovirus concentrations.

Comparison of Dry and Wet Weather *Calicivirus* (Norovirus) Results

The results indicate that the percentage of norovirus detections during wet weather were greater than the dry weather detections. The percentage of adenovirus detections at the North Side waterway segment increased from 4% during dry weather to 44% during wet weather. The percentage of adenovirus detections at the Stickney waterway segment increased from 12% during dry weather to 63% during wet weather. The percentage of norovirus detections at the Calumet waterway segment increased from 4% during dry weather to 17% during wet weather. In addition, the concentrations detected during wet weather sampling are generally greater than the dry weather concentrations.

Wastewater Disinfection

According to *WERF* (2005), disinfection is warranted in situations where direct human contact in the immediate vicinity of an outfall is possible or where effluent is discharged to areas involving the production of human food. Disinfection is warranted in situations where its application leads to a reduction in the risk of disease transmission. As illustrated by post-disinfection re-growth of bacteria, relatively poor virucidal performance, and generation of persistent disinfection by products (DBPs), it is not clear that wastewater disinfection always yields improved effluent or receiving water quality (*WERF*, 2005). The effectiveness of the following disinfection technologies were evaluated for the risk assessment study:

- Ultra Violet (UV)
- Ozonation
- Chlorination/Dechlorination

The effectiveness of disinfection is a complex function of several variables including type and dose of disinfectant, type and concentration of microorganisms, contact time, and water quality characteristics. In most cases, pilot-studies and other considerations guide the selection process. If available, published data regarding pathogen inactivation achieved by disinfection are typically used to estimate the concentration of pathogens in disinfected wastewater.

There is great variability in the performance and uncertainty in the efficacy of disinfection (see Table ES-1). There are many unanswered questions with respect to disinfection efficiency data for microbial indicators and pathogens. Therefore, it is uncertain if disinfection designed to remove indicators can be effective in the removal of pathogens and in the reduction of pathogen risks.

In applying any disinfectant, it is important to strike a balance between risks associated with microbial pathogens and those associated with DBPs. DBPs are persistent chemicals, some of which have relevant toxicological characteristics. The inventory of DBPs that have the potential to cause adverse health effects is large and highly variable among publicly owned treatment works (POTW) effluents.

The human health effects associated with chemical contaminants that are influenced or produced as a result of disinfection operations tend to be chronic in nature. Therefore, the development of a risk assessment for exposure to chemical constituents, including DBPs, is far more complex than the microbial risk assessment. Risk assessments of wastewater disinfection should consider microbial and chemical quality. The health effects of disinfectants are generally evaluated by epidemiological studies and/or toxicological studies using laboratory animals (*WERF*, 2005).

Microbial Risk Assessment

Microbial risk assessment techniques were used to quantitatively assess the health risks for the use of recreational waters that receive effluent discharges. The goal of the study was to determine the expected number of illnesses associated with designated usage of the waterways both with and without disinfection of WRP effluent. A probabilistic analysis was employed that used input assumptions drawn from site-specific and scientific literature sources. Risks were estimated for recreational users participating in activities involving different levels of exposure in dry, wet, or a combination of weather events over the course of a recreational year.

Microbial Risk Methodology

Risk assessment inputs were drawn extensively from site-specific data and were developed using state-of-the-science methodology to accurately represent recreational user exposure conditions and risks. Recreational survey studies were used to provide insight on the types and frequency of recreational exposure expected in the waterway. For quantitative risk analysis, the UAA study was used as the primary source for exposure use data for the CWS. As a part of the UAA, the CWS was divided into three major waterway segments each associated with a single WRP. Recreational use was divided into high (canoeing), medium (fishing) and low (pleasure boating) exposure activities. UAA survey data was used to estimate the proportion of recreational users participating in each receptor scenario along each waterway segment.

Exposure parameters were developed as distributional parameters for each receptor scenario as inputs to the exposure model. These parameters include incidental ingestion rates and exposure duration. Selection of input distributions relied on literature derived sources, site-specific use information and professional judgment.

Dose-response parameters define the mathematical relationship between the dose of a pathogenic organism and the probability of infection or illness in exposed persons. Dose-response data are typically derived from either controlled human feeding studies or reconstruction of doses from outbreak incidences. In human feeding trials volunteers are fed pathogens in different doses and the percentage of subjects experiencing the effect (either illness or infection) are calculated. While feeding trials can provide useful dose-response analysis data, studies are usually performed in healthy individuals given high levels of a single strain. Epidemiological outbreak studies provide responses on a larger cross-section of the population but dose reconstruction is often problematic. Dose-response data was developed from regulatory documents, industry white papers and peer reviewed literature.

Concentrations of pathogens in the waterway were selected for each simulation from the entire dataset of dry and wet weather samples collected. The proportion of dry and wet weather samples utilized were weighted to account for the proportion of dry and wet weather days in a typical Chicago recreational season.

Microbial Risk Results

Results of the risk assessment demonstrate that risks to recreational users under various weather and use scenarios is low and within the EPA recommended risk limits for primary contact exposure. The highest rates of illness were associated with recreational use on the Stickney and North Side waterway segments and the lowest illness rate on the Calumet waterway segment. Illness rates were higher under wet weather conditions than under dry weather conditions (see Table ES-2). The results demonstrate that the expected illness rates for receptors were all below the proposed EPA limit of 14 illnesses per 1000 exposure events for freshwater recreational use including immersion/swimming activities.

Risks were also calculated individually for each of the three different classes of recreational use that span the range of exposures reported in the UAA survey in proportion to the frequency of use for each waterway segment. The recreational activity with the highest potential for illness was fishing while that with the lowest potential for illness was pleasure boating. Which recreational activity results in the greatest number of affected users, however, depends on both the proportion of users engaged in that activity and the pathogen load in that waterway segment. For example, in the North Side segment, 33.7% of the gastrointestinal illnesses are predicted to result from canoeing, but canoeing accounts for only 20% of the users of the North Side waterway. In the Stickney and Calumet segments, the predicted illnesses were predominantly from fishing and boating due to the low frequency of canoeists in these waterway segments. To further evaluate the risk stratified by the recreational use activity, risk per 1000 exposure events were computed separately for canoeing, boating, and fishing recreational uses (see Table ES-3). As expected, the highest risks were associated with recreational use by the highest exposure group (i.e. canoeing). However, for each waterway the risks associated with the highest exposure use are below the proposed EPA limit of 14 illnesses per 1000 exposure events for freshwater recreational use including immersion/swimming activities.

For the North Side and Stickney waterway segments, the majority of predicted illnesses were the result of concentrations of viruses, *E. coli* and *Giardia*. For the Calumet waterway the risks are generally lower with multiple organisms contributing to overall risk. Secondary transmission for these pathogens resulted in an approximately two-fold increase in population illness associated with the primary recreational user illnesses. However, secondary transmission rates are higher for the North Side and Stickney waterway segments where the highly communicable norovirus is a dominant pathogen. Secondary transmission considers spread from individuals who may become infected but not ill, a common condition for a number of these pathogens.

Effect of Effluent Disinfection on Pathogen Microbial Risks

The results of this study demonstrate that disinfection of WRP effluents will have a negligible effect on risk for recreational users of the waterway. The effects of various

disinfection techniques on risk reduction were estimated for combined wet and dry weather days. Dry weather sampling data was used to estimate the waterway load that would be affected by disinfection. Wet weather sampling data was assumed to encompass both effluent loading (attenuated by disinfection) and non-point discharges to the waterway (e.g. CSO, pumping stations, stormwater outfalls). Disinfection of the effluent outfall was predicted to result in a decrease in effluent pathogen loads but have a much lower effect on overall pathogen concentrations in the waterway (see Table ES-4). This is because the sampling data shows that a large proportion of the pathogen load results from sources other than the WRP effluent. Disinfection results in effluent pathogen risk decreasing from a low level to essentially zero but has little impact in waterway pathogen concentrations affected by current or past wet weather conditions. These results suggest that disinfection of effluent has little impact on the overall illness rates from recreational use of the CWS.

Non-Gastrointestinal Microbial Risks

Although *Pseudomonas aeruginosa* is not a pathogen that is linked to gastrointestinal illness, this pathogen has been linked to recreational illness outbreaks involving dermal (folliculitis), eye, and ear (otitis externia) infections. For this reason, the levels of *Pseudomonas aeruginosa* were evaluated under the sampling program for this risk assessment. However, quantitative evaluation of the risk for this pathogen is problematic. There are no published dose-response relationships for *Pseudomonas aeruginosa*. Without a clear dose-response relationship there is no way to establish the expected illness level associated with any particular waterway concentration. The dermal pathway for estimating exposure to *Pseudomonas aeruginosa* is also problematic. Ear and eye infections associated with contact by *Pseudomonas aeruginosa* contaminated water are typically associated with full immersion activities. Since these types of activities are not permitted or designated uses of the CAW the incidence of ear and eye exposures are expected to be low and as the result of accidental or intentional misuse of the waterway. *Pseudomonas* related folliculitis commonly requires a break in the skin from a preexisting cut, open sore or scrape as an entry point for infection. Immunocompetent individuals without skin abrasions rarely develop folliculitis by

exposure to intact skin. For these reasons, a quantitative evaluation of risks is not feasible.

A qualitative review of the wet and dry weather data, however, may provide some insight on the relative risk from *Pseudomonas* exposure. Comparison of the waterway level to the outfall levels may also provide an indication on the effectiveness that a disinfection step may have on *Pseudomonas* levels in the waterway. Wet weather levels are higher than dry weather conditions. Perhaps more importantly, the outfall samples show lower levels of *Pseudomonas* than the corresponding wet weather samples. This suggests that the major inputs for *Pseudomonas* in the waterways are sources other than the WRP effluent. Therefore, disinfection of the WRP effluent would have minor effects on the overall loading of *Pseudomonas* in the waterway and risks associated with recreational exposure to this pathogen.

Sensitivity Analysis

A sensitivity analysis was conducted to identify the contribution of each input distribution to the variance of the resulting risk estimates. The actual pathogen dose levels from the combined wet and dry weather assessment were used. Results from the sensitivity analysis indicate that the incidental ingestion rates and weather are the largest contributors to the North Side waterway segment. Recreational user type followed by incidental ingestion rate, exposure duration and weather contributes the most to the variance for the Stickney and Calumet waterway segments.

Conclusions

The results from this study indicate that, despite elevated levels of fecal indicator bacteria, the concentrations of actual pathogenic organisms in the waterway are low. Given the low pathogen levels in the waterway, there is a low probability of developing gastrointestinal illness even in areas of the CWS in close proximity to the District's non-disinfected WRP effluents. For the designated recreational uses evaluated, the risks of developing illness, both with and without disinfection for each waterway segments, are below the EPA guideline of 14 illnesses per 1,000 exposures for fresh water recreation

including immersion and swimming. The pathogen concentrations within the waterway are largely a result of non-WRP derived wet weather inputs. Disinfection of the WRP effluent would have marginal impact on CWS pathogen concentrations. These results confirm that current health risks to CWS recreators are low and disinfection of treated wastewater effluent would have little impact on the overall gastrointestinal illness rates.

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EXECUTIVE SUMMARY

TABLES

Table ES-1. Summary of Pathogen Disinfection Efficiencies

Pathogen	Ozonation	UV Disinfection	Chlorination/Dechlorination
E. coli	4 log (Note 1); 1.3 log-4.5 log (Note 2)	4 log (Note 8)	> 4 log (Note 8)
Pseudomonas aeruginosa	2 log (Note 2)	4 log (Note 8)	> 4 log (Note 8)
Salmonella	4 log (Note 1)	3-4 log (Note 10)	Not Available
Enterococci	Not Available	Not Available	More resistant than *E. coli* (Note 8)
Cryptosporidium	0.57 log-2.67 log (Note 2)	3 log (Note 3)	0.2 log-3 log (Note 1)
Giardia	1.57 log-2.7 log (Note 2)	2 log (Note 10)	0.5 log (Note 1)
Total Enteric Viruses	5 log (Note 2)	0.32 log-3.61 log (Note 8)	5 log (Note 4)
Calicivirus	2 log (Note 5)	4 log (Note 7)	2 log (Note 5)
Adenovirus	4 log (Note 9)	1 log- 4 log (Note 6)	2-4 log (Note 11)

Notes:

(1) EPA (1999)
(2) Paraskeva and Graham (2002)
(3) Clancy (2004)
(4) Nelson et al. (undated)
(5) Health Canada (2004)
(6) Gerba et al. (2002)
(7) Thurston-Enriquez et al. (2003)
(8) *WERF* (2005)
(9) Thurston-Enriquez et al. (2005); results obtained in buffered disinfectant demand free water at 5°C and pH 7. These conditions may not be representative of wastewater.
(10) Chang et al. (1985)
(11) Thurston-Enriquez et al. (2003a)

Table ES-2
Total Expected Primary Illnesses per 1,000 Exposures under Combined Dry and Wet Weather Using Different Effluent Disinfection Techniques

Exposure Input	Waterway		
	North Side	**Stickney**	**Calumet**
Dry Weather	0.36	1.28	0.10
Wet Weather	2.78	2.34	0.36
Combined Weather Samples	1.55	1.77	0.21

Note:
Includes all primary gastrointestinal illnesses from *E. coli*, *Salmonella*, total enteric viruses, adenoviruses, *Giardia*, and *Cryptosporidium* expected from the waterway exposures. Waterway concentration inputs for the simulations were randomly selected (bootstrap sampled) from datasets that include the indicated sample sets.

Table ES-3
Estimated Illness Rates Assuming Single Recreational Use with No Effluent Disinfection

	Illnesses per 1,000 Exposures for Combined Wet and Dry Weather Samples		
Recreational Use	**North Side**	**Stickney**	**Calumet**
Canoeing	2.45	3.19	0.52
Fishing	1.42	1.90	0.31
Pleasure Boating	0.66	1.05	0.14

Note:
Includes all primary gastrointestinal illnesses from *E. coli*, *Salmonella*, total enteric viruses, adenoviruses, *Giardia*, and *Cryptosporidium* expected from the waterway exposures.

Table ES-4
Effect of Disinfection on Expected Recreational Illnesses per 1000 Exposures

	Waterway		
	North Side	**Stickney**	**Calumet**
No Disinfection	1.53	1.74	0.20
UV Irradiation	1.32	1.48	0.17
Ozone	1.45	1.65	0.19
Chlorination	1.43	1.63	0.19

Note:
Estimates based on geometric mean pathogen concentrations and central tendency estimates for exposure assumptions. Waterway pathogen concentrations were developed by the difference in wet and dry disinfected concentrations. Includes all primary gastrointestinal illnesses from *E. coli*, *Salmonella*, total enteric viruses, adenoviruses, *Giardia*, and *Cryptosporidium* expected from the waterway exposures.

EXECUTIVE SUMMARY

FIGURES

FIGURE ES-1

CHICAGO WATERWAY SYSTEM - DRY WEATHER SAMPLING LOCATIONS

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

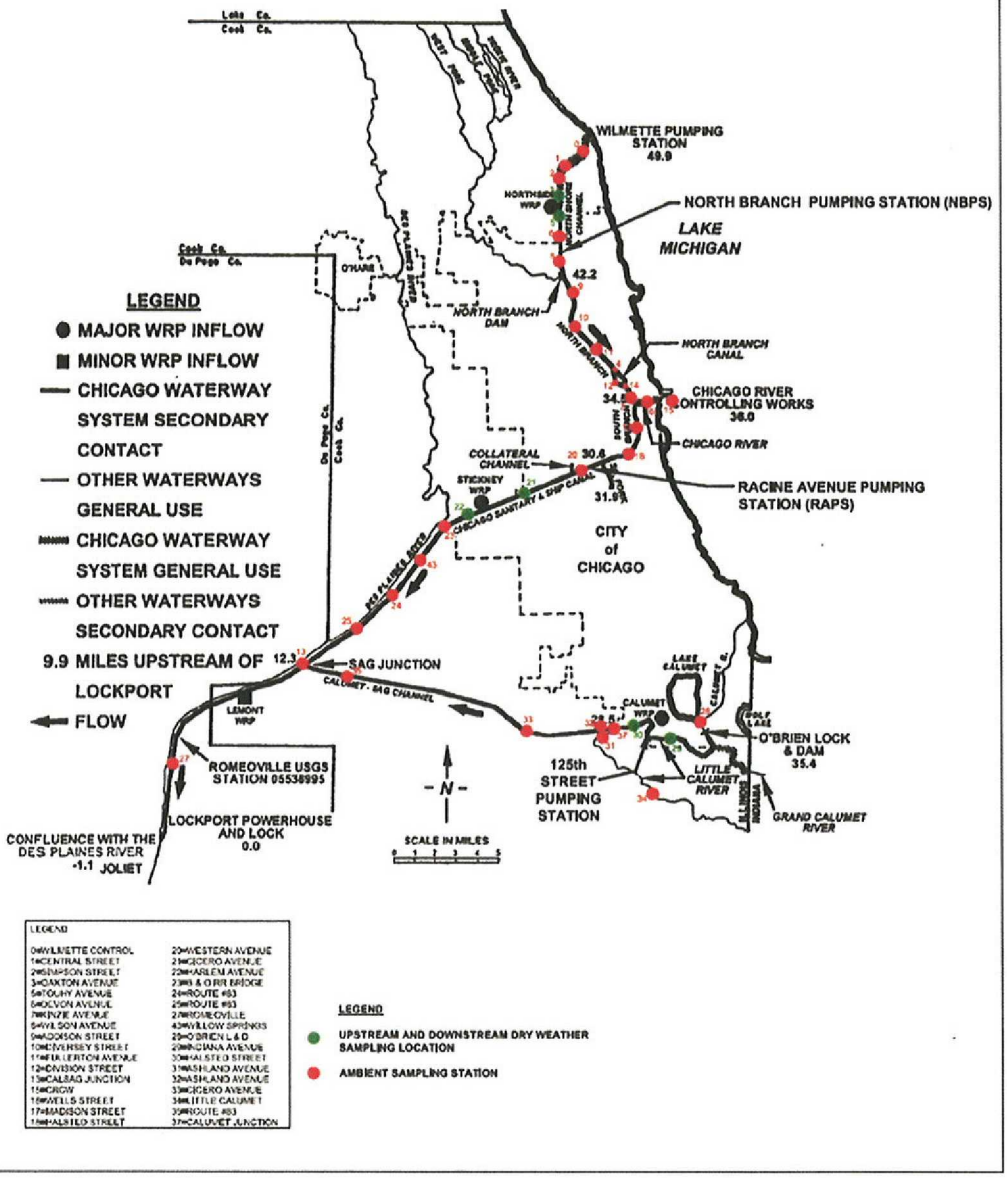
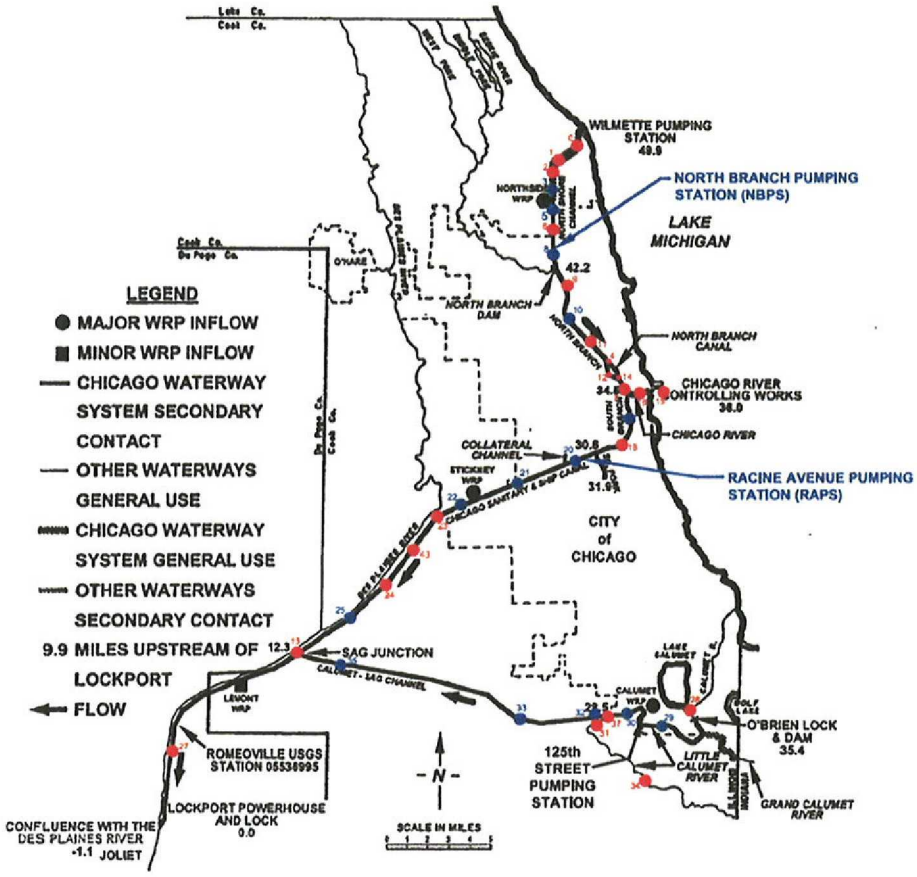


FIGURE ES-2

CHICAGO WATERWAY SYSTEM - WET WEATHER SAMPLING LOCATIONS

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO



LEGEND

- MAJOR WRP INFLOW
- MINOR WRP INFLOW
- CHICAGO WATERWAY SYSTEM SECONDARY CONTACT
- OTHER WATERWAYS GENERAL USE
- CHICAGO WATERWAY SYSTEM GENERAL USE
- OTHER WATERWAYS SECONDARY CONTACT
- 9.9 MILES UPSTREAM OF LOCKPORT
- FLOW

LEGEND

0=WILMETTE CONTROL	20=DAMEN AVENUE
1=CENTRAL STREET	21=CICERO AVENUE
2=SIMPSON STREET	22=HARLEM AVENUE
3=DAMTON AVENUE	23=I & O RR BRIDGE
5=TOUHY AVENUE	24=ROUTE #83
6=DEVON AVENUE	25=ROUTE #83
7=KNZIE AVENUE	27=ROMEOVILLE
8=WILSON AVENUE	43=YELLOW SPRINGS
9=ADDISON STREET	28=O'BRIEN L & D
10=DIVERSEY PARKING	29=INDIANA AVENUE
11=FULLERTON AVENUE	30=HALSTED STREET
12=DIVISION STREET	31=ASHLAND AVENUE
13=CALSAG JUNCTION	32=ASHLAND AVENUE
14=CRCW	33=CICERO AVENUE
15=WELLS STREET	34=LITTLE CALUMET
17=MADISON STREET	35=ROUTE #83
18=HALSTED STREET	37=CALUMET JUNCTION

LEGEND

- AMBIENT SAMPLING STATION
- UPSTREAM AND DOWNSTREAM WET WEATHER SAMPLING LOCATION

1. INTRODUCTION

The Metropolitan Water Reclamation District of Greater Chicago (MWRDGC or District) has retained The Geosyntec Team, which includes Geosyntec Consultants (Geosyntec) and its subcontractors: Patterson Environmental Consultants (PEC); Cecil Lue-Hing & Associates (CLHA); Dr. Charles Gerba of the University of Arizona (UA); Hoosier Microbiological Laboratory, Inc. (HML); and Clancy Environmental Consultants, Inc. (CEC) to perform a Risk Assessment of Human Health Impacts of Disinfection Vs. No Disinfection of the Chicago Area Waterways System (CWS).

The CWS consists of 78 miles of canals, which serve the Chicago area for two principal purposes: the drainage of urban storm water runoff and treated municipal wastewater effluents from the District's three major water reclamation plants (WRP) (North Side, Stickney and Calumet), and the support of commercial navigation (see Figure 1-1). Approximately 75 percent of the length of the CWS includes manmade canals where no waterway existed previously, and the remainder includes natural streams that have been deepened, straightened and/or widened to such an extent that reversion to the natural state is not possible (MWRDGC, 2004).

The CWS has two river systems: the Calumet River System and the Chicago River System. The Calumet River System is 23.1 miles in length and includes the Calumet-Sag Channel (CSC) and the Little Calumet River (LCR). The Chicago River System consists of 55.1 miles of waterways and includes the Chicago River, Chicago Sanitary and Ship Canal (CSSC), North Branch, North Branch Canal (NBC), North Shore Channel (NSC), South Branch and South Fork (MWRDGC, 2004).

By 1972, most states had adopted bacterial water quality standards, and beginning with the early enforcement of the National Pollutant Discharge Elimination System (NPDES) most municipal sewage treatment facilities were required to meet effluent bacterial standards. These effluent bacterial standards were generally met through effluent disinfection by chlorination. In 1972, the Illinois Pollution Control Board (IPCB) adopted year-round effluent and water quality bacterial standards of 400 (effluent) and

200 (water quality) fecal coliform colony forming units (CFU) per 100 mL, respectively (MSDGC, 1984).

In 1973, the U.S. Environmental Protection Agency (EPA) incorporated a 400 CFU per 100 mL fecal coliform secondary effluent standard for all municipal wastewater treatment facilities. The fecal coliform standards in both the effluents and receiving water bodies were clearly intended to prevent or minimize the transmission of pathogens to persons ingesting or coming in contact with waters which receive the treated wastewater (MSDGC, 1984). In 1976, EPA deleted the fecal coliform standard from its definition of secondary treatment, stating that the benefits achieved by disinfection should be weighed against the environmental risks and costs (MSDGC, 1984).

In 1977, the Illinois Environmental Protection Agency (IEPA) proposed revisions to the existing IPCB fecal coliform effluent and water quality standards. The IEPA submitted these changes to the IPCB for approval. The IPCB held administrative public hearings (designated R77-12D) to gather testimony regarding these proposed revisions. In 1984, the Illinois Appellate Court affirmed the IPCB in its revised regulations, which eliminated chlorination of effluents discharged to secondary contact waters (MSDGC, 1984).

In 1986, EPA published *Ambient Water Quality Criteria for Bacteria-1986.* This document contains EPA's recommended water quality criteria for bacteria to protect bathers in recreational waters. The EPA (1986) document identifies the maximum concentrations of *Escherichia coli (E. coli)* and *enterococci* allowable in fresh and marine recreational waters. In 1997, EPA established the Beaches Environmental Assessment and Coastal Health (BEACH) Program to reduce risks to human health caused by exposure to pathogens in recreational waters. The BEACH Act of 2000 amended the Clean Water Act (CWA) by adding Section 303(i)(1)(A), which requires that:

> *Not later than [April 10, 2004], each State having coastal recreation waters shall adopt and submit to the Administrator water quality criteria and standards for the coastal recreation waters of the State for those pathogens and pathogen indicators for which the Administrator has published criteria under §304(a).*

Furthermore, the BEACH Act added Section 502(21) to the CWA, which defines "coastal recreation waters" to include the Great Lakes and marine coastal estuaries that are designated by States under CWA Section 303(c) for **swimming, bathing, surfing, or similar water contact activities**. The requirements of the BEACH Act do not apply to the CWS.

The IEPA has conducted a Use Attainability Analysis (UAA) of the CWS in accordance with 40 CFR 131.10(d). The UAA report has proposed water quality standards for the CWS based on the *Ambient Water Quality Criteria for Bacteria*-1986 (EPA, 1986) and EPA guidance (EPA, 2003). In order to assist IEPA in evaluating the proposed bacterial water quality standards, the District commissioned qualified consultants (research scientists and water quality experts) to conduct a peer review of the EPA's Water Quality Criteria for Bacteria – 1986 and the November 2003 draft implementation guidance document (EPA, 1986 and 2003). The findings of the expert review panel indicated that there is no scientific basis for developing protective bacteria standards for the designated recreational uses of the CWS (MWRDGC, 2006). One of the recommendations from the expert review panel report was that more science is needed before bacteria criteria can be established for effluent dominated urban waterways (MWRDGC, 2006). To address this recommendation, the District proposed a microbial risk assessment study to determine health impacts of recreational use of the CWS assuming disinfected and non-disinfected effluents from the North Side, Stickney, and Calumet WRPs.

The results of this microbial risk assessment will be evaluated and compared against the IEPA-proposed bacteria standards for the CWS. The following bacteria standards were proposed by the UAA report to protect identified uses of the CWS effective 1 March 2010:

- The incidental contact recreational waters shall not exceed a 30-day geometric mean for *E. coli* of 1,030 CFU/100 mL, which is applicable to the CSSC from its junction with the South Branch of the Chicago River to California Avenue, and North Side and Calumet waterways.

- The non-contact recreational waters shall not exceed a 30-day geometric mean for *E. coli* of 2,740 CFU/100 mL, which is applicable to the Calumet River and Lower Des Plaines River from its confluence with the CSSC locations.
- Currently, there are no bacteria standards for the non-recreational waters applicable to the CSSC from California Avenue to the confluence of the Des Plaines River location.

The IEPA rejected these proposed standards and instead proposed WRP effluent fecal coliform standards of 400 CFU/100 mL. The IEPA also required effluent disinfection in order to achieve this standard. Over time, there have been major improvements in water quality, altered land use and additional public access along the CWS. Such improvements and conditions have produced both greater opportunity and heightened public interest in environmental and recreational uses within and along the waterways. Currently, the waterways are used for recreational boating, canoeing, fishing and other streamside recreational activities. These waterways also provide aquatic habitat for wildlife. About 70 percent of the annual flows in the CWS are from the discharge of treated municipal wastewater effluent from the District's WRPs (MWRDGC, 2004).

The IEPA along with other federal, state and local agencies has initiated a multi-year, comprehensive evaluation of the waterways known as the UAA, to identify future uses of the waterways for commercial and recreational activities. Treated, but non-disinfected wastewater effluent is one of several sources that contribute to the presence of indicator bacteria and pathogens in the waterways. Other pathogen sources include the following (http://www.ChicagoAreaWaterways.org):

- Faulty sewage disposal systems
- Combined and sanitary sewer overflows
- Wild and domestic animal waste
- Illegal discharges to drains and sewers
- Storm water runoff

- Treated, but non-disinfected wastewater effluent

The UAA Stakeholders evaluating the CWS have agreed that swimming and other primary contact recreation should not be considered as a viable designated use for the CWS because of physical limitations due to the configuration of the embankments and safety hazards. The Geosyntec Team has relied on UAA existing recreational use survey data for the CWS. Where possible, The Geosyntec Team supplemented the data with information presented in the technical literature.

1.1 Project Objective and Project Tasks

The main objective of this risk assessment study was to evaluate the human health impact of continuing the current practice of not disinfecting the effluents from the District's Calumet, North Side, and Stickney WRPs versus initiating disinfection of the effluent at these three WRPs. This Risk Assessment Study includes two phases: Phase I dry weather risk assessment and Phase II wet weather risk assessment. The dry weather risk assessment sampling was completed in the summer of 2005. The climatic conditions during the 2005 sampling period were not suitable for conducting wet weather sampling. The wet weather sampling took place between June and October of 2006. Dry and wet weather microbial sampling results of the surface water in the CWS and the WRP effluents formed the basis for the risk assessment. The dry and wet weather microbial results were integrated to enable an evaluation of the potential impacts of disinfection on overall risks associated with the recreational use of the waterway.

To accomplish the main project objective, The Geosyntec Team completed the following project tasks:

1. Prepared Dry and Wet Weather Sampling and Analysis Plans (SAPs) and Quality Assurance Project Plans (QAPPs) to generate microbial analytical results that formed the basis of the microbial risk assessment
2. Provided field training to the District's sampling personnel
3. Completed a Microbial Risk Assessment, including:
    a. Literature review of pathogen disinfection effectiveness

b. Microbial exposure assessment by literature review
c. Microbial infection dose-response analysis by literature review
d. Microbial risk characterization of three waterway segments: North Side, Stickney and Calumet

Geosyntec prepared Dry and Wet Weather SAPs and QAPPs in collaboration with the District and the Geosyntec team of experts. The SAP documented the sampling locations, procedures and acceptable wet weather sampling criteria and triggers, including but not limited to rainfall depth, duration, intensity and antecedent dry period. The dry weather QAPP was applicable to the samples collected during wet weather, because the same pathogens were analyzed by the same laboratories both for dry and wet weather. However, the wet weather QAPP specified additional requirements for pathogen samples regarding sample dilution, filtration volume, and reporting requirements.

1.2 Report Organization

This report summarizes the results of the microbial risk assessment based on dry and wet weather sampling and analytical results. Section 2 discusses microbial sampling and analysis. Section 3 presents microbial analytical results. Section 4 discusses wastewater disinfection. Section 5 presents the dry and wet weather microbial risk assessment results.

1.3 References

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SECTION 1

FIGURES

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

CHICAGO WATERWAY SYSTEM

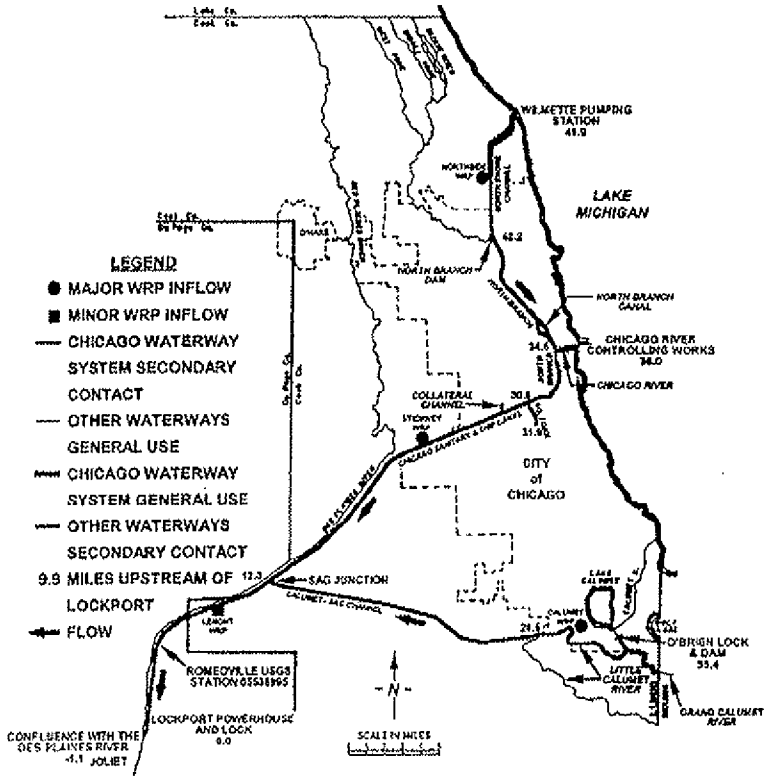

FIGURE NO: 1-1

Geosyntec consultants

CHICAGO, ILLINOIS

PROJECT NO.:	NCP2003.8000	FIGURE NO.:	1
DATE:	11 NOV 2007	FILE NO.:	FIG 1 - CWS

2. MICROBIAL SAMPLING AND ANALYSIS

One of the components of the risk assessment was to conduct sampling and analysis of the CWS. This section discusses the field sampling procedures used to ensure the collection of representative data during dry and wet weather sampling. Dry weather sampling was conducted between July and September 2005 in accordance with the procedures in the SAP and QAPP for the CWS (Geosyntec, 2005). Wet weather sampling was conducted between June and October 2006 in accordance with the procedures in the Wet Weather SAP and QAPP for the CWS (Geosyntec, 2006).

Dr. Charles Gerba of the University of Arizona provided on-site training to the District personnel on sample collection procedures. MWRDGC personnel collected the samples using the District's boats at the designated sampling locations using the procedures in the SAP and QAPP.

2.1 Rationale for Indicator and Pathogenic Microorganism Selection

The primary objective of the microbial examination of the CWS was the detection of fecal pollution that may be excreted in the feces of humans and animals. The direct detection of pathogenic bacteria, viruses, and protozoa requires costly and time-consuming procedures and well-trained technicians. In addition, there are no standard methods available to detect each pathogen possibly present in the CWS.

This study focused on the detection of microorganisms typically present in the feces of humans and other warm-blooded animals, as indicators of fecal pollution. Hence, a group of EPA-approved indicator microorganisms, such as *E. coli*, *enterococci*, and fecal coliform was selected. In addition, pathogens representative of those present in the wastewater that are also of public health concern were selected. Some of these microorganisms were identified by Mead et al. (1999) and WERF (2004).

Table 2-1 presents a summary of the microorganisms selected for this microbial risk assessment study. The rationale for selecting the pathogens for this microbial risk assessment study included the following criteria:

- The pathogens selected are associated with documented outbreaks of disease, including gastrointestinal and respiratory diseases and infections
- There are EPA-approved methods or laboratory standard operating procedures (SOPs) available for the measurement of the selected pathogens.

2.2 Sampling Objectives

The objective of the sampling was to determine the concentrations of the following indicators and pathogens during the 2005 (dry weather) and 2006 (wet weather) recreational seasons:

- Enteric viruses: i) total culturable viruses, (ii) viable adenovirus; and (iii) *Calicivirus*
- Infectious *Cryptosporidium parvum* and viable *Giardia lamblia*
- *Salmonella* spp.
- *Pseudomonas aeruginosa*
- Fecal coliforms
- *E. coli*
- *Enterococci*

2.2.1 Dry Weather Sampling Objectives

The specific objectives of dry weather sampling were as follows:

1. Evaluate the impact of the treated effluent from the District's three major WRPs (North Side, Stickney, and Calumet) on the microbial quality of the CWS.
2. Estimate health risks to recreational users of the CWS due to incidental contact pathogen exposure under dry weather conditions.
3. Quantify any reduction of risk that would result from disinfection of WRP effluents during dry weather.

During the 2005 dry weather sampling, samples were taken at locations upstream, downstream and at the outfalls of the Stickney, Calumet and North Side WRPs (see Figure 2-1). The sampling plan provided a detailed sampling strategy, including sampling locations, the number of samples and sampling frequency. Five dry weather

sampling events took place over a 5-week period, which began the week of 26 July 2005. Seventy five (75) samples were collected (five events at each of the three [3] WRPs; 5 samples per event at each WRP). The number of samples collected during dry weather sampling at each location is summarized in Table 2-2.

2.2.2 Wet Weather Sampling Objectives

The specific objectives of wet weather sampling were as follows:

1. Evaluate the impact of wet weather flow on the microbial quality of the WRP outfalls.
2. Evaluate the impact of combined sewer overflows (CSOs) on the microbial quality of the CWS.
3. Estimate health risks to recreational users of the CWS due to incidental contact pathogen exposure under wet weather conditions.
4. Quantify any reduction of risk that would result from disinfecting WRP effluents during wet weather.

It has been established in the technical literature that wet weather contributes significantly to the microbial load in surface water due to surface runoff and CSOs. Several sources contribute to the microbial load in the waterway during wet weather: CSOs, discharges from storm drains, overland runoff, land use activities (such as agriculture and construction), erosion, and habitat destruction.

A total of nine (9) sampling events took place during the 2006 wet weather recreational season between the months of June and October 2006. Three (3) sampling events took place at each of the North Side, Stickney and Calumet WRPs. The sampling plan provided a detailed sampling strategy, including sampling locations, the number of samples and sampling frequency. Based on the sampling locations outlined in Section 2.2.1, the number of samples collected during wet weather sampling at each location are summarized in Table 2-2. The wet weather sampling program included fifty (50) samples for each of the pathogens discussed above.

Sampling protocols and methods of analysis were specified according to EPA-approved methods where possible. When EPA-approved methods were not available, laboratory SOPs were used.

2.3 Field Sampling Procedures

This section discusses: (1) microbial sampling locations; (2) sample collection equipment, material and procedures; (3) sample identification; (4) sample custody; (5) sample packaging, shipment and tracking; (6) waste management; and (7) health and safety procedures.

2.3.1 Microbial Sampling Locations

Samples were taken at locations upstream, downstream, and at the outfalls of the Stickney, Calumet, and North Side WRPs. In selecting the sampling locations the following factors were also considered: 1) locations of pumping stations for combined sewer outflows; 2) recreational navigation; and 3) commercial navigation (barge traffic). Boat traffic, especially commercial barge traffic, can have a significant effect on the water quality in the CWS through re-suspension of sediment containing attached microorganisms. In accordance with MWRDGC sampling procedures, when there was barge traffic during the sampling events the sampling stopped and commenced 30 minutes after the barge passed. The sampling personnel recorded traffic of recreational boats and barges during sampling.

The Stickney WRP discharges to the CSSC; the Calumet WRP discharges to the LCR that in turn discharges to CSC, and the North Side WRP discharges to the NSC (see Figure 2-1). The following sections present the physical description of the above-mentioned waterways and the sampling locations.

Physical Description of the CSSC

This 31.1 mile long man-made channel has many different shapes and sizes. Its alignment is straight throughout its length, except for four bends near Harlem Avenue, LaGrange and Romeoville Roads, and in Lockport (see Figure 2-1). Downstream of the

Lockport Powerhouse and Lock (LP&L), a reach of 1.1 miles, the depth is 10 feet and the width is 200 feet. Upstream of the LP&L, the depth varies from 20 to 27 feet. The reach immediately upstream of the LP&L, 2.4 miles in length, varies in width from 160 to 300 feet. The east bank of this reach is a vertical concrete wall. The west bank varies from a vertical rock wall to a steep rock hill embankment. The next 14.6 miles of the CSSC have vertical concrete or rock walls 160 feet apart. The last 13.0 miles have a trapezoidal shape, 220 feet wide, with steep earth or rock side slopes. There are several areas with vertical rock walls in this last reach.

Physical Description of the CSC and LCR

The Calumet WRP discharges to the LCR. The LCR, 6.9 miles in length, has been deepened and widened from its original natural condition. It has few vertical rock walls and most of the banks are earthen side slopes. There are several changes in alignment, with one full 180-degree bend west of Indiana Avenue. LCR's width varies from 250 to 750 feet and its depth is generally 12 feet in the center part of the channel. The width of LCR at the point of the Calumet WRP outfall discharge was measured by the District to be 750 feet, but it diminishes rapidly to 375 feet.

A man-made channel, the CSC is 16.2 miles long with a generally trapezoidal shape, 225 feet wide and approximately 10 feet deep. In some sections, the north bank is a vertical wall. The alignment is generally straight with three bends near Crawford, Ridgeland and Western Avenues (see Figure 2-1).

Physical Description of the NSC

This man-made channel is 7.7 miles in length and is straight throughout except for four bends in alignment near Devon and Central Avenues and Emerson and Linden Streets (see Figure 2-1). It has steep earthen side slopes and a width of 90 feet. The depth varies from 5 to 10 feet.

2.3.1.1 Dry Weather Sampling Locations

A subset of the District's Ambient Water Quality Monitoring (AWQM) sampling stations employed by the MWRDGC along the 78 miles of the CWS was used for this study.

Three monitoring stations were chosen for each of the WRPs, one upstream of the outfall, one downstream, and the WRP outfall itself. The sampling locations were surveyed by MWRDGC sampling personnel using the GPS system available on the District's boat.

Upstream Sampling Locations

The upstream locations at each WRP were situated at the nearest AWQM sampling station upstream of the WRP. These locations are as follows:

1. NSC-Oakton Avenue, also known as WW-102 (see Sampling Location 3 on Figure 2-1) – 8,200 feet or 1.6 miles from the WRP.
2. CSSC-Cicero Avenue, also known as WW-75 (see Sampling Location 21 on Figure 2-1) – 6,300 feet or 1.2 miles from the WRP.
3. CSC-Indiana Avenue, also known as WW-56 (see Sampling Location 29 on Figure 2-1) – 2,800 feet or 0.53 miles from the WRP.

Downstream Sampling Locations

The downstream locations were selected to be the nearest established District monitoring station that are no less than 10 to 15 waterway widths from the outfall. For the CSSC, the waterway width downstream of the outfall is 220 feet, resulting in 15 waterway widths of 3,300 feet or 0.625 miles. For the CSC, the waterway width downstream of the outfall ranges from 750 feet at the point of discharge to LCR to 375 ft. This results in 15 waterway widths ranging from 11,250 feet (~2 miles) to 5,625 feet (~1 mile). For the NSC the waterway width downstream of the outfall is 90 feet, resulting in 15 waterway widths of 1,350 feet or 0.225 miles. The approximate downstream locations were as follows:

1. NSC-Touhy Avenue, also known as WW-36 (see Sampling Location 5 on Figure 2-1) – 2,800 feet or 0.53 miles from the WRP.
2. CSSC-Harlem Avenue, also known as WW-41 (see Sampling Location 22 on Figure 2-1) – 9,500 feet or 1.8 miles from the WRP.
3. CSC-Halsted Street, also known as WW-76 (see Sampling Location 32 on Figure 2-1) – 5,800 feet or 11 miles from the WRP.

2.3.1.2 Wet Weather Sampling Locations

A subset of the District's AWQM stations employed by the MWRDGC along the 78 miles of the CWS was used for wet weather sampling. Nine wet weather sampling events (three at each of the North Side, Stickney and Calumet WRPs) were conducted during the recreational period between 6 June and 17 October 2006. During each sampling event, samples were collected by District personnel at several locations upstream and downstream of the Stickney, Calumet and North Side WRPs (see Figure 2-2). Outfall samples were also collected during each sampling event at the Calumet WRP. One sample was also collected at the outfalls of North Side and Stickney WRPs during the last sampling event at each of these WRPs. The sampling locations were situated at the nearest MWRDGC AWQM sampling station. At the North Side, samples were also collected near each of the North Branch Pumping Station (NBPS) or Wilson Avenue sampling station, depending on the level of turbulence near the NBPS. In addition, at Stickney, samples were collected near the Racine Avenue Pumping Station (RAPS). The exact sampling location proximal to the pumping stations was decided by the boat captain based on the level of turbulence and other logistical and safety considerations.

A larger number of sampling locations was used during wet weather sampling. The wet weather locations were spaced at significantly larger distances away from the WRPs to account for the contributions of storm water runoff, CSO outfalls, and pumping stations. In summary, wet weather samples were collected at the following locations:

Upstream of Stickney WRP at the CSSC

1. CSSC-Damen Avenue, also known as WW-40 (see Sampling Location 20 on Figure 2-2)-29,400 feet or 5.6 miles from the WRP
2. CSSC-Cicero Avenue, also known as WW-75 (see Sampling Location 21 on Figure 2-2)-8,200 feet or 1.6 miles from the WRP
3. RAPS outfall (the sample was collected from Bubbly Creek at 35th Street)-32,800 feet or 6.2 miles from the WRP

Downstream of Stickney WRP at the CSSC

1. CSSC-Harlem Avenue, also known as WW-41 (see Sampling Location 22 on Figure 2-2)-9,500 feet or 1.8 miles from the WRP.
2. CSSC-Route 83, also known as WW-42 (see Sampling Location 25 on Figure 2-2)-61,500 feet or 11.7 miles from the WRP.

Upstream of the Calumet WRP at the LCR

1. Little Calumet-Indiana Avenue, also known as WW-56 (see Sampling Location 29 on Figure 2-2)-6,300 feet or 1.2 miles from the WRP.

Downstream of the Calumet WRP at the LCR and CSC

1. Little Calumet-Halsted Street, also known as WW-76 (see Sampling Location 30 on Figure 2-2)-5,800 feet or 1.1 miles from the WRP
2. CSC-Ashland Avenue, also known as WW-58 (see Sampling Location 32 on Figure 2-2)-11,400 feet or 2.2 miles from the WRP
3. CSC-Cicero Avenue, also known as WW-59 (see Sampling Location 33 on Figure 2-2)-33,800 feet or 6.4 miles from the WRP
4. CSC-Route 83, also known as WW-43 (see Sampling Location 35 on Figure 2-2), 37,500 feet or 7.1 miles from the WRP.

Upstream of the North Side WRP at the NSC

1. NSC-Oakton Avenue, also known as WW-102 (see Sampling Location 3 on Figure 2-2)-2,800 feet or 0.53 miles from the WRP

Downstream of the North Side WRP at the NSC and Chicago River

1. NSC-Touhy Avenue, also known as WW-36 (see Sampling Location 5 on Figure 2-2)-2,800 feet or 0.53 miles from the WRP
2. NBPS or North Branch-Wilson Avenue, also known as WW-37 (see Sampling Location 8 on Figure 2-2)-21,600 feet or 4.09 miles from the WRP
3. North Branch-Diversey Parkway, also known as WW-73 (see Sampling Location 10 on Figure 2-2)-36,400 feet or 6.9 miles from the WRP.
4. South Branch-Madison Street, also known as WW-39 (see Sampling Location 17 on Figure 2-2)-52,600 feet or 9.96 miles from the WRP.

2.3.2 Sample Collection Equipment, Materials and Procedures

At each location during both dry and wet weather sampling, field parameters such as pH and temperature were measured and recorded in the field sample collection forms, which

are included in Appendix A-1 (dry weather sampling forms) and Appendix A-2 (wet weather sampling forms). In addition, the following information was recorded on the sample collection form (see Appendices A-1 and A-2):

- WRP name
- WRP address
- Sampler name
- Sample ID
- Sample location ID
- Sample location name
- Sample collection date/time
- Sample volume
- Requested analysis
- Observations

The District used disinfected and sterilized sampling equipment at each sampling location and for each sampling event. The equipment was sterilized by scrubbing with warm detergent solution and exposing to bleach (minimum of a 0.5% solution of bleach and water) for at least 30 minutes at ambient temperature. The equipment was rinsed with sterilized deionized water and placed in an area free of potential pathogen contamination until dry. Deionized water was sterilized by autoclaving at 121°C.

The details of dry and wet weather sampling are discussed in the following sections.

Dry Weather Sample Collection Equipment, Materials and Procedures

At each sampling station a total of six samples were taken at three locations across the width of the waterway. Sampling was conducted upstream of the boat (at the bow). At each location a sample was taken at the surface and another at one-meter depth. The samples from the three locations at the surface were combined to make a composite sample. Also, the samples from the three locations at one-meter depth were combined to make a composite sample. For virus and protozoa samples that require filtration, the following procedure was followed: At each location upstream and downstream of the WRP, the three samples at the surface were composited by filtering 1/3 of the required volume at each location. Similarly, at each location upstream and downstream of the

WRP, the three samples at 1-meter depth were composited by filtering 1/3 of the required volume at each location.

The exception to this protocol is the outfall samples. Four grab samples were taken over a period of six hours at the WRP outfall. These four grab samples were combined to make one composite sample. The composite sample was used as the source of samples for bacteria by pouring the collected water into the appropriate sample containers. For protozoa and virus samples, the composite sample was filtered using the procedures described below.

During each sampling event, 15 samples were collected. Each sample was analyzed for bacteria, viruses and protozoa. For the five sampling events a total of 75 samples were collected.

Wet Weather Sample Collection Equipment, Materials and Procedures

The District and Geosyntec developed a strategy for determining which rain events were appropriate for wet weather sampling. Samples were collected during the wet weather event or immediately after. The following criteria were evaluated to develop the strategy (EPA, 1999):

1. Minimum amount of precipitation
2. Duration of precipitation
3. Antecedent Period (minimum 72 hours of dry weather)

The District monitored pending wet weather using the internet, public media and the District's Waterway Control Center (WCC). Each business day that wet weather was in the forecast, at approximately 10:00 a.m., the designated District personnel conferred by conference call regarding the potential for significant wet weather (SWW) over the following 24-hour period. SWW was defined as a forecast with 0.5 inch or greater rainfall. In addition to discussing the forecast, the location, status and work schedule of the two boats required for sampling was reviewed. District notified Geosyntec of the potential for sampling following the daily conference calls when appropriate.

When there was the potential for SWW, the District contacted the WCC for wet weather updates. When rainfall of more than 0.1 inch had fallen at any WCC rain gauge within the CSO service area and the 0.5 inch or greater expectation remained, the boat crew supervisor was notified of the situation by the designated District person. When 0.3 inches of rainfall had fallen at any WCC rain gauge in the CSO service area, the designated District person contacted the appropriate treatment plant operator to determine if any CSO outfall tide gate alarms had occurred or if there had been pumping to the river at either the 125th Street Pumping Station, NBPS or Racine Avenue RAPS.

After the decision was made to call out the boat crew, the District's laboratory sampling manager contacted Geosyntec to inform them that a sampling event had been initiated.

Grab wet weather samples were collected at the center of the channel because during the 2005 dry weather sampling good mixing conditions were visually observed across the relatively narrow channel. Therefore, no significant differences were expected across the channel during wet weather. Wet weather samples were collected only at the surface of the CWS. There was no statistical difference between samples collected at the surface and at 1-meter depth as shown by the 2005 dry weather sampling results (see Section 3 for details).

In addition, effluent (outfall) samples were collected during wet weather sampling to evaluate whether the increased flow through the WRPs during wet weather may affect the pathogen concentrations in the effluent of the District's North Side, Stickney, and Calumet WRPs. Four grab samples were taken over a period of six hours at each WRP outfall. These four grab samples were combined to make one composite sample. The composite sample was used as the source of samples for bacteria by pouring the collected water into the appropriate sample containers. For protozoa and virus samples, a composite filtered sample was collected using the procedures described below.

Table 2-3 summarizes the dry and wet weather WRP flows (million gallons per day [MGD]) during the 2005 and 2006 sampling events. The table also summarizes the pumping station discharge volumes (million gallons [MG]) during the wet weather sampling events. The data in Table 2-3 indicate that the effluent discharge flows are

significantly higher during wet weather at each WRP. The data also indicate that the CSO volumes are significantly higher at the RAPS (near the Stickney WRP) than the NBPS (near the North Side WRP) and the 125th Street Pumping Station (near the Calumet WRP). In addition, the data indicate that during the 2006 wet weather sampling, the NBPS and the RAPS discharged CSOs during two of the three sampling events at each WRP. At the Calumet WRP the 125th Street Pumping Station discharged during one of the three sampling events, which is a very unusual occurrence. Based on the District's experience, the 125th Street Pumping Station discharges about once every ten years.

The following sections discuss (i) virus sampling in accordance to EPA (1996); (ii) bacteria sampling according to EPA (1986; 2002; 2003; 2003a) and the Standard Methods for the Examination of Water and Wastewater (1998); and protozoa sampling according to EPA (2001; 2003).

2.3.2.1 Virus Sampling

Sampling for viruses was conducted according to EPA (1996) using the virus adsorption-elution (VIRADEL) method for recovering human enteric viruses from water matrices. Positively charged cartridge filters (Virosorb® 1MDS cartridge, Cuno Inc. Meriden, CT) were used to concentrate viruses from water. Figure 2-3 presents a typical filter apparatus (EPA, 1996). Gloves were changed if they touched human skin or handled components that may be contaminated (i.e. boat surfaces). Procedures for sample packaging and shipment are discussed in Section 2.3.5.

During the 2005 dry weather sampling, at each location upstream and downstream of the WRP, the three samples at the surface were composited by filtering $^1/_3$ of the required volume at each location. Similarly, the 1-meter depth samples were composited by filtering $^1/_3$ of the required volume at each location. Approximately 300-L of upstream and downstream samples were filtered at each location during dry and wet weather sampling. In addition, approximately 100-L samples were filtered at the outfall. The outfall samples were composited over a six hour period by filtering ¼ of the required volume every 1.5 hours.

During the 2006 wet weather sampling at each location upstream and downstream of the WRP, virus samples were collected by filtering the required volume near the center of the channel. Because of the relatively high turbidity of the surface water, pre-filter modules were used routinely during wet weather sampling.

2.3.2.2 Bacteria Sampling

During dry weather sampling, at each location upstream and downstream of the WRP, the three samples at the surface were composited by collecting $^1/_3$ of the required volume at each location. Similarly, the samples at 1-meter depth were composited by collecting $^1/_3$ of the required volume at each location. The samples were collected using a sampling pump and attaching a weight to the sampling tubing to lower it to the surface and 1-meter depth, respectively. The sample container was filled using an aseptic technique and leaving at least 1 inch of head space to allow for mixing of the sample before analysis. The container was closed immediately after the sample was collected.

During wet weather sampling, two sample containers were used for bacteria samples. A 10-L cubitainer was used for *Salmonella* spp. and one 10-L cubitainer was used for the other bacteria analyzed. The sample container was filled using an aseptic technique and leaving at least 1 inch of head space. The container was closed immediately after the sample was collected.

Immediately following sample collection, the sample container lid was tightened, labeled with water-proof ink and clear tape was placed over the sample label. The sample container was then placed in a ziplock bag, wrapped with bubble wrap or paper towels (to prevent freezing) and placed upright in the cooler with ice. Fresh ice was placed in the cooler immediately prior to shipment. Procedures for sample packaging and shipment are discussed in Section 2.3.5.

2.3.2.3 *Cryptosporidium* and *Giardia* Sampling

Cryptosporidium and *Giardia* sampling was performed by EPA Method 1623 using field filtration. Method 1623 has been validated only for laboratory filtration. However, recent guidance in EPA (2003), entitled "Source Water Monitoring Guidance Manual for

Public Water Systems for the Long Term 2 Enhanced Surface Water Treatment Rule. EPA 815-D-03-005. June," indicates that field filtration is acceptable. Field filtration was performed using Pall Gelman Envirochek™ HV capsule filters, which are acceptable filtration systems. During the first dry weather sampling event at the Calumet Waterway System, 10-L samples were field filtered for protozoa analysis. During the remaining dry and wet weather events, 20-L samples were field filtered for protozoa analysis.

During dry weather, four bulk water matrix spike (MS) samples were collected for *Cryptosporidium* and *Giardia*, which were spiked in the laboratory and analyzed. The matrix spike (MS) test in EPA method 1623 entails analysis of a separate sample aliquot spiked with 100 to 500 oocysts to determine the effect of the matrix on the method's oocyst recovery. One MS sample was analyzed for every 20 samples (or 5% of the total samples) as required by the method. The MS results were used collectively to assess overall recovery and variability for EPA Method 1623. The MS sample results were not used to adjust *Cryptosporidium* and *Giardia* recoveries at any sampling location.

During wet weather, two bulk water MS samples for *Cryptosporidium* and *Giardia* were collected, spiked in the laboratory and analyzed. MS samples were collected near the NBPS at Wilson Avenue and at RAPS. During dry weather sampling, four MS samples were collected: one at each of the WRPs and one downstream of the Calumet WRP. Before collection of the bulk MS sample, temperature and pH were measured. Turbidity and specific conductance or conductivity (SC) of field samples were also measured at the District's laboratory. The MS samples were collected immediately after the field-filtered samples by filling two 10-L cubitainers directly from the pump tubing.

The cubitainer cap was tightened, labeled (see Section 2.3.3) and placed in the shipping cooler with ice. The ice was replaced with fresh ice before shipping. Sample packaging, shipment and tracking procedures are discussed in Section 2.3.5.

2.3.3 Sample Identification

Samples were identified on the sample container with a separate identification label. All labeling was done in indelible/waterproof ink. Each securely affixed label included the following information:

- Sample ID, which included:
  - WRP identification (Stickney, North Side, Calumet)
  - Sampling location (upstream, downstream, outfall)
  - Sampling depth (surface or 1-meter)
  - Date of sample collection

In addition, the sample label included the following:

- Time of sample collection
- Sampler's name or initials
- Required analytical method
- Sample type (i.e., composite, grab)
- Preservation requirement (i.e. ice)

2.3.4 Sample Custody

After collection and identification, samples were maintained under chain-of-custody procedures. Proper sample custody procedures were used to ensure that samples were obtained from the locations stated and that they reached the laboratory without alteration. A sample was considered to be in a person's custody if the sample was:

- in a person's actual possession;
- in view after being in a person's possession;
- locked so that no one can tamper with it after having been in physical custody; or
- in a secured area, restricted to authorized personnel.

The District sampling personnel were the field sample custodians and were responsible for ensuring sample custody until the samples were transferred to a courier or to the laboratory. All samples were accompanied by a Chain-of-Custody Record. When transferring samples, the individuals relinquishing and receiving the samples signed and dated the record. Shipping bills were kept as receipt of shipment. Airbills were retained as part of the permanent documentation. Before shipping the samples, one of the three Chain-of-Custody carbon copies was kept as part of the permanent documentation.

When the samples were received by the laboratory, a designated laboratory person checked all incoming samples for integrity and noted any observations on the original Chain-of-Custody Record. Each sample was logged into the laboratory system by assigning it a unique laboratory sample number. This number and the field sample identification number were recorded on the laboratory report.

The laboratory maintained a file of all the documents (e.g., Chain-of-Custody forms) pertinent to sample custody and sample analysis protocols. For Chain-of-Custody forms, the laboratory maintained a file copy, and the completed original was returned to the project manager as a part of the final analytical report.

2.3.5 Sample Packaging, Shipment, and Tracking

After labeling, all samples were stored in ice-filled coolers until shipment to the laboratory. At the end of each day the samples were packed for shipment.

2.3.5.1 Sample Packaging

Two large plastic trash bags were inserted into the shipping cooler to create a double liner. Immediately before packing the cooler, fresh ice was put into several Ziploc bags. The Ziploc bags were sealed by expelling as much air as possible and securing the top with tape. The samples were placed into the shipping container with ice around the sample bag. A temperature sample was also placed in the cooler (e.g., extra sample bottle) for measuring sample temperature upon receipt at the laboratory. The liner bags were closed by twisting the top of each bag and tying it in a knot.

The chain of custody form was completed, signed and dated, before being placed and sealed inside a Ziploc bag, which was taped under the cooler lid. A copy of the sample collection form was faxed to the laboratory the day after sample collection. The cooler lid near the horizontal joints was sealed with duct tape. The lid was also secured by taping the cooler at each end, perpendicular to the seal. The coolers were also affixed with security labels taped over opposite ends of the lid.

2.3.5.2 Shipping and Tracking

The protozoa samples were shipped to CEC on the day of collection or on the morning of the following day using United Parcel Service. The bacteria and virus samples were hand-delivered to HML. Due to the relatively short holding time of bacteria samples it was decided to hand-deliver the samples to ensure that they would be analyzed within the holding time requirements.

The District Field Sampling Managers kept track of the CEC sample shipment by using the airbill number on the shipper's copy of the airbill, using the shipping company's web page, or by contacting the shipping company over the phone.

2.3.6 Waste Management

Each laboratory was responsible for complying with all federal, state and local regulations governing waste management, particularly the biohazard and hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling the releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations was also required. Samples, reference materials, and equipment known or suspected to have viable pathogens attached or contained were sterilized prior to disposal.

2.3.7 Health and Safety

The sampling was performed in accordance with MWRDGC health and safety procedures.

2.4 Quality Assurance/ Quality Control Procedures

This section discusses the quality assurance/quality control (QA/QC) procedures that were used for the analysis of surface water and outfall samples. The QA/QC procedures discussed are in accordance with the requirements of the analytical methods specified in Section 2.4.1.

2.4.1 Microbial Methods of Analyses

Sampling and analysis of microbial samples were conducted in accordance with the procedures described at http://epa.gov/microbes and in Standard Methods for the Examination of Water and Wastewater (Standard Methods, 1998). The microbial methods of analysis include the following:

- Enteric viruses: i) (total culturable viruses) using the methods described in the ICR Microbial Laboratory Manual, EPA 600/R-95/178 (EPA, 1996); ii) adenovirus; and iii) *Calicivirus*. The samples for total culturable viruses were analyzed by HML and the samples for adenovirus and *Calicivirus* were analyzed by the UA Laboratory. Adenovirus and *Calicivirus* were determined using the UA SOPs. There are no EPA-approved methods for viable *Calicivirus*. The method used involves a Polymerase Chain Reaction (PCR) method that offers an estimate of the virus concentration, but does not determine or confirm viability. *Calicivirus* is a family of human and animal viruses. For this risk assessment study *Calicivirus* refers to human *Caliciviruses*, specifically the genus norovirus.
- Infectious *Cryptosporidium parvum* and viable *Giardia lamblia* were determined using EPA Method 1623 (EPA, 2001) in conjunction with cell culture infectivity for the *Cryptosporidium* and viability staining (DAPI-PI) for the *Giardia*. The samples for protozoa were analyzed by CEC.
- *Salmonella* spp. using Standard Method 9260D (Standard Methods, 1998)
- *Pseudomonas aeruginosa* using Standard Method 9213E (Standard Methods, 1998)
- Fecal coliforms using Standard Method 9222D (Standard Methods, 1998)
- *E. coli* using EPA Method 1103.1 (EPA, 2002)
- *Enterococci* using EPA Method 1106.2 (EPA, 2001a)

2.4.2 Data Quality Objectives

Data quality objectives (DQO) are qualitative statements that specify the quality of the data required to generate valid data for the risk assessment calculations. DQOs are based on the ultimate use of the data to be collected; therefore, different data uses may require different levels of data quality (EPA, 1998; EPA, 2002a). Two analytical levels address various data uses and the QA/QC effort and methods required for this project to achieve the desired level of quality. These two levels are discussed below:

1) DQO Level 2 (On-site Analyses): DQO Level 2 provides rapid results and a better level of data quality than Level 1. This level is used for on-site analytical measurement data using the District's YSI Datasonds Model 6600 and includes pH and temperature.
2) DQO Level 3 (Off-site Analyses using EPA-approved Methods, Standard Methods (1998) or laboratory SOPs): DQO Level 3 provides data that will be used in the risk assessment calculations. Off-site analyses of viruses, bacteria, and protozoa are subject to Level 3 DQOs.

The following sections discuss the QA/QC procedures of the analyses to be performed off-site. The on-site analyses met Level 2 DQOs. On-site analyses were conducted in accordance with the manufacturer's operations and maintenance manual.

The overall QA objective was to implement procedures for sampling, chain-of-custody, laboratory analysis, and reporting that would provide valid and complete data results. The following sections discuss specific requirements for QA/QC procedures: laboratory internal QC checks; equipment calibration; equipment maintenance; corrective actions; data reduction, validation, and reporting; and archiving examination results.

2.4.3 QA/QC Procedures

Implementation of the QA/QC procedures was established through the following steps:

- The District Project Manager ensured that each field team member was familiar with the SAP and QAPP prior to implementation of field activities.

- The District Project Manager and Geosyntec QA Manager regularly provided a QA review of field activities, field notebooks and forms to ensure that all procedures were followed.
- Both the Geosyntec Project Manager and QA Manager identified laboratories with national certifications that routinely analyze for the pathogens specified in the sampling plan.
- The Geosyntec Project Manager and QA Manager verified that the laboratories have a written description of their QA activities, a QA plan describing the QA management of day-to-day routine operations. In addition, The Geosyntec Team conducted telephone interviews and on-site visits to audit the laboratories for this project.
- The laboratories were required to adhere to defined quality assurance procedures to ensure that generated analytical data are scientifically valid and are of known and acceptable precision and specificity.

The latest EPA-approved methods and Standard Methods were used to perform the analyses for this project.

2.4.3.1 Laboratory Internal QC

The laboratories performed all QC procedures that were required by the analytical methods. The dry and wet weather analytical reports of HML, CEC and UA are included in Appendices: B-1 and B-2; C-1 and C-2; and D-1 and D-2, respectively. The laboratories were also required to comply with the requirements in EPA (1978) as required by the analytical methods. In addition, the University of Arizona Microbiological Laboratory was also required to comply with the requirements in EPA (2004). The laboratories were also required to implement the corrective actions required if the QC criteria were not met. Data that did not meet the internal QC criteria was flagged and the laboratory documented the reason(s) for the nonconformance. All samples were analyzed within holding time requirements.

Bacteria QC

The dry and wet weather bacteria analytical results are included in Appendices B-1 and B-2, respectively. Bacteria sample results met the QC specifications set forth in the

approved methods described above. Each batch (or lot, if commercially prepared) of dilution/rinse water was checked for sterility by adding 50 mL of water to 50 mL of a double-strength non-selective broth (e.g., tryptic soy, trypticase soy, or tryptose broth). The water was incubated at 35°C ± 0.5°C and checked for growth after 24 hours and 48 hours (or for the longest incubation time specified in the method).

To test sterility of newly prepared media prior to the analysis of field samples, one plate per each media batch was incubated at the appropriate temperature for 24 and 48 hours (or for the longest incubation time specified in the method) and checked for growth. For each new lot or batch of medium, the analytical procedures and integrity of the medium was checked before use by testing with known positive and negative control cultures. Preparation blanks were analyzed to detect potential contamination of dilution/rinse water during the course of analyses. A membrane filtration (MF) preparation blank was performed at the beginning and the end of each filtration series by filtering 20-30 mL of dilution water through the membrane filter and testing for growth. For the most probable number (MPN) technique, a volume of sterilized, buffered water was analyzed exactly like a field sample each day samples were analyzed. The preparation blank was incubated with the sample batch and observed for growth of the target organism.

Cryptosporidium and *Giardia* QC

The following QC samples were analyzed for *Cryptosporidium* and *Giardia*: MS, ongoing precision and recovery (OPR), and method blanks; the results are presented in Appendices C-1 and C-2. The method blank test in EPA Method 1623 consists of analysis of an unspiked reagent water sample to test for contamination. The OPR in EPA Method 1623 entails analysis of a reagent water sample spiked with 100 to 500 oocysts to demonstrate ongoing acceptable performance. The MS test in EPA Method 1623 entails analysis of a separate sample aliquot spiked with 100 to 500 oocysts to determine the effect of the matrix on oocyst recovery.

For dry weather samples, four MS samples were analyzed for the 75 samples collected (or 5% of the total samples). One MS sample was collected at each of the three WRP

outfalls. One MS sample was collected downstream of the Calumet WRP that was sampled during the first sampling event.

For wet weather samples, two MS samples were analyzed for the 50 samples collected (or about 5% of the total samples). One MS sample was collected near the NBPS at Wilson Avenue. A second MS sample was collected at RAPS. MS results were within the acceptance criteria specified in EPA Method 1623. The MS sample results were not used to adjust *Cryptosporidium* and *Giardia* recoveries at any sampling location.

During dry weather, cyst and oocyst recoveries for the surface water MS samples were 52% and 61%, respectively. The *Giardia* cysts recovery for the outfall MS sample was 29.8% and the *Cryptosporidium* oocysts recovery was 27.7%.

During wet weather, the recovery rates of seeded *Giardia* and *Cryptosporidium* in the Stickney RAPS MS sample (Stickney – RAPS-MS-080306) were 46.5% and 89.1%, respectively. For the North Side MS sample (North Side –DNS-WW-37 – 062606 – MS), the *Giardia* and *Cryptosporidium* recovery rates for the matrix spike were 151% and 77.7%, respectively.

During dry weather, no oocysts or cysts were detected in method blanks analyzed indicating no contamination in the spiking or sample processing procedures. Mean cyst recovery for OPR samples was 51.0 ± 27% (n=5) with recoveries ranging from 24.6 to 96.4%. The mean oocyst recovery for OPR samples was 61.1 ± 17% with recoveries ranging from 40.4 to 84.3%. All recoveries were well within the acceptance criteria specified for OPR samples in Method 1623 (EPA, 2003).

During wet weather, no oocysts or cysts were detected in method blanks analyzed indicating no contamination in the spiking or sample processing procedures. The cyst recoveries for OPR samples ranged from 33.5 to 84.4%. The oocyst recoveries for OPR samples ranged from 33.2 to 89.1%. The lowest OPR recoveries for cysts (33.5%) and oocysts (33.2%) were measured during the analysis of the 26 June 2006 North Side samples. A calculation error when preparing the oocyst working suspension resulted in a tenfold reduction in the concentration of oocysts used in the spiking trials. While the

OPR recoveries for the 26 June 2006 North Side analysis were relatively lower than the ones typically obtained by CEC, they were still within acceptance criteria established by EPA validation trials. Overall, all recoveries were well within the acceptance criteria specified for OPR samples in Method 1623 (EPA, 2003).

Virus QC

The dry and wet weather analytical results for viruses are presented in Appendices B-1 and B-2, and D-1 and D-2, respectively. For the determination of total culturable viruses the laboratories run a negative and positive assay control with every group of subsamples inoculated into cell cultures. The laboratories performed a negative assay control (NAC) by inoculating Blue Green Monkey Kidney (BGMK) cell culture with a volume of sodium phosphate buffer (pH = 7 to 7.5) equal to the inoculation volume. This culture served as a negative control. The laboratories performed a positive assay control (PAC) by diluting attenuated poliovirus type 3 (from the high titered QC stock) in sodium phosphate buffer (pH = 7 to 7.5) to give a concentration of 20 Plaque Forming Units (PFU) per inoculation volume. The laboratories inoculated a BGM culture with a volume of diluted virus solution equal to the inoculation volume. This control provided a measure for continued sensitivity of the cell cultures to virus infection.

University of Arizona QA/QC Physical Measures: Two PCR workstations, with non-circulating air and ultraviolet (UV) light were used to ensure clean areas. All the areas for the analysis were physically separate. All the reagents were prepared in a separate room from the samples. Both rooms had positive pressure from the main laboratory to reduce contamination. Each room has a workstation, the reagents were only opened in the workstation, and the samples were opened only in their respective workstations. The workstations were cleaned with 10% bleach solution and the UV light was turned on for at least 30 minutes prior to sample handling. Different equipment was used in each room and not used in other areas (e.g. pipets, pipet tips and lab coats were exclusively used for each room). The PCR thermocyclers are contained in another room outside the main laboratory. The PCR product was only open in the workstation designated for samples

and in the electrophoresis room (negative pressure isolates this room from the main laboratory).

RNA free water was used as a negative control. The Reverse Transcriptase (RT) and PCR reagent was mixed in the workstation in the room for reagents. The lab coat, pipet tips, pipet aid, coolers and tubes used were exclusively for this room. The samples for RNA extraction were opened in a biological type II hood. The tube with RNA extracted from the samples was opened only in the workstation located in the sample RNA extraction room. All the equipment for RNA extraction and for handling the samples was used exclusively for this function. The samples were centrifuged before opening in order to reduce the potential for aerosol formation. One negative control for each 5 samples was performed for the RNA extraction; also one negative control was run for the PCR.

2.4.3.2 Equipment Calibration

Each instrument was calibrated following the specific manufacturer's recommendations. Laboratory instruments were calibrated prior to each use or on a scheduled, periodic basis as specified in the analytical methods.

2.4.3.3 Equipment Maintenance

Equipment maintenance and repair was performed as required for each instrument. Preventive maintenance for all equipment included inspection before use, cleaning as necessary during use, and thorough cleaning and inspection after use.

2.4.3.4 Corrective Actions

Corrective actions for the analytical laboratories included the following:

- Re-analyses of *Calicivirus* and adenovirus samples to verify the results; the relatively long holding times of the virus samples permitted the reanalysis.
- Re-sampling and re-analysis of samples took place for the second dry sampling event because UPS failed to deliver the original samples on time.
- Evaluation and amendment of sampling procedures for protozoa samples after the first dry sampling event to increase the sample volume to 20 L, instead of 10 L as originally planned.

- The first wet weather MS sample collected at RAPS on 10 June 2006 was not used because only 10 L of sample was collected. The correct volume of MS sample (20 L) was collected at RAPS during the 3 August 2007 sampling event.
- Flagging the results of certain bacteria samples as "estimated" because they were based on a number of colonies outside the ideal or preferred range. However, the uncertainty of the results in the risk assessment is acceptable and the flagged results are usable.

Data Reduction, Validation, and Reporting

Reduction of analytical results was done by reviewing the calculations recorded on analytical data sheets. The laboratory QA manager verified that the appropriate analytical methods were followed and the data were calculated properly. The laboratory QA Managers validated the data by comparing the raw data to the reported results. In addition, the results of calibration and internal QA/QC checks were compared with the project acceptance criteria to assess the usefulness of the data.

The dry and wet weather analytical reports of HML, CEC and University of Arizona for both dry and wet weather sampling are included in Appendices: B-1, B-2; C-1, C-2; and D-1 and D-2, respectively. The laboratory analytical reports contain the following information:

- raw data, including results of calibration and internal QC checks;
- analytical data results;
- units of measurement;
- client and sample identification;
- sample analysis dates;
- summary of any problems encountered;
- QC data (MS, blanks, OPRs); and
- QA reviewer's signature

2.5 References

Center for Disease Control (CDC), Microbial Contaminant Candidate List (www.epa.gov/safewater/ccl/ccl2.html#microbial)

EPA, Undated, Microbial Contaminant Candidate List (www.epa.gov/safewater/ccl/ccl2.html#microbial)

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EPA, 2002, Method 1103.1: *Escherichia coli* (*E. coli*) in Water Membrane Filtration Using membrane-Thermotolerant *Escherichia coli* Agar (mTEC), EPA-821-R-2-020. September.

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EPA, 2003a, Implementation Guidance for Ambient Water Quality Criteria for Bacteria, EPA-823-B-03-xxx. November. Draft.

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Geosyntec, 2006, Wet Weather Sampling Plan and Analysis and Quality Assurance Project Plan for the Chicago Area Waterway System, May.

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Standard Methods for the Examination of Water and Wastewater, 1998, 20th Edition. Method 9222D. Fecal Coliform Membrane Filter Procedure; Method 9213E. Membrane Filter Technique for *Pseudomonas aeruginosa*; Method 9260B. General Quantitative Isolation and Identification Procedures for *Salmonella*; Method 9260D. Quantitative *Salmonella* Procedures.

Water Environment Research Foundation (WERF), 2004. Evaluation of Microbial Risk Assessment Techniques and Applications.

World Health Organization (WHO), 1993, Guidelines for Drinking Water Quality, Second Edition, Volume 1 Recommendations.

SECTION 2

TABLES

Table 2-1. Major Waterborne Pathogenic Microorganisms Selected for the Microbial Risk Assessment

Pathogen	*Major Reservoir*	*Disease (see Note)*
Bacteria		
E. coli	Human/animal feces	Gastroenteritis
Salmonella	Human/animal feces	Typhoid, Paratyphoid fever, Salmonellosis
Pseudomonas	Water/wastewater/soil	Otitis externa and infections of open skin wounds
Virus		
Adenoviruses	Human feces	Gastroenteritis, pharyngitis, eye and nose infections
Enteroviruses	Human feces	Gastroenteritis, meningitis, rash, febrile illness, respiratory infections
Calicivirus	Human feces	Gastroenteritis
Protozoa		
Giardia	Human/animal feces	Giardiasis
Cryptosporidium	Human/animal feces	Cryptosporidiasis

Note:

The information presented in the table was obtained from the following sources:

Center for Disease Control (CDC), Microbial Contaminant Candidate List

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., and Tauze, R.V. (1999). Food Related Illness and Death in the U.S. Emerg. Infect. Dis. (5)5, 607-625.

World Health Organization (WHO), 1993. Guidelines for drinking Water Quality, Second Edition, Volume 1 recommendations

Table 2-2. Summary of Dry and Wet Weather Samples

WRP	Upstream samples/event	Downstream samples/event	Pumping Station samples/event	No. of Events	Outfall	Total No. of Samples
DRY WEATHER						
Stickney	2	2	0	5	5	25
Calumet	2	2	0	5	5	25
North Side	2	2	0	5	5	25
			Total Number Of Dry Weather Samples			**75**
WET WEATHER						
Stickney	2	2	1	3	1	16
Calumet	1	4	0	3	3	18
North Side	1	3	1	3	1	16
			Total Number Of Wet Weather Samples			**50**

Table 2-3. Summary of Dry and Wet Weather WRP Flows (MGD) and Pumping Station Discharge Volumes (MG) Provided by MWRDGC

Dry Weather Sampling Date	Dry WRP Flow (MGD)	Wet Weather Sampling Date	Pumping Station Discharge Volume (MG)	Wet WRP Flow (MGD)
North Side				
7/28/2005	210	6/26/2006	33[1]	397
8/4/2005	226	8/3/2006	115[2]	386
8/18/2005	270	9/23/2006	No Pumping Station Discharge	388
8/25/2005	219			
9/1/2005	201			
Stickney				
8/1/2005	544	6/10/2006	238[3]	1261
8/3/2005	627	8/3/2006	655[4]	1160
8/17/2005	566	10/11/2006	No Pumping Station Discharge	939
8/24/2005	659			
8/31/2005	447			
Calumet				
7/26/2005	221	8/24/2006	No Pumping Station Discharge	294
8/2/2005	157	8/29/2006	37[5]	473
8/16/2005	159	10/17/2006	No Pumping Station Discharge	461
8/23/2005	178			
8/30/2005	164			

Notes:

1. The pumping station discharged 33 MG in 2 hours and 45 minutes
2. The pumping station discharged 115 MG in 11 hours and 15 minutes (between 2 and 3 August 2006)
3. The pumping station discharged 238 MG in 7 hours and 25 minutes
4. The pumping station discharged 655 MG in 14 hours and 55 minutes (between 2 and 3 August 2006)
5. The pumping station discharged 37 MG in 3 hours and 23 minutes

SECTION 2

FIGURES

FIGURE 2-1

CHICAGO WATERWAY SYSTEM - DRY WEATHER SAMPLING LOCATIONS

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

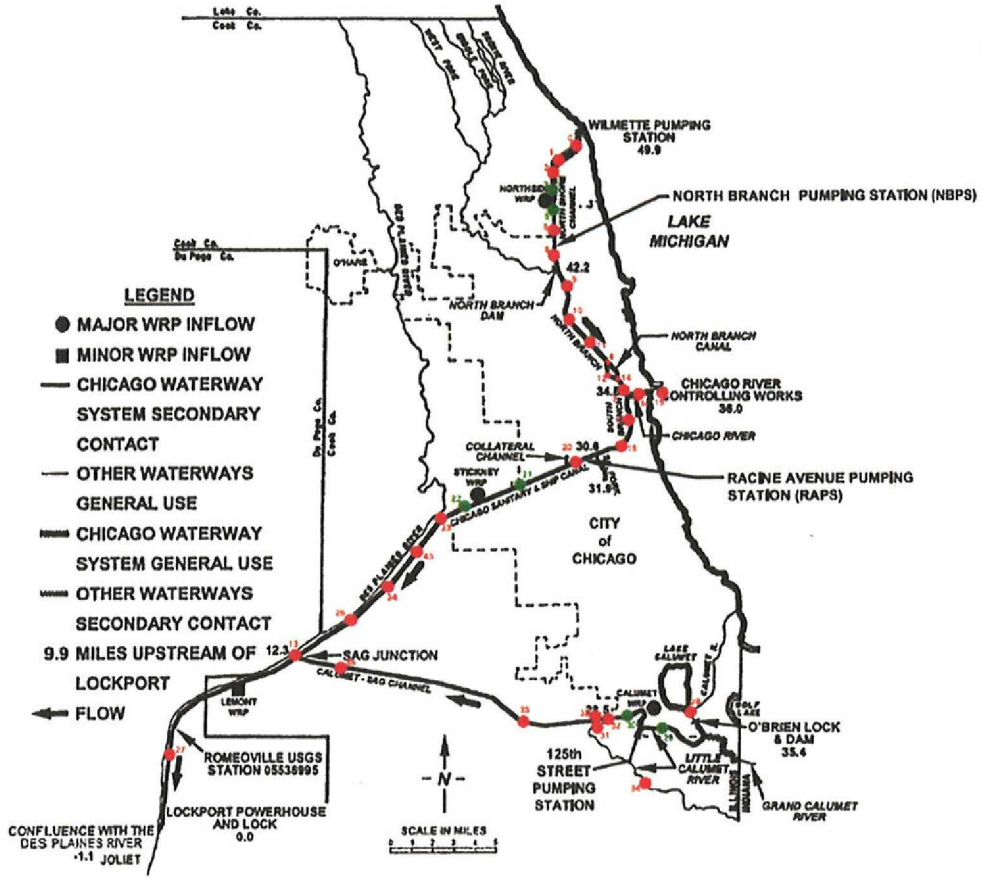


FIGURE 2-2

CHICAGO WATERWAY SYSTEM - WET WEATHER SAMPLING LOCATIONS

METROPOLITAN WATER RECLAMATION DISTRICT OF GREATER CHICAGO

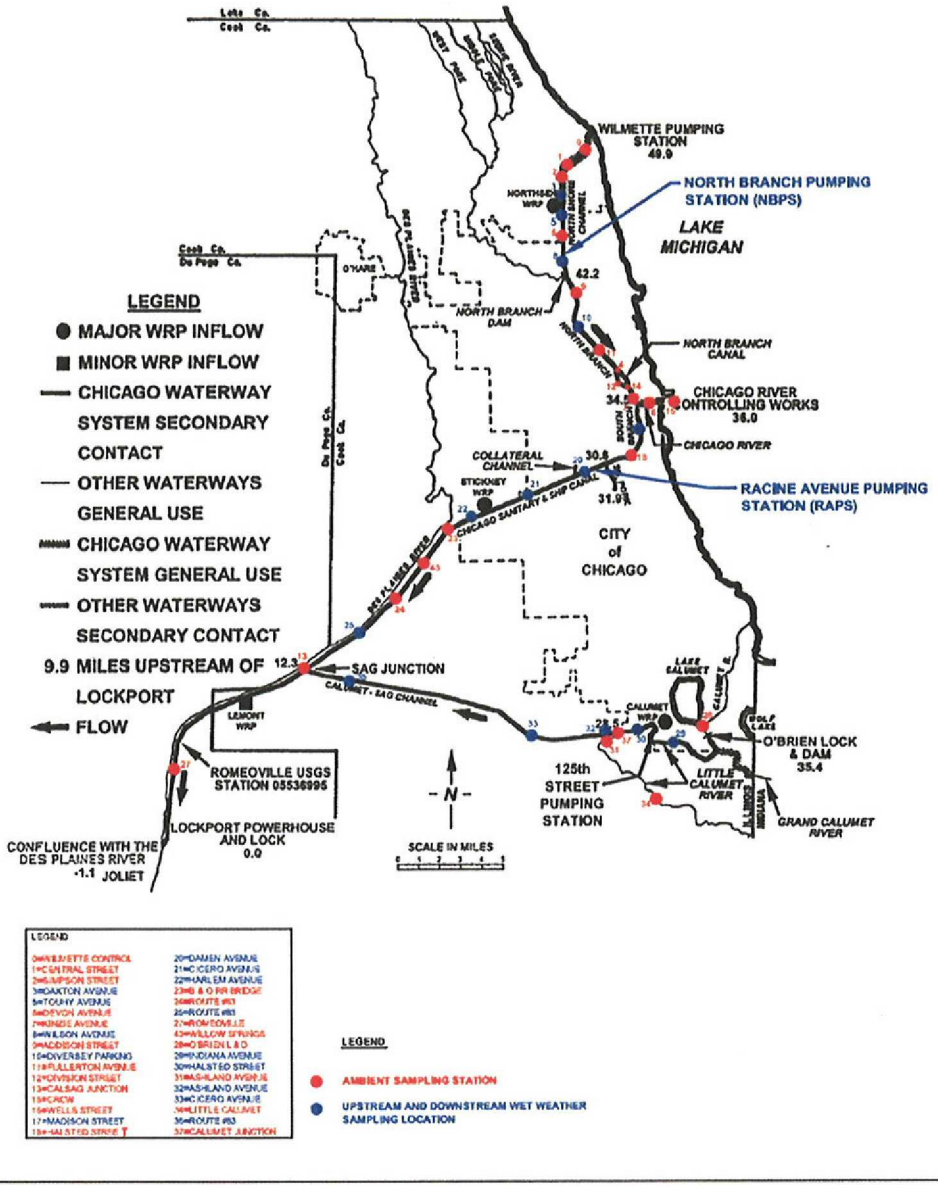
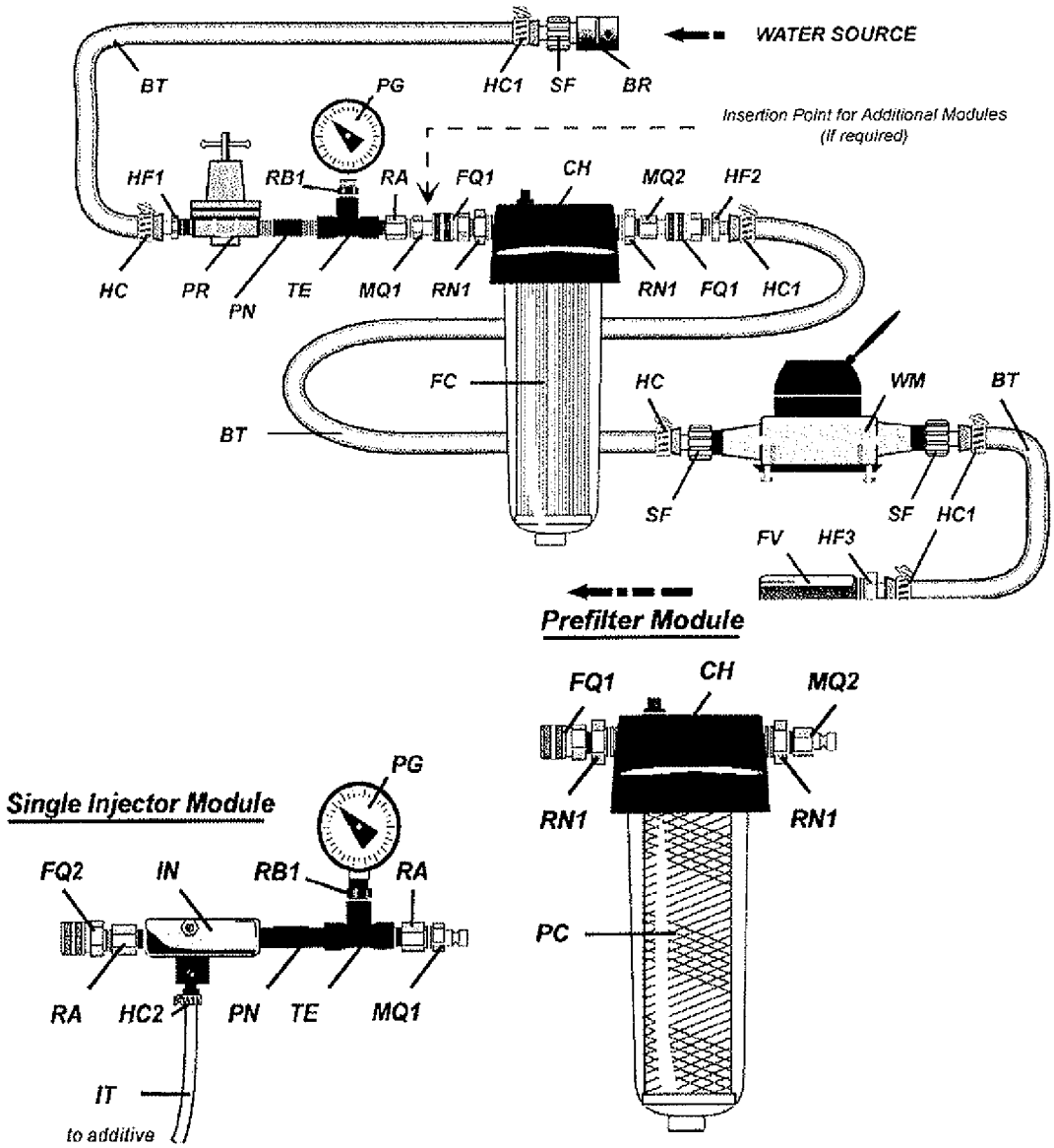


Figure 2-3. Typical Filter Apparatus



BR — Backflow Regulator
SF — Swivel Female
BT — Braided Tubing
HC — Hose Clamps
HFI — Hose Fitting
PR — Pressure Regulator
PN — PVC Nipple
TE — PVC TEE
RB — Reducing Bushing
PG — Pressure Gauge
RA — Reducing Adaptor
MQI — Male Quick Connects
FQ1 — Female Quick Connects
RN1 — Reducing Nipples
CH — Cartridge Housing
FC — Filter Cartridge
MQ2 — Male Quick Connects
HF2 — Hose Fitting
WM — Water Meter
HF3 — Hose Fitting
FV — Flow Control Valve
PC—Prefilter Cartridge

3. ANALYTICAL RESULTS

Five (5) dry weather samples were collected at each designated location upstream, downstream and at the outfall of each of the North Side, Stickney, and Calumet WRPs between 28 July and 1 September 2005. Three (3) wet weather samples were collected at each designated location upstream and downstream of each of the North Side, Stickney, and Calumet WRPs between 10 June and 17 October 2006. In addition, three (3) wet weather outfall samples were collected at the Calumet WRP and one (1) wet weather sample was collected at each of the North Side and Stickney WRPs. Section 2 discusses in detail the sampling locations at each WRP.

During dry weather, both surface and 1-meter depth samples were taken at the upstream and downstream monitoring locations. During wet weather, all samples were collected near the surface of the waterway. The samples were analyzed for three major groups of indicator and pathogenic microorganisms including bacteria, protozoa, and viruses. The dry and wet weather laboratory reports summarizing the analytical results are included in the following Appendices:

- Appendices B-1 and B-2 include the HML reports documenting the results of bacteria and total enteric viruses for dry and wet weather, respectively.
- Appendices C-1 and C-2 include the CEC reports documenting the results of protozoa (*Cryptosporidium* and *Giardia*) for dry and wet weather, respectively.
- Appendices D-1 and D-2 include the UA reports documenting the results of *Calicivirus* and adenovirus for dry and wet weather, respectively.

3.1 Bacteria Results

Bacteria samples were analyzed for the following microorganisms:

- *Enterococci*
- *Escherichia coli*
- Fecal coliforms
- *Pseudomonas aeruginosa*
- *Salmonella* spp.

Bacteria were the most abundant microbial species detected in the waterway compared to viruses and protozoa during both dry and wet weather events. A summary of the dry weather analytical results is presented in Tables 3-1a through 3-1c for the North Side, Stickney, and Calumet WRPs, respectively. A summary of the wet weather analytical results is presented in Tables 3-1d through 3-1f for the North Side, Stickney, and Calumet WRPs, respectively. The results were analyzed and evaluated statistically using the Minitab computing software and the procedures in Helsel and Hirsch (2002) and Helsel (2005).

3.1.1 Analysis of Variance (ANOVA)

During dry weather, at each upstream (UPS) and downstream (DNS) monitoring location, two samples were collected, one at the surface and another at 1-m depth. At each effluent location, only one composited sample per event was collected. The purpose of collecting upstream and downstream sample data at two different depths was to determine if pathogen concentrations varied significantly over the channel's vertical cross-section, as would be the case if the WRPs' effluent plumes did not achieve complete downstream mixing. An Analysis of Variance (ANOVA) analysis was conducted to evaluate this question.

For dry weather, histograms were developed for *Enterococcus, E. coli* and fecal coliform only, since these parameters had the greatest frequency of detection. These histograms are shown in Figures 3-1 through 3-3 for the North Side, Stickney, and Calumet WRPs (note the log scale on the y-axis). Nine separate charts (three locations [UPS, DNS and OUTFALL] and three bacteria parameters for each location [*E. coli, Enterococcus* and fecal coliform]) are provided for each WRP. Each histogram shows the concentration of bacteria vs. the sampling date. For each instream monitoring location, two sample (surface and 1-m depth) results are shown for each sample date.

ANOVA tests were performed for the dry weather results to determine differences of bacteria concentrations by site (i.e., North Side, Stickney, and Calumet), by location (i.e., UPS and DNS), and by depth (i.e., surface and 1-m depth). This analysis was only conducted on *E. coli*, fecal coliform, and *Enterococcus* data as these groups had the most

statistically significant (by percent detect) datasets. *E. coli,* fecal coliform, and *Enterococcus* were detected at a frequency ranging from 99 to 100%, while *Pseudomonas aeruginosa* was detected in 75% of the samples and *Salmonella* spp. in only 13% of the samples. Each factor (site, location, and depth) was tested to see if it was a cause of statistically significant differences in bacteria concentrations, alone or in combination with these factors. As such, a total of seven statistics were tested for the null hypothesis that pathogen concentrations are not statistically different at a significance level of 5%. The results of the ANOVA analysis are shown on Figures 3-4 to 3-6 for dry weather *E. coli*, fecal coliform, and *Enterococcus,* respectively.

The dry weather results obtained are consistent for all bacteria groups in that there is a significant difference between concentrations by site (North Side, Stickney and Calumet), and by location (UPS and DNS). This finding is consistent with a physical understanding of the waterway system, that different sites have varying loading and dilution conditions which results in varying concentrations, and that bacteria concentrations will generally increase downstream of the WRP outfalls compared to the upstream locations.

All bacteria groups in dry weather samples also showed no statistically significant difference in concentration by depth. That is, based on the dry weather results for each microbial group, depth does not appear to be a significant factor, either alone or in combination with the other factors (site and location). This finding is consistent with the understanding that upstream and downstream monitoring locations are well mixed vertically. These conclusions are based on the high (i.e., >1) F (indicator of variability) values and the low (i.e., <0.05) P (probability of statistical significance) values for the site (WRP), location (UPS, DNS, OUTFALL), and site and location (in combination) factors.

The charts of dry weather bacteria concentrations versus site, location, and depth (see Figures 3-4 to 3-6) also graphically demonstrate the significance of the first two factors, but not the last. For instance, downstream concentrations at North Side are generally greater than Stickney, which are greater than Calumet. Also, downstream concentrations are consistently greater than upstream (consistent with our previous findings). However,

surface concentrations are not consistently greater or lower than 1-m depth concentrations.

The results of the wet weather data ANOVA analysis are shown on Figures 3-7 to 3-11 for *E. coli*, fecal coliform, *Enterococcus*, *P. aeruginosa* and *Salmonella* spp., respectively. During wet weather sampling no samples were collected at 1-meter depth. Wet weather *E. coli* and *Enterococcus* data are significantly different by site (i.e. North Side, Stickney and Calumet waterway) only. Fecal coliform, *P. aeruginosa* and *Salmonella* spp. do not differ by site or any other factor. Unlike the dry weather bacteria data, the wet weather bacteria data do not differ by location (UPS vs. DNS).

The results of the dry and wet weather ANOVA analysis are shown on Figures 3-12 to 3-15 for *E. coli*, fecal coliform, *Enterococcus* and, *P. aeruginosa*, respectively. Although an ANOVA was not performed on the *P. aeruginosa* dry weather data due to the limited number of detections, the additional data in the wet weather sampling allows us to pool the data to evaluate the factors of interest (e.g. site, weather). For this analysis the non-detects were replaced with fixed detection limit values which may affect the variance estimates. Statistical estimates may be biased in cases where an ANOVA is conducted with highly censored datasets. Dry and wet weather combined bacteria data (*E. coli*, *Enterococcus*, *P. aeruginosa*) are significantly different by site (i.e. North Side, Stickney and Calumet waterway) and weather (dry and wet). Fecal coliform differs by weather only (not by site). The *Salmonella* spp. dry weather results had statistically insignificant detections and therefore an ANOVA analysis of both the dry and wet weather results was not performed. In summary, Figures 3-12 through 3-15 illustrate that unlike the dry weather data, the combined dry and wet weather bacteria do not differ by location (UPS vs. DNS).

Attachment A summarizes correlations between indicator bacteria levels and pathogens under dry weather and wet weather conditions at the CWS. Recent studies indicate that there is a poor correlation between indicator bacteria levels and levels of human pathogenic bacteria, viruses and protozoa (Noble *et al.*, 2006; Noble and Fuhrman *et al.*, 2001; Hardwood *et al.*, 2005; Jiang *et al.*, 2001, and Hörman *et al.*, 2004). The

Geosyntec Team is not aware of any published results in the technical review literature that indicate statistically significant correlations between indicator bacteria and protozoa or virus pathogens.

3.1.2 Geometric Means

Table 3-2a summarizes the dry weather bacteria geometric mean concentrations at different locations. Figures 3-16, 3-17 and 3-18 show the geometric mean results graphically for North Side, Stickney and Calumet, respectively. The geometric mean values for the censored datasets (i.e., datasets containing below detection results) were computed using a maximum likelihood method. Bacteria concentration data with censoring greater than 80% are considered statistically insignificant, and therefore no geometric mean values were computed (see results for *Salmonella* spp.) (Helsel, 2005). These tabulated results confirm that the dry weather microbial concentrations tend to increase immediately downstream of the WRPs. The results in Table 3-2a also indicate that the fecal coliform concentrations upstream of the North Side and Stickney WRPs were greater than the IEPA proposed effluent limit of 400 CFU/100 mL.

Table 3-2b summarizes the wet weather bacteria geometric mean concentrations at different locations. Figure 3-19 is a graphical presentation of the wet weather geometric means at each sampling location (UPS, DNS, OUTFALL) at the North Side, Stickney and Calumet WRPs. The wet weather results indicate that most of the North Side and Stickney geometric mean bacteria concentrations upstream and downstream of the WRPs are higher than the outfall concentrations. Also, the wet weather concentrations at Stickney and North Side are greater than Calumet. Fecal coliform and *E. coli* wet weather concentrations are greater than the other bacteria geometric means at each sampling location at all WRPs. The results in Table 3-2b also indicate that the wet weather fecal coliform concentrations upstream of the North Side, Stickney and Calumet WRPs were above the IEPA proposed effluent limit of 400 CFU/100 mL.

Figure 3-20 presents a comparison between dry and wet weather geometric mean concentrations (including OUTFALL, UPS and DNS locations) at each WRP. The figure indicates that the wet weather concentrations are significantly greater than the dry

weather concentrations at each WRP waterway. The most significant differences are observed at the North Side and Stickney waterways. In addition, the following observations can be made regarding the geometric mean results in Figure 3-20:

- The geometric mean concentrations of *Salmonella* spp. were low in both dry and wet weather conditions. The *Salmonella* spp. concentrations in the UPS and DNS samples were similar during wet weather conditions at the North Side, Stickney, and Calumet segments of the waterway.
- The *enterococci* concentration was lower than *E. coli* and fecal coliform concentrations under wet weather conditions.
- *P. aeruginosa* wet weather concentrations were slightly higher than the dry weather levels. However, the effluent samples show lower levels of *P. aeruginosa* than the corresponding upstream and downstream wet weather samples.

3.1.3 Percentile Box Plots

Semi-log box plots were created to graphically demonstrate the central tendencies and variability of the various bacteria datasets. Each box indicates the 25th, 50th, and 75th percentile values. The spatial (UPS, DNS, Outfall) percentile box plots for the dry weather results are shown in Figures 3-21 through 3-23. No box plots were prepared for dry weather *Salmonella* results as most of these datasets were statistically insignificant (i.e., non-detect frequency >80%). For dry weather results, the box plots again show concentrations increasing downstream, except for *P. aeruginosa* at Stickney and Calumet, and *Enterococcus* at Calumet. *P. aeruginosa* percentile results are highly influenced by non-detect results, therefore downstream increases can not be seen in these box plots; geometric mean values (generated using the maximum likelihood method) are better indicators of this trend for significantly censored datasets.

For dry weather results, the box plots demonstrate a modest spread of the concentration data around the median (around 1 log between the 1st and 3rd quartiles), as well as the occasionally significant skewedness (in log space) of these results (as indicated by the relative box and whisker heights above and below the median values). Moreover, all the box plots consistently show that downstream concentrations exhibit less variability than upstream concentrations.

An examination of the spatial variability of the wet weather data did not reveal any discernable trends. Therefore, the box plots were used to evaluate any temporal trends that may be attributable to the different weather conditions and the occurrence or non-occurrence of discharges from the pumping stations. The percentile temporal box plots for the wet weather results are shown in Figures 3-24 through 3-26. These figures illustrate the central tendencies and variabilities at the various bacteria data sets as a function of time. Each box indicates the 25th, 50th and 75th percentile values of the logarithmic bacteria concentrations at each WRP (including UPS, DNS, and Outfall concentrations).

The plots indicate that the occurrence of pumping station discharges resulted in elevated concentrations of bacteria in the Stickney and Calumet waterway, except for *Salmonella*. The occurrence of pumping station discharges took place on 10 June 2006 and 3 August 2006 at RAPS, near the Stickney WRP and on 29 August 2006 at the 125th Street Pumping Station near the Calumet WRP. The NBPS discharged on 26 June 2006 and 3 August 2006, but not on 23 September 2006. The large variability of the North Side bacteria results is probably masking the effect of the pumping station discharge.

3.2 Protozoa Analytical Results

Dry and wet weather samples were analyzed for the presence of *Cryptosporidium* oocysts and *Giardia* cysts using EPA Method 1623 or a modified version for wastewater samples. In addition, a portion of each sample was analyzed for the presence of infectious oocysts and viable cysts using cell culture techniques and vital dyes, respectively. The following sections discuss enumeration and viability results for *Cryptosporidium* and *Giardia*.

3.2.1 Enumeration Results

Dry weather enumeration results from samples collected at the North Side facility are presented in Table 3-3a. *Giardia* cysts (cysts) were detected in all outfall samples with concentrations ranging from 0.6 to 4.6/L. Cysts were detected in all downstream samples with the exception of those collected 8/18/05. Cyst concentrations in the downstream samples ranged from 0.3 to 3/L. Cysts were detected in four (4) of 10 upstream samples

at concentrations ranging from 0.2 to 3.6/L. *Cryptosporidium* oocysts (oocysts) were detected in three (3) of five (5) outfall samples, one (1) of 10 upstream samples and six (6) of 10 downstream samples. Oocyst concentrations ranged from 0.1 to 1.0/L in downstream samples where they were detected.

Dry weather enumeration results for samples collected at the Stickney plant are presented in Table 3-3b. Cysts were detected in all outfall samples analyzed from the Stickney plant with concentrations ranging from 0.4 to 4.9/L. Cysts were not detected in the upstream samples collected on 8/1/05. Cysts were detected in the upstream samples collected in the last four sampling events at concentrations ranging from 0.1 to 0.3/L when detected. Cyst concentrations in the downstream samples ranged from 0.2 to 1.1/L when detected. Cysts were not detected in two (2) of 10 downstream samples analyzed. Cysts were detected in all samples (upstream, downstream and outfall) collected at the Stickney plant on 8/24/05. *Cryptosporidium* oocysts were detected in three (3) of five (5) outfall samples analyzed at concentrations ranging from 0.1 to 0.6/L. Oocysts were detected in only one upstream sample (of 10 analyzed) at 0.3 oocysts/L, and in three (3) of 10 downstream samples analyzed at concentrations ranging from 0.2 to 0.5 oocysts/L.

Dry weather enumeration results for samples collected at the Calumet waterway and outfall are presented in Table 3-3c. *Giardia* cysts were detected in four (4) of five (5) outfall samples collected at the Calumet WRP. Where cysts were detected, the concentrations ranged from 0.6 to 2.2/L in the outfall samples. Cysts were not detected in any of the upstream samples. In downstream samples cyst concentrations ranged from 0.3 to 0.6 cysts/L, when detected. *Cryptosporidium* oocysts were detected in one (1) of five (5) outfall samples at a concentration of 0.4 oocysts/L. Oocysts were not detected in any of the samples collected in the first three sampling rounds. No oocysts were detected in the upstream samples collected on 8/23/05, but were present in the downstream samples collected that day at a concentration of 0.2 oocysts/L. For samples collected on 8/30/05, oocysts were detected in the upstream surface and in both (surface and 1-meter depth) downstream samples. Oocyst concentrations in these samples ranged from 0.3 to 0.5 oocysts/L. No oocysts or cysts were detected in the samples received that exhibited signs of freezing (collected on 8/2/05).

Wet weather enumeration results from samples collected at the North Side designated locations are presented in Table 3-3d. The results indicate that the concentrations of *Cryptosporidium* oocysts ranged from <0.2 to 1.6 oocysts/L. The MS sample at this location contained *Cryptosporidium* oocysts ranging from 0.8 to 3 oocysts/L. The concentrations of *Giardia* cysts ranged from <0.3 to 49.5 cysts/L. The MS sample at this location contained *Giardia* cysts ranging from 5.3 to 48.9 cysts /L. Sections 2.3.2.3 and 2.4.3.1 provide details on the analysis of the MS samples.

Wet weather enumeration results from samples collected at the Stickney designated locations are presented in Table 3-3e. The results indicate that the concentrations of *Cryptosporidium* oocysts ranged from <0.2 to 0.8 oocysts/L. The MS sample at this location contained *Cryptosporidium* oocysts ranging from 3 to 25 oocysts/L. The concentrations of *Giardia* cysts ranged from <0.2 to 5.4 cysts/L. The MS sample at this location contained *Giardia* cysts ranging from 7 to 53 cysts/L. Sections 2.3.2.3 and 2.4.3.1 provide details on the analysis of the MS samples.

Wet weather enumeration results from samples collected at the Calumet designated locations are presented in Table 3-3f. The results indicate that the concentrations of *Cryptosporidium* oocysts ranged from <0.2 to 6.3 oocysts/L. No MS sample was collected at the Calumet waterway. The concentrations of *Giardia* cysts ranged from <0.2 to 8.5 cysts/L.

Overall, the concentrations of *Cryptosporidium* oocysts and *Giardia* cysts were greater during wet weather compared to dry weather sampling. Also, the frequency of detection was greater.

3.2.2 Detection of Infectious *Cryptosporidium* Oocysts Using Cell Culture

This section describes the procedure that was used to determine infectious *Cryptosporidium* oocysts in the samples collected in this study. Control *Cryptosporidium parvum (C. parvum)* oocysts obtained from Waterborne, Inc. were inoculated to confluent monolayers of human ileocaecal adenocarcinoma (HCT-8) cells at concentrations ranging from 0 to approximately 10^4 oocysts. The oocyst age at the time of inoculation ranged

from 3 to 40 days old (post shedding) and demonstrated infection rates starting at 3.2% and dropping to 0.6% as the oocysts aged in the positive controls analyzed. It has been reported that freshly purified oocysts inoculated to monolayers of HCT8 cells routinely demonstrate infection rates of less than 10% when fresh (< 1 week) and decline rapidly within 1 month of age (Rochelle et al., 2001). Method blanks and heat-inactivated controls yielded no infections. One to two infectious foci were detected in three (3) of four (4) seeded OPR samples and two (2) of four (4) seeded MS samples. The theoretical number of *Cryptosporidium* oocysts applied to monolayers for these samples ranged from 160 to 172 oocysts, and based on infection rates obtained in these trials one would expect to find 0 to 5 infectious foci. For dry weather samples, no infectious oocysts were detected in the portions of each unseeded sample analyzed.

Similarly, for wet weather samples, no infectious *Cryptosporidium* oocysts were detected in the field samples analyzed with one exception: Calumet-DNS-WW-58-082406 had 1 infectious foci. Also, a total of 3 infectious foci were detected in the 26 June 2006 MS sample from the North Side (North Side-DNS-WW-37-062606-MS). Five (5) subsamples of the MS sample were analyzed. Only two (2) of the five (5) subsamples contained infectious oocysts; one subsample contained two (2) and the other contained one (1) infectious oocyst. However, none of the samples collected at the North Side waterway on the same date contained infectious oocysts.

Overall, the combined wet and dry weather percentage of infectious foci is estimated to be approximately 2.4% (3 of 125 samples [75 dry weather and 50 wet weather samples] contained foci).

3.2.3 *Giardia* Viability Results

The inclusion, or exclusion, of the fluorogenic dyes in these protozoa may indicate the integrity of the cell wall and therefore, its viability. Inclusion of propidium iodide (PI) in *Giardia muris* cysts was reported by Schupp and Erlandsen (1987) to indicate non-viable cysts. To demonstrate the cysts were not viable, 14 to 21 day old mice were infected with PI positive cysts at levels of 5×10^3 cysts per mouse and 5×10^4 cysts per mouse. After 11 days no infections were noted in the animals. Conversely, cysts that were fluorescein

diacetate (FDA) positive were capable of causing *Giardia*sis in 100% of the mice infected at seeding levels of 1 x 10^3 cysts per mouse. Smith and Smith (1989) reported that the FDA consistently overestimated cyst viability in human isolates of *Giardia intestinalis* while PI under-estimated non-viable cysts when compared to *in vitro* excystation. One of the human isolates could not be stained with either FDA or PI. The authors did conclude that PI could be used to determine the lower limit of non-viability in environmental samples where low numbers of cysts are expected.

Thiriat *et al.* (1998) reported using 4',6'-diamidino-2-phenylindole (DAPI)/PI to assess viability of cysts recovered in *Giardia* positive stool samples from humans and sewage. When the authors compared FDA/PI, DAPI/PI and eosin exclusion, the FDA/PI and eosin exclusion procedures seemed to over-estimate cyst viability. These findings are similar to those reported by Smith and Smith (1989) and Kasprzak and Majewska (1983), respectively. CEC used the DAPI/PI method for determining cyst viability for these environmental samples.

Giardia cysts were detected using FITC-mAb and were then examined for DAPI characteristics and were scored as DAPI positive or negative (see the CEC reports in Appendices C-1 and C-2). DAPI positive *Giardia* cysts may contain 0 to 4 sky blue nuclei or diffuse staining of the nuclei or cytoplasmic staining, while cysts exhibiting no internal staining are scored as DAPI negative. Cysts were then examined for inclusion of PI and were scored as PI positive or PI negative. Internal morphology of each cyst was examined using Normarski optics. Cysts exhibiting good morphology had a smooth appearance and were refractive and the cytoplasm had not pulled away from the cell wall. Internal features such as axonemes, median bodies, ventral disks or nuclei may be discernable in these organisms. Cysts exhibiting poor morphology were slightly to very grainy in appearance or the contents of the cell were shrunken and pulled away from the cell wall. Internal structures were sometime evident in these organisms. Cysts scored as empty exhibited excellent fluorescence with FITC-mAb, were DAPI negative, and had no internal cell contents. However, the thickness of the cell wall was examined to make a determination of identification. Most algal cells have much thicker cell walls and are easily ruled out as being *Giardia* cysts.

Also, PI staining is not a consistent measure of cyst viability. Sauch *et al.* (1991), state that the PI procedure is not satisfactory for determining viability of *Giardia muris* cysts. In addition, it must be noted that it is common to observe empty cysts that do not take up the PI stain. The method for determination of viability of *Giardia* cysts has not been validated, therefore the results must be considered as a further characterization of *Giardia* by this staining method.

For dry weather, most *Giardia* cysts found in the samples at all sites were PI positive indicating non-viability. Outfall samples at the North Side (see Table 3-4a) and Stickney (see Table 3-4b) WRPs contained a higher level of viable cysts compared to Calumet (see Table 3-4c). Viable cysts were also found in downstream samples at the North Side (see Table 3-4a) and Stickney (see Table 3-4b) waterways. While levels of potentially viable *Giardia* cysts may pose a public health risk, it is important to note that not all viable organisms are capable of causing infection.

The average dry weather percentage of viable *Giardia* cysts found in each waterway segment, including outfall and in-stream concentrations, is provided below:

- Calumet: *Giardia* viability=10%
- Stickney: *Giardia* viability=21%
- North Side: *Giardia* viability=26%

The average dry weather percentage of viable *Giardia* cysts found in the outfall only of each WRP is provided below:

- Calumet Outfall: *Giardia* viability=10%
- Stickney Outfall: *Giardia* viability=47%
- North Side Outfall: *Giardia* viability=51%

Wet weather samples contained viable *Giardia* cysts at each waterway (see Tables 3-4d through 3-4f). Viable cysts were also found in upstream samples at North Side (see Table 3-4d) and Stickney (see Table 3-4e) WRPs.

The average wet weather percentage of viable *Giardia* cysts found in each waterway segment, including outfall and in-stream concentrations, are provided below:

- Calumet: *Giardia* viability=10%
- Stickney: *Giardia* viability=47%
- North Side: *Giardia* viability=49%

The average wet weather percentage of viable *Giardia* cysts found in the outfall only of each WRP is provided below:

- Calumet Outfall: *Giardia* viability=10%
- Stickney Outfall: *Giardia* viability=50%
- North Side Outfall: *Giardia* viability=42%

These results indicate that the Calumet waterway under both dry and wet weather contained the smallest percentage (10%) of viable *Giardia* cysts compared to Stickney and North Side.

3.3 Virus Analytical Results

Enteric virus samples were analyzed for: i) total culturable viruses using the method described in the ICR Microbial Laboratory Manual, EPA 600/R-95/178; and ii) adenovirus and *Calicivirus*. Adenovirus and *Calicivirus were* determined using UA SOPs. There are no published assays for viable *Calicivirus*. The method involves a PCR assay that estimates the virus concentration, but does not determine or confirm viability. The infectivity of the virus cannot be determined by the PCR method. Therefore, the number of genomes in a volume of water was determined using the most probable number (MPN) method. The virus concentration was estimated by recording the presence of the viral genomes, but does not determine or confirm viability. *Calicivirus* is a family of human and animal viruses. For this risk assessment it was assumed that *Calicivirus* refers to human *Caliciviruses*, specifically the genus norovirus.

Adenovirus and norovirus samples were sent as concentrates to the Environmental Virology Laboratory, Department of Soil, Water and Environmental Science at the UA from HML and received by Pat Gundy, laboratory director of cell culture.

Assay on the PCL/PRF/5 cell line was done because adenoviruses will grow in this cell line. Adenoviruses are believed to be more common in sewage than enteroviruses, and have been a cause of recreational waterborne illness. Adenoviruses do not produce cytopathogenic effects (CPE) in the BGM cell line, thus the need to use another cell line to assess their occurrence. Since enteroviruses and other enteric viruses can grow in PCL/PRF/5 cells, PCR was used to confirm the presence of adenoviruses in the cell culture in which CPE was observed.

Norovirus detection was done by RT-PCR (reverse transcriptase polymerase chain reaction) since it is an RNA virus. Adenovirus is a DNA virus so only PCR is needed for its detection. While PCR cannot be used to determine the infectivity of the virus, the number of genomes in a volume of water can be estimated by using the most probable number (MPN) method. Generally, the ratio of genomes (virions) to cell culture infectivity units is 1:100 to 1:46,000 (Ward *et al.* 1984; Gerba personal observations).

3.3.1 Enteric Viruses

HML analyzed the culturable enteric virus samples using the EPA (1996) method in EPA/600/4-84/013(014) (see Section 2.4). The laboratory analytical report is included in Appendix B. Tables 3-5a through 3-5c present a summary of the dry weather total enteric virus analytical results for the North Side, Stickney and Calumet WRPs. Tables 3-5d through 3-5f present a summary of the wet weather total enteric virus analytical results for the North Side, Stickney and Calumet WRPs, respectively. Tables 3-9 and 3-10 summarize the percentage of dry and wet weather samples, respectively with virus detections and the range of concentrations detected.

The dry weather results indicate that a relatively small number of samples (17 of 75 samples or 23%) had detectable concentrations of enteric viruses (see Table 3-9). Eight (8) of 25 dry weather samples (29%) upstream, downstream and at the outfall of the

North Side WRP had detectable enteric virus concentrations. The detectable concentrations upstream ranged from 1.04 to 3.25 MPN/100L. The detectable concentrations downstream ranged from 2.12 to 16.07 MPN/100L. The outfall concentrations ranged from 1.72 MPN/100L to 24.73 MPN/100L.

Six (6) of 25 dry weather samples (24%) upstream and downstream of the Stickney WRP had detectable virus concentrations (see Table 3-9). The detectable concentrations upstream ranged from 1.03 to 3.25 MPN/100L. The detectable concentrations downstream ranged from 1.02 to 1.03 MPN/100L. There were no detectable viruses at the outfall.

Only three (3) of 25 dry weather samples (12%), one at each upstream, downstream and outfall location of the Calumet WRP had detectable concentrations of viruses (see Table 3-9). The upstream concentration was 1.04 MPN/100L; the downstream concentration was 1.04 MPN/100L; the outfall concentration was 1.28 MPN/100L.

During the North Side wet weather sampling, 11 of 16 samples (69%) had detectable enteric virus concentrations (see Table 3-10). The detectable concentrations upstream ranged from 1 to 12 MPN/100L. The detectable downstream concentrations ranged from 1 to 28 MPN/100L. Only one (1) wet weather outfall concentration was collected at the North Side WRP that had an enteric virus concentration 1MPN/100L. Due to safety concerns, the discharge of the NBPS was sampled at the nearest downstream location: North Side-DNS-WW-37 and had only one detection of 1 MPN/100L.

During the Stickney wet weather sampling, 14 of 16 samples (88%) had detectable enteric virus concentrations (see Table 3-10). The detectable concentrations upstream ranged from 2 to 28 MPN/100L. The detectable downstream concentrations ranged from 1 to 9 MPN/100L. Only one (1) wet weather outfall sample was collected at the Stickney WRP that had an enteric virus concentration of 10 MPN/100L. All three (3) RAPS samples had detectable concentrations of total enteric viruses ranging between 1 and 63 MPN/100L. The highest concentration of 63 MPN/100L was detected during the 3 August 2006 sampling event when RAPS discharged 655 MG in 14 hours and 55 minutes of operation.

During the Calumet wet weather sampling, 14 of 18 samples (77%) had detectable enteric virus concentrations (see Table 3-10). The detectable concentrations upstream ranged from 1 to 9 MPN/100L. The detectable downstream concentrations ranged from 1 to 85 MPN/100L. Two (2) of the three (3) wet weather outfall samples collected at the Calumet WRP had detectable enteric virus concentrations ranging from 10 to 32 MPN/100L.

Table 3-11 presents a comparison between dry and wet weather percentage of virus sample detections. The results indicate that the percentage of enteric virus detections during wet weather were greater than the dry weather detections. The percentage of enteric virus detections at the North Side waterway segment increased from 29% during dry weather to 69% during wet weather. The percentage of virus detections at the Stickney waterway segment increased from 24% during dry weather to 88% during wet weather. The percentage of enteric virus detections at the Calumet waterway segment increased from 12% during dry weather to 77% during wet weather. In addition, the concentrations detected during wet weather sampling are generally greater than the dry weather concentrations.

3.3.2 Adenovirus

Table 3-6 presents a summary of the culturable virus and adenovirus dry weather analytical results. Table 3-8 summarizes the wet weather culturable virus and adenovirus analytical results.

Of 75 dry weather samples, 42 or 56% demonstrated the presence of detectable virus by assay in the PCL/PRF/5 cell line. Of 42 samples that were cell culture positive, adenoviruses were detected in 31 or about 74% of the samples by PCR. Enteroviruses or other enteric viruses were probably responsible for the observed CPE in the other samples or the CPE of other viruses could have masked the presence of adenoviruses i.e. the other enteric viruses were in higher concentrations.

During the North Side dry weather sampling, 12 of 25 samples (48%) had detectable adenovirus virus concentrations (see Tables 3-6 and 3-9). The detectable concentrations

upstream ranged from 1.5 to 2.94 MPN/100L. The detectable downstream concentrations ranged from 5.03 to 27.6 MPN/100L. The outfall concentrations ranged from 45.1 to 256 MPN/100L.

During the Stickney dry weather sampling, 13 of 25 samples (52%) had detectable adenovirus concentrations (see Tables 3-6 and 3-9). The detectable concentrations upstream ranged from 11 to 117 MPN/100L. The detectable downstream concentrations ranged from 1.39 to 112 MPN/100L. The detectable outfall concentrations ranged from 7.99 to 36.9 MPN/100L.

During the Calumet dry weather sampling, six (6) of 25 samples (24%) had detectable adenovirus concentrations (see Tables 3-6 and 3-9). There were no detectable concentrations upstream of the Calumet WRP. The detectable downstream concentrations ranged from 1.31 MPN/100L to 3.35 MPN/100L. The outfall concentrations ranged from 7.52 to 15.5 MPN/100L.

Of 50 wet weather samples, 42 or 84% demonstrated the presence of infectious virus by assay in the PCL/PRF/5 cell line and had adenoviruses confirmed by PCR. Enteroviruses or other enteric viruses were probably responsible for the observed CPE in the other samples or the CPE of other viruses could have masked the presence of adenoviruses i.e. the other enteric viruses were in higher concentrations.

During the North Side wet weather sampling, 14 of 16 samples (88%) had detectable adenovirus concentrations (see Tables 3-8 and 3-10). The detectable concentrations upstream ranged from 20.7 to 2,890 MPN/100L. The detectable downstream concentrations ranged from 105 to 2,870 MPN/100L. Only one (1) wet weather outfall sample was collected at the North Side WRP that had an adenovirus concentration of 121MPN/100L. Several of the upstream and downstream locations had concentrations greater than the outfall. Due to safety concerns, the discharge of NBPS was sampled at the nearest downstream location: North Side-DNS-WW-37 that had concentrations ranging from 66.7 to 199 MPN/100L.

During the Stickney wet weather sampling, 15 of 16 samples (94%) had detectable adenovirus concentrations (see Tables 3-8 and 3-10). The detectable concentrations upstream ranged from 3.5 to 1,280 MPN/100L. The detectable downstream concentrations ranged from 4.37 to 1,180 MPN/100L. Only one wet weather outfall sample was collected at the Stickney WRP that had an adenovirus concentration 1,308 MPN/100L. All three (3) RAPS samples had detectable concentrations of adenovirus ranging between 49.7 and 1,560 MPN/100L. The highest adenovirus concentration of 1,560 MPN/100L was detected during the 3 August 2006 sampling event when RAPS discharged 655 MG in 14 hours and 55 minutes of operation.

During the Calumet wet weather sampling, 13 of 18 samples (72%) had detectable adenovirus concentrations (see Tables 3-8 and 3-10). There was only one (1) detectable concentration upstream of 14.7 MPN/100L. The detectable downstream concentrations ranged from 6.24 MPN/100L to >3,277 MPN/100L. All three (3) wet weather outfall samples collected at the Calumet WRP had detectable adenovirus concentrations ranging from 10 to 355 MPN/100L.

Table 3-11 presents a comparison between dry and wet weather percentage of virus sample detections. The results indicate that the percentage of adenovirus detections during wet weather were greater than the dry weather detections. The percentage of adenovirus detections at the North Side waterway segment increased from 48% during dry weather to 87.5% during wet weather. The percentage of adenovirus detections at the Stickney waterway segment increased from 52% during dry weather to 94% during wet weather. The percentage of adenovirus detections at the Calumet waterway segment increased from 24% during dry weather to 72% during wet weather. In addition, the concentrations detected during wet weather sampling are generally greater than the dry weather concentrations.

3.3.3 *Calicivirus* (Norovirus)

In the absence of cell culture methods, the norovirus concentrations were estimated by the RT-PCR method. However, several limiting factors need to be considered in the use of RT-PCR results. First, the detection of viral genomes in water by standard RT-PCR

methods does not provide information about the infectivity of the viruses in question, which impedes a meaningful health risk evaluation when high-virus concentrations are obtained in samples. Second, the high sensitivity of RT-PCR for routine monitoring of norovirus has not been validated and standardized to demonstrate the reliability, sensitivity, and accuracy of the technique.

Table 3-7 presents a summary of the dry weather *Calicivirus* or norovirus analytical results. Table 3-8 summarizes the wet weather *Calicivirus* or norovirus analytical results. During dry weather, norovirus was only detected in 5 samples or about 7% of the 75 samples. During the North Side dry weather sampling, only one outfall sample (1 of 25 samples [4%]) had a detectable norovirus concentration of 35,000 PCR MPN/100L (see Tables 3-7 and 3-9). The greatest concentration was observed in an outfall sample at the North Side WRP (North Side Outfall-80405). The greater concentration of *Calicivirus* or norovirus observed in this sample may be due to the fact that only duplicates per dilution in the MPN assay could be performed because of reassay difficulties reducing the precision of this analysis. In addition, of the five norovirus samples with MPN assays, this sample was the only one that had a positive result in the highest dilution. The combination of these factors could have resulted in the relatively high MPN value of this sample. Therefore, the high *Calicivirus* concentration in the subject sample is likely an artifact of these factors and it appears to be an outlier.

During the Stickney dry weather sampling, three (3) of 25 samples (12%) had detectable norovirus concentrations (see Tables 3-7 and 3-9). The detectable concentrations upstream ranged from 181 to 511 PCR MPN/100L. There was only one (1) detectable downstream concentration of 176 PCR MPN/100L. During the dry weather sampling, the Stickney WRP outfall did not have any detectable norovirus concentrations.

During the Calumet dry weather sampling, only one (1) outfall sample (one [1] of 25 samples [4%]) had a detectable norovirus concentration of 781 PCR MPN/100L (see Tables 3-7 and 3-9). Norovirus infection is most common in the winter and that may explain the low concentration of norovirus observed in this study (Gerba, 2006).

During wet weather, *Calicivirus* or norovirus were only detected in 20 samples or 40% of the 50 samples. The greatest concentration of norovirus was observed at RAPS upstream of the Stickney WRP. During the North Side wet weather sampling, seven (7) of 16 samples (44%) had detectable norovirus concentrations (see Tables 3-8 and 3-10). There were no detectable concentrations of norovirus upstream of the North Side WRP. The detectable downstream concentrations ranged from 66.9 to 3,930 PCR MPN/100L. Only one (1) wet weather outfall sample was collected at the North Side WRP; it did not have a detectable norovirus concentration. Therefore, the concentrations of norovirus downstream of the WRP may be attributable to sources other than the outfall. Due to safety concerns, the discharge of the North Branch Pumping Station was sampled at the nearest downstream location: North Side-DNS-WW-37 that had one detectable concentration of 99.1 PCR MPN/100L, during the 3 August 2007 wet weather sampling event. The pumping station discharged a large volume of wastewater of about 115 MG in 11 hours and 15 minutes, between 2 and 3 August 2006.

During the Stickney wet weather sampling, 10 of 16 samples (63%) had detectable norovirus concentrations (see Tables 3-8 and 3-10). The detectable concentrations upstream ranged from 58.2 to 1,150 PCR MPN/100L. The detectable downstream concentrations ranged from 60 to 1,930 PCR MPN/100L. Only one (1) wet weather outfall sample was collected at the Stickney WRP, which had a norovirus concentration of 682 PCR MPN/100L. Two (2) of the three (3) RAPS samples had detectable concentrations of norovirus ranging between 2,590 and 5,700 PCR MPN/100L. The highest concentration of 5,700 PCR MPN/100L was detected during the 10 June 2006 sampling event when RAPS discharged 238 MG in 7 hours and 25 minutes.

During the Calumet wet weather sampling, three (3) of 18 samples (17%) had detectable norovirus concentrations (see Tables 3-8 and 3-10). There were no detectable norovirus concentrations upstream of the WRP. There was only one (1) detectable downstream concentration of 85.3 PCRMPN/100L during the 29 August 2006 sampling event. Two (2) of the three (3) wet weather outfall samples collected at the Calumet WRP had detectable norovirus concentrations ranging from 337 to 651 PCR MPN/100L.

Table 3-11 presents a comparison between dry and wet weather percentage of virus sample detections. The results indicate that the percentage of norovirus detections during wet weather were greater than the dry weather detections. The percentage of adenovirus detections at the North Side waterway segment increased from 4% during dry weather to 44% during wet weather. The percentage of adenovirus detections at the Stickney waterway segment increased from 12% during dry weather to 63% during wet weather. The percentage of norovirus detections at the Calumet waterway segment increased from 4% during dry weather to 17% during wet weather. In addition, the concentrations detected during wet weather sampling are generally greater than the dry weather concentrations.

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SECTION 3

TABLES

Table 3-1b. Summary of the Dry Weather Stickney Bacteria Results-Continued

****Note of Deviation:**
The dilutions for the *Pseudomonas aeruginosa* testing began at dilutions which did not yield desirable results; the minimum detection limit was too high or plates were overgrown with other competing bacteria and mold growth. Therefore, the dilutions were ultimately changed to 100 mL, 10 mL, and 1 mL of sample to accommodate. These dilutions are implemented from this point forward for the Stickney sampling location.

Stickney-82405

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	1,500 cfu/100mL [E]	700 cfu/100mL [E]	600 cfu/100mL [E]	270 cfu/100mL	14,600 cfu/100mL
E. coli	3,000 cfu/100mL [E]	2,000 cfu/100mL [E]	17,000 cfu/100mL [E]	19,000 cfu/100mL [E]	34,000 cfu/100mL
Enterococci	32 cfu/100mL [E]	44 cfu/100mL	490 cfu/100mL	550 cfu/100mL	1,010 cfu/100mL [E]
Salmonella	<1 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL
Fecal Coliform	2,000 cfu/100mL [E]	7,000 cfu/100mL [E]	47,000 cfu/100mL	42,000 cfu/100mL	33,000 cfu/100mL

****Note of Deviation:**
The dilutions for the *Salmonella* testing began at 100 mL, 10 mL, and 1 mL of sample in a series of five each. Changes to the dilutions were made at the request of Geosyntec Consultants. The dilutions were changed to 1 L and 100 mL of sample in a series of five each and are implemented from this point forward for the Stickney sampling location.

Stickney-83105

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	140 cfu/100mL	10 cfu/100mL	200 cfu/100mL [E]	100 cfu/100mL [E]	3,700 cfu/100mL [E]
E. coli	10 cfu/100mL [E]	40 cfu/100mL [E]	8,000 cfu/100mL [E]	8,000 cfu/100mL [E]	21,000 cfu/100mL
Enterococci	2 cfu/100mL [E]	4 cfu/100mL [E]	480 cfu/100mL	280 cfu/100mL	5,000 cfu/100mL [E]
Salmonella	<1 MPN/1L	<1 MPN/1L	0.62 MPN/1L	<1 MPN/1L	<1 MPN/1L
Fecal Coliform	2,000 cfu/100mL [E]	190 cfu/100mL [E]	23,000 cfu/100mL	22,000 cfu/100mL	45,000 cfu/100mL

***E – Indicates the reported value is an Estimated Count. The number of colonies counted did not fall into the recommended limits of 20-80 cfu / filter for *E. coli* and 20-60 cfu / filter for Fecal Coliform and *Enterococci*. For *Pseudomonas aeruginosa* it indicates mold interference, or one of the dilutions did not confirm.**

Table 3-1a. Summary of the Dry Weather North Side Bacteria Results

North Side-72805

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	200 cfu/100mL	300 cfu/100mL	1,600 cfu/100mL	3,000 cfu/100mL	3,600 cfu/100mL
E. coli	200 cfu/100mL	70 cfu/100mL [E]	20,000 cfu/100mL	14,000 cfu/100mL [E]	31,000 cfu/100mL
Enterococci	80 cfu/100mL	40 cfu/100mL	570 cfu/100mL	640 cfu/100mL [E]	1,950 cfu/100mL [E]
Salmonella	<1 MPN/100mL	<1 MPN/100mL	< 1MPN/100mL	<1 MPN/100mL	<1 MPN/100mL
Fecal Coliform	910 cfu/100mL [E]	970 cfu/100mL [E]	37,000 cfu/100mL	52,000 cfu/100mL	28,000 cfu/100mL

North Side-80405

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	<100 cfu/100mL	40 cfu/100mL	70 cfu/100mL [E]	10 cfu/100mL	400 cfu/100mL [E]
E. coli	630 cfu/100mL	40 cfu/100mL [E]	26,000 cfu/100mL	13,000 cfu/100mL [E]	16,000 cfu/100mL [E]
Enterococci	82 cfu/100mL	28 cfu/100mL [E]	1,000 cfu/100mL [E]	1,680 cfu/100mL [E]	1,000 cfu/100mL [E]
Salmonella	<1 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL
Fecal Coliform	3,000 cfu/100mL [E]	30 cfu/100mL [E]	50,000 cfu/100mL	37,000 cfu/100mL	55,000 cfu/100mL

North Side-81805

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	600 cfu/100mL [E]	700 cfu/100mL [E]	1,800 cfu/100mL [E]	600 cfu/100mL [E]	700 cfu/100mL [E]
E. coli	20 cfu/100mL [E]	710 cfu/100mL	6,000 cfu/100mL [E]	21,000 cfu/100mL	30,000 cfu/100mL
Enterococci	104 cfu/100mL	126 cfu/100mL [E]	4,000 cfu/100mL [E]	1,140 cfu/100mL [E]	6,000 cfu/100mL [E]
Salmonella	<1 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL	0.9 MPN/100mL	<1 MPN/100mL
Fecal Coliform	50 cfu/100mL [E]	1,000 cfu/100mL [E]	16,000 cfu/100mL [E]	41,000 cfu/100mL	45,000 cfu/100mL

Table 3-1a. Summary of the Dry Weather North Side Bacteria Results-Continued

****Note of Deviation:**
The dilutions for the *Pseudomonas aeruginosa* testing began at dilutions which did not yield desirable results; the minimum detection limit was too high or plates were overgrown with other competing bacteria and mold growth. Therefore, the dilutions were ultimately changed to 100 mL, 10 mL, and 1 mL of sample to accommodate. These dilutions are implemented from this point forward for the North Side sampling location.

North Side-82505

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	500 cfu/100mL [E]	2,500 cfu/100mL [E]	700 cfu/100mL [E]	700 cfu/100mL [E]	900 cfu/100mL [E]
E. coli	7,000 cfu/100mL [E]	220 cfu/100mL	8,000 cfu/100mL [E]	50,000 cfu/100mL	32,000 cfu/100mL
Enterococci	146 cfu/100mL [E]	62 cfu/100mL	1,010 cfu/100mL [E]	580 cfu/100mL	740 cfu/100mL [E]
Salmonella	<1 MPN/100mL	<1 MPN/100mL	2.2 MPN/100mL	1.3 MPN/100mL	<1 MPN/100mL
Fecal Coliform	6,000 cfu/100mL [E]	4,010 cfu/100mL [E]	26,000 cfu/100mL	45,000 cfu/100mL	44,000 cfu/100mL

****Note of Deviation:**
The dilutions for the *Salmonella* testing began at 100 mL, 10 mL, and 1 mL of sample in a series of five each. Changes to the dilutions were made at the request of Geosyntec Consultants. The dilutions were changed to 1 L and 100 mL of sample in a series of five each and are implemented from this point forward for the Northside sampling location.

North Sides-90105

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	27,700 cfu/100mL [E]	15,800 cfu/100mL [E]	11,800 cfu/100mL [E]	4,700 cfu/100mL [E]	1,700 cfu/100mL [E]
E. coli	2,000 cfu/100mL [E]	150 cfu/100mL [E]	32,000 cfu/100mL	6,000 cfu/100mL [E]	27,000 cfu/100mL
Enterococci	24 cfu/100mL [E]	22 cfu/100mL [E]	810 cfu/100mL [E]	810 cfu/100mL [E]	920 cfu/100mL [E]
Salmonella	<1 MPN/1L	<1 MPN/1L	<1 MPN/1L	2.1 MPN/1L	1.7 MPN/1L
Fecal Coliform	790 cfu/100mL	450 cfu/100mL	33,000 cfu/100mL	49,000 cfu/100mL	45,000 cfu/100mL

***E – Indicates the reported value is an Estimated Count. The number of colonies counted did not fall into the recommended limits of 20-80 cfu / filter for *E. coli* and 20-60 cfu / filter for Fecal Coliform and *Enterococci*. For *Pseudomonas aeruginosa* it indicates mold interference or one of the dilutions did not confirm.**

Table 3-1b. Summary of the Dry Weather Stickney Bacteria Results

Stickney-80105

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	<100 cfu/100mL	100 cfu/100mL	<100 cfu/100mL	<100 cfu/100mL	1,000 cfu/100mL
E. coli	1,000 cfu/100mL [E]	550 cfu/100mL	2,000 cfu/100mL [E]	3,000 cfu/100mL [E]	14,000 cfu/100mL [E]
Enterococci	36 cfu/100mL [E]	40 cfu/100mL	28 cfu/100mL [E]	28 cfu/100mL [E]	2,530 cfu/100mL [E]
Salmonella	<1 MPN/100mL	<1 MPN/100mL	< 1MPN/100mL	<1 MPN/100mL	<1 MPN/100mL
Fecal Coliform	430 cfu/100mL	4,000 cfu/100mL [E]	1,210 cfu/100mL [E]	5,000 cfu/100mL [E]	32,000 cfu/100mL

Stickney-80305

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	90 cfu/100mL	580 cfu/100mL	<10 cfu/100mL	20 cfu/100mL	1,180 cfu/100mL
E. coli	140 cfu/100mL [E]	<1,000 cfu/100mL [E]	9,000 cfu/100mL [E]	7,000 cfu/100mL [E]	53,000 cfu/100mL
Enterococci	6 cfu/100mL [E]	10 cfu/100mL [E]	68 cfu/100mL	34 cfu/100mL [E]	2,640 cfu/100mL [E]
Salmonella	<1 MPN/100mL	<1 MPN/100mL	1.38 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL
Fecal Coliform	550 cfu/100mL	790 cfu/100mL [E]	14,000 cfu/100mL [E]	22,000 cfu/100mL	50,000 cfu/100mL

Stickney-81705

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	<10 cfu/100mL	<10 cfu/100mL	<10 cfu/100mL	<10 cfu/100mL	800 cfu/100mL [E]
E. coli	1,000 cfu/100mL [E]	50 cfu/100mL [E]	36,000 cfu/100mL	13,000 cfu/100mL [E]	39,000 cfu/100mL
Enterococci	54 cfu/100mL	6 cfu/100mL [E]	204 cfu/100mL [E]	92 cfu/100mL	980 cfu/100mL [E]
Salmonella	<1 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL
Fecal Coliform	660 cfu/100mL [E]	690 cfu/100mL [E]	32,000 cfu/100mL	45,000 cfu/100mL	240,000 cfu/100mL

Table 3-1c. Summary of the Dry Weather Calumet Bacteria Results

Calumet-72605

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	300 cfu/100mL	200 cfu/100mL	<100 cfu/100mL	<100cfu/100mL	<100 cfu/100mL
E. coli	130 cfu/100mL [E]	110 cfu/100mL [E]	1,000 cfu/100mL [E]	1,540 cfu/100mL [E]	5,000 cfu/100mL [E]
Enterococci	10 cfu/100mL [E]	50 cfu/100mL [E]	30 cfu/100mL [E]	70 cfu/100mL [E]	690 cfu/100mL [E]
Salmonella	<1 MPN/100mL	<1 MPN/100mL	< 1MPN/100mL	<1 MPN/100mL	<1 MPN/100mL
Fecal Coliform	530 cfu/100mL	60 cfu/100mL [E]	1,300 cfu/100mL [E]	4,000 cfu/100mL [E]	22,000 cfu/100mL

Calumet-80205

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	<100 cfu/100mL	<100 cfu/100mL	<100 cfu/100mL	<100 cfu/100mL	<100 cfu/100mL
E. coli	180 cfu/100mL [E]	170 cfu/100mL [E]	1,600 cfu/100mL [E]	1,480 cfu/100mL [E]	12,000 cfu/100mL [E]
Enterococci	32 cfu/100mL [E]	32 cfu/100mL [E]	42 cfu/100mL	42 cfu/100mL	1,700 cfu/100mL [E]
Salmonella	<1 MPN/100mL	<1 MPN/100mL	< 1MPN/100mL	<1 MPN/100mL	<1 MPN/100mL
Fecal Coliform	210 cfu/100mL	320 cfu/100mL	890 cfu/100mL [E]	2,000 cfu/100mL [E]	45,000 cfu/100mL

Calumet-81605

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	30 cfu/100mL	10 cfu/100mL	160 cfu/100mL	440 cfu/100mL	300 cfu/100mL [E]
E. coli	220 cfu/100mL	30 cfu/100mL [E]	1,680 cfu/100mL [E]	1,000 cfu/100mL [E]	29,000 cfu/100mL
Enterococci	44 cfu/100mL	160 cfu/100mL	58 cfu/100mL	50 cfu/100mL	1,470 cfu/100mL [E]
Salmonella	<1 MPN/100mL	<1 MPN/100mL	0.20 MPN/100mL	0.45 MPN/100mL	0.20 MPN/100mL
Fecal Coliform	50 cfu/100mL [E]	130 cfu/100mL [E]	8,000 cfu/100mL [E]	14,000 cfu/100mL [E]	41,000 cfu/100mL

Table 3-1c. Summary of the Dry Weather Calumet Bacteria Results-Continued

****Note of Deviation:**
The dilutions for the *Pseudomonas aeruginosa* testing began at dilutions which did not yield desirable results; the minimum detection limit was too high or plates were overgrown with other competing bacteria and mold growth. Therefore, the dilutions were ultimately changed to 100 mL, 10 mL, and 1 mL of sample to accommodate. These dilutions are implemented from this point forward for the Calumet sampling location.

Calumet-82305

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. aeruginosa	<10 cfu/100mL	90 cfu/100mL	20 cfu/100mL	<10 cfu/100mL	9 cfu/100mL
E. coli	70 cfu/100mL [E]	80 cfu/100mL [E]	4,000 cfu/100mL [E]	4,000 cfu/100mL [E]	3,000 cfu/100mL [E]
Enterococci	46 cfu/100mL	30 cfu/100mL [E]	32 cfu/100mL [E]	40 cfu/100mL	510 cfu/100mL
Salmonella	<1 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL	<1 MPN/100mL
Fecal Coliform	70 cfu/100mL [E]	190 cfu/100mL [E]	10,000 cfu/100mL [E]	2,200 cfu/100mL [E]	48,000 cfu/100mL

****Note of Deviation:**
The dilutions for the *Salmonella* testing began at 100 mL, 10 mL, and 1 mL of sample in a series of five each. Changes to the dilutions were made at the request of Geosyntec Consultants. The dilutions were changed to 1 L and 100 mL of sample in a series of five each and are implemented from this point forward for the Calumet sampling location.

Calumet-83005

Test	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
P. Aeruginosa	2,520 cfu/100mL	500 cfu/100mL	2,050 cfu/100mL	1,030 cfu/100mL	5,300 cfu/100mL
E. coli	10 cfu/100mL [E]	20 cfu/100mL [E]	610 cfu/100mL	390 cfu/100mL	100,000 cfu/100mL [E]
Enterococci	62 cfu/100mL	68 cfu/100mL	82 cfu/100mL	210 cfu/100mL	1,440 cfu/100mL [E]
Salmonella	<1 MPN/1L	<1 MPN/1L	<1 MPN/1L	<1 MPN/1L	<1 MPN/1L
Fecal Coliform	530 cfu/100mL	200 cfu/100mL	8,000 cfu/100mL [E]	1,600 cfu/100mL [E]	290,000 cfu/100mL

***E – Indicates the reported value is an Estimated Count. The number of colonies counted did not fall into the recommended limits of 20-80 cfu / filter for *E. coli* and 20-60 cfu / filter for Fecal Coliform and *Enterococci*. For *Pseudomonas aeruginosa* it indicates mold interference, or one of the dilutions did not confirm.**

Table 3-1d. Summary of the Wet Weather North Side Bacteria Results

North Side-62606

Test	UPS-WW-102	DNS-WW-36	DNS-WW-37	DNS-WW-73	DNS-WW-39
P. aeruginosa	6,000 cfu/100mL	8,400 cfu/100mL[E]	2,600 cfu/100mL[E]	7,400 cfu/100mL	4,600 cfu/100mL
E. coli	18,000 cfu/100mL[E]	12,000 cfu/100mL	33,000 cfu/100mL	27,000 cfu/100mL	40,000 cfu/100mL
Enterococci	9,400 cfu/100mL	8,400 cfu/100mL	13,000 cfu/100mL[E]	14,000 cfu/100mL[E]	12,000 cfu/100mL[E]
Salmonella	3.40 MPN/1L	1.11 MPN/1L	28.9 MPN/1L	33.4 MPN/1L	1.64 MPN/1L
Fecal Coliform	42,000 cfu/100mL	54,000 cfu/100mL	53,000 cfu/100mL	44,000 cfu/100mL	110,000 cfu/100mL[E]

North Side-80306

Test	UPS-WW-102	DNS-WW-36	DNS-WW-37	DNS-WW-73	DNS-WW-39
P. aeruginosa	6,200 cfu/100mL	4,000 cfu/100mL	5,000 cfu/100mL	6,300 cfu/100mL	1,700 cfu/100mL[E]
E. coli	36,000 cfu/100mL	13,000 cfu/100mL[E]	27,000 cfu/100mL	41,000 cfu/100mL	34,000 cfu/100mL[E]
Enterococci	18,000 cfu/100mL[E]	5,800 cfu/100mL	9,800 cfu/100mL	7,400 cfu/100mL	5,400 cfu/100mL
Salmonella	0.77 MPN/1L	3.46 MPN/1L	4.81 MPN/1L	2.66 MPN/1L	16.22 MPN/1L
Fecal Coliform	580,000 cfu/100mL	62,000 cfu/100mL[E]	180,000 cfu/100mL[E]	280,000 cfu/100mL	400,000 cfu/100mL

North Side-92306

Test	UPS-WW-102	DNS-WW-36	DNS-WW-37	DNS-WW-73	DNS-WW-39	Outfall
P. aeruginosa	8,200 cfu/100mL	7,400 cfu/100mL	4,800 cfu/100mL	4,800 cfu/100mL	4,000 cfu/100mL	800 cfu/100mL
E. coli	22,000 cfu/100mL	17,000 cfu/100mL[E]	34,000 cfu/100mL	51,000 cfu/100mL	26,000 cfu/100mL	21,000 cfu/100mL
Enterococci	8,600 cfu/100mL	3,400 cfu/100mL[E]	34,000 cfu/100mL	38,000 cfu/100mL	8,000 cfu/100mL	3,000 cfu/100mL
Salmonella	10.4 MPN/1L	1.00 MPN/1L	1.13 MPN/1L	1.92 MPN/1L	1.83 MPN/1L	0.54 MPN/1L
Fecal Coliform	66,000 cfu/100mL	56,000 cfu/100mL	70,000 cfu/100mL	72,000 cfu/100mL	230,000 cfu/100mL	22,000 cfu/100mL

Table 3-1d. Summary of the Wet Weather North Side Bacteria Results-Continued

*E – Indicates the reported value is an Estimated Count as follows:

E. coli - the number of colonies counted did not fall within the recommended limits of 20-80 cfu / filter.

Fecal Coliform and *Enterococci* - the number of colonies counted did not fall within the recommended limits of 20-60 cfu/filter.

P. aeruginosa - the number of colonies counted did not fall within the recommended limits of 20-80 cfu / filter, one of the dilutions did not confirm or mold interference.

Table 3-1e. Summary of the Wet Weather Stickney Bacteria Results

Stickney-61006

Test	UPS-WW-40	UPS-WW-75	RAPS	DNS-WW-41	DNS-WW-42
P. aeruginosa	13,000 cfu/100mL	42,000 cfu/100mL	49,000 cfu/100mL	6,000 cfu/100mL[E]	29,000 cfu/100mL
E. coli	42,000 cfu/100mL	160,000 cfu/100mL[E]	300,000 cfu/100mL	46,000 cfu/100mL	410,000 cfu/100mL
Enterococci	11,000 cfu/100mL[E]	30,000 cfu/100mL	200,000 cfu/100mL	52,000 cfu/100mL	100,000 cfu/100mL[E]
Salmonella	0.43 MPN/1L	0.37 MPN/1L	2.30 MPN/1L	0.14 MPN/1L	1.33 MPN/1L
Fecal Coliform	80,000 cfu/100mL[E]	460,000 cfu/100mL	450,000 cfu/100mL	300,000 cfu/100mL	1,060,000 cfu/100mL[E]

****Note of Deviation:**
Due to sample filtration, a portion of the *Salmonella* dilutions were out of the 24 hour recommended holding time, specifically the following:
Stickney-UPS-WW-40-61006, the 2L dilution, 4 out of 5 exceeded 24 hours; Stickney-UPS-WW-75-61006, the 2L dilution, 2 out of 5 exceeded 24 hours; Stickney-RAPS-61006, the 2L dilution, 4 out of 5 exceeded 24 hours; Stickney-RAPS-61006, the 1L dilution, 1 out of 5 exceeded 24 hours; Stickney-DNS-WW-41-61006, the 2L dilution, 1 out of 5 exceeded 24 hours.

Stickney-80306

Test	UPS-WW-40	UPS-WW-75	RAPS	DNS-WW-41	DNS-WW-42
P. aeruginosa	15,000 cfu/100mL	7,800 cfu/100mL	75,000 cfu/100mL	6,400 cfu/100mL	42,000 cfu/100mL
E. coli	280,000 cfu/100mL	360,000 cfu/100mL	480,000 cfu/100mL	160,000 cfu/100mL	100,000 cfu/100mL[E]
Enterococci	52,000 cfu/100mL	60,000 cfu/100mL	260,000 cfu/100mL	42,000 cfu/100mL	51,000 cfu/100mL
Salmonella	1.24 MPN/1L	0.63 MPN/1L	0.35 MPN/1L	0.95 MPN/1L	4.90 MPN/1L
Fecal Coliform	3,440,000 cfu/100mL[E]	2,540,000 cfu/100mL[E]	11,700,000 cfu/100mL[E]	1,400,000 cfu/100mL[E]	540,000 cfu/100mL

****Note of Deviation:**
Due to sample filtration, a portion of the *Salmonella* dilutions were out of the 24 hour recommended holding time.
Specifically, Stickney-RAPS-80306; the 2L dilution, 5 out of 5 exceeded 24 hours.

Table 3-1e. Summary of the Wet Weather Stickney Bacteria Results-Continued

Stickney-101106

Test	UPS-WW-40	UPS-WW-75	RAPS	DNS-WW-41	DNS-WW-42	Outfall
P. Aeruginosa	1,000 cfu/100mL[E]	1,200 cfu/100mL[E]	500 cfu/100mL[E]	5,200 cfu/100mL	200 cfu/100mL[E]	6,800 cfu/100mL
E. coli	2,000 cfu/100mL[E]	2,000 cfu/100mL[E]	2,000 cfu/100mL[E]	28,000 cfu/100mL	3,000 cfu/100mL[E]	14,000 cfu/100mL
Enterococci	<200 cfu/100mL	1,000 cfu/100mL[E]	1,800 cfu/100mL[E]	14,000 cfu/100mL[E]	600 cfu/100mL[E]	9,800 cfu/100mL
Salmonella	20.0 MPN/1L	1.74 MPN/1L	0.41 MPN/1L	1.70 MPN/1L	0.71 MPN/1L	3.07 MPN/1L
Fecal Coliform	1,000 cfu/100mL[E]	10,000 cfu/100mL[E]	8,000 cfu/100mL[E]	64,000 cfu/100mL	10,000 cfu/100mL[E]	39,000 cfu/100mL

****Note of Deviation:**
Due to sample filtration, a portion of the Salmonella dilutions were out of the 24 hour recommended holding time, specifically the following: Stickney-UPS-WW-40-101106, the 2L dilution, 2 out of 5 exceeded 24 hours; and Stickney-RAPS-101106, the 2L dilution, 3 out of 5 exceeded 24 hours.

All samples in the data sets passed QAP and details may be reviewed on each raw data report. Each raw data report contains the required positive and negative control information, as well as sterility checks that were performed. Information is also provided on the sample temperature and incubation period, as defined in each procedure. Pertinent logs have also been provided in this final report. This testing was completed by Keri Howell, Katy Howell, Julie Birdsong and Dustin Smith.

***E – Indicates the reported value is an Estimated Count as follows:**

***E. coli* - the number of colonies counted did not fall within the recommended limits of 20-80 cfu / filter.**

Fecal Coliform and *Enterococci* - the number of colonies counted did not fall within the recommended limits of 20-60 cfu / filter.

***P. Aeruginosa* - the number of colonies counted did not fall within the recommended limits of 20-80 cfu / filter, one of the dilutions did not confirm or mold interference.**

Table 3-1f. Summary of the Wet Weather Calumet Bacteria Results

Calumet-82406

Test	UPS-WW-56	DNS-WW-76	DNS-WW-58	DNS-WW-59	DNS-WW-43	Outfall
P. Aeruginosa	1,400 cfu/100mL[E]	4,100 cfu/100mL	1,300 cfu/100mL[E]	3,200 cfu/100mL	9,000 cfu/100mL	2,000 cfu/100mL
E. coli	<200 cfu/100mL	<200 cfu/100mL	3,400 cfu/100mL[E]	<200 cfu/100mL	2,000 cfu/100mL[E]	6,000 cfu/100mL[E]
Enterococci	<100 cfu/100mL	800 cfu/100mL[E]	1,400 cfu/100mL[E]	2,600 cfu/100mL[E]	5,600 cfu/100mL	2,400 cfu/100mL[E]
Salmonella	6.53 MPN/1L	0.37 MPN/1L	1.43 MPN/1L	0.064 MPN/1L	1.27 MPN/1L	1.08 MPN/1L
Fecal Coliform	2,000 cfu/100mL[E]	4,000 cfu/100mL[E]	21,000 cfu/100mL	5,000 cfu/100mL[E]	14,000 cfu/100mL[E]	4,000 cfu/100mL

Calumet-82906

Test	UPS-WW-56	DNS-WW-76	DNS-WW-58	DNS-WW-59	DNS-WW-43	Outfall
P. aeruginosa	3,700 cfu/100mL	4,600 cfu/100mL	22,000 cfu/100mL	24,000 cfu/100mL	21,000 cfu/100mL	3,200 cfu/100mL
E. coli	770 cfu/100mL	40,000 cfu/100mL	65,000 cfu/100mL	52,000 cfu/100mL	170,000 cfu/100mL[E]	15,000 cfu/100mL
Enterococci	1,400 cfu/100mL[E]	12,000 cfu/100mL	46,000 cfu/100mL	56,000 cfu/100mL	40,000 cfu/100mL	5,800 cfu/100mL
Salmonella	12.2 MPN/1L	0.88 MPN/1L	0.46 MPN/1L	0.46 MPN/1L	0.37 MPN/1L	0.21 MPN/1L
Fecal Coliform	22,000 cfu/100mL	200,000 cfu/100mL[E]	140,000 cfu/100mL[E]	44,000 cfu/100mL	28,000 cfu/100mL[E]	69,000 cfu/100mL

Calumet-101706

Test	UPS-WW-56	DNS-WW-76	DNS-WW-58	DNS-WW-59	DNS-WW-43	Outfall
P. aeruginosa	1,300 cfu/100mL	2,300 cfu/100mL	28,000 cfu/100mL	2,800 cfu/100mL	1,300 cfu/100mL	15,000 cfu/100mL
E. coli	140 cfu/100mL[E]	7,800 cfu/100mL	12,000 cfu/100mL[E]	3,600 cfu/100mL[E]	1,200 cfu/100mL[E]	16,000 cfu/100mL[E]
Enterococci	260 cfu/100mL[E]	1,300 cfu/100mL[E]	6,600 cfu/100mL	1,700 cfu/100mL[E]	2,500 cfu/100mL	5,800 cfu/100mL
Salmonella	0.54 MPN/1L	1.20 MPN/1L	2.03 MPN/1L	20.5 MPN/1L	1.08 MPN/1L	1.76 MPN/1L
Fecal Coliform	600 cfu/100mL[E]	27,000 cfu/100mL	17,000 cfu/100mL[E]	7,800 cfu/100mL	3,400 cfu/100mL[E]	58,000 cfu/100mL

****Note of Deviation:**
Due to sample filtration, a portion of the *Salmonella* dilutions were out of the 24 hour recommended holding time. Specifically, Calumet UPS-WW-56-101706; the 2L dilution, 3 out of 5 exceeded 24 hours.

Table 3-1f. Summary of the Wet Weather Calumet Bacteria Results-Continued

*E – Indicates the reported value is an Estimated Count as follows:

E. coli - the number of colonies counted did not fall within the recommended limits of 20-80 cfu / filter.

Fecal Coliform and *Enterococci* - the number of colonies counted did not fall within the recommended limits of 20-60 cfu / filter.

P. aeruginosa - the number of colonies counted did not fall within the recommended limits of 20-80 cfu / filter, one of the dilutions did not confirm or mold interference.

Table 3-2a. Dry Weather Geometric Mean Bacteria Concentrations (in CFU/100 mL; *Salmonella* in MPN/100 mL)

Site	Location	Sampling dates	*E. coli*	Fecal coliform	*Enterococcus*	*Pseudomonas aeruginosa*	*Salmonella*
North Side	UPS	7/28/05 - 9/1/05	273	713	58	665	S.I.D. *
	Outfall	7/28/05 - 9/1/05	26,413	42,411	1,514	1,091	S.I.D. *
	DNS	7/28/05 - 9/1/05	15,710	36,687	1,007	999	0.316
Stickney	UPS	8/1/05 - 8/31/05	254	1,061	14	62	S.I.D. *
	Outfall	8/1/05 - 8/31/05	29,042	56,391	2,013	2,195	S.I.D. *
	DNS	8/1/05 - 8/31/05	9,043	17,491	127	31	0.09
Calumet	UPS	7/26/05 - 8/30/05	71	170	43	67	S.I.D. *
	Outfall	7/26/05 - 8/30/05	13,917	56,287	1,048	65	0.112
	DNS	7/26/05 - 8/30/05	1,370	3,520	55	49	0.113

Note:
* S.I.D. = Statistically Insignificant Data. Most samples (more than 80%) had concentrations below the analytical detection limit of 1 MPN/100mL for dry weather samples. Therefore, the geometric mean was not estimated.

Table 3-2b. Wet Weather Geometric Mean Bacteria Concentrations (in CFU/100 mL; *Salmonella* in MPN/L)

Site	Location	Sampling dates	*E. coli*	*Enterococcus*	Fecal coliform	*Pseudomonas aeruginosa*	*Salmonella*
North Side	UPS	6/26/06-9/23/06	24,262	11,347	117,399	6,723	3.00
	Outfall	9/23/06	20,952	3,011	22,026	796	0.54
	DNS	6/26/06-9/23/06	27,106	10,327	100,962	4,675	3.61
Stickney	UPS	6/10/06-10/11/06	45,101	13,920	172,819	8,049	1.04
	Outfall	10/11/06	14,045	9,799	38,949	6,768	3.06
	DNS	6/10/06-10/11/06	54,176	21,340	231,345	6,053	1.01
Calumet	UPS	8/24/06-10/17/06	279	331	2,981	1,888	3.50
	Outfall	8/24/06-10/17/06	11,309	4,330	25,168	4,583	0.74
	DNS	8/24/06-10/17/06	6,073	5,473	19,165	5,914	0.86

Table 3-3a. Dry Weather Indigenous *Cryptosporidium* Oocysts and *Giardia* Cysts in Samples Collected at the North Side Waterway Segment

Sample Site	Sample Volume Collected (L)	Sample Volume Analyzed (L)	No.of *Giardia* Cysts Detected in Volume Analyzed	No. of *Giardia* Cysts/L	No. of *Cryptosporidium* Oocysts Detected in Volume Analyzed	No. of *Cryptosporidium* Oocysts/L
North Side - Outfall 7/28/05	20	6.7	6	0.9	0	<0.2
North Side –UPS - 1 Meter 72805	18.9	6.3	1	0.2	0	<0.2
North Side – UPS- Surface 72805	18.9	6.3	1	0.2	0	<0.2
North Side - DNS - 1 Meter 72805	18.9	6.3	7	1.1	0	<0.2
North Side - DNS - Surface 72805	18.9	6.3	3	0.5	0	<0.2
North Side - Outfall 8-4-05	20	6.7	26	3.9	1	0.1
North Side - UPS - 1 Meter 80405	18.9	9.4	0	0.0	0	<0.1
North Side – UPS- Surface 80405	18.9	9.4	0	0.0	2	0.2
North Side - DNS - 1 Meter 80405	18.9	6.3	2	0.3	0	<0.2
North Side - DNS - Surface 80405	18.9	6.3	3	0.5	1	0.2
North Side - Outfall 8-18-05	20	6.7	4	0.6	0	<0.2
North Side - UPS - 1 Meter 81805	18.9	1.2	0	0.0	0	<0.8
North Side – UPS- Surface 81805	18.9	1.0	0	0.0	0	<1.0
North Side –DNS - 1 Meter 81805	18.9	9.4	0	0.0	0	<0.2
North Side - DNS - Surface 81805	18.9	6.3	0	0.0	1	0.1
North Side - Outfall 8-25-05	20	6.7	14	2.1	4	0.6
North Side - UPS - 1 Meter 82505	18.9	1.0	0	0.0	0	<1.0
North Side – UPS- Surface 82505	18.9	6.3	2	0.3	0	<0.2
North Side - DNS - 1 Meter 82505	18.9	3.2	2	0.6	1	0.3
North Side - DNS - Surface 82505	18.9	6.3	10	1.6	6	1.0
North Side - Outfall 9-1-05	20	6.7	31	4.6	1	0.1
North Side - UPS - 1 Meter 090105	18.9	1.1	4	3.6	0	<0.9
North Side – UPS- Surface 090105	18.9	6.3	0	0.0	0	<0.2
North Side - DNS - 1 Meter 090105	18.9	6.3	4	0.6	3	0.5
North Side - DNS - Surface 090105	18.9	6.3	19	3.0	4	0.6

Table 3-3b. Dry Weather Indigenous *Cryptosporidium* Oocysts and *Giardia* Cysts in Samples Collected at the Stickney Waterway Segment

Sample Site	Sample Volume Collected (L)	Sample Volume Analyzed (L)	No. of *Giardia* Cysts Detected in Volume Analyzed	No. of *Giardia* Cysts/L	No. of *Cryptosporidium* Oocysts Detected in Volume Analyzed	No. of *Cryptosporidium* Oocysts /L
Stickney – Outfall 7-27-05[1]	-	-	-		-	
Stickney - UPS - 1 Meter 72705[1]	-	-	-		-	
Stickney - UPS- Surface 72705[1]	-	-	-		-	
Stickney - DNS - 1 Meter 72705[1]	-	-	-		-	
Stickney - DNS – Surface 72705	18.9	6.3	4	0.6	0	<0.2
Stickney – Outfall 8-1-05	18.9	6.3	5	0.8	0	<0.2
Stickney -UPS - 1 Meter 8105	18.9	6.3	0	<0.2	0	<0.2
Stickney - UPS- Surface 8105	18.9	6.3	0	<0.2	0	<0.2
Stickney - DNS - 1 Meter 8105	18.9	6.3	0	<0.2	0	<0.2
Stickney - DNS – Surface 8105	18.9	6.3	1	0.2	0	<0.2
Stickney – Outfall 8-3-05	20	6.7	5	0.7	1	0.1
Stickney - UPS - 1 Meter 80305	18.9	6.3	2	0.3	0	<0.2
Stickney - UPS- Surface 80305	18.9	6.3	0	<0.2	0	<0.2
Stickney - DNS - 1 Meter 80305	18.9	6.3	3	0.5	0	<0.2
Stickney - DNS – Surface 80305	18.9	6.3	1	0.2	0	<0.2
Stickney – Outfall 8-17-05	20	6.7	3	0.4	0	<0.2
Stickney - UPS - 1 Meter 81705	18.9	6.3	0	<0.2	0	<0.2
Stickney - UPS- Surface 81705	18.9	6.3	1	0.2	0	<0.2
Stickney - DNS - 1 Meter 81705	18.9	6.3	3	0.5	0	<0.2
Stickney - DNS – Surface 81705	18.9	6.3	0	<0.2	0	<0.2
Stickney – Outfall 8-24-05	20	6.7	33	4.9	4	0.6
Stickney - UPS - 1 Meter 082405	18.9	9.4	1	0.1	0	<0.10
Stickney - UPS- Surface 082405	18.9	6.3	1	0.2	2	0.3
Stickney - DNS - 1 Meter 082405	18.9	6.3	7	1.1	3	0.5
Stickney - DNS – Surface 082405	18.9	6.3	7	1.1	1	0.2
Stickney – Outfall 8/31/05	20	6.7	5	0.7	1	0.1
Stickney - UPS - 1 Meter 83105	18.9	6.3	0	<0.2	0	<0.2
Stickney - UPS- Surface 83105	18.9	6.3	1	0.2	0	<0.2
Stickney - DNS - 1 Meter 83105	18.9	6.3	1	0.2	0	<0.2
Stickney - DNS – Surface 83105	18.9	6.3	4	0.6	1	0.2

1. Samples were not analyzed because the corresponding bacteria samples were not delivered on time by UPS.

Table 3-3c. Dry Weather Indigenous *Cryptosporidium* Oocysts and *Giardia* Cysts in Samples Collected at the Calumet Waterway Segment

Sample Site	Sample Volume Collected (L)	Sample Volume Analyzed (L)	No. of *Giardia* Cysts Detected in Volume Analyzed	No. of *Giardia* Cysts/L	No. of *Cryptosporidium* Oocysts Detected in Volume Analyzed	No. of *Cryptosporidium* Oocysts/L
Calumet – Outfall –7/26/05	10	5	6	1.2	0	<0.2
Calumet - UPS - 1 Meter 72605	10	3.3	0	<0.3	0	<0.3
Calumet - UPS- Surface 72605	10	3.3	0	<0.3	0	<0.3
Calumet - DNS - 1 Meter 72605	10	3.3	2	0.6	0	<0.3
Calumet - DNS – Surface 72605	10	3.3	2	0.6	0	<0.3
Calumet – Outfall 8/2/05[1]	20	10.0	0	<0.1	0	<0.1
Calumet - UPS - 1 Meter 8205[1]	18.9	6.3	0	<0.2	0	<0.2
Calumet - UPS- Surface 8205[1]	18.9	6.3	0	<0.2	0	<0.2
Calumet - DNS - 1 Meter 8205[1]	18.9	9.4	0	<0.1	0	<0.1
Calumet - DNS – Surface 8205[1]	18.9	9.4	0	<0.1	0	<0.1
Calumet – Outfall 8/16/05	20	10.0	22	2.2	0	<0.1
Calumet - UPS - 1 Meter 081605	18.9	9.4	0	<0.1	0	<0.1
Calumet - UPS- Surface 081605	18.9	9.4	0	<0.1	0	<0.1
Calumet - DNS - 1 Meter 081605	18.9	6.3	0	<0.2	0	<0.2
Calumet - DNS – Surface 081605	18.9	6.3	2	0.3	0	<0.2
Calumet – Outfall 8/23/05	20	6.7	4	0.6	3	0.4
Calumet - UPS - 1 Meter 82305	18.9	9.4	0	<0.1	0	<0.1
Calumet - UPS- Surface 82305	18.9	9.4	0	<0.1	0	<0.1
Calumet - DNS - 1 Meter 82305	18.9	6.3	0	<0.2	1	0.2
Calumet - DNS – Surface 82305	18.9	6.3	0	<0.2	1	0.2
Calumet – Outfall 8/30/05	20	6.7	4	0.6	0	<0.2
Calumet - UPS - 1 Meter 83005	18.9	6.3	0	<0.2	0	<0.2
Calumet - UPS- Surface 83005	18.9	6.3	0	<0.2	3	0.5
Calumet - DNS - 1 Meter 83005	18.9	6.3	3	0.5	3	0.5
Calumet - DNS – Surface 83005	18.9	6.3	0	<0.2	2	0.3

1. One filter capsule and the temperature blank were received in the laboratory partially frozen. District was notified that samples should not be analyzed especially since viability/infectivity assay would not yield useful information.

Table 3-3d. Wet Weather Indigenous *Cryptosporidium* Oocysts and *Giardia* Cysts in Samples Collected at the North Side Waterway Segment

Sample Site	Sample Volume Collected (L)	Aliquot ID (Volume in L)	Total Sample Volume Analyzed	No. of *Giardia* Cysts Detected in Volume Analyzed	No. of *Giardia* Cysts/L	No. of *Cryptosporidium* Oocysts Detected in Volume Analyzed	No. of *Cryptosporidium* Oocysts /L
North Side-UPS-WW-102-062606	18.9	NA[1]	6.3	34	5.4	0	< 0.2
North Side-DNS-WW-36 - 062606	18.9	A (3.15)	6.3	145	46.0	3	1.0
		B (3.15)		156	49.5	4	1.3
North Side –DNS-WW-37 - 062606	18.9	A (3.15)	6.3	6	1.9	0	< 0.3
		B (3.15)		20	6.3	4	1.3
North Side –DNS-WW-37 – 062606 - MS	20.0	A (1.33)	6.7	7	5.3	1	0.8
		B (1.33)		60	45.1	3	2.3
		C (1.33)		38	28.6	2	1.5
		D (1.33)		52	39.1	2	1.5
		E (1.33)		65	48.9	4	3.0
North Side – DNS-WW-73-062606	18.9	NA[1]	6.3	72	11.4	3	0.5
North Side –DNS-WW-39-062606	18.9	NA[1]	6.3	10	1.6	3	0.5
North Side – UPS-WW-102- 080306	18.9	NA[1]	6.3	11	1.7	0	<0.2
North Side – DNS-WW-36 - 080306	18.9	NA[1]	6.3	31	4.9	1	0.2
North Side-DNS-WW-37 - 080306	18.9	NA[1]	3.15 (A)	5	1.6	2	0.6
			3.15 (B)	16	5.1	0	<0.3
North Side –DNS-WW-73 - 080306	18.9	NA[1]	6.3	31	4.9	1	0.2
North Side – DNS-WW-39-080306	18.9	NA[1]	6.3	48	7.6	10	1.6
North Side-UPS-WW-102-092306	18.9	NA[1]	6.3	7	1.1	7	1.1
North Side-DNS-WW-36 - 092306	18.9		6.3	24	3.8	4	0.6
North Side –DNS-WW-37 - 092306	18.9	A (3.15)	6.3	0	<0.3	0	<0.3
		B (3.15)		2	0.6	0	<0.3
North Side – DNS-WW-73-092306	18.9	A (3.15)	6.3	1	0.3	0	<0.3
		B (3.15)		2	0.6	0	<0.3
North Side –DNS-WW-39-092306	18.9	A (3.15)	6.3	4	1.3	3	1.0
		B (3.15)		4	1.3	4	1.3
North Side – Outfall - 092306	20	A (3.3)	6.6	3	0.9	1	0.3
		B (3.3)		1	0.3	2	0.6

1. Not applicable. Entire sample was analyzed in one aliquot.

Table 3-3e. Wet Weather Indigenous *Cryptosporidium* Oocysts and *Giardia* Cysts in Samples Collected at the Stickney Waterway Segment

Sample Site	Sample Volume Collected (L)	Aliquot ID (Volume in L)	Total Sample Volume Analyzed	No. of *Giardia* Cysts Detected in Volume Analyzed	No. of *Giardia* Cysts/L	No. of *Cryptosporidium* Oocysts Detected in Volume Analyzed	No. of *Cryptosporidium* Oocysts /L
Stickney – UPS-WW-40-061006	18.9	NA[1]	6.3	0	<0.2	0	<0.2
Stickney – UPS – WW-75-061006	18.9	NA[1]	6.3	7	1.1	1	0.2
Stickney – RAPS – 061006	18.9	NA[1]	6.3	10	1.6	0	<0.2
Stickney – RAPS – MS- 061006[1]	NA[2]	NA[1]	NA	NA	NA	NA	NA
Stickney – DNS –WW- 41-061006	18.9	NA[1]	6.3	14	2.2	0	<0.2
Stickney – DNS-WW-42-061006	18.9	NA[1]	6.3	4	0.6	1	0.2
Stickney – UPS-WW-40-080306	18.9	NA[1]	6.3	8	1.3	5	0.8
Stickney – UPS – WW-75-080306	18.9	NA[1]	6.3	16	2.5	3	0.5
Stickney – RAPS – 080306	22.6[1]	NA[1]	3.8	4	1.0	1	0.3
Stickney – RAPS – MS- 080306	12.0	NA[1]	1.0 (A)	7	7.0	3	3.0
		NA[1]	1.0 (B)	30	30.0	25	25.0
		NA[1]	1.0 (C)	32	32.0	10	10.0
		NA[1]	1.0 (D)	53	53.0	9	9.0
Stickney – DNS –WW- 41-080306	18.9	NA[1]	6.3	11	1.7	3	0.5
Stickney – DNS-WW-42-080306	18.9	NA[1]	6.3	4	0.6	2	0.3
Stickney – UPS-WW-40-101106	18.9	NA[1]	6.3	7	1.1	1	0.2
Stickney – UPS – WW-75-101106	18.9	NA[1]	6.3	1	0.2	0	<0.2
Stickney – RAPS – 101106	18.9	NA[1]	6.3	13	2.1	4	0.6
Stickney – DNS –WW- 41-101106	18.9	NA[1]	6.3	15	2.4	5	0.8
Stickney – DNS-WW-42- 101106	18.9	NA[1]	6.3	6	1.0	0	<0.2
Stickney – Outfall - 101106	20.0	NA[1]	6.7	36	5.4	4	0.6

1. Not applicable. Entire sample was analyzed in one aliquot

2. Matrix spike was not analyzed due to insufficient volume collected.

Table 3-3f. Wet Weather Indigenous *Cryptosporidium* Oocysts and *Giardia* Cysts in Samples Collected at the Calumet Waterway Segment

Sample Site	Sample Volume Collected (L)	Aliquot ID (Volume in L)	Total Sample Volume Analyzed	No. of Giardia Cysts Detected in Volume Analyzed	No. of Giardia Cysts/L	No. of Cryptosporidium Oocysts Detected in Volume Analyzed	No. of Cryptosporidium Oocysts /L
Calumet Outfall -082406	20	NA[1]	3.35 (A)	6	1.8	1	0.3
		NA[1]	3.35 (B)	1	0.3	0	<0.3
Calumet – UPS-WW56-082406	18.9	NA[1]	6.3	0	<0.2	0	<0.2
Calumet – DNS-WW76-082406	18.9	NA[1]	6.3	0	<0.2	0	<0.2
Calumet – DNS-WW58-082406	18.9	NA[1]	3.15(A)	1	0.3	0	<0.3
		NA[1]	3.15 (B)	0	<0.3	1	0.3
Calumet – DNS-WW59-082406	18.9	NA[1]	3.15 (A)	0	<0.3	0	<0.3
		NA[1]	3.15 (B)	0	<0.3	0	<0.3
Calumet – DNS-WW43-082406	18.9	NA[1]	3.15 (A)	0	<0.3	0	<0.3
		NA[1]	3.15 (B)	0	<0.3	0	<0.3
Calumet Outfall -082906	20	NA[1]	2.23 (A)	7	3.1	6	2.7
		NA[1]	2.23 (B)	19	8.5	14	6.3
		NA[1]	2.23(C)	14	6.3	10	4.5
Calumet – UPS-WW56-082906	18.9	NA[1]	3.15 (A)	0	<0.3	0	<0.3
		NA[1]	3.15 (B)	0	<0.3	0	<0.3
Calumet – DNS-WW76-082906	18.9	NA[1]	6.3	0	<0.2	0	<0.2
Calumet – DNS-WW58-082906	18.9	NA[1]	1.05 (A)	0	<1.0	1	1.0
		NA[1]	1.05 (B)	0	<1.0	0	<1.0
		NA[1]	1.05 (C)	0	<1.0	3	2.9
		NA[1]	1.05 (D)	0	<1.0	0	<1.0
		NA[1]	1.05 (E)	0	<1.0	0	<1.0
		NA[1]	1.05 (F)	0	<1.0	0	<1.0

Table 3-3f. Wet Weather Indigenous *Cryptosporidium* Oocysts and *Giardia* Cysts in Samples Collected at the Calumet Waterway Segment (Continued)

Sample Site	Sample Volume Collected (L)	Aliquot ID (Volume in L)	Total Sample Volume Analyzed	No. of *Giardia* Cysts Detected in Volume Analyzed	No. of *Giardia* Cysts/L	No. of *Cryptosporidium* Oocysts Detected in Volume Analyzed	No. of *Cryptosporidium* Oocysts/lL
Calumet – DNS-WW59-082906	18.9	NA[1]	1.05 (A)	0	<1.0	0	<1.0
		NA[1]	1.05 (B)	0	<1.0	0	<1.0
		NA[1]	1.05 (C)	0	<1.0	0	<1.0
		NA[1]	1.05 (D)	0	<1.0	0	<1.0
		NA[1]	1.05 (E)	0	<1.0	0	<1.0
		NA[1]	1.05 (F)	0	<1.0	0	<1.0
		NA[1]					
Calumet – DNS-WW43-082906	18.9	NA[1]	3.15 (A)	0	<0.3	2	0.6
		NA[1]	3.15 (B)	0	<0.3	2	0.6
Calumet Outfall -101706	20	NA[1]	0.8 (A)	2	2.5	0	<1.2
		NA[1]	0.8 (B)	2	2.5	0	<1.2
Calumet – UPS-WW56-101706	18.9	NA[1]	1.6 (A)	0	<0.6	0	<0.6
		NA[1]	1.6 (B)	0	<0.6	0	<0.6
Calumet – DNS-WW76-101706	18.9	NA[1]	6.3	3	0.5	2	0.3
Calumet – DNS-WW58-101706	18.9	NA[1]	1.6(A)	0	<0.6	2	1.2
		NA[1]	1.6 (B)	0	<0.6	0	<0.6
Calumet – DNS-WW59-101706	18.9	NA[1]	3.15 (A)	0	<0.3	0	<0.3
		NA[1]	3.15 (B)	1	0.3	1	0.3
Calumet – DNS-WW43-101706	18.9	NA[1]	3.15 (A)	1	0.3	1	0.3
		NA[1]	3.15 (B)	0	<0.3	0	<0.3

1. Not applicable. Entire sample was analyzed in one aliquot.

Table 3-4a. Dry Weather Viability Results of *Giardia* Cysts Using Fluorogenic Dyes in Samples Collected at the North Side Waterway Segment

Sample ID	Volume Analyzed (L)	Viable Cysts		Non-viable Cysts				Totals	
		DAPI+ Good	DAPI- Good	DAPI+ Poor	DAPI-Poor	DAPI+/PI+	Empty	Viable	Non-viable
North Side - Outfall 7/28/05	6.7	0	2	0	0	2	1	2	3
North Side -UPS - 1 Meter 72805	6.3	0	0	0	0	0	0	0	0
North Side - UPS- Surface 72805	6.3	0	0	0	3	0	5	0	8
North Side - DNS - 1 Meter 72805	6.3	0	1	0	4	0	1	1	5
North Side - DNS - Surface 72805	6.3	0	2	0	2	1	0	1	3
North Side - Outfall 8-4-05	6.7	4	1	1	4	1	1	5	7
North Side - UPS - 1 Meter 80405	4.7	0	0	0	0	0	3	0	3
North Side - UPS- Surface 80405	4.7	0	0	0	0	0	0	0	0
North Side - DNS - 1 Meter 80405	6.3	0	0	0	0	0	0	0	0
North Side - DNS - Surface 80405	6.3	1	0	0	0	3	0	1	3
North Side - Outfall 8-18-05	6.7	4	13	0	1	13	2	17	16
North Side - UPS - 1 Meter 81805	1.2	0	0	0	0	0	0	0	0
North Side - UPS- Surface 81805	1.0	0	0	0	0	0	0	0	0
North Side -DNS - 1 Meter 81805	4.7	0	5	0	0	1	2	5	3
North Side - DNS - Surface 81805	6.3	0	1	0	0	5	0	1	5
North Side - Outfall 8-25-05	6.7	1	12	1	0	3	3	13	7
North Side - UPS - 1 Meter 82505	1.0	0	0	0	0	0	0	0	0
North Side - UPS- Surface 82505	6.3	0	1	0	0	1	0	1	1
North Side - DNS - 1 Meter 82505	3.2	0	0	0	0	1	0	0	1
North Side - DNS - Surface 82505	6.3	0	4	0	0	4	0	4	4
North Side - Outfall 9-1-05	6.7	0	4	0	2	8	5	4	15
North Side - UPS - 1 Meter 090105	1.0	0	0	0	0	1	7	0	8
North Side - UPS- Surface 090105	6.3	0	0	0	0	0	0	0	0
North Side - DNS - 1 Meter 090105	6.3	0	1	0	0	5	5	1	10
North Side - DNS - Surface 090105	6.3	0	0	0	0	8	5	0	13

Table 3-4b. Dry Weather Viability Results of *Giardia* Cysts Using Fluorogenic Dyes in Samples Collected at the Stickney Waterway Segment

Sample ID	Volume Analyzed (L)	Viable Cysts		Non-viable Cysts				Totals	
		DAPI+ Good	DAPI- Good	DAPI+ Poor	DAPI- Poor	DAPI+/PI+	Empty	Viable	Non-viable
Stickney - DNS – Surface 72705	6.3	0	4	0	1	26	0	4	27
Stickney – Outfall 8-1-05	6.3	1	1	0	0	1	0	2	1
Stickney -UPS - 1 Meter 8105	6.3	2	0	0	0	0	0	2	0
Stickney - UPS- Surface 8105	6.3	0	0	0	0	0	2	0	2
Stickney - DNS - 1 Meter 8105	6.3	0	0	0	0	0	0	0	0
Stickney - DNS – Surface 8105	6.3	1	0	0	0	1	1	1	2
Stickney – Outfall 8-3-05	6.7	0	0	0	0	0	0	0	0
Stickney - UPS - 1 Meter 80305	6.3	0	0	0	1	1	2	0	4
Stickney - UPS- Surface 80305	6.3	0	0	0	2	0	1	0	3
Stickney - DNS - 1 Meter 80305	6.3	3	0	1	1	4	0	3	6
Stickney - DNS – Surface 80305	6.3	0	0	0	0	0	1	0	1
Stickney – Outfall 8-17-05	6.7	6	19	3	1	12	1	25	17
Stickney - UPS - 1 Meter 81705	6.3	1	0	0	0	1	1	1	2
Stickney - UPS- Surface 81705	6.3	0	0	0	0	2	1	0	3
Stickney - DNS - 1 Meter 81705	6.3	4	2	3	1	10	1	6	15
Stickney - DNS – Surface 81705	6.3	1	1	0	0	13	1	2	14
Stickney – Outfall 8-24-05	6.7	6	10	1	0	13	0	16	14
Stickney - UPS - 1 Meter 082405	4.7	0	0	0	1	2	0	0	3
Stickney - UPS- Surface 082405	6.3	0	0	0	0	3	0	0	3
Stickney - DNS - 1 Meter 082405	6.3	0	1	0	0	2	0	1	2
Stickney - DNS – Surface 082405	6.3	0	2	0	0	6	0	2	6
Stickney – Outfall 8/31/05	6.7	0	1	0	0	10	4	1	14
Stickney - UPS - 1 Meter 83105	6.3	0	0	0	0	3	7	0	10
Stickney - UPS- Surface 83105	6.3	0	0	0	0	1	1	0	2
Stickney - DNS - 1 Meter 83105	6.3	0	1	0	0	1	2	1	3
Stickney - DNS – Surface 83105	6.3	0	0	0	0	4	6	0	10

Table 3-4c. Dry Weather Viability Results of *Giardia* Cysts Using Fluorogenic Dyes in Samples Collected at the Calumet Waterway Segment

Sample ID	Volume Analyzed (L)	Viable Cysts		Non-viable Cysts				Total	
		DAPI+ Good	DAPI- Good	DAPI+Poor	DAPI- Poor	DAPI+/PI+	Empty	Viable	Non-viable
Calumet – Outfall –7/26/05	2.5	1	1	0	1	4	0	2	5
Calumet - UPS - 1 Meter 72605	3.3	0	0	0	0	0	0	0	0
Calumet - UPS- Surface 72605	3.3	0	0	0	0	0	0	0	0
Calumet - DNS - 1 Meter 72605	3.3	0	0	0	0	1	0	0	1
Calumet - DNS – Surface 72605	3.3	0	0	0	0	0	0	0	0
Calumet – Outfall 8/2/05[1]	5.0	0	0	0	0	4	0	0	4
Calumet - UPS - 1 Meter 8205[1]	6.3	0	0	0	0	0	0	0	0
Calumet - UPS- Surface 8205[1]	6.3	0	0	0	0	0	0	0	0
Calumet - DNS - 1 Meter 8205[1]	4.7	0	0	0	0	0	4	0	4
Calumet - DNS – Surface 8205[1]	4.7	0	0	0	0	0	0	0	0
Calumet – Outfall 8/16/05	5.0	0	0	0	0	4	0	0	4
Calumet - UPS - 1 Meter 081605	4.7	0	0	0	0	0	0	0	0
Calumet - UPS- Surface 081605	4.7	0	0	0	0	1	0	0	1
Calumet - DNS - 1 Meter 081605	6.3	0	1	0	0	4	1	1	5
Calumet - DNS – Surface 081605	6.3	0	1	0	0	2	1	1	3
Calumet – Outfall 8/23/05	6.7	0	0	0	0	0	0	0	0
Calumet - UPS - 1 Meter 82305	4.7	0	0	0	0	0	0	0	0
Calumet - UPS- Surface 82305	4.7	0	0	0	0	0	0	0	0
Calumet - DNS - 1 Meter 82305	6.3	0	0	0	0	2	0	0	2
Calumet - DNS – Surface 82305	6.3	0	0	0	0	0	0	0	0
Calumet – Outfall 8/30/05	6.7	0	0	0	0	0	0	0	0
Calumet - UPS - 1 Meter 83005	6.3	0	0	0	0	1	0	0	1
Calumet - UPS- Surface 83005	6.3	0	0	0	0	0	0	0	0
Calumet - DNS - 1 Meter 83005	6.3	0	1	0	0	1	1	1	2
Calumet - DNS – Surface 83005	6.3	0	0	0	0	4	2	0	6

Note:

1. Samples in this shipment were received partially frozen and results must be interpreted with caution.

Table 3-4d. Wet Weather Viability Results of *Giardia* Cysts Using Fluorogenic Dyes in Samples Collected at the North Side Waterway Segment

Sample ID	Volume Analyzed (L)	Viable Cysts		Non-viable Cysts				Totals	
		DAPI+ Good	DAPI- Good	DAPI+ Poor	DAPI- Poor	DAPI+/PI+	Empty	Viable	Non-viable
North Side-UPS-WW-102-062606	6.3	1	10	0	0	4	0	11	4
North Side-DNS-WW-36 - 062606	3.15	0	14	2	2	49	0	14	53
	3.15	1	15	1	3	46	0	16	50
North Side –DNS-WW-37 - 062606	3.15	0	3	0	1	6	0	3	7
	3.15	0	1	0	1	4	1	1	6
North Side –DNS-WW-37 – 062606 - MS	1.33	2	21	0	4	23	1	23	28
	1.33	0	4	0	6	18	0	4	24
	1.33	1	14	0	6	27	0	15	33
	1.33	2	13	0	10	14	0	15	24
	1.33	0	14	3	12	19	0	14	34
North Side – DNS-WW-73-062606	6.3	2	29	0	3	15	0	31	18
North Side –DNS-WW-39-062606	6.3	1	10	0	3	8	0	11	11
North Side –UPS-WW-102 –080306	6.3	11	5	0	5	19	0	16	24
North Side –DNS–WW 36 – 080306	6.3	7	15	2	0	13	25	22	40
North Side –DNS–WW 37 – 080306	3.15	0	10	0	0	4	11	10	15
	3.15	0	14	0	0	2	3	14	5
North Side –DNS–WW 73 – 080306	6.3	6	15	2	0	12	19	21	33
North Side –DNS–WW 39 – 080306	6.3	3	5	0	0	3	0	8	3
North Side-UPS-WW-102-092306	6.3	5	0	1	1	11	0	5	13
North Side-DNS-WW-36 - 092306	6.3	7	17	2	0	1	0	24	3
North Side –DNS-WW-37 - 092306	3.15	1	0	0	0	1	0	1	1
	3.15	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
North Side – DNS-WW-73-092306	3.15	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	3.15	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
North Side –DNS-WW-39-092306	3.15	1	1	0	0	0	0	2	0
	3.15	1	0	0	0	1	0	1	1
North Side – Outfall - 092306	3.3	1	1	0	0	2	0	2	2
	3.3	0	1	0	1	1	0	1	2

Note:

1. ND = No cysts detected in the portion of samples analyzed.

Table 3-4e. Wet Weather Viability Results of *Giardia* Cysts Using Fluorogenic Dyes in Samples Collected at the Stickney Waterway Segment

Sample ID	Volume Analyzed (L)	Viable Cysts			Non-viable Cysts			Totals	
		DAPI+ Good	DAPI- Good	DAPI+ Poor	DAPI- Poor	DAPI+/PI+	Empty	Viable	Non-viable
Stickney – UPS-WW-40-061006	6.3	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
Stickney – UPS – WW-75-061006	6.3	1	3	0	1	3	0	4	4
Stickney – RAPS – 061006	6.3	7	22	1	2	18	0	29	21
Stickney – DNS –WW- 41-061006	6.3	3	20	0	1	6	0	23	7
Stickney – DNS-WW-42-061006	6.3	1	1	0	0	1	0	2	1
Stickney – UPS – WW-40-080306	6.3	4	10	0	0	10	0	14	10
Stickney – UPS – WW-75-080306	6.3	10	8	0	0	27	0	18	27
Stickney – RAPS – 080306	3.7	2	8	2	1	17	0	10	20
Stickney – RAPS – MS – 080306	1.0	1	6	1	13	7	0	7	21
	1.0	1	4	0	4	5	1	5	10
	1.0	2	7	0	6	4	3	9	13
	1.0	3	12	1	2	13	0	15	16
Stickney – DNS –WW- 41-080306	6.3	8	8	0	0	9	0	16	9
Stickney – DNS-WW-42- 080306	6.3	2	3	1	0	6	0	5	7
Stickney – UPS-WW-40-101106	6.3	0	1	0	1	0	1	1	2
Stickney – UPS – WW-75-101106	6.3	3	2	0	1	10	1	5	12
Stickney – RAPS – 101106	6.3	3	6	0	3	20	0	9	23
Stickney – DNS –WW- 41-101106	6.3	2	5	2	0	18	0	7	20
Stickney – DNS-WW-42-101106	6.3	0	1	1	0	0	0	1	1
Stickney – Outfall - 101106	6.7	7	4	0	0	10	1	11	11

Note:

1. ND = No cysts detected in the portion of samples analyzed.

Table 3-4f. Wet Weather Viability Results of *Giardia* Cysts Using Fluorogenic Dyes in Samples Collected at the Calumet Waterway Segment

Sample ID	Volume Analyzed (L)	Viable Cysts			Non-viable Cysts			Totals	
		DAPI+ Good	DAPI- Good	DAPI+ Poor	DAPI- Poor	DAPI+/PI+	Empty	Viable	Non-viable
Calumet – Outfall Composite –082406	3.35	0	0	1	0	2	1	0	4
	3.35	0	1	0	0	1	1	1	2
Calumet – UPS- WW 56 – 082406	6.3	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
Calumet – DNS – WW 76-082406	6.3	0	1	0	0	2	0	1	2
Calumet – DNS – WW 58 – 082406	3.15	0	1	0	0	2	0	1	2
	3.15	0	0	0	0	1	0	0	1
Calumet – DNS – WW 59 – 082406	3.15	1	0	0	0	1	0	1	1
	3.15	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
Calumet – DNS – WW 43 - 082406	3.15	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	3.15	0	0	0	0	0	2	0	2
Calumet – Outfall Composite –082906	2.23	1	0	0	0	2	3	1	5
	2.23	0	0	1	1	3	3	0	8
	2.23	0	0	0	0	2	3	0	5
Calumet – UPS- WW 56 – 082906	3.15	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	3.15	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
Calumet – DNS – WW 76-082906	6.3	1	1	0	0	18	0	2	18
Calumet – DNS – WW 58 – 082906	1.05 (A)	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	1.05 (B)	0	0	0	0	2	0	0	2
	1.05 (C)	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	1.05 (D)	0	0	0	0	3	0	0	3
	1.05 (E)	0	0	0	0	2	0	0	2
	1.05 (F)	0	0	0	0	1	0	0	1
Calumet – DNS – WW 59 – 082906	1.05 (A)	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	1.05 (B)	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	1.05 (C)	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	1.05 (D)	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	1.05 (E)	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	1.05 (F)	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
Calumet – DNS – WW 43 - 082906	3.15 (A)	0	0	0	0	2	0	0	2
	3.15 (B)	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]

Table 3-4f. Wet Weather Viability Results of *Giardia* Cysts Using Fluorogenic Dyes in Samples Collected at the Calumet Waterway Segment (Continued)

Sample ID	Volume Analyzed (L)	Viable Cysts			Non-viable Cysts			Totals	
		DAPI+ Good	DAPI- Good	DAPI+ Poor	DAPI- Poor	DAPI+ PI+	Empty	Viable	Non-viable
Calumet – Outfall Composite –101706	0.8	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	0.8	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
Calumet – UPS- WW 56 – 101706	1.6	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
	1.6	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
Calumet – DNS – WW 76-101706	6.3	5	0	1	0	8	1	5	10
Calumet – DNS – WW 58 – 101706	1.6	0	0	0	1	1	0	0	2
	1.6	0	0	0	0	1	0	0	1
Calumet – DNS – WW 59 – 101706	3.15	0	0	0	0	2	0	0	2
	3.15	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]	ND[1]
Calumet – DNS – WW 43 - 101706	3.15	0	0	0	0	1	0	0	1
	3.15	0	0	0	1	0	0	0	1

Note:

1. ND = No cysts detected in the portion of samples analyzed.

Table 3-5a. Summary of the North Side Dry Weather Enteric Virus Results

Enteric Virus	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
North Side-72805	<1 MPN/100L	<1 MPN/100L	<1MPN/100L	<1 MPN/100L	<1.17/100L
North Side-80405	<1 MPN/100L	<1 MPN/100L	<1MPN/100L	<1 MPN/100L	1.72/100L
North Side-81805	<1 MPN/100L	<1 MPN/100L	3.27 MPN/100L	2.12 MPN/100L	<1.28/100L
North Side-82505	3.25 MPN/100L	1.04 MPN/100L	8.72 MPN/100L	16.07 MPN/100L	24.73/100L
North Side-90105	<1 MPN/100L	<1 MPN/100L	<1 MPN/100L	<1 MPN/100L	<1.23/100L

Table 3-5b. Summary of the Stickney Dry Weather Enteric Virus Results

Enteric Virus	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
Stickney-80105	<1 MPN/100L	<1 MPN/100L	<1MPN/100L	<1 MPN/100L	<2 MPN/100L
Stickney-80305	<1 MPN/100L	<1 MPN/100L	<1MPN/100L	<1 MPN/100L	<1.19/100L
Stickney-81705	<1 MPN/100L	1.03 MPN/100L	<1MPN/100L	1.02 MPN/100L	<1.27/100L
Stickney-82405	3.25 MPN/100L	2.13 MPN/100L	1.03 MPN/100L	1.03 MPN/100L	<1.3/100L
Stickney-83105	<1 MPN/100L	<1 MPN/100L	<1 MPN/100L	<1 MPN/100L	<1.21/100L

Table 3-5c. Summary of the Calumet Dry Weather Enteric Virus Results

Enteric Virus	UPS-1Meter	UPS-Surface	DNS-1Meter	DNS-Surface	Outfall
Calumet-72605	<1 MPN/100L	<1 MPN/100L	<1MPN/100L	<1 MPN/100L	<1.27 MPN/100L
Calumet-80205	<1 MPN/100L	<1 MPN/100L	<1MPN/100L	<1 MPN/100L	<1.28 MPN/100L
Calumet-81605	<1 MPN/100L	<1 MPN/100L	<1MPN/100L	<1 MPN/100L	1.28 MPN/100L
Calumet-82305	<1 MPN/100L	<1 MPN/100L	1.04 MPN/100L	<1 MPN/100L	<1.20 MPN/100L
Calumet-83005	<1 MPN/100L	1.04 MPN/100L	<1 MPN/100L	<1 MPN/100L	<1.28 MPN/100L

Table 3-5d. Summary of the North Side Wet Weather Enteric Virus Results

Enteric Virus	UPS-WW-102	DNS-WW-36	DNS-WW-37	DNS-WW-73	DNS-WW-39	Outfall
North Side-62606	1 MPN/100L	<1 MPN/100L	<1 MPN/100L	7 MPN/100L	9 MPN/100L	See Note 1
North Side-80306	1 MPN/100L	<1 MPN/100L	<1 MPN/100L	<1 MPN/100L	6 MPN/100L	See Note 1
North Side-92306	12 MPN/100L	7 MPN/100L	1 MPN/100L	12 MPN/100L	28 MPN/100L	1 MPN/100L

Note:

1. Prior to 24 August 2006, the outfall location was not collected. All sampling events after 24 August 2006 included an outfall location.

Table 3-5e. Summary of the Stickney Wet Weather Enteric Virus Results

Enteric Virus	UPS-WW-40	UPS-WW-75	RAPS	DNS-WW-41	DNS-WW-42	Outfall
Stickney-61006	<1 MPN/100L	<1 MPN/100L	1 MPN/100L	1 MPN/100L	2 MPN/100L	See Note 1
Stickney-80306	10 MPN/100L	28 MPN/100L	63 MPN/100L	9 MPN/100L	7 MPN/100L	See Note 1
Stickney-101106	3 MPN/100L	2 MPN/100L	6 MPN/100L	6 MPN/100L	6 MPN/100L	10 MPN/100L

Note:

1. Prior to 24 August 2006, the outfall location was not collected. All sampling events after 24 August 2006 included an outfall location.

Table 3-5f. Summary of the Calumet Wet Weather Enteric Virus Results

Enteric Virus	UPS-WW-56	DNS-WW-76	DNS-WW-58	DNS-WW-59	DNS-WW-43	Outfall
Calumet-82406	2 MPN/100L	1 MPN/100L	<1 MPN/100L	<1 MPN/100L	<1 MPN/100L	<1 MPN/100L
Calumet-82906	1 MPN/100L	5 MPN/100L	32 MPN/100L	3 MPN/100L	85 MPN/100L	10 MPN/100L
Calumet-101706	9 MPN/100L	10 MPN/100L	18 MPN/100L	7 MPN/100L	6 MPN/100L	32 MPN/100L

Table 3-6. Dry Weather Cell Culture Assay and Adenovirus Results

Virus Sample ID	Total Culturable Virus		Total MPN/100L	PCR Confirmation	Adenovirus[1] MPN/100L
	1st Passage	*2nd Passage*			
Calumet-UPS-1meter-72605	negative	negative	<1		neg
Calumet-UPS-surface-72605	negative	negative	<1		neg
Calumet-DNS-1meter-72605	negative	positive	3.21	neg	neg
Calumet-DNS-surface-72605	negative	positive	1.09	neg	neg
Calumet-Outfall-72605	negative	positive	7.52	pos	7.52
North Side-UPS-1meter-72805	negative	negative	<1		neg
North Side-UPS-surface-72805	negative	negative	<1		neg
North Side-DNS-1meter-72805	negative	positive	13.9	neg	neg
North Side-DNS-surface-72805	negative	positive	18.4	pos	18.4
North Side-Outfall-72805	positive	positive	135	pos	135
Stickney-UPS-1meter-80105	negative	positive	108	neg	neg
Stickney-UPS-surface-80105	negative	positive	117	pos	117
Stickney-DNS-1meter-80105	negative	positive	112	pos	112
Stickney-DNS-surface-80105	negative	positive	110	pos	110
Stickney-Outfall-80105	negative	positive	7.99	pos	7.99
Calumet-UPS-1meter-80205	negative	positive	1.21	neg	neg
Calumet-UPS-surface-80205	negative	negative	<1		neg
Calumet-DNS-1meter-80205	negative	negative	<1		neg
Calumet-DNS-surface- 80205	negative	negative	<1		neg
Calumet-Outfall- 80205	negative	positive	12.6		neg
Stickney-UPS- surface-80305	negative	positive	3.6	neg	neg
Stickney-UPS- 1meter-80305	negative	positive	11	pos	11
Stickney-DNS- surface-80305	negative	positive	1.67	pos	1.67
Stickney-DNS- 1meter-80305	negative	positive	6.22	pos	6.22
Stickney-Outfall-80305	negative	positive	18	pos	18
North Side-UPS-surface-80405	negative	negative	<1		neg
North Side-UPS- 1meter-80405	negative	negative	<1		neg
North Side-DNS- surface-80405	positive	positive	11.2	pos	11.2
North Side-DNS- 1meter-80405	positive	positive	9.84	pos	9.84
North Side-Outfall-80405	positive	positive	256	pos	256
Calumet-UPS-surface-81605	negative	negative	<1		neg
Calumet-UPS-1meter-81605	negative	negative	<1		neg
Calumet-DNS-surface-81605	negative	negative	<1		neg
Calumet-DNS-1meter- 81605	negative	positive	1.31	pos	1.31
Calumet-Outfall- 81605	negative	positive	3.21	neg	neg
Stickney-UPS-surface-81705	negative	negative	<1		neg
Stickney-UPS-1meter-81705	negative	negative	<1		neg
Stickney-DNS-surface-81705	negative	positive	1.72	pos	1.72
Stickney-DNS-1meter- 81705	negative	negative	<1		neg
Stickney-Outfall- 81705	negative	negative	<1		neg

Table 3-6. Dry Weather Cell Culture Assay and Adenovirus Results-Continued

Virus Sample ID	Total Culturable Virus		Total MPN/100L	PCR Confirmation	Adenovirus[1] MPN/100L
	1st Passage	*2nd Passage*			
North Side-UPS-surface-81805	negative	negative	<1		neg
North Side-UPS-1meter-81805	negative	positive	1.5	pos	1.5
North Side-DNS-surface-81805	negative	positive	12.4	pos	12.4
North Side-DNS-1meter-81805	negative	positive	10.8	pos	10.8
North Side-Outfall- 81805	negative	negative	<1		neg
Calumet-UPS-surface-82305	negative	negative	<1		neg
Calumet-UPS-1meter-82305	negative	negative	<1		neg
Calumet-DNS-surface-82305	negative	positive	3.35	pos	3.35
Calumet-DNS-1meter- 82305	negative	positive	1.36	neg	neg
Calumet-Outfall- 82305	negative	positive	14.5	neg	14.5
Stickney-UPS-surface-82405	negative	negative	<1		neg
Stickney-UPS-1meter-82405	negative	negative	<1		neg
Stickney-DNS-surface-82405	negative	positive	7.4	neg	neg
Stickney-DNS-1meter- 82405	positive	positive	28.7	pos	28.7
Stickney-Outfall- 82405	positive	positive	36.9	pos	36.9
North Side-UPS-surface-82505	negative	positive	2.94	pos	2.94
North Side-UPS-1meter-82505	negative	negative	<1		neg
North Side-DNS-surface-82505	negative/T[2]	positive	5.03	pos	5.03
North Side-DNS-1meter-82505	positive	positive	27.6	pos	27.6
North Side-Outfall- 82505	negative	positive	45.1	pos	45.1
Calumet-UPS-surface-83005	negative	negative	<1		neg
Calumet-UPS-1meter-83005	negative	negative	<1		neg
Calumet-DNS-surface-83005	negative	positive	6.24	neg	neg
Calumet-DNS-1meter- 83005	negative	positive	3.05	pos	3.05
Calumet-Outfall- 83005	negative	positive	15.5	pos	15.5
Stickney-UPS-surface-83105	negative	negative	<1		neg
Stickney-UPS-1meter-83105	negative	negative	<1		neg
Stickney-DNS-surface-83105	negative	positive	1.39	pos	1.39
Stickney-DNS-1meter- 83105	negative	negative	<1		neg
Stickney-Outfall- 83105	negative	positive	8.38	pos	8.38
North Side-UPS-1meter-90105	negative	negative	<1		neg
North Side-UPS-surface-90105	negative	negative	<1		neg
North Side-DNS-1meter-90105	negative	negative	<1		neg
North Side-DNS- surface-90105	negative	negative	<1		neg
North Side-Outfall- 90105	negative	negative	<1		neg

Note:

1. Of 75 dry samples, 42 demonstrated the presence of detectable virus in the PCL/PRF/5 cell line. Adenoviruses we confirmed only in 31 of the 42 samples by PCR. Enteroviruses or other enteric viruses were probably responsible for the observed CPE in the other samples or the CPE of other viruses could have masked the presence of adenoviruses.
2. Sample concentrate toxic to cells; entire content of flask frozen and re-assayed. Toxicity was not the result of virus in the sample.
3. neg = negative
   Pos = positive

Table 3-7. Dry Weather Norovirus (*Calicivirus*) Results

Virus Sample ID	Results	Viral concentration	Equivalent volume assayed	Viral concentration
	(positive/negative)	(PCR results)	liters	MPN PCR units/ 100 liters
Calumet-UPS-1meter-72605	negative	-	0.24	
Calumet-UPS-surface-72605	negative	-	0.24	
Calumet-DNS-1meter-72605	negative	-	0.23	
Calumet-DNS-surface-72605	negative	-	0.26	
Calumet-Outfall-72605	negative	-	0.09	
North Side-UPS-1meter-72805	negative	-	0.20	
North Side-UPS-surface-72805	negative	-	0.18	
North Side-DNS-1meter-72805	negative	-	0.19	
North Side-DNS-surface-72805	negative	-	0.20	
North Side-Outfall-72805	negative	-	0.08	
Stickney-UPS-1meter-80105	negative		0.24	
Stickney-UPS-surface-80105	negative	-	0.23	
Stickney-DNS-1meter-80105	negative	-	0.23	
Stickney-DNS-surface-80105	negative	-	0.23	
Stickney-Outfall-80105	negative	-	0.11	
Calumet-UPS-1meter-80205	negative	-	0.28	
Calumet-UPS-surface-80205	negative	-	0.23	
Calumet-DNS-1meter-80205	negative	-	0.23	
Calumet-DNS-surface- 80205	negative	-	0.21	
Calumet-Outfall- 80205	negative	-	0.10	
Stickney-UPS- surface-80305	negative	-	0.20	
Stickney-UPS- 1meter-80305	negative	-	0.20	
Stickney-DNS- surface-80305	negative	-	0.20	
Stickney-DNS- 1meter-80305	negative	-	0.20	
Stickney-Outfall-80305	negative	-	0.08	
North Side-UPS-surface-80405	negative	-	0.21	
North Side-UPS- 1meter-80405	negative	-	0.18	
North Side-DNS- surface-80405	negative	-	0.23	
North Side-DNS- 1meter-80405	negative	-	0.26	
North Side-Outfall-80405	**positive**	+	**0.20**	**See Note 1**
Calumet-UPS-surface-81605	negative	-	0.21	
Calumet-UPS-1meter-81605	negative	-	0.22	
Calumet-DNS-surface-81605	negative	-	0.22	
Calumet-DNS-1meter- 81605	negative	-	0.23	
Calumet-Outfall- 81605	**positive**	+	**0.19**	**781**
Stickney-UPS-surface-81705	**positive**	+	**0.41**	**511**
Stickney-UPS-1meter-81705	negative	-	0.27	
Stickney-DNS-surface-81705	negative	-	0.19	
Stickney-DNS-1meter- 81705	negative	-	0.22	
Stickney-Outfall- 81705	negative	-	0.10	

Note:

1. The *Calicivirus* concentration at this location was estimated to be 35,000 MPN/PRC Units/100 liter. The greater concentration of *Calicivirus* observed in this sample compared to the other samples may be due to the fact that only two duplicates per dilution in the MPN assay could be performed because of reassay difficulties, therefore reducing the precision of the analysis. In addition, of the five norovirus samples with MPN assays, this sample was the only one that had a positive result in the highest dilution. The combination of these factors could have resulted in the relatively high MPN value of this sample. Therefore, the high *Calicivirus* concentration in the subject sample is likely and artifact of these factors and appears to be an outlier.

Table 3-7. Dry Weather Norovirus (*Calicivirus*) Results (Continued)

Virus Sample ID	Results	Viral Concentration	Equivalent Volume Assayed	Viral Concentration
	(positive/negative)	(PCR results)	liters	MPN PCR units/ 100 liters
North Side-UPS-surface-81805	negative	-	0.20	
North Side-UPS-1meter-81805	negative	-	0.20	
North Side-DNS-surface-81805	negative	-	0.21	
North Side-DNS-1meter- 81805	negative	-	0.20	
North Side-Outfall- 81805	negative	-	0.10	
Calumet-UPS-surface-82305	negative	-	0.24	
Calumet-UPS-1meter-82305	negative	-	0.27	
Calumet-DNS-surface-82305	negative	-	0.22	
Calumet-DNS-1meter- 82305	negative	-	0.22	
Calumet-Outfall- 82305	negative	-	0.08	
Stickney-UPS-surface-82405	negative	-	0.20	
Stickney-UPS-1meter-82405	negative	-	0.21	
Stickney-DNS-surface-82405	**positive**	+	**0.42**	**176**
Stickney-DNS-1meter- 82405	negative	-	0.20	
Stickney-Outfall- 82405	negative	-	0.10	
North Side-UPS-surface-82505	negative	-	0.21	
North Side-UPS-1meter-82505	negative	-	0.20	
North Side-DNS-surface-82505	negative	-	0.21	
North Side-DNS-1meter- 82505	negative	-	0.21	
North Side-Outfall- 82505	negative	-	0.08	
Calumet-UPS-surface-83005	negative	-	0.22	
Calumet-UPS-1meter-83005	negative	-	0.21	
Calumet-DNS-surface-83005	negative	-	2.17	
Calumet-DNS-1meter- 83005	negative	-	0.28	
Calumet-Outfall- 83005	negative	-	0.10	
Stickney-UPS-surface-83105	**positive**	+	**0.41**	**181**
Stickney-UPS-1meter-83105	negative	-	0.19	
Stickney-DNS-surface-83105	negative	-	0.20	
Stickney-DNS-1meter- 83105	negative	-	0.21	
Stickney-Outfall- 83105	negative	-	0.09	
North Side-UPS-1meter-90105	negative	-	0.20	
North Side-UPS-surface-90105	negative	-	0.21	
North Side-DNS-1meter-90105	negative	-	0.20	
North Side-DNS-surface- 90105	negative	-	0.21	
North Side-Outfall- 90105	negative	-	0.09	

Table 3-8. Wet Weather Cell Culture Assay/Adenovirus and Norovirus (*Calicivirus*) Results

Virus Sample ID	Virus		Cell Culture		Adenovirus[1]	Norovirus PCR		
	1st Pass	2nd Pass	MPN/ 100L	PCR	MPN/100L	Result	MPN PCR Units/100L	PCR eq. volume assayed (L)
Stickney-UPS-WW-40-061006	pos	pos	661	pos	661	pos	1,150	0.37
Stickney-UPS-WW-75-061006	neg	pos	4.46	neg	neg	neg	< 5.8	0.37
Stickney-RAPS-061006	neg	pos	135	pos	135	pos	5,700	0.42
Stickney-DNS-WW-41-061006	pos	pos	6.5	pos	6.5	pos	1,930	0.39
Stickney-DNS-WW-42-061006	pos	pos	39.2	pos	39.2	pos	1,310	0.32
North Side-UPS-WW102-062606	pos	pos	2,890	pos	2,890	neg	< 5.8	0.43
North Side-DNS-WW36-062606	pos	pos	2,770	pos	2,770	neg	< 5.8	0.45
North Side-NBPS-WW37-062606	pos	pos	148	pos	148	neg	< 5.8	0.39
North Side-DNS-WW73-062606	pos	pos	2,870	pos	2,870	neg	< 5.8	0.43
North Side-DNS-WW-39-062606	pos	pos	328	pos	328	pos	3,930	0.38
North Side-UPS-WW102-080306	neg	pos	20.7	pos	20.7	neg	< 5.8	0.40
North Side-DNS-WW-36-080306	neg	pos	871	neg	neg	pos	149	0.42
North Side-NBPS-DNS-WW37-080306	pos	pos	66.7	pos	66.7	pos	99.1	0.36
North Side-DNS-WW73-080306	pos	pos	974	pos	974	neg	< 5.8	0.25
North Side-DNS-WW-39-080306	pos	pos	332	pos	332	pos	243	0.38
Stickney-UPS-WW-40-080306	pos	pos	332	pos	332	neg	< 5.8	0.38
Stickney-UPS-WW-75-080306	pos	pos	1,280	pos	1,280	neg	< 5.8	0.45
Stickney-RAPS-080306	pos	pos	1,560	pos	1,560	pos	2,590	0.36
Stickney-DNS-WW-41-080306	pos	pos	57.4	pos	57.4	neg	< 5.8	0.42
Stickney-DNS-WW-42-080306	pos	pos	1,180	pos	1,180	pos	74.2	0.48
Calumet-UPS-WW-56-082406	**neg**	**pos**	**54.1**	**neg**	**neg**	**neg**	**< 5.8**	**0.44**
Calumet-DNS-WW-76-082406	**neg**	**pos**	**128**	**pos**	**128**	**neg**	**< 5.8**	**0.44**
Calumet-DNS-WW-58-082406	**neg**	**pos**	**28.9**	**pos**	**28.9**	**neg**	**< 5.8**	**0.44**
Calumet-DNS-WW-59-082406	**neg**	**pos**	**128**	**neg**	**neg**	**neg**	**< 5.8**	**0.44**
Calumet-DNS-WW-43-082406	**neg**	**pos**	**8.77**	**neg**	**neg**	**neg**	**< 5.8**	**0.44**
Calumet-Outfall-082406	**neg**	**pos**	**10.0**	**pos**	**10.0**	**neg**	**< 5.8**	**0.19**
Calumet-UPS-WW-56-082906	pos	pos	14.7	pos	14.7	neg	< 5.8	0.39
Calumet-DNS-WW-76-082906	pos	pos	548	pos	548	neg	< 5.8	0.44
Calumet-DNS-WW-58-082906	pos	pos	344	pos	344	pos	85.3	0.36
Calumet-DNS-WW-59-082906	pos	pos	44.9	pos	44.9	neg	< 5.8	0.44
Calumet-DNS-WW-43-082906	pos	pos	>3,277	pos	>3,277	neg	< 5.8	0.38
Calumet-Outfall-082906	neg	pos	117	pos	117	pos	651	0.19

Table 3-8. Wet Weather Cell Culture Assay/Adenovirus and Norovirus (*Calicivirus*) Results (Continued

Virus Sample ID	Virus		Cell Culture		Adenovirus[1]	Norovirus PCR		
	1st Pass	2nd Pass	MPN/ 100L	PCR	MPN/100L	Result	MPN PCR Units/100L	PCR eq. volume assayed (L)
North Side-UPS-WW102-092306	pos	pos	115	neg	neg	neg	< 5.8	0.42
North Side-DNS-WW-36-092306	pos	pos	110	pos	110	pos	393	0.44
North Side-NBPS-WW-37-092306	pos	pos	199	pos	199	neg	< 5.8	0.45
North Side-DNS-WW-73-092306	pos	pos	303	pos	303	pos	128	0.48
North Side-DNS-WW-39-092306	pos	pos	105	pos	105	pos	66.9	0.53
North Side –Outfall 092306	neg	pos	121	pos	121	neg	< 5.8	0.21
Stickney-UPS-WW-40-101106	pos	pos	3.5	pos	3.5	neg	< 5.8	0.52
Stickney-UPS-WW-75-101106	pos	pos	4.16	pos	4.16	pos	58.2	0.52
Stickney-RAPS-101106	pos	pos	49.7	pos	49.7	neg	< 5.8	0.51
Stickney-DNS-WW-41-101106	pos	pos	288	pos	288	pos	60	0.50
Stickney-DNS-WW-42-101106	pos	pos	4.37	pos	4.37	pos	783	0.49
Stickney Outfall 101106	neg	pos	1,308	pos	1,308	pos	682	0.21
Calumet-UPS-WW-56-101706	neg	pos	3.06	neg	neg	neg	< 5.8	0.60
Calumet-DNS-WW-76-101706	pos	pos	1,118	pos	1,118	neg	< 5.8	0.59
Calumet-DNS-WW-58-101706	pos	pos	271	pos	271	neg	< 5.8	0.53
Calumet-DNS-WW-59-101706	pos	pos	6.24	pos	6.24	neg	< 5.8	0.60
Calumet-DNS-WW-43-101706	neg	pos	21	neg	neg	neg	< 5.8	0.60
Calumet-Outfall-101706	pos	pos	355	pos	355	pos	337	0.21

Note:

1. All 50 wet weather samples demonstrated the presence of infectious viruses assay in the PCL/PRF15 cell line. Adenoviruses were confirmed in 42 of the samples by PCR. Enteroviruses or other enteric viruses were probably responsible for the observed CPE in the other samples, or the CPE of the other viruses could have masked the presence of adenoviruses.
2. The samples in bold print had severe toxicity problems in three of the six and inconsistent results on another two. The University of Arizona analyst believes that there was something in the sample that was probably interfering with the virus replication, as well as causing enough toxicity to affect the cells ability to provide reliable results. The MPN numbers were calculated with only two dilutions instead of three, and they were the analysts best estimate based on the fact that we did not see any toxicity in the highest dilution. The fact that this set was all negative for PCR supports this, as there was probably some interference here as well.
3. pos = positive
4. neg = negative

Table 3-9. Summary of Dry Weather Virus Detections (%) and Detectable Concentration Ranges

Virus	North Side	Stickney	Calumet
Enteric	8/25[1] (29%)[2]	6/25[1] (24%)[2]	3/25[1] (12%)[2]
Upstream[3]	1.04-3.25 MPN/100L	1.03-3.25 MPN/100L	1.04 MPN/100L
Downstream[3]	2.12 -16.07 MPN/100L	1.02-1.03 MPN/100L	1.04 MPN/100L
Outfall[3]	1.72 - 24.73 MPN/100L	Not Detected	1.28 MPN/100L
Adenovirus	12/25[1] (48%)[2]	13/25[1] (52%)[2]	6/25[1] (24%)[2]
Upstream[3]	1.5-2.94 MPN/100L	11-117 MPN/100L	Not Detected
Downstream[3]	5.03-27.6 MPN/100L	1.39-112 MPN/100L	1.31-3.35 MPN/100L
Outfall[3]	45.1-256 MPN/100L	7.99 -36.9 MPN/100L	7.52-15.5 MPN/100L
Norovirus	1/25[1] (4%)[2]	3/25[1] (12%)[2]	1/25[1] (4%)[2]
Upstream[3]	Not Detected	181-511PCR MPN/100L	Not Detected
Downstream[3]	Not Detected	176 PCR MPN/100L	Not Detected
Outfall[3]	See Note 4	Not Detected	781 PCR MPN/100L

Notes:

1. The ratio represents the number of samples with detections of viruses over the total number of samples collected and analyzed
2. The number in parentheses represents the percentage of samples with virus detections
3. The detectable concentration ranges at each sampling location are shown
4. The *Calicivirus* concentration at this location was estimated to be 35,000 MPN/PCR Units/100 liter. The greater concentration of *Calicivirus* observed in this sample compared to the other samples may be due to the fact that only duplicates per dilution in the MPN assay could be performed because of reassay difficulties, therefore reducing the precision of the analysis. In addition, of the five norovirus samples with MPN assays, this sample was the only one that had a positive result in the highest dilution. The combination of these factors could have resulted in the relatively high MPN value of this sample. Therefore, the high *Calicivirus* concentration in the subject sample is likely and artifact of these factors and appears to be an outlier.

Table 3-10. Summary of Wet Weather Virus Detections (%) and Detectable Concentration Ranges

Virus	North Side	Stickney	Calumet
Enteric	11/16[1] (69%)[2]	14/16[1] (88%)[2]	14/18[1] (77%)[2]
Upstream[3]	1-12 MPN/100L	2-28 MPN/100L	1-9 MPN/100L
Downstream[3]	1-28 MPN/100L	1-9 MPN/100L	1-85 MPN/100L
Outfall[3]	1 MPN/100L	10 MPN/100L	10-32 MPN/100L
PS[3]	<1-1 MPN/100L	1-63 MPN/100L	Not Sampled[5]
Adenovirus	14/16[1] (88%)[2]	15/16[1] (94%)[2]	13/18[1] (72%)[2]
Upstream[3]	20.7-2,890 MPN/100L	3.5-1,280 MPN/100L	14.7 MPN/100L
Downstream[3]	105-2,870 MPN/100L	4.37-1,180 MPN/100L	6.24->3,277 MPN/100L
Outfall[3]	121 MPN/100L	1,308 MPN/100L	10-355 MPN/100L
PS[3,4]	66.7- 199 MPN/100L	49.7-1,560 MPN/100L	Not Sampled[5]
Norovirus	7/16[1] (44%)[2]	10/16[1] (63%)[2]	3/18[1] (17%)[2]
Upstream[3]	Not Detected	58.2-1,150 PCR MPN/100L	Not Detected
Downstream[3]	66.9-3,930 PCR MPN/100L	66.9-1,930 PCR MPN/100L	85.3 PCR MPN/100L
Outfall[3]	Not Detected	682 PCR MPN/100L	337-651 PCR MPN/100L
PS[3]	99.1 PCR MPN/100L	2,590-5,700 PCR MPN/100L	Not Sampled[5]

Notes:

1. The ratio represents the number of samples with detections of viruses over the total number of samples collected and analyzed
2. The number in parentheses represents the percentage of samples with virus detections
3. The detectable concentration ranges at each sampling location are shown
4. Due to safety concerns, the discharge of the North Branch Pumping Station was sampled at the nearest downstream location: North Side-DNS-WW-37
5. The Calumet Pumping Station was not sampled, because historically it did not discharge during rain events

Table 3-11. Comparison of Percent (%) Virus Detections During Dry and Wet Weather

Virus	North Side	Stickney	Calumet
Enteric			
Dry	8/25 (29%)	6/25 (24%)	3/25 (12%)
Wet	11/16 (69%)	14/16 (88%)	14/18 (77%)
Adenovirus			
Dry	12/25 (48%)	13/25 (52%)	6/25 (24%)
Wet	14/16 (87.5%)	15/16 (94%)	13/18 (72%)
Norovirus			
Dry	1/25 (4%)	3/25(12%)	1/25 (4%)
Wet	7/16 (44%)	10/16(62.5%)	3/18 (17%)

SECTION 3

FIGURES

Figure 3-1. North Side Dry Weather Bacteria Histograms

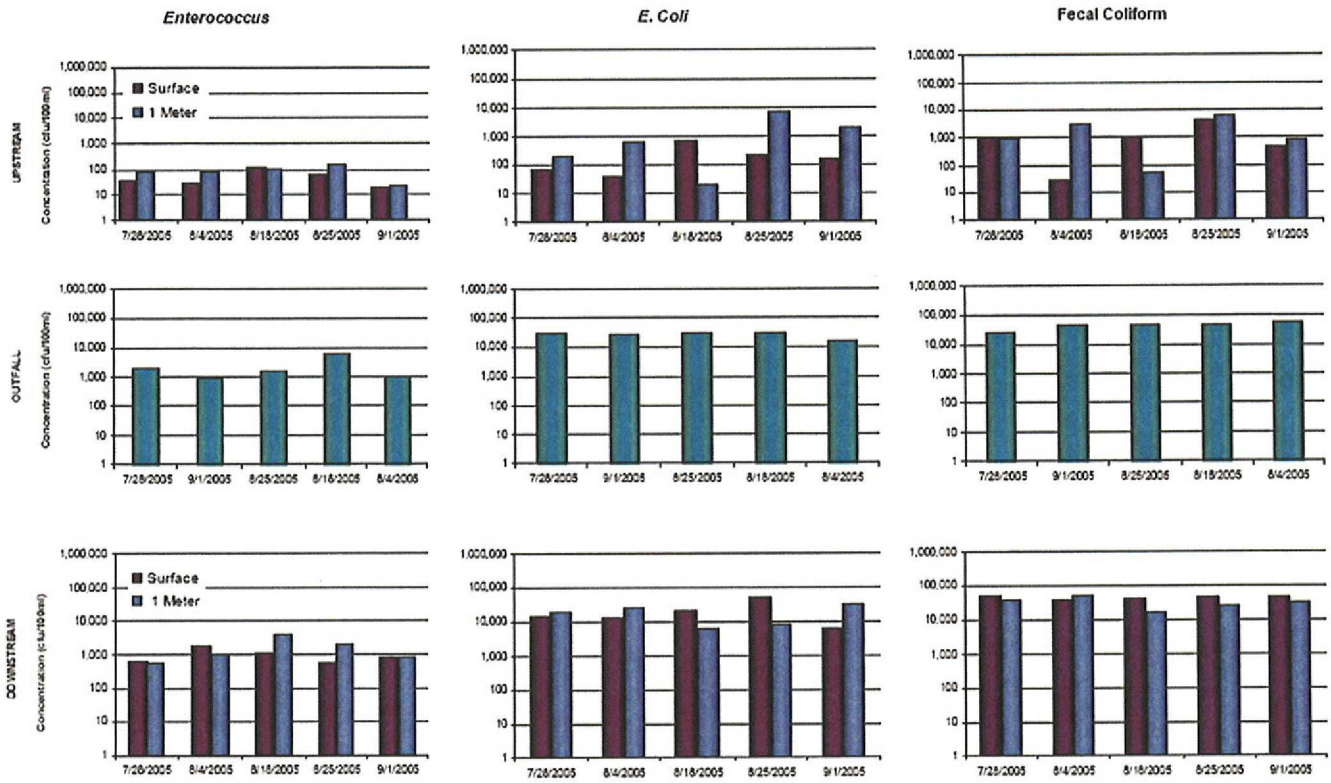


Figure 3-2. Stickney Dry Weather Bacteria Histograms

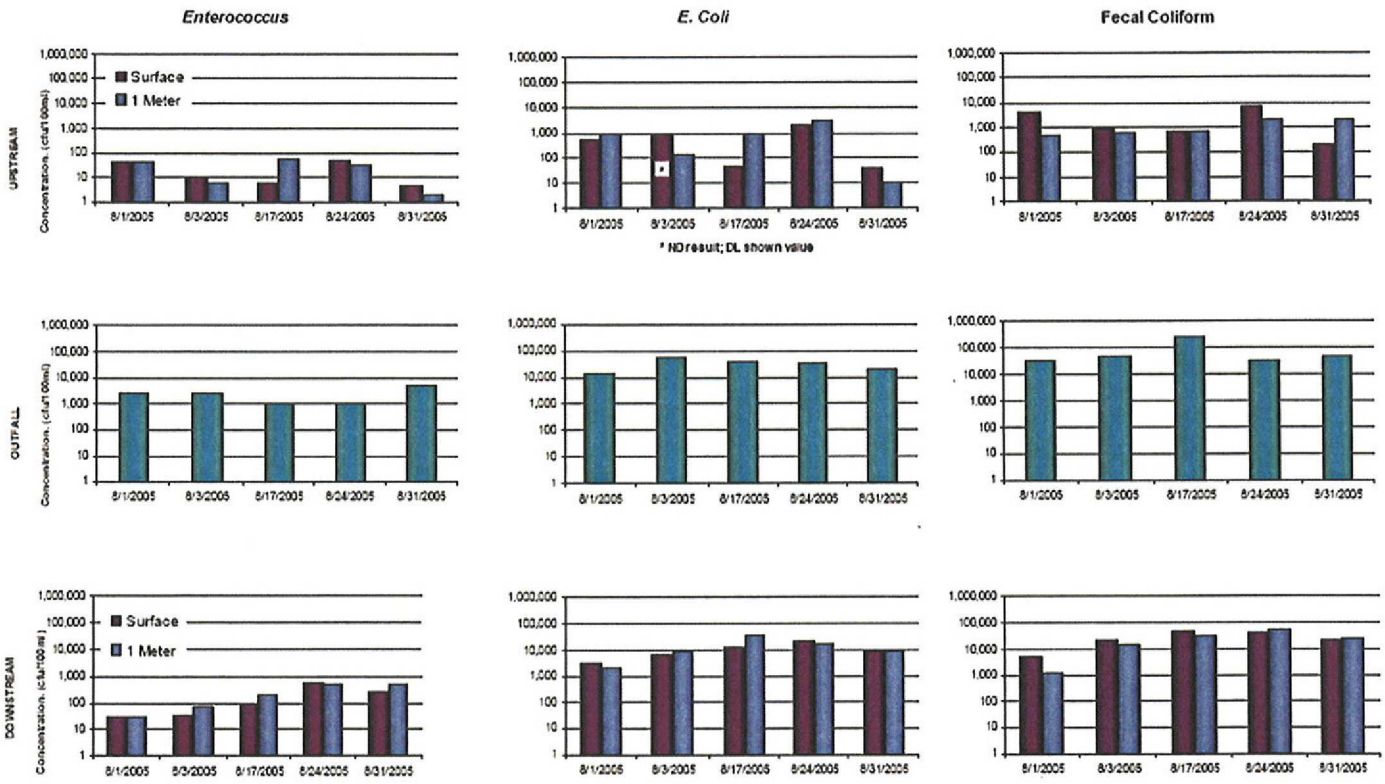


Figure 3-3. Calumet Dry Weather Bacteria Histograms

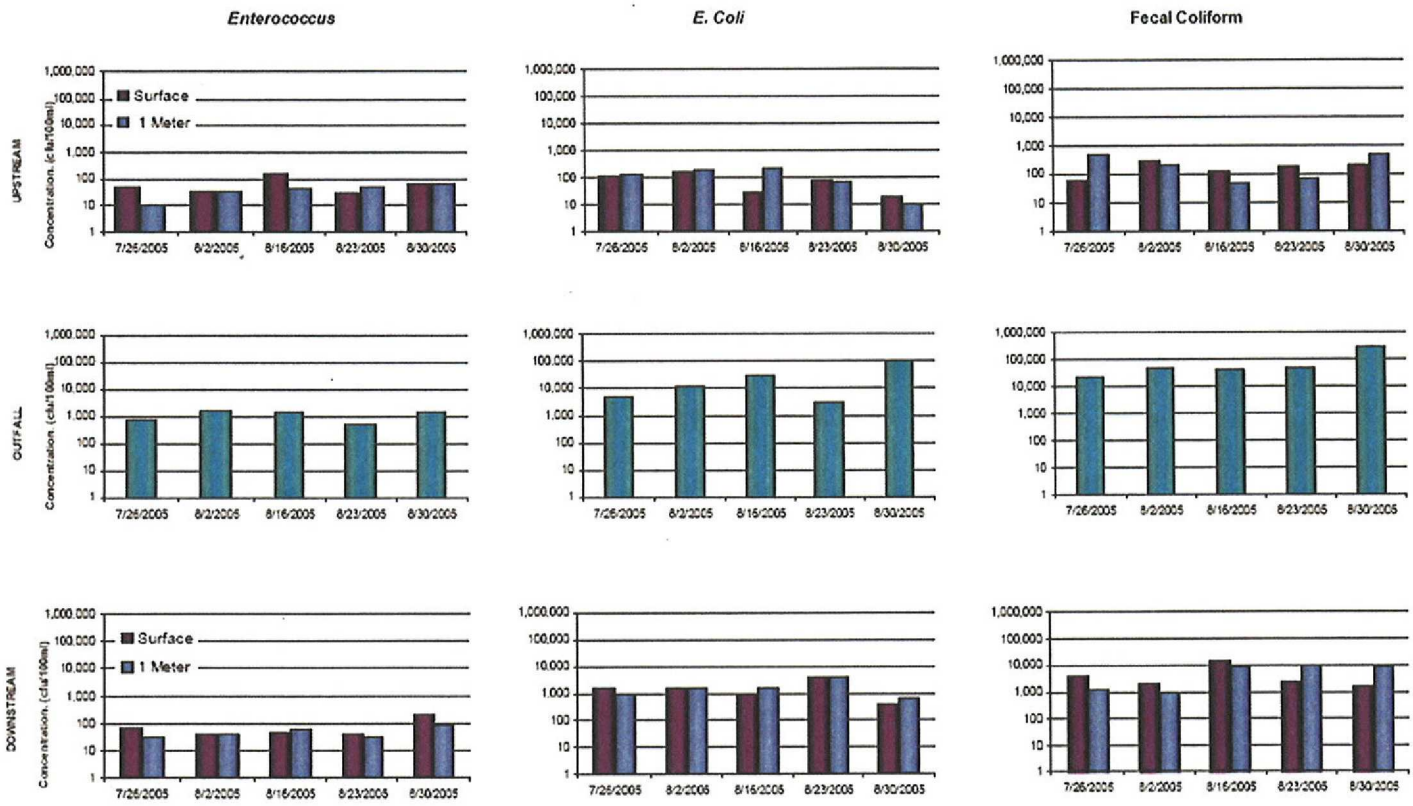


Figure 3-4. ANOVA Results: Dry Weather *E. coli* (EC)- vs Site, Location, Depth

```
Factor     Type   Levels  Values
Site       fixed       3  Calumet, Northside, Stickney
Location   fixed       2  DNS, UPS
Depth      fixed       2  1 Meter, Surface

Analysis of Variance for EC

Source                DF          SS          MS       F      P
Site                   2   901313363   450656682    8.34  0.001
Location               1  1647980042  1647980042   30.51  0.000
Depth                  1     7245375     7245375    0.13  0.716
Site*Location          2   716607163   358303582    6.63  0.003
Site*Depth             2    20971530    10485765    0.19  0.824
Location*Depth         1         202         202    0.00  0.998
Site*Location*Depth    2    42337503    21168752    0.39  0.678
Error                 48  2592462880    54009643
Total                 59  5928918056

S = 7349.13   R-Sq = 56.27%   R-Sq(adj) = 46.25%

Means

Depth      N      EC
1 Meter   30  6283.3
Surface   30  5580.3

Location   N      EC
DNS       30  11177
UPS       30    686
```

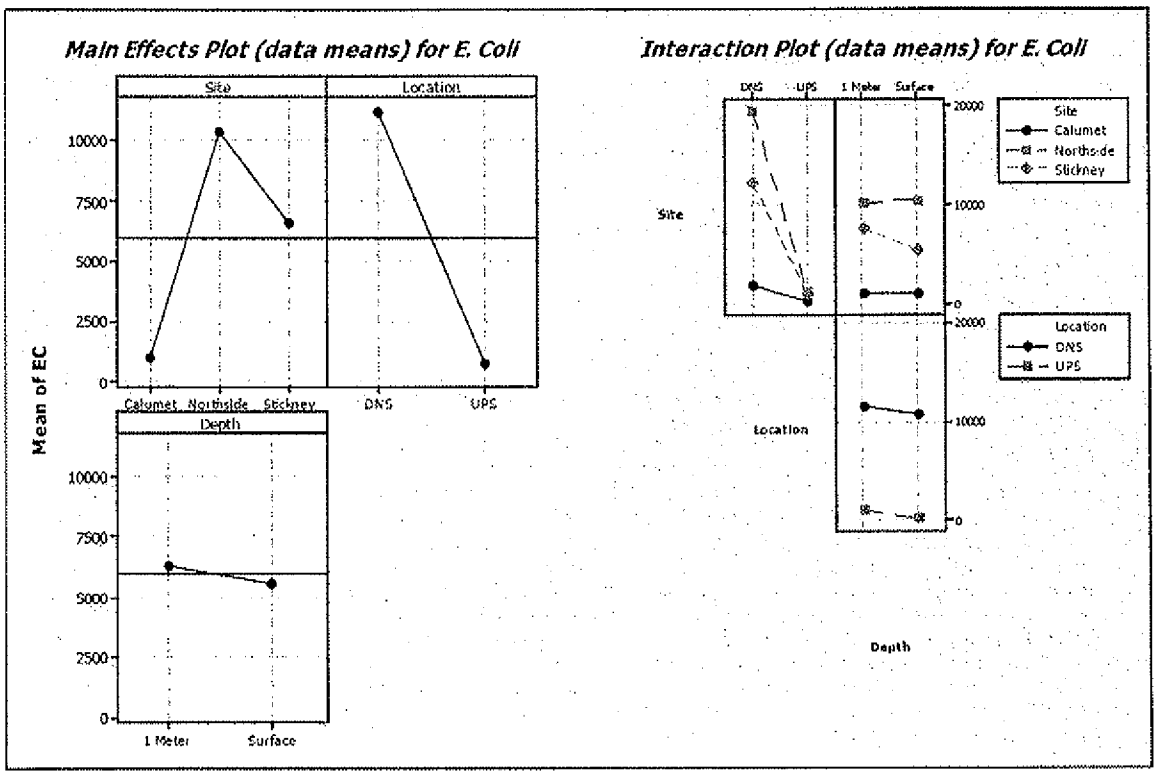


Figure 3-5. ANOVA Results: Dry Weather Fecal coliform (FC) - vs Site, Location, Depth

```
Factor  Type  Levels  Values
Site    fixed    3  Calumet, North Side, Stickney
Location  fixed    2  DNS, UPS
Depth  fixed    2  1 Meter, Surface

Analysis of Variance for FC

Source          DF       SS       MS     F     P
Site             2  3104793643  1552396822  22.36  0.000
Location         1  7115308202  7115308202  102.49  0.000
Depth            1  103097042  103097042  1.49  0.229
Site*Location      2  2567400003  1283700002  18.49  0.000
Site*Depth         2  97949503  48974752  0.71  0.499
Location*Depth     1  91637042  91637042  1.32  0.256
Site*Location*Depth  2  135756543  67878272  0.98  0.384
Error           48  3332361920  69424207
Total           59  16548303890

S = 8332.12  R-Sq = 79.86%  R-Sq(adj) = 75.25%

Means

Depth  N  FC
1 Meter  30  10839
Surface  30  13461

Location  N  FC
DNS     30  23040
UPS     30  1260
```

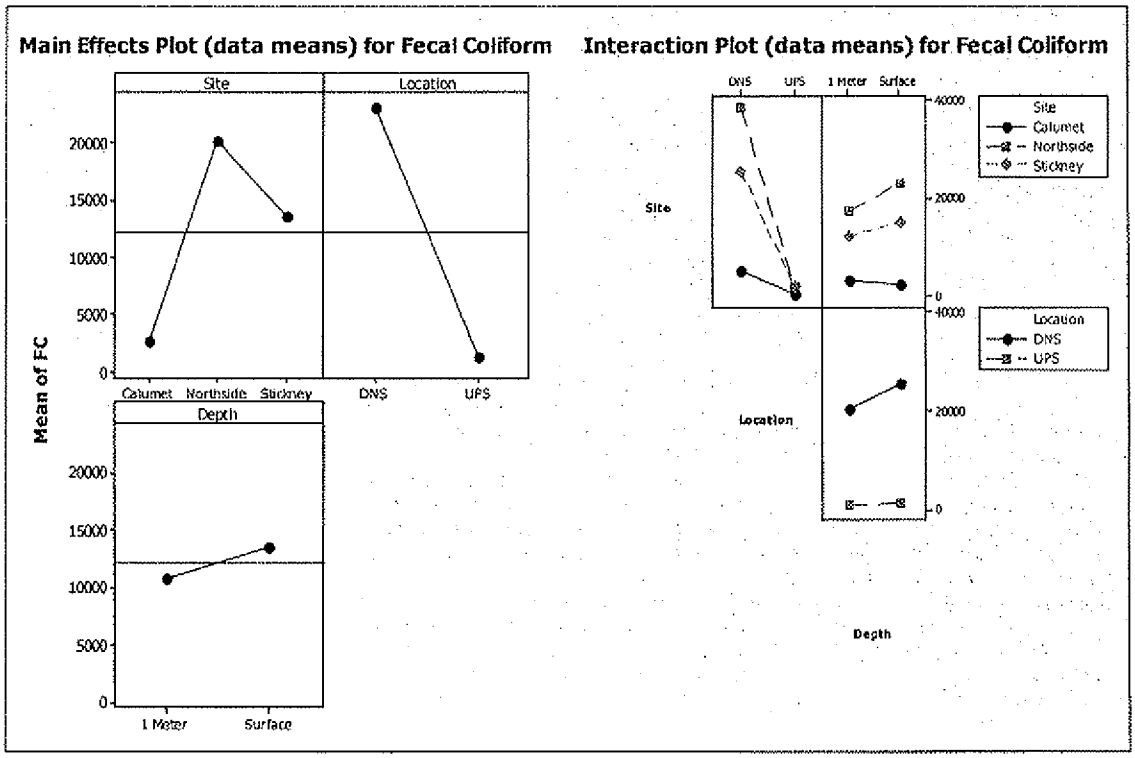


Figure 3-6. ANOVA Results: Dry Weather *Enterococcus* (EN)- vs Site, Location, Depth

```
Factor     Type    Levels  Values
Site       fixed        3  Calumet, Northside, Stickney
Location   fixed        2  DNS, UPS
Depth      fixed        2  1 Meter, Surface


Analysis of Variance for EN

Source                DF        SS        MS       F       P
Site                   2   4952167   2476083   12.90   0.000
Location               1   3777042   3777042   19.64   0.000
Depth                  1    232379    232379    1.21   0.277
Site*Location          2   4279671   2139835   11.13   0.000
Site*Depth             2    465794    232897    1.21   0.306
Location*Depth         1    223016    223016    1.16   0.286
Site*Location*Depth    2    356439    178219    0.93   0.402
Error                 48   9211734    191911
Total                 59  23498241

S = 438.077   R-Sq = 60.80%   R-Sq(adj) = 51.81%

Means

Depth      N      EN
1 Meter   30  355.80
Surface   30  231.33

Location   N      EN
DNS       30  537.73
UPS       30   49.40
```

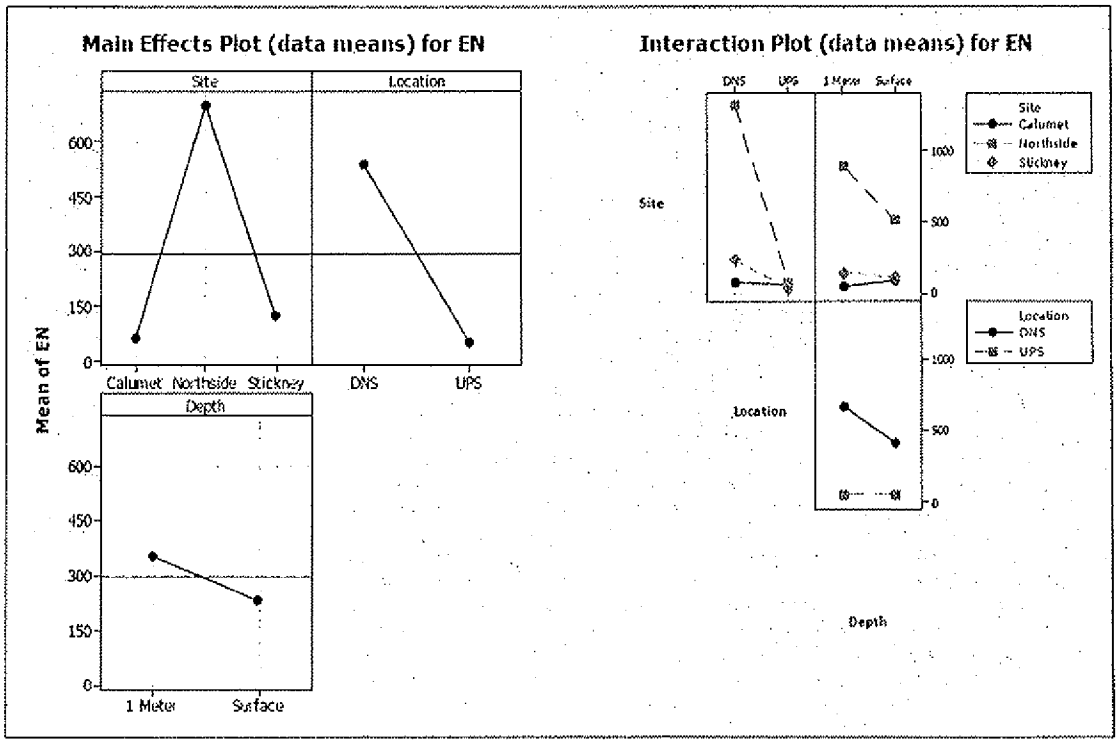


Figure 3-7. ANOVA Results: Wet Weather *E. coli* (EC) -vs Site, Location

```
Factor     Type    Levels  Values
Site       fixed        3  Calumet, Northside, Stickney
Location   fixed        2  DNS, UPS
```

```
Analysis of Variance for EC-Result, using Adjusted SS for Tests
```

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	2	1.74458E+11	1.42868E+11	71434162422	6.90	0.003
Location	1	1777951817	464805788	464805788	0.04	0.833
Site*Location	2	11788688654	11788688654	5894344327	0.57	0.570
Error	39	4.03612E+11	4.03612E+11	10349013607		
Total	44	5.91636E+11				

```
S = 101730   R-Sq = 31.78%   R-Sq(adj) = 23.03%
```

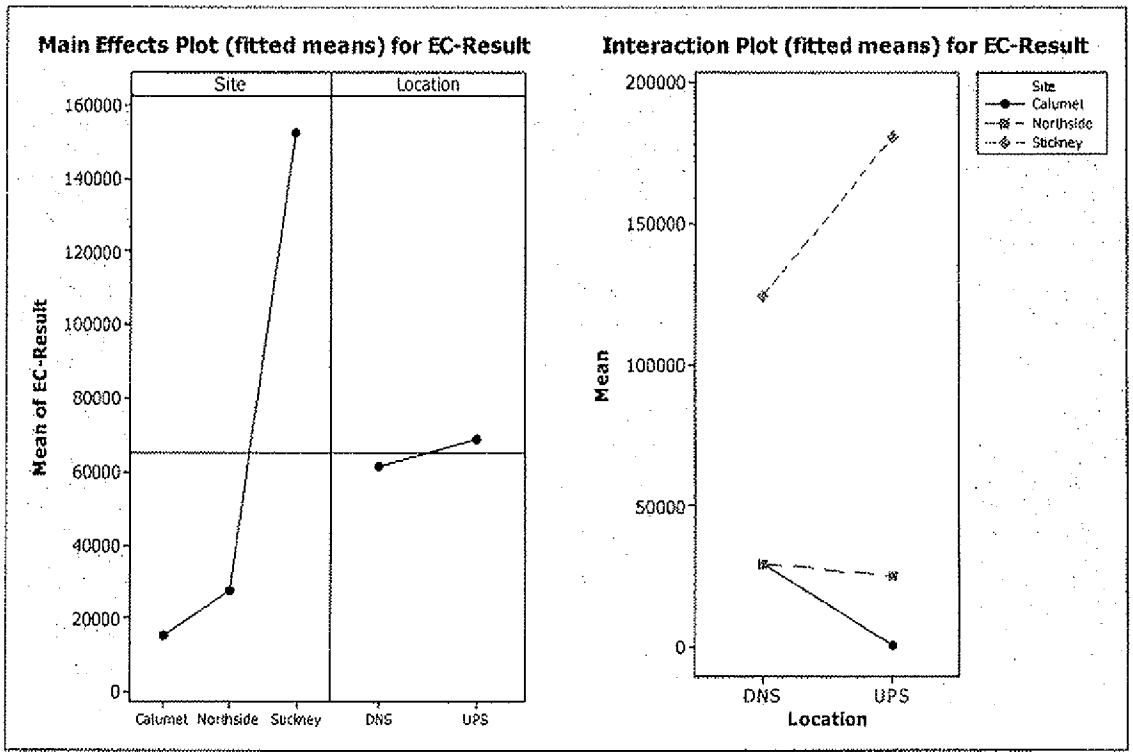


Figure 3-8. ANOVA Results: Wet Weather Fecal Coliform (FC)-vs Site, Location

Factor	Type	Levels	Values
Site	fixed	3	Calumet, Northside, Stickney
Location	fixed	2	DNS, UPS

Analysis of Variance for FC-Result, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	2	1.90477E+13	1.22816E+13	6.14080E+12	2.02	0.147
Location	1	3.72912E+12	2.23229E+12	2.23229E+12	0.73	0.397
Site*Location	2	4.54975E+12	4.54975E+12	2.27487E+12	0.75	0.480
Error	39	1.18731E+14	1.18731E+14	3.04438E+12		
Total	44	1.46057E+14				

S = 1744815 R-Sq = 18.71% R-Sq(adj) = 8.29%

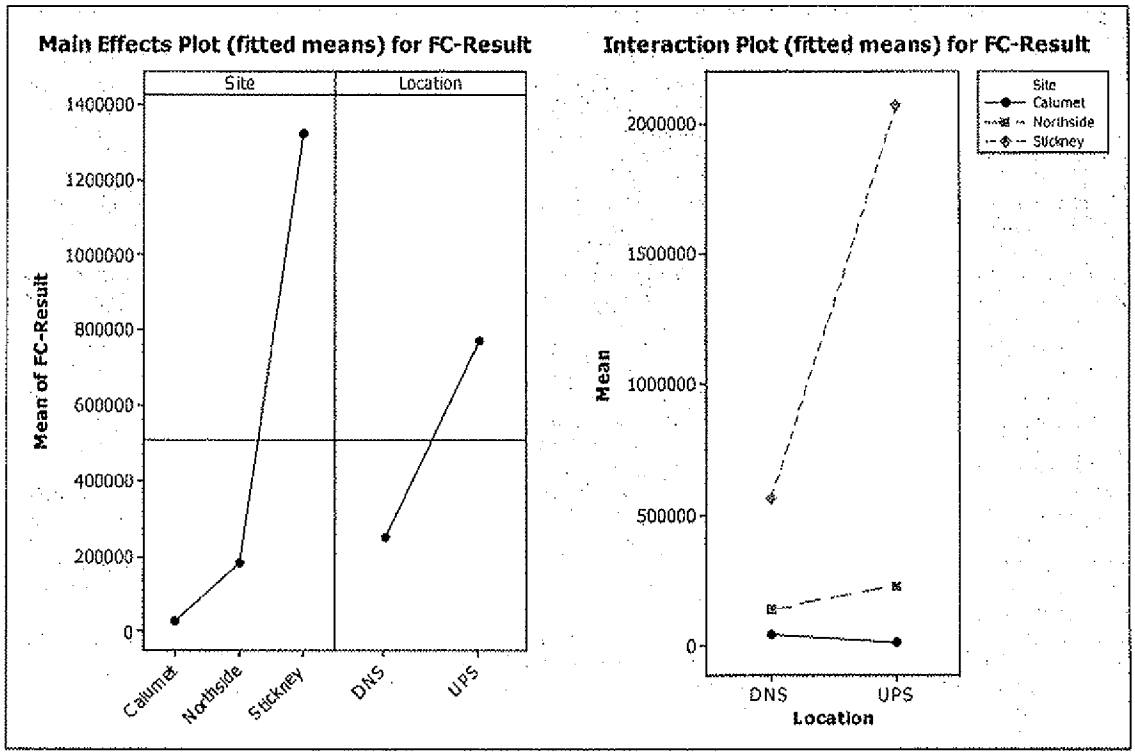


Figure 3-9. ANOVA Results: Wet Weather *Enterococcus* (EN)- vs Site, Location

Factor	Type	Levels	Values
Site	fixed	3	Calumet, Northside, Stickney
Location	fixed	2	DNS, UPS

Analysis of Variance for EN-Result, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	2	21100315538	17315997821	8657998910	3.99	0.027
Location	1	343398722	86249900	86249900	0.04	0.843
Site*Location	2	2421177249	2421177249	1210588625	0.56	0.577
Error	39	84707916456	84707916456	2171997858		
Total	44	1.08573E+11				

S = 46604.7 R-Sq = 21.98% R-Sq(adj) = 11.98%

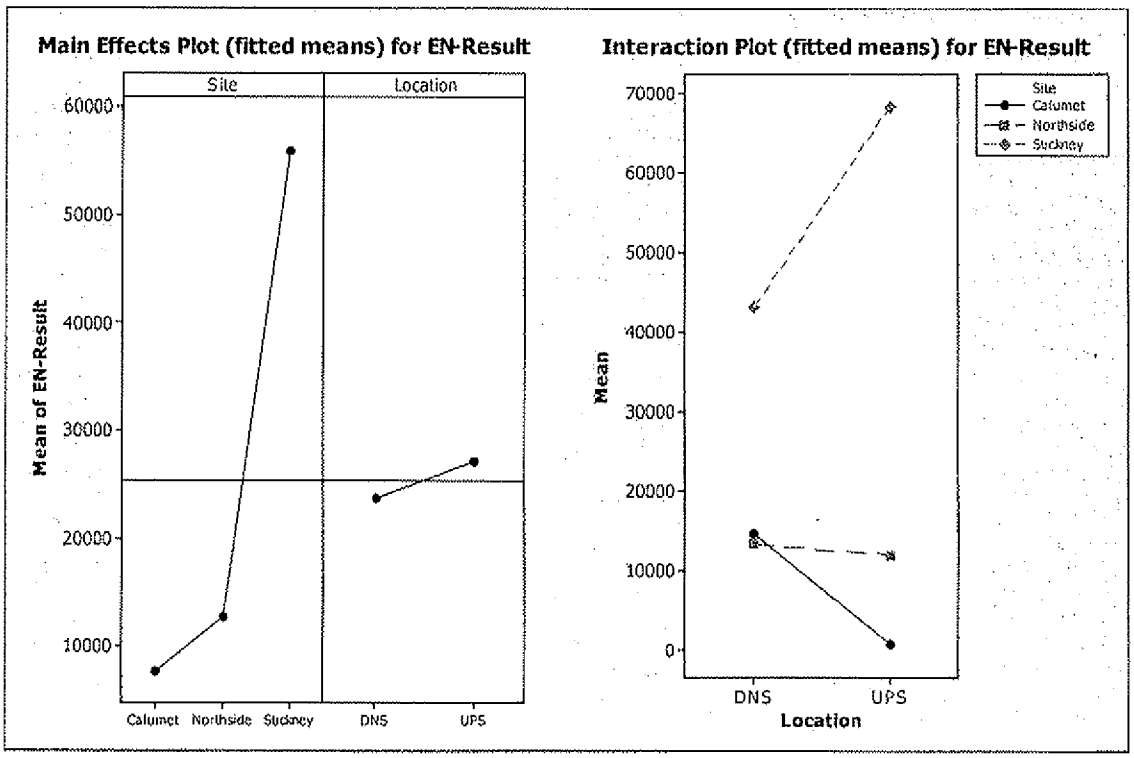


Figure 3-10. ANOVA Results: Wet Weather *Pseudomonas aeruginosa* (PA)- vs Site, Location

```
Factor     Type    Levels  Values
Site       fixed        3  Calumet, Northside, Stickney
Location   fixed        2  DNS, UPS
```

```
Analysis of Variance for PA-Result, using Adjusted SS for Tests
```

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	2	1642899111	1323778254	661889127	3.15	0.054
Location	1	20243048	1950694	1950694	0.01	0.924
Site*Location	2	372838063	372838063	186419032	0.89	0.420
Error	39	8203498889	8203498889	210346125		
Total	44	10239479111				

```
S = 14503.3   R-Sq = 19.88%   R-Sq(adj) = 9.61%
```

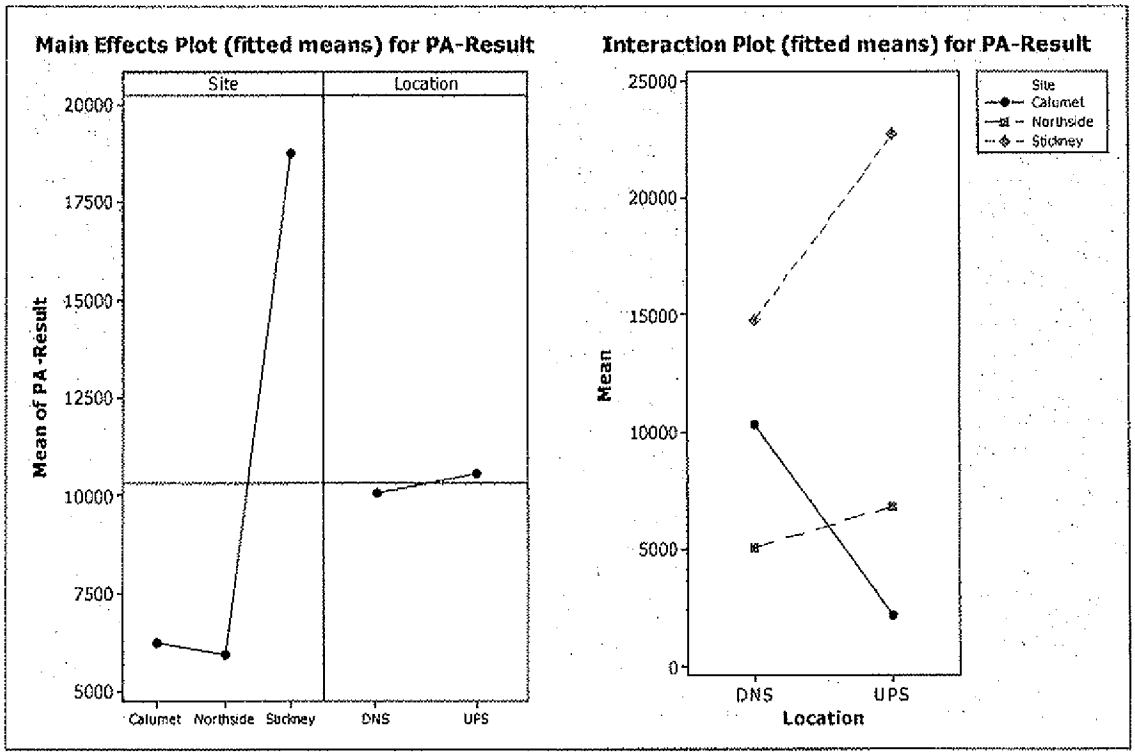


Figure 3-11. ANOVA Results: Wet Weather *Salmonella* (SA)—vs Site, Location

```
Factor     Type   Levels  Values
Site       fixed       3  Calumet, Northside, Stickney
Location   fixed       2  DNS, UPS


Analysis of Variance for SA-Result, using Adjusted SS for Tests

Source          DF   Seq SS   Adj SS   Adj MS     F      P
Site             2   218.75   101.99    50.99  0.87  0.426
Location         1     5.16     3.70     3.70  0.06  0.803
Site*Location    2    65.37    65.37    32.69  0.56  0.577
Error           39  2283.06  2283.06    58.54
Total           44  2572.34


S = 7.65114   R-Sq = 11.25%   R-Sq(adj) = 0.00%
```

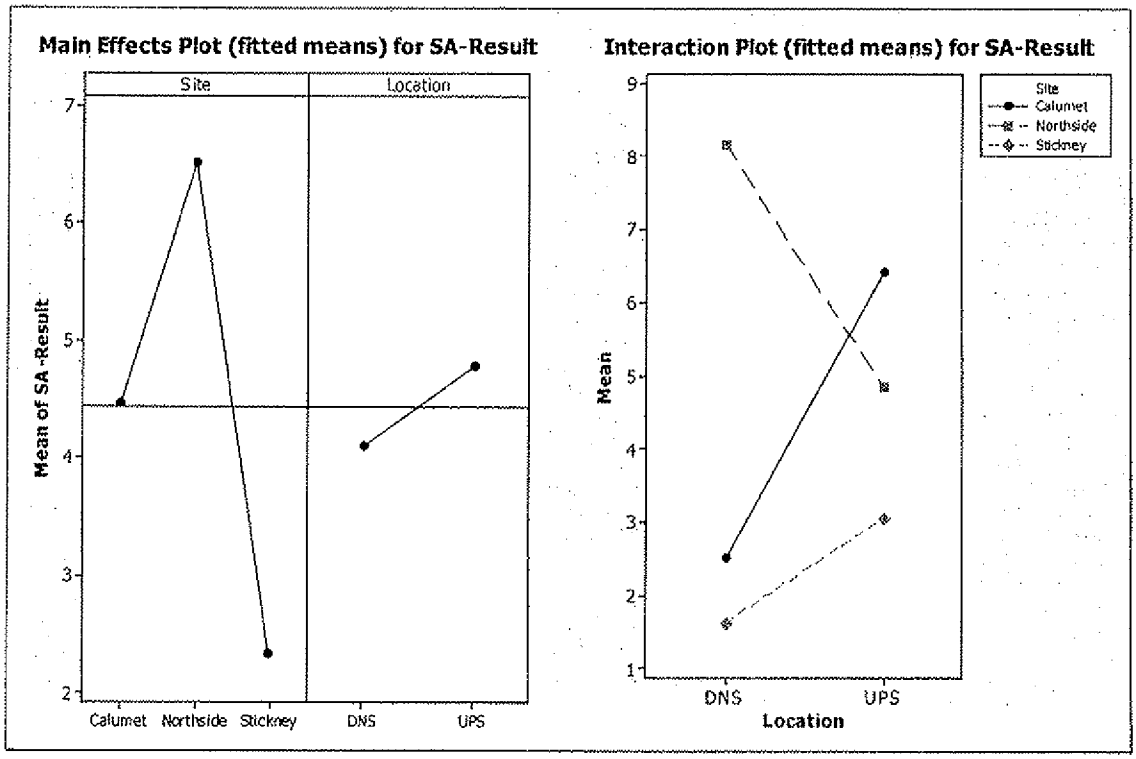


Figure 3-12. ANOVA Results: Dry and Wet Weather *E. coli* (EC) -vs Site, Location, Weather

Factor	Type	Levels	Values
Site	fixed	3	Calumet, Northside, Stickney
Location	fixed	2	DNS, UPS
Weather	fixed	2	Dry, Wet

Analysis of Variance for EC-Result, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	2	77039918173	90650182856	45325091428	10.38	0.000
Location	1	5081666	44432364	44432364	0.01	0.920
Weather	1	1.09478E+11	73586212295	73586212295	16.84	0.000
Site*Location	2	2885045618	6643241215	3321620607	0.76	0.470
Site*Weather	2	97308166973	86712312287	43356156143	9.92	0.000
Location*Weather	1	2687853662	1714937779	1714937779	0.39	0.532
Site*Location*Weather	2	8488919292	8488919292	4244459646	0.97	0.382
Error	93	4.06275E+11	4.06275E+11	4368543529		
Total	104	7.04167E+11				

S = 66095.0 R-Sq = 42.30% R-Sq(adj) = 35.48%

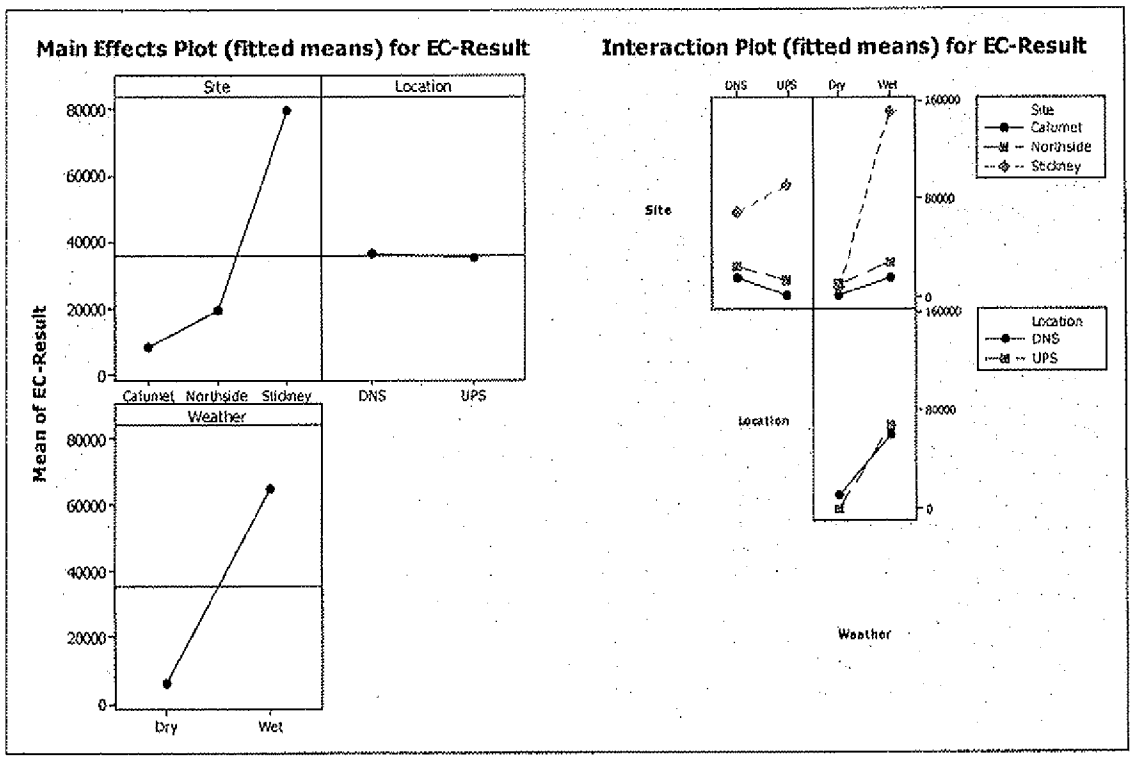


Figure 3-13. ANOVA Results: Dry and Wet Weather Fecal coliforms (FC)-vs Site, Location, Weather

Factor	Type	Levels	Values
Site	fixed	3	Calumet, Northside, Stickney
Location	fixed	2	DNS, UPS
Weather	fixed	2	Dry, Wet

Analysis of Variance for FC-Result, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	2	8.21628E+12	7.64653E+12	3.82326E+12	2.99	0.055
Location	1	1.48286E+12	1.33176E+12	1.33176E+12	1.04	0.310
Weather	1	8.95674E+12	5.19114E+12	5.19114E+12	4.07	0.047
Site*Location	2	1.72380E+12	2.79905E+12	1.39952E+12	1.10	0.338
Site*Weather	2	9.51074E+12	7.55820E+12	3.77910E+12	2.96	0.057
Location*Weather	1	2.14690E+12	1.57231E+12	1.57231E+12	1.23	0.270
Site*Location*Weather	2	2.83182E+12	2.83182E+12	1.41591E+12	1.11	0.334
Error	93	1.18735E+14	1.18735E+14	1.27672E+12		
Total	104	1.53604E+14				

S = 1129918 R-Sq = 22.70% R-Sq(adj) = 13.56%

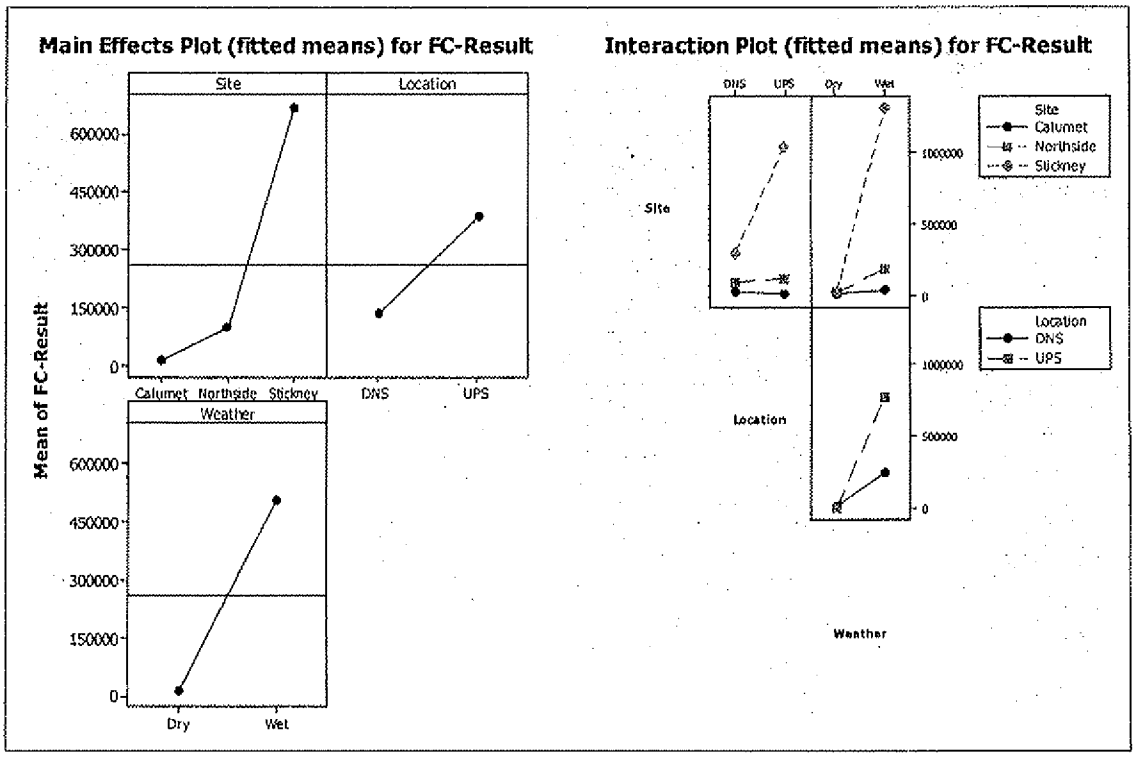


Figure 3-14. ANOVA Results: Dry and Wet Weather *Enterococcus* (EN)-vs Site, Location, Weather

Factor	Type	Levels	Values
Site	fixed	3	Calumet, Northside, Stickney
Location	fixed	2	DNS, UPS
Weather	fixed	2	Dry, Wet

Analysis of Variance for EN-Result, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	2	8930767038	10628391514	5314195757	5.83	0.004
Location	1	47827470	41458991	41458991	0.05	0.832
Weather	1	20324722837	13256441835	13256441835	14.55	0.000
Site*Location	2	709926440	1517836660	758918330	0.83	0.438
Site*Weather	2	11609334268	10803606740	5401803370	5.93	0.004
Location*Weather	1	169279329	72737410	72737410	0.08	0.778
Site*Location*Weather	2	1501755019	1501755019	750877509	0.82	0.442
Error	93	84717922360	84717922360	910945402		
Total	104	1.28012E+11				

S = 30181.9 R-Sq = 33.82% R-Sq(adj) = 25.99%

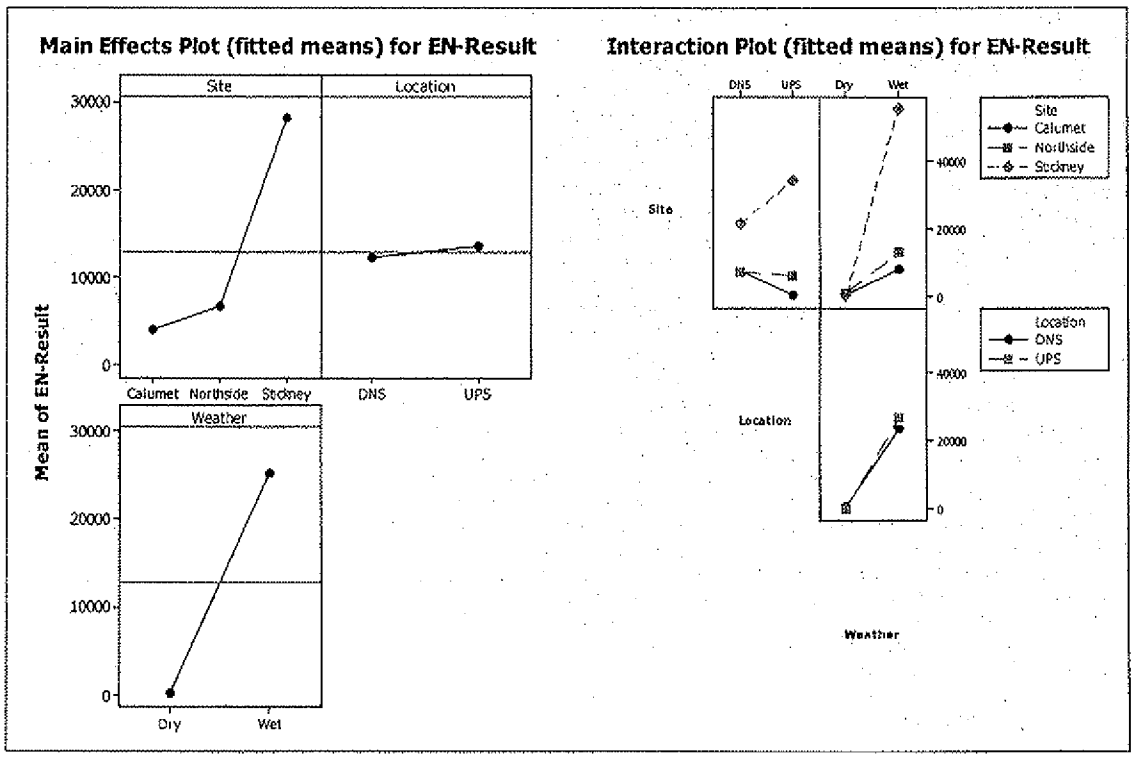


Figure 3-15. ANOVA Dry and Wet Weather Results : *Pseudomonas aeruginosa* (PA)-vs Site, Location, Weather

Factor	Type	Levels	Values
Site	fixed	3	Calumet, Northside, Stickney
Location	fixed	2	DNS, UPS
Weather	fixed	2	Dry, Wet

Analysis of Variance for PA-Result, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	2	441616973	631491193	315745596	3.22	0.044
Location	1	10667259	9235164	9235164	0.09	0.760
Weather	1	2589159499	1656144308	1656144308	16.89	0.000
Site*Location	2	217156362	253295666	126647833	1.29	0.280
Site*Weather	2	1182143499	1108234022	554117011	5.65	0.005
Location*Weather	1	732611	620943	620943	0.01	0.937
Site*Location*Weather	2	232796854	232796854	116398427	1.19	0.310
Error	93	9119832219	9119832219	98062712		
Total	104	13794105276				

S = 9902.66 R-Sq = 33.89% R-Sq(adj) = 26.07%

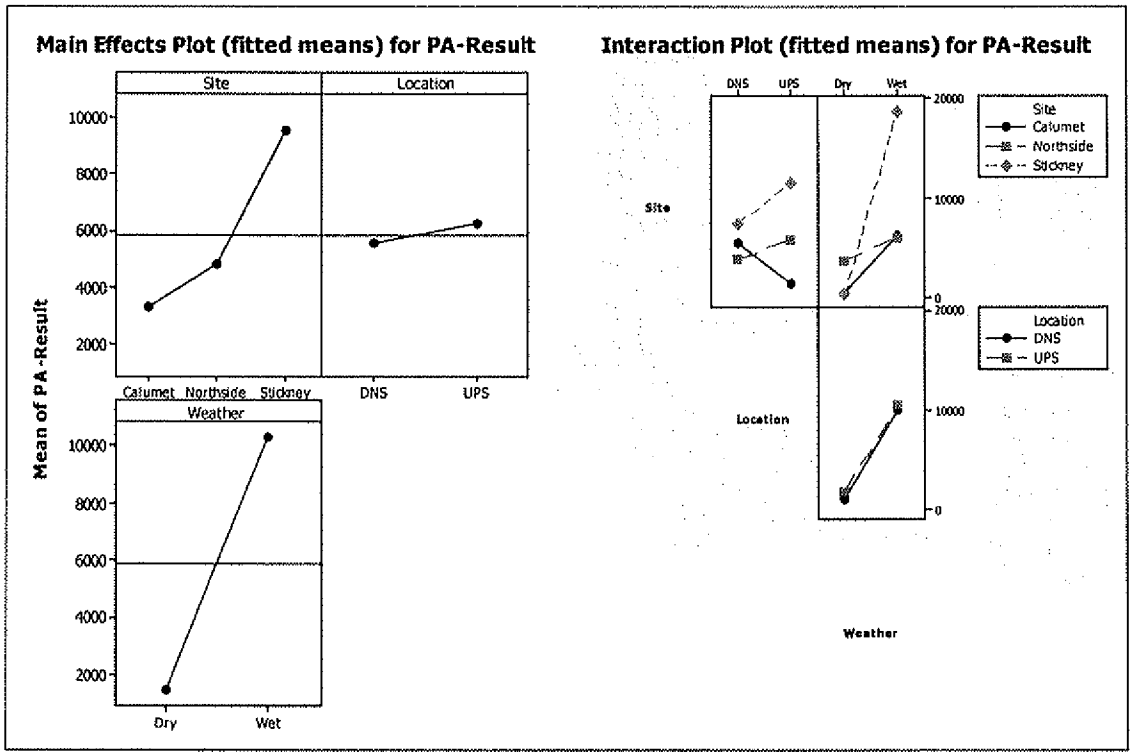
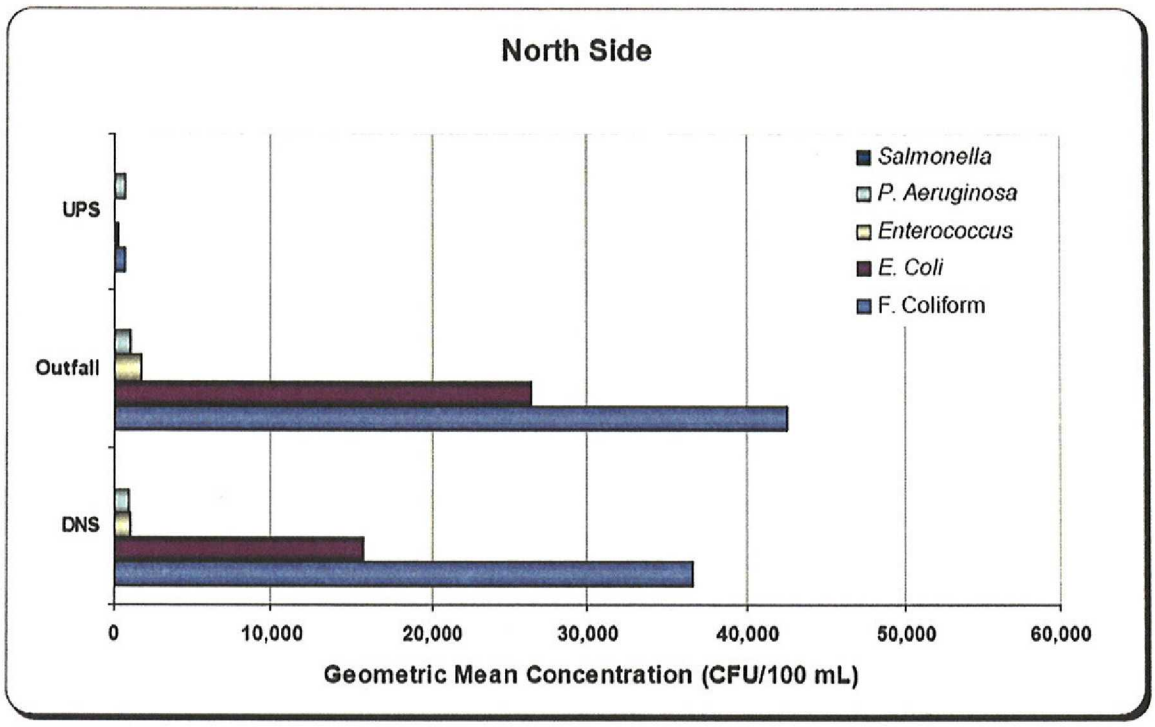


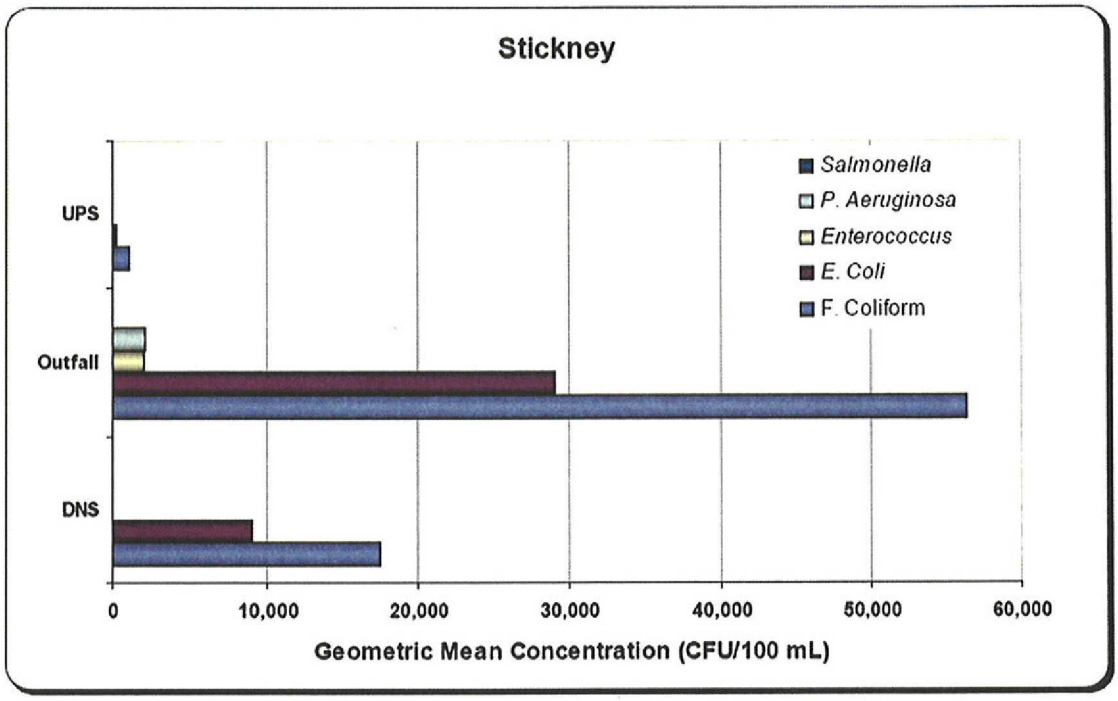
Figure 3-16. Geometric Mean Dry Weather Bacteria Concentrations at North Side



Note:

The units for *Salmonella* are in MPN/100 mL

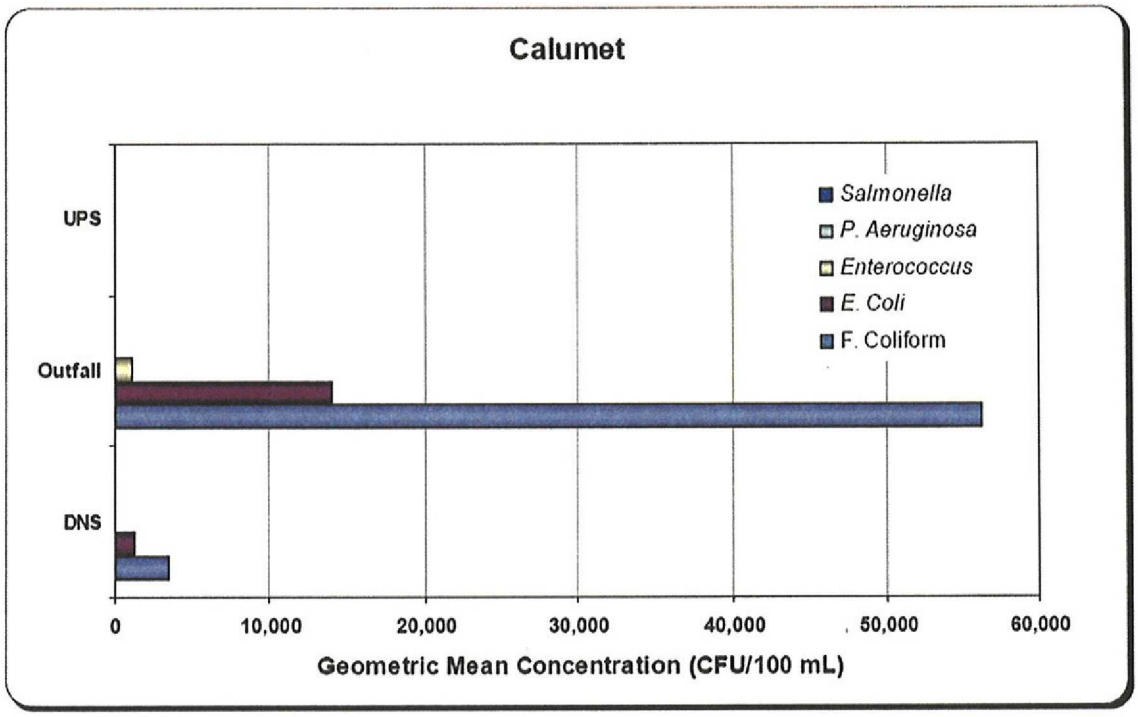
Figure 3-17. Geometric Mean Dry Weather Bacteria Concentrations at Stickney



Note:

The units for *Salmonella* are in MPN/100 mL

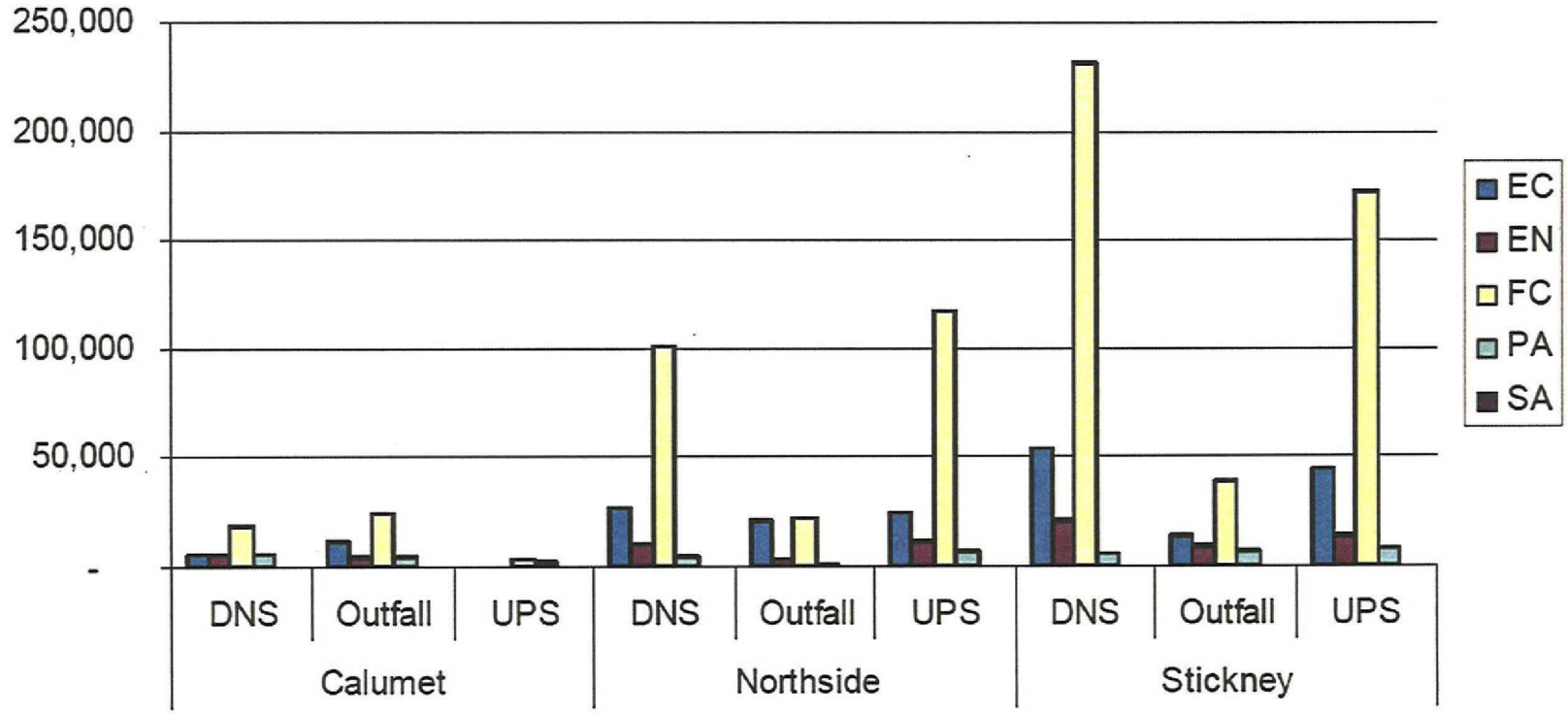
Figure 3-18. Geometric Mean Dry Weather Bacteria Concentrations at Calumet



Note:

The units for *Salmonella* are in MPN/100 mL

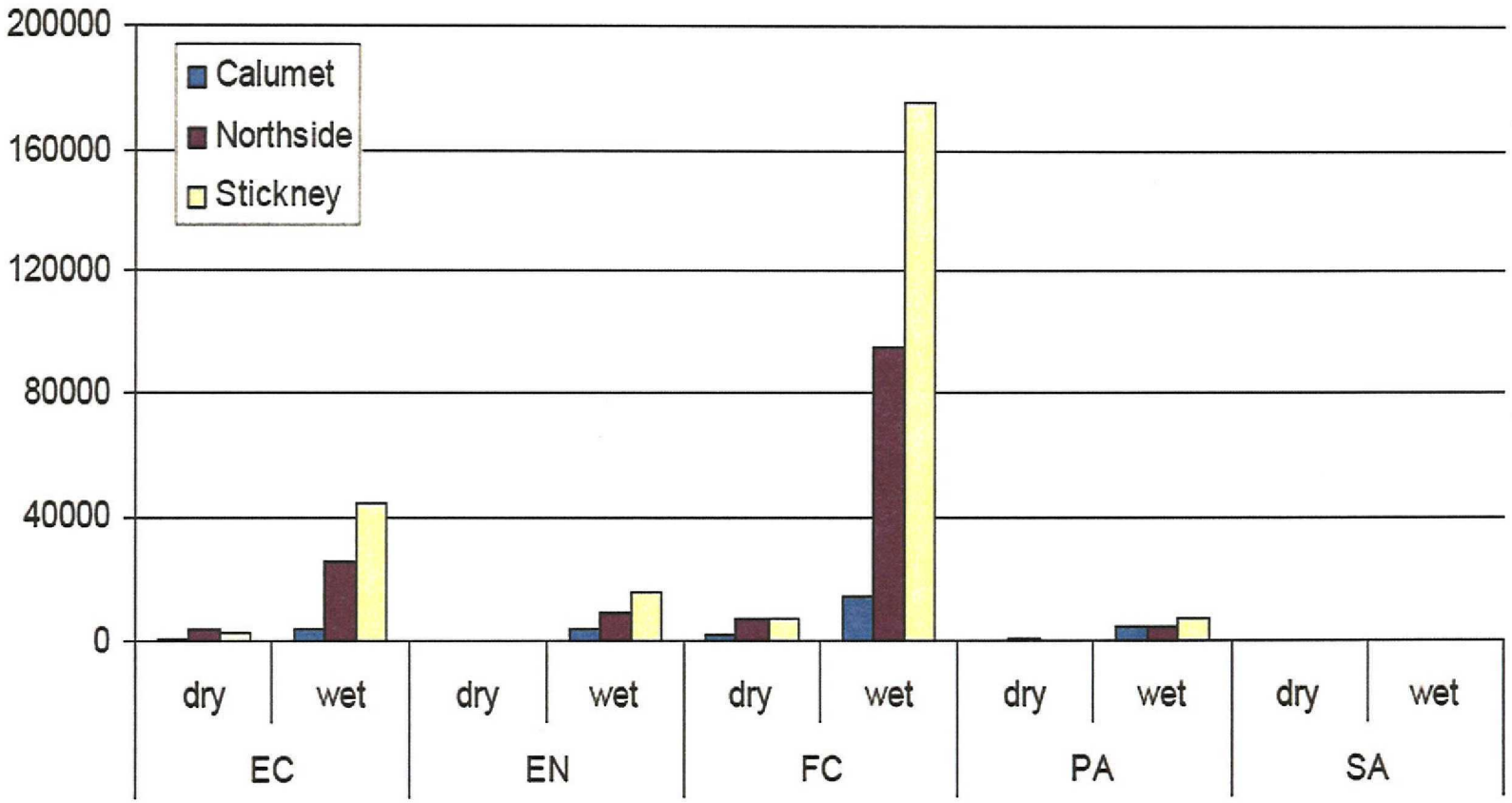
Figure 3-19. Wet Weather Geometric Mean Bacteria Concentrations by Location (UPS, DNS, OUTFALL) at North Side, Stickney and Calumet WRPs (cfu/100mL; *Salmonella* in MPN/L)



Notes:

UPS=Upstream
DNS=Downstream
PA=*Pseudomonas aeruginosa*
FC=Fecal coliforms
EC=*E. coli*
EN=*Enterococci*
SA=*Salmonella*

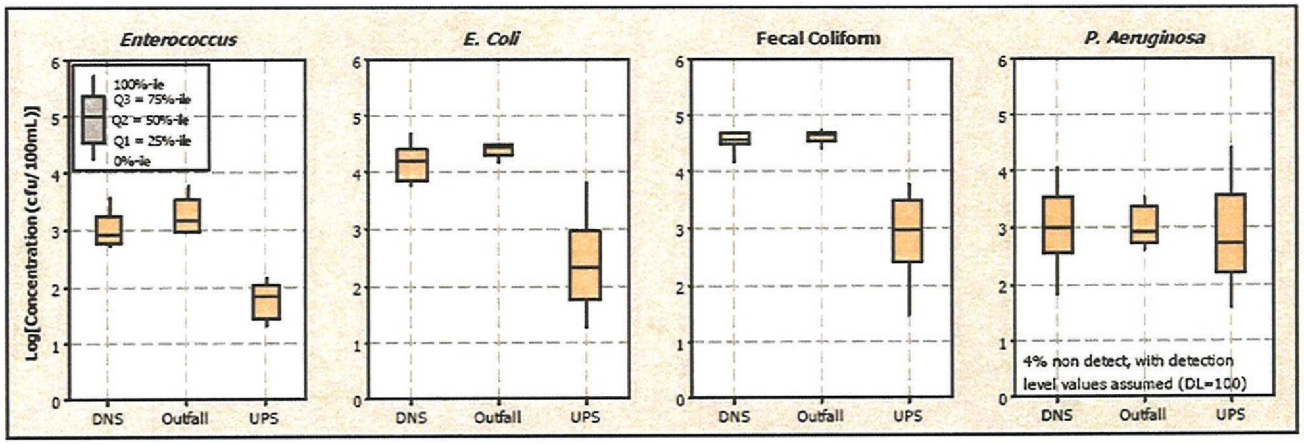
Figure 3-20. Dry and Wet Weather Geometric Mean Bacteria Concentrations by WRP (including OUTFALLS, UPS, DNS) (cfu/100mL; *Salmonella* in MPN/L)



Notes:

UPS=Upstream
DNS=Downstream
PA=*Pseudomonas aeruginosa*
FC=Fecal coliforms
EC=*E. coli*
EN=*Enterococci*
SA=*Salmonella*

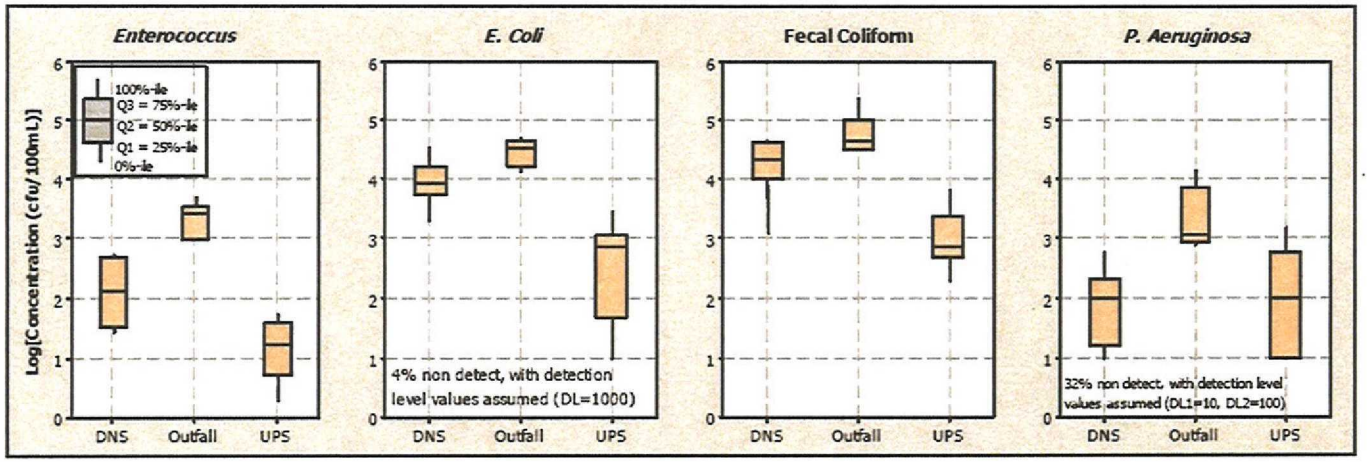
Figure 3-21. North Side Dry Weather Spatial Box Plots of Bacteria Concentrations



Notes:

UPS = Upstream
DNS = Downstream

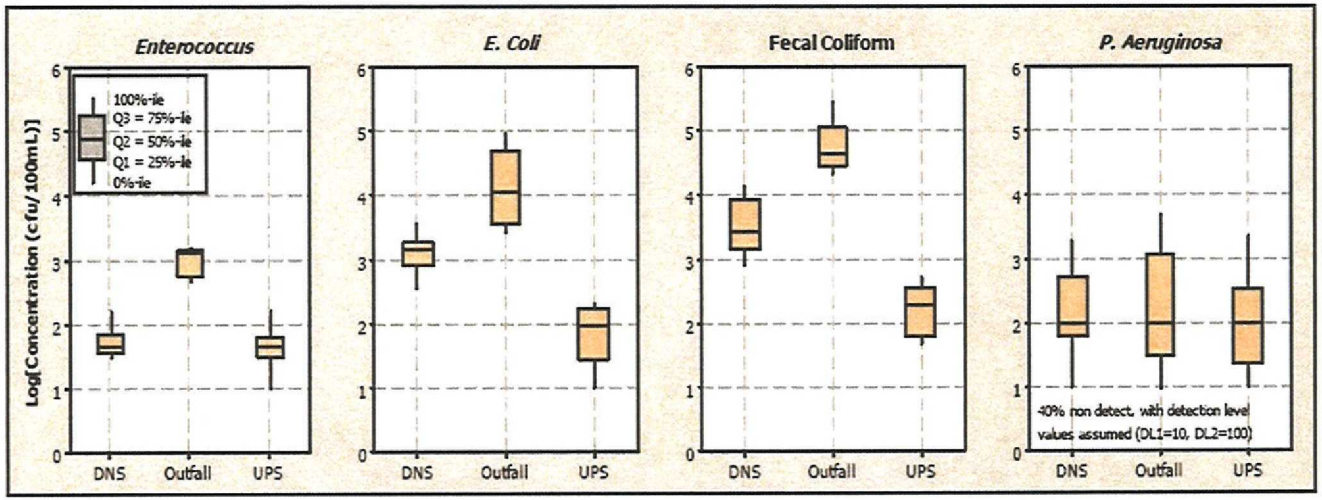
Figure 3-22. Stickney Dry Weather Spatial Box Plots of Bacteria Concentrations



Notes:

UPS = Upstream
DNS = Downstream

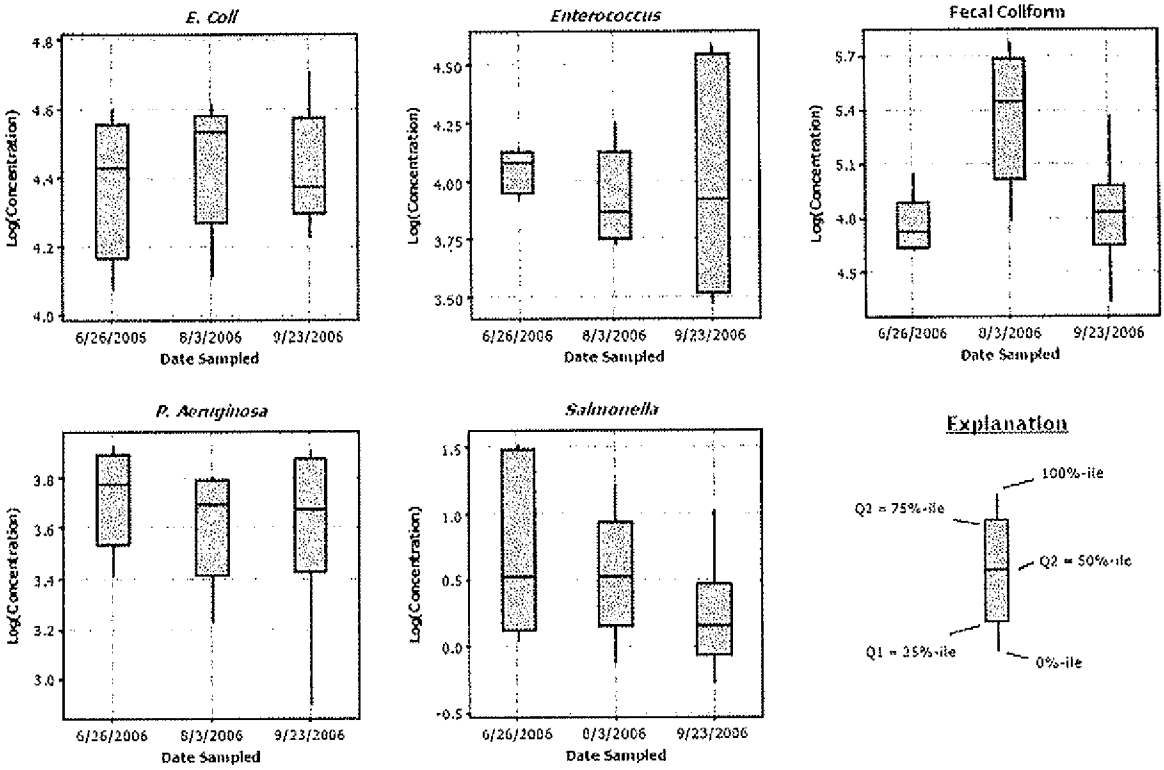
Figure 3-23. Calumet Dry Weather Spatial Box Plots of Bacteria Concentration



Notes:

UPS = Upstream
DNS = Downstream

Figure 3-24. North Side Wet Weather Temporal Percentile Box Plots of Bacteria Concentrations

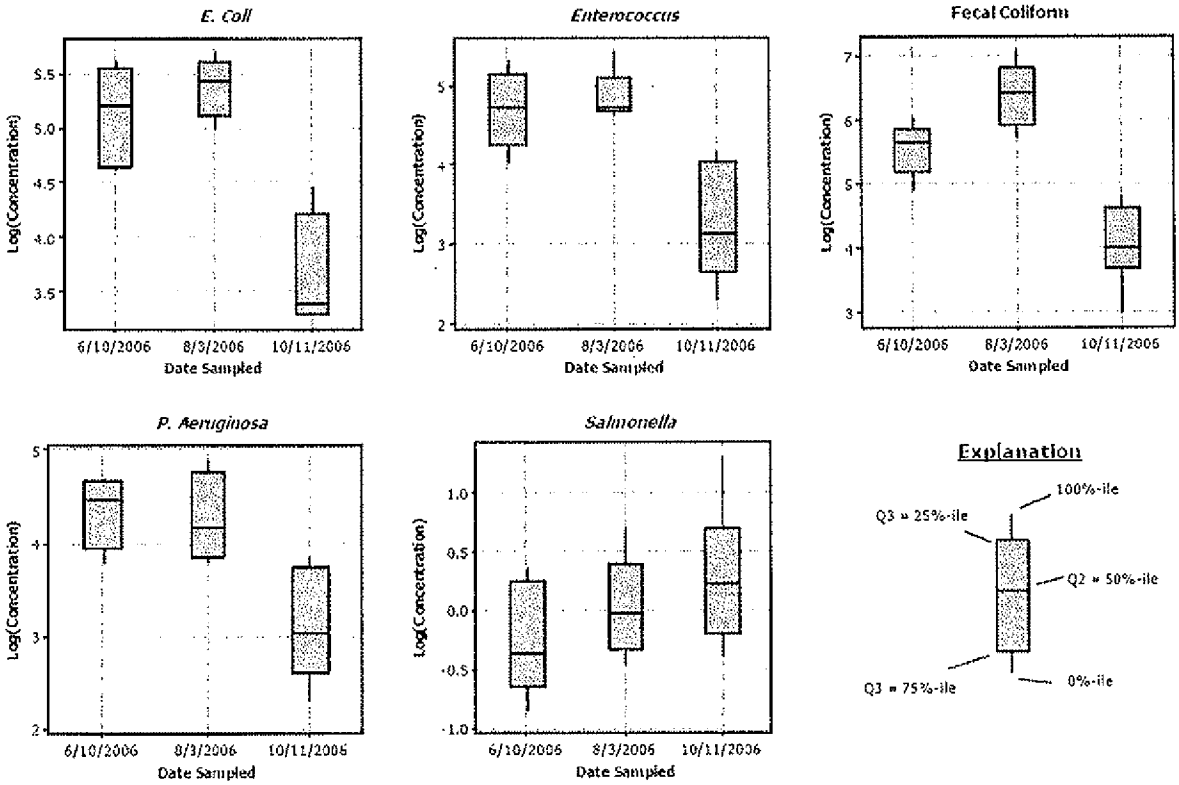


Notes:

UPS=Upstream
DNS=Downstream
PA=*Pseudomonas aeruginosa*
FC=Fecal coliforms
EC=*E. coli*
EN=*Enterococci*
SA=*Salmonella*

Figure 3-25. Stickney Wet Weather Temporal Percentile Box Plots of Bacteria Concentrations

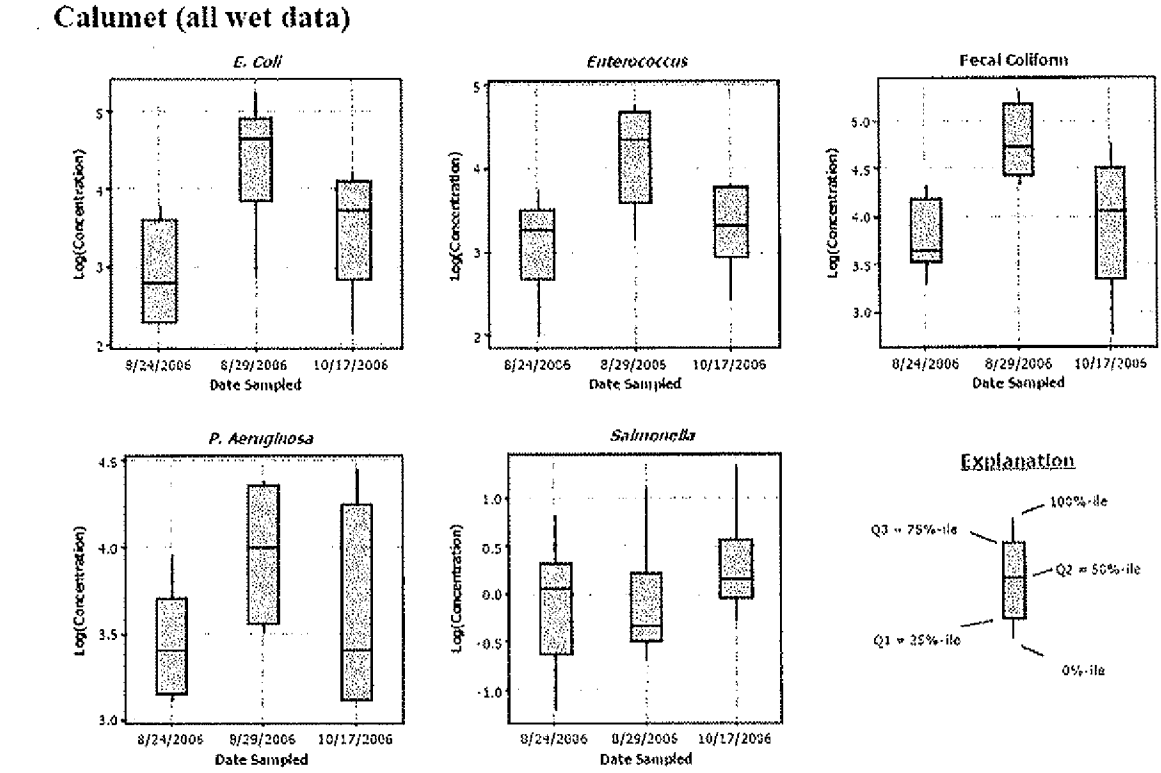
Stickney (all wet data)



Notes:

UPS=Upstream
DNS=Downstream
PA=*Pseudomonas aeruginosa*
FC=Fecal coliforms
EC=*E. coli*
EN=*Enterococci*
SA=*Salmonella*

Figure 3-26. Calumet Wet Weather Temporal Percentile Box Plots of Bacteria Concentrations



Notes:

UPS=Upstream
DNS=Downstream
PA=*Pseudomonas aeruginosa*
FC=Fecal coliforms
EC=*E. coli*
EN=*Enterococci*
SA=*Salmonella*

4. DISINFECTION

Disinfection is the destruction or otherwise inactivation of disease causing pathogenic microorganisms, including bacteria, viruses, and protozoa. Major disinfection mechanisms include: (1) damage to the cell wall, (2) alteration of cell permeability, (3) alteration of the colloidal nature of the protoplasm, and (4) inhibition of enzyme activity. Oxidizing agents, such as chlorine, can alter the chemical arrangement of enzymes and deactivate the enzyme. Radiation and ozone alter the colloidal nature of the protoplasm, producing a lethal effect (Metcalf & Eddy, 1991; Montgomery, 1985).

Disinfection is most commonly accomplished by the use of (1) chemical agents, (2) physical agents, and (3) radiation. Chlorine is the most commonly used chemical disinfectant. In addition, chloramines and chlorine dioxide can be used. Ozone is a highly effective disinfectant and its use is increasing. Ultra violet (UV) radiation is a physical disinfectant. UV radiation was originally used for high quality water supplies but is increasingly being used for wastewater disinfection. Chlorination and UV irradiation are the most prevalent forms of wastewater disinfection in the United States (Metcalf & Eddy, 1991; Montgomery, 1985; *WERF*, 2005). Table 4-1 presents a summary of disinfectant characteristics.

The following disinfection technologies have been evaluated by the District's consultants as candidate disinfection alternatives for the North Side, Stickney and Calumet WRPs (MWRDGC, 2005):

- Chlorination/dechlorination
- UV
- Ozonation

The District's evaluation criteria included: (1) long-term and short-term performance, (2) cost, (3) formation of disinfection by-products, and (4) public acceptance criteria. Chlorination/dechlorination is the most common disinfection method practiced in publicly owned treatment works (POTWs) in the State of Illinois. Dechlorination is needed to meet the District's National Pollutant Discharge Elimination (NPDES) effluent discharge limit of 0.05 mg/L for residual chlorine (Lue-Hing, 2005). Therefore, chlorination without dechlorination will not be considered in the evaluation of human risk assessment.

A large volume of scientific research has been conducted to assess whether municipal wastewater effluents need to be disinfected, and if so, how it should be accomplished. *WERF* (2005) concludes that it is not clear that wastewater disinfection should be practiced in all cases. Decisions regarding the need for effluent disinfection must be made on a site-specific basis. According to *WERF* (2005), disinfection is warranted in situations where direct human contact in the immediate vicinity of an outfall is possible or where effluent is discharged to areas involving the production of human food. Disinfection is warranted in situations where its application leads to a reduction in the risk of disease transmission. As illustrated by post-disinfection regrowth of bacteria, relatively poor virucidal behavior, and generation of persistent disinfection by-products (DBPs), it is not clear that wastewater disinfection always yields improved effluent or receiving water quality (*WERF*, 2005).

The following sections discuss chlorination/dechlorination, ozonation and UV effluent disinfection characteristics.

4.1 Chlorination/Dechlorination

Chlorination is widely used for wastewater disinfection in the United States. Although there are widespread differences in the susceptibility of various pathogens,

the general order of decreasing chlorine disinfection effectiveness are bacteria, viruses, and then protozoa (EPA, 1999).

Turbidity, color, inorganic, and organic nitrogenous compounds, iron, manganese, hydrogen sulfide, and total organic carbon have been shown to consistently and negatively influence chlorine disinfection efficiency. Chlorine-based disinfection of wastewater can be influenced by: (1) disinfectant concentration, (2) contact time, (3) pH, (4) temperature, and (5) physiological status of the target microbes (Montgomery, 1985).

Done properly, chlorination following secondary treatment will inactivate more than 99% of the pathogenic bacteria in the effluent. Viruses, and parasites found in municipal wastewater, whether primary or secondary, are characterized as being much more resistant and have different sensitivities to chlorination. When comparing the FC $\log_{10}$ reduction values following disinfection with chlorine, there was some variability between samples from different facilities. There appears to be no seasonal explanation for this variability; rather, it is likely that changes in the microbiological, chemical, and physical components of the wastewater streams were responsible for the observed variations in disinfection efficacy (*WERF*, 2005; EPA, 1999).

Results from the primary treatment of sewage coupled with chlorine disinfection demonstrated that *enterococci* were more resistant to chlorination than *E.coli*. Also, both bacteria were inactivated more rapidly than the viruses examined. There are currently no data to demonstrate that *Giardia* cysts are inactivated during chlorine-based disinfection of secondary effluents. Studies on infectivity of *Cryptosporidium* have found no inactivation due to chlorination of even highly treated wastewaters (*WERF*, 2005).

Chlorine disinfection can inactivate some viruses in wastewater, but not as effectively as it does in drinking water because of interference by dissolved organics and suspended particulates. Unless ammonia-nitrogen is removed from wastewater (e.g. through nitrification), the predominant form of chlorine will be chloramines, which are generally regarded as being less effective against viruses and parasites than free chlorine (*WERF*, 2005; EPA, 1999).

Chlorination beyond the break point to obtain free chlorine is required to kill many of the viruses of concern. To minimize the effects of the potentially toxic chlorine residuals on the environment, it is necessary to dechlorinate wastewater treated with chlorine. Dechlorination is necessary to reduce effluent toxicity because residual free chlorine and chloramines can cause acute toxicity effects in receiving waters (Sedlak and Pehlivanoglou, 2004). Traditional dechlorination is accomplished by adding sodium bisulfite, followed by discharge to the environment. Other dechlorination reagents include: sulfur dioxide, sodium metabisulfite, sodium sulfite, sodium thiosulfate, ammonium bisulfite, and ammonium thiosulfate (Sedlak and Pehlivanoglou, 2004).

The reactions between bisulfite [S (IV)] and free chlorine, or bisulfite and inorganic combined chlorine are extremely rapid. However, less is known about the kinetics of reactions between bisulfite and organic combined chlorine. Studies have indicated that some organic chloramines are recalcitrant to S (IV)-based dechlorination and may cause toxicity in dechlorinated wastewater effluent. This suggests that organic chloramines might pose toxicity risks. Likewise, little is known on the fate of S(IV) in natural waters. Also, some organic-N compounds (e.g., propionamilide) may be recalcitrant to biodegradation. Some chlorinated organic-N compounds have been observed to be resistant to traditional dechlorination using S (IV). Studies have shown that dechlorination was capable of removing 87% to 98% of residual chlorine, but the remainder, which may exceed regulatory limits, was very slowly reduced. The

dechlorination rate and extent are likely to depend on the structure of the organic-N precursors. Chlorinated secondary organic amines and peptides have been shown to be important contributors to S (IV)-resistant residual chlorine. Studies have shown that some organic-N-chloramines were dechlorinated slowly by sulfite, with half lives of >20 minutes. Studies have also shown that the dechlorination rate constants of N-chloropeptides were 1 to 2 orders of magnitude smaller than those for NH_2Cl and some aliphatic organic chloramines (*WERF*, 2005; Jensen, 1997; Sedlak and Pehlivanoglu, 2004).

4.2 Ozone

Ozonation is considered a viable alternative to chlorination, especially where dechlorination may be required. Because ozone dissipates rapidly and decomposes to oxygen, ozone residuals will normally not be found in the effluent discharged into the receiving water. However, some researchers have reported that ozonation can produce some unstable, toxic, mutagenic and/or carcinogenic compounds (EPA, 2002).

In the context of wastewater treatment, the high reactivity of ozone makes it appropriate for disinfection, color removal, the degradation or conversion of organic micropollutants, the conversion of chemical oxygen demand (COD), and effluent oxygenation. The effectiveness of ozone disinfection depends on the ozone dose, the quality of the effluent, the ozone demand, and the transfer efficiency of the ozone system (EPA, 2002).

The disinfection dose (i.e., the dose of ozone that achieves certain microbiological standards in a municipal effluent) is expressed as the transferred (or absorbed) mass of ozone per liter of effluent in mg/L. The ozone dose is described by the CT product, where C is the concentration of dissolved (residual) ozone measured at the outlet of the contact chamber (in milligrams per liter) and T is the contact time between the residual ozone and water (in minutes). The physicochemical quality of the effluent is

particularly influential in determining the effectiveness of disinfection and the ozone dose required to achieve a specific performance (Paraskeva and Graham, 2002).

Attempts have been made to establish empirical relationships or formulas to predict the total or fecal coliform (FC) inactivation by ozonation in terms of organic and inorganic species, such as COD, TSS, and nitrite-nitrogen (NO_2 - N). A close linear relationship (R = 0.95) has been established between the logarithm of FC survival (counts remaining/initial counts) and the COD of the influent wastewater to the ozonation chamber, although this was for a very narrow ozone dose range (8 to 10 mg/L) (Paraskeva and Graham, 2002).

Ozone has been found to be very effective at inactivating a wide range of microorganisms and is generally believed to be more effective than chlorine. The mechanism of bacterial inactivation by ozone is thought to occur by general inactivation of the whole cell. Thus, ozone causes damage to the cell membrane, to the nucleic acids, and to certain enzymes (Paraskeva and Graham, 2002).

Ozone is particularly effective against viruses. The mechanism of viral inactivation involves coagulation of the protein and oxidation of the nucleobases forming the nucleic acid. Studies have shown that a 5 mg/L dose and 5-minute contact time were sufficient to achieve a 5-log removal of the highly resistant virus, MS2 bacteriophage. Compared with chlorine and UV irradiation, ozone required a shorter contact time to achieve the same inactivation level (Paraskeva and Graham, 2002).

4.3 UV

UV radiation at a wavelength of around 254 nm penetrates the cell wall of microorganisms and is absorbed by cellular material, including nucleic acids (DNA and RNA), which either prevents replication or causes death of the cell to occur. The effectiveness of UV is largely dependent on the applied UV dose, suspended solids

content, UV transmittal, non-disinfected microbial concentration, and the degree of association of microorganisms with particles (EPA, 2003).

The UV dose is commonly defined as the product of radiation intensity and exposure time, also known as contact time, T. A proper dosage of UV radiation has been shown to be an effective disinfectant for several microorganisms while not contributing to the formation of toxic compounds. However, certain chemical compounds may be altered by the UV radiation and additional investigation into this occurrence is warranted (Andrew, 2005; *WERF*, 2005; EPA, 2003).

Because the only UV radiation effective in destroying microorganisms is the one that reaches the microorganisms, the wastewater must be relatively free of turbidity that can absorb the UV energy and shield the microorganisms. It has been reported that UV light is not an effective disinfectant for wastewaters that contain high total suspended solids concentrations. Because UV light is not a chemical agent, no toxic residuals are produced (EPA, 2003).

UV disinfection is reportedly characterized by the following advantages over chlorine (Lazarova and Savoye, 2004):

1. UV efficiency for protozoa of concern (*Cryptosporidium parvum* and *Giardia lamblia*) is significantly greater than chlorine efficiency.
2. Proven ability to disinfect pathogenic bacteria and most viruses. There were no significant differences between the efficacy of chlorine and UV radiation as a disinfectant for the reduction of FC.
3. The formation of harmful by-products by UV is negligible at conventional UV doses.

4. Proven effectiveness in meeting federal wastewater effluent standards based on the reduction of indicator organisms in the finished effluents to meet permitted effluent discharge limits.
5. Increased safety compared to the storage and handling of chlorine.
6. Increasing costs of chlorination due to regulations curbing chlorine discharge limits, thus, mandating dechlorination, and
7. UV technology has become increasingly more reliable and predictable with regard to performance.

Improvements in the lamp and ballast technology has led to the use of medium pressure UV sources for disinfection applications, thus, expanding the range of water qualities that can be treated with UV radiation (EPA, 2003).

4.4 Disinfection By-products (DBPs) and Residuals

Most disinfectants are strong oxidants, and can generate oxidants (such as hydroxyl free radicals) as by-products that react with organic and inorganic compounds in water to produce DBPs. The production of DBPs depends on the amounts and types of precursors in the water. Natural organic matter (NOM) is the principal precursor of organic DBP formation (EPA, 1999).

In applying any disinfectant, it is important to strike a balance between risks associated with microbial pathogens and those associated with DBPs. DBPs are persistent chemicals, some of which have relevant toxicological characteristics. The inventory of DBPs that have the potential to express adverse health effects is large and highly variable among POTW effluents. Moreover, the human health effects associated with chemical contaminants that are influenced or produced as a result of disinfection operations tend to be chronic in nature. Therefore, the development of a risk assessment for exposure to chemical constituents, including DBPs, is far more complex

than the microbial risk assessment. Risk assessments of wastewater disinfection should consider microbial and chemical quality (*WERF*, 2005).

The issue of balancing chemical and microbial risks was the subject of a series of conferences on the safety of water disinfection organized by the International Life Science Institute. The conference sessions provided a forum for scientists from the disciplines of toxicology, chemistry, epidemiology, water treatment technology, public health and risk assessment, to discuss recent advances in health effects of DBPs of both chlorination and alternative disinfectants. The following conclusions were reached on microbial versus chemical risks of DBPs (Falwell et al., 1997):

- Limited information is available concerning health risks from wastewater DBPs
- Human exposure to DBPs raises the concern that even small risks could have public health significance
- Chemical risks increase with disinfectant dosages
- Chemical risks don't start from zero, due to the presence of background organic constituents in wastewater
- More information is available for chlorine DBPs than other disinfectants
- There is a scarcity of quantitative risk assessment of the relative risks of chemical and microbial constituents

Chlorination DBP concentrations vary seasonally and are typically greatest in the summer and early fall for several reasons (EPA, 1999):

- The rate of DBP formation increases with increasing temperature
- The nature of organic DBP precursors varies with season
- Due to warmer temperatures, chlorine demand may be greater during summer months, requiring higher dosages to maintain disinfection efficiency

Table 4-2 is a list of DBPs and disinfection residuals that may be a concern for human health. The table includes both the disinfectant residuals and the specific products produced by the disinfectants of interest. These contaminants of concern are grouped into four distinct categories, and include disinfectant residuals, inorganic by-products, organic oxidation by-products, and halogenated organic by-products.

The health effects of disinfectants are generally evaluated by epidemiological studies and/or toxicological studies using laboratory animals. Table 4-3 indicates the cancer classifications of both disinfectants and DBPs, as of January 1999. The classification scheme used by EPA is shown at the bottom of Table 4-3. The EPA classification scheme for carcinogenicity weighs both animal studies and epidemiologic studies, but places greater weight on evidence of carcinogenicity in humans.

The following sections discuss chlorination DBPs and ozonation DBPs. UV disinfection results in negligible DBPs and is not discussed further.

4.4.1 Chlorination DBPs and Residuals

Certain organic constituents in wastewater form chlorination by-products including chloroform, and chlorinated aliphatic and aromatic compounds. Trihalomethanes (THM), mainly chloroform ($CHCl_3$), bromodichloromethane ($CHBrCl_2$), dibromochloromethane ($CHBr_2Cl$), and carbon tribromide ($CHBr_3$) account for the majority of by-products on a weight basis. Haloacetic acids are the next most significant fraction, accounting for about 25% of DBPs. Aldehydes account for about 7% of DBPs (Viessman and Hammer, 1993; EPA, 1999).

In 2002, EPA published a national study on the occurrence of DBPs in drinking water. More than 500 DBPs have been reported in the technical literature, but only a limited number of them have been studied for adverse health effects. Approximately 50 DBPs are denoted as "high priority" for drinking waters and include such compounds as MX

[3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone], brominated forms of MX (BMXs), halonitromethanes, iodo-trihalomethanes, and many brominated species of halomethanes, haloacetonitriles, haloketones, and haloamides (EPA, 2002).

An EPA (2002) study found that the use of disinfectants other than chlorination does not necessarily limit the formation of all halogenated DBPs, and can even result in increased concentrations of some. Halogenated furanones, including MX and brominated MX (BMX) analogues, were widely observed at relatively high concentrations, up to 310 ng/L. Water treatment plants with the highest MX and BMX levels were plants that used chlorine dioxide for primary disinfection, probably due to the inability of chlorine dioxide to destroy MX precursors as ozone does (EPA, 2002).

Pre-ozonation, in some cases, was found to increase the formation of trihalonitromethanes. A number of brominated organic acids were identified, with most being observed in water treatment plants that had significant bromide levels in their source area. One of the high priority DBPs, 3,3-dichloropropenoic acid, was found in several finished waters, providing further evidence that haloacids with longer chains are prevalent DBPs. Dihaloacetaldehydes and brominated analogues of chloral hydrate (trichloroacetaldehyde) were detected in many samples, as were mono-, di-, tri-, and/or tetraspecies of halomethanes and haloketones. A newly-identified class of DBPs, haloamides, were also found at significant levels (EPA, 2002).

Carbon tetrachloride was also found and it could be a DBP or a contaminant from the cleaning process of chlorine cylinders, before they are filled (EPA, 2002). Another finding of the EPA study was the discovery of iodoacid by-products. These iodoacids and iodobutanal were formed as DBPs in a high-bromide water from a treatment plant that uses chloramines for disinfection. Brominated acids, and another brominated ketone (1-bromo-1,3,3-trichloropropan) were also identified for the first time.

In most cases where chloramination was used, the DBPs were relatively stable. When free chlorine was used, THMs and other DBPs, including haloacetic acids, increased in concentration both in actual and simulated distribution systems. Haloacetonitriles were generally chemically stable and increased in concentration in distribution systems, but many of the haloketones were found to degrade. Halonitromethanes and dihaloacetaldehydes were found to be stable. MX and MX analogues were sometimes stable, and sometimes degraded but not to non-detectable levels. In several facilities BMXs were stable.

4.4.2 Ozonation DBPs and Residuals

The heterogeneous nature of municipal wastewaters and the relatively high cost of ozone application make it unlikely that organic substrates can be completely degraded (to carbon dioxide and water) by ozone treatment. This has led to concerns over the presence of intermediate by-product compounds that may be of toxicological significance. The reactivity of ozone with humic substances has also received considerable attention in recent years because such substances are found in natural and polluted waters, and are known to influence ozone decomposition and the occurrence of secondary radicals.

Ozone causes substantial structural changes to humic substances such as: strong and rapid decrease in color and UV-absorbance resulting from a loss of aromaticity and depolymerization; a small reduction in total organic carbon (TOC); a slight decrease in the high apparent molecular weight fractions and a slight increase in the smaller fractions, a significant increase of the carboxylic fractions; and the formation of ozone by-products (Paraskeva and Graham, 2002). By-products such as aldehydes, ketones, acids, and other species can be formed upon ozonation of wastewater. The primary aldehydes that have been measured are: formaldehyde, acetaldehyde, glyoxyl, and methyl glyoxal. The total aldehyde concentration in drinking water disinfected with

ozone depends on the TOC concentration and the applied ozone to organic carbon ratio. Aldehydes with higher molecular weights have also been reported. The primary carboxylic acids measured include (formic, acetic, glyoxylic, pyruvic, and ketomalinic acids). Table 4-4 presents principal known by-products of ozonation (Paraskeva and Graham, 2002; EPA, 1999).

A significant concern associated with ozone disinfection in drinking water is the potential of halogenated substances such as bromate, a possible carcinogen, and brominated organics (including bromoform) arising from the reaction of ozone and bromide. In contrast, the potential formation of brominated components in the field of wastewater treatment has received comparatively little research attention. The scarcity of information concerning the formation of ozonation by-products in wastewater effluents clearly indicates that further investigations are necessary on this subject (Paraskeva and Graham, 2002).

Ozonation of wastewater containing bromide ions can produce brominated by-products, the brominated analogues of the chlorinated DBPs. Bromate ion formation is an important consideration for waters containing more than 0.10 mg/L bromide ion. These brominated by-products include bromate ion, bromoform, the brominated acetic acids and acetonitriles, bromopicrin, and cyanogen bromide (if ammonia is present). An ozone dose of 2 mg/L produced 53 μg/L of bromoform and 17 μg/L of dibromoacetic acid in a water containing 2 mg/L of bromide ion. Ozonation of the same water spiked with 2 mg/L bromide ion showed cyanogen bromide formation of 10 μg/L. Furthermore, ozone may react with the hypobromite ion to form bromate ion, a probable human carcinogen. Bromate ion concentrations in ozonated water of up to 60 μg/L have been reported. Note that the amount of bromide ion incorporated into the measured DBPs accounts for only one-third of the total raw water bromide ion concentration. This indicates that other brominated DBPs exist that are not yet identified (EPA, 1999).

The presence of residual ozone concentrations following ozonation can be toxic to many forms of aquatic life. The tolerance to ozone varies with the type of organism, the period of exposure and its age. Even very small residual ozone concentrations can cause mortality in fish and larvae (Paraskeva and Graham, 2002).

In the context of wastewater disinfection, however, residual ozone concentrations are believed to be short-lived and to have decayed before the final discharge of the effluent to the receiving water system. For low residual ozone doses arising from typical disinfection conditions (i.e., 0.2 to 1.0 mg O_3/L), the time required for ozone decay to below detectable concentrations was between 20 seconds and 2 minutes. Toxicity studies of disinfected municipal wastewater effluents using *Ceriodaphnia dubia* indicated that toxicity results were site-specific and seasonal, but confirmed that ozone had the ability to change the toxicity of the effluent, either by increasing or decreasing it (Paraskeva and Graham, 2002).

Studies using fish and crustaceans as test organisms did not result in any changes in the toxicity of a secondary effluent after ozonation. Changes in effluent mutagenicity were found to be site-specific (Paraskeva and Graham, 2002). Several researchers reported that ozone did not induce mutagenicity in a secondary municipal effluent, and they presented evidence that ozone could reduce the mutagenicity of the effluent. Other researchers found that ozone at low doses (2.5 to 3 O_3 mg/L) produced a low level of mutagenicity in samples of secondary effluent taken in both summer and winter; no mutagenicity was recorded in untreated effluent samples (Paraskeva and Graham, 2002).

4.5 Disinfection Effectiveness

The effectiveness of disinfection is a complex function of several variables including type and dose of disinfectant, type and concentration of microorganisms, contact time,

and water quality characteristics. In most cases, pilot-studies and other considerations guide the selection process. The overall behavior of a disinfection system will be affected by (non-disinfected) effluent composition, the type of disinfectant applied, the design of the disinfection system, and the operating conditions. For example, the presence or absence of nitrogenous compounds (organic or inorganic) can have a profound effect on chlorine-based systems. Chlorinated forms of these compounds are generally less effective disinfectants than free chlorine. Moreover, inorganic and organic nitrogenous compounds represent important precursors to DBP formation, as discussed in detail in the previous section. Nitrogenous compounds can also have an adverse effect on UV disinfection systems as UV-absorbing compounds (*WERF*, 2005).

The effectiveness of the disinfectants will be influenced by the nature and condition of the microorganisms. For example, viable growing bacteria cells are killed easily. In contrast, bacterial spores are extremely resistant and many of the chemical disinfectants normally used will have little or no effect (*WERF*, 2005).

Wastewater characteristics other than microbiological components also influence disinfectant efficiency. Among these are turbidity, organics, disinfectant scavengers, pH and temperature. Particulates responsible for turbidity can surround and shield microorganisms from disinfectant action. Organic materials can decrease disinfection efficiency, by one or more of the following mechanisms:

- Adhering to cell surfaces and hindering attack by the disinfectant
- Reacting with the disinfectant, to form compounds with weaker germicidal properties
- Reacting with the disinfectant, to form toxic by-products

Compounds such as iron, manganese, hydrogen sulfide, cyanides, and nitrates can decrease the disinfection efficiency as they are rapidly oxidized by and thereby deplete the disinfectant. This reaction of inorganic compounds with disinfectant, such as chlorine, creates a demand that must be met before the disinfectant can act on the microorganisms.

The pH of the water affects the chemical form of the disinfectant in aqueous solution, and can influence microbial destruction. For example, the most active chlorine species for disinfection is hypochlorous acid (HOCl), which predominates in water if the pH is less than 7. Temperature affects the reaction rate of the disinfection process, such as diffusion of the disinfectant through cell walls or the reaction rate with key enzymes, and can influence the rate of disinfection (Montgomery, 1985).

The following sections discuss: (1) bacteria disinfection efficiency, (2) protozoa disinfection efficiency, and (3) virus disinfection efficiency.

4.5.1 Bacteria Disinfection Efficiency

The current regulatory focus of wastewater disinfection is on fecal coliform (FC) and *E.coli* bacteria. State and federal regulations require monitoring of the FC indicator group of bacteria in wastewater treatment facility effluents. These regulations are designed to assess the microbiological contamination following contact or ingestion of the effluent or receiving waters (MWRDGC, 2005a).

Disinfectant efficiencies used in wastewater treatment processes are commonly evaluated using the FC group. FC removal or reduction, expressed as the difference between the log values of FC concentration prior to and following treatment, is a commonly used parameter for characterization of disinfection efficacy. However, there is little information about the correlation between these indicator organisms and pathogens, particularly in terms of long-term behavior. Also, many of the pathogenic

bacteria are not culturable. In fact, less than 1% of the microorganisms in natural water and soil samples are cultured in viable count procedures. If available, published data regarding pathogen inactivation achieved by disinfection are typically used to estimate the concentration of pathogens in disinfected wastewater (*WERF*, 2005).

Recent research results provide a detailed characterization of the effects of common disinfectants (chlorine, UV radiation and ozone) on wastewater bacteria, in terms of initial response to disinfectant exposure, changes in bacterial community post-exposure, and the nature and extent of bacterial physiological damage resulting from exposure to these disinfectants (*WERF*, 2005).

Chlorine is an extremely effective disinfectant for inactivating bacteria, including *E. coli* and *Pseudomonas aeruginosa.* Data presented in the technical literature indicate that UV irradiation and chlorination/dechlorination, when applied with the goal of complying with conventional effluent discharge regulations, are similar in terms of their ability to inactivate water-borne bacteria, although total bacterial populations generally recover to a greater extent in chlorinated effluents than in UV irradiated effluents. Also, the conditions that are used to accomplish indicator bacteria inactivation based on chlorination/dechlorination are relatively ineffective for control of waterborne viruses, as compared with UV irradiation (*WERF*, 2005).

Both pilot-plant studies and results from operating plants have shown that ozone effectively removes fecal and total coliforms, as well as enteric viruses from secondary effluents. Typical disinfection doses, contact times, and residual ozone concentrations required for the reduction of indicator organisms, based upon pilot-plant studies and operating plants are presented in Table 4-5.

Studies have also shown the effect of small concentrations of dissolved ozone (i.e., 0.6 µg/L) on *E.coli*. *E.coli* levels were reduced by 4 logs (99.99 percent removal) in less

than 1 minute with an ozone residual of 9 µg/L at a temperature of 12°C. *E.coli* is one of the most sensitive types of bacteria to ozone disinfection. Furthermore, significant differences in ozone disinfection efficiency have been found among the Gram-negative bacillae, including *E.coli* and other pathogens such as *Salmonella*, which are all sensitive to ozone inactivation. The Gram-positive cocci (*Staphylococcus* and *Streptococcus*), the Gram-positive bacillae (*Bacillus*), and the Mycobacteria are the most resistant forms of bacteria to ozone disinfection. Sporular bacteria forms are always far more resistant to ozone disinfection than vegetative forms, but all are easily destroyed by relatively low levels of ozone (EPA, 1999).

An important factor affecting long-term disinfection efficacy is re-growth potential. After disinfection, some sub-lethally damaged bacteria may be able to repair disinfectant-induced damage. Together with organisms that retain viability following disinfection, it is possible for the microbial community to re-grow. Experiments were conducted to assess the long-term effects of chlorination/dechlorination and UV irradiation on indigenous bacterial communities. These experiments were designed to provide information regarding the effects of disinfectant exposure on bacteria at time scales well beyond those represented by conventional methods, where disinfected effluent samples are collected and assayed for viable indicator bacteria immediately after treatment (*WERF*, 2005).

Based on re-growth conditions and FC (indicator) to total bacteria ratio, the long-term outcome of disinfection processes can be divided into the nine scenarios illustrated in Figure 4-1. From this figure, the effectiveness of a disinfection process can be evaluated based upon variations in the total bacterial community and the pathogenic fraction. Cases for which disinfection is not effective against pathogenic bacteria are indicated by red. Cases for which disinfection efficacy is not clear are indicated by gray.

For example, cases (c), (g), and (i) in Figure 4-1 may represent a positive effect of disinfection since they imply a reduction in pathogenic bacteria. Cases (a), (b), (d), and (e) in Figure 4-1 represent an adverse effect of disinfection since pathogenic bacteria concentrations are not reduced. In cases (f) and (h) in Figure 4-1, it is difficult to judge disinfection efficacy as judgment of antibacterial efficacy requires additional information, such as the concentration of pathogenic bacteria or indicator microorganisms.

To evaluate if disinfection is effective in reducing bacterial risk, it is necessary to consider re-growth and pathogen ratios. Under conditions of abundant substrate supply, rapidly-growing microorganisms usually dominate populations. This is true in municipal wastewater treatment facilities, where the abundance of available organic substrates favors the growth of rapidly dividing bacteria, such as coliforms and pseudomonads. These dominant microbial populations in sewage, which gain a competitive advantage because of their high intrinsic growth rates, are rapidly displaced in competition with other microbial populations of receiving waters as the concentration of organic compounds diminishes, owing to natural attenuation mechanisms, such as degradation and dilution. Under lower nutrient conditions, a more diverse community of slowly growing bacteria is favored (*WERF*, 2005).

Experimental results from chlorination/dechlorination and UV disinfection studies indicate that these processes can result in reduced FC concentrations compared to the initial concentration, even after re-growth. In addition, the following conclusions were drawn (*WERF*, 2005):

1. FC, when used as an indicator, may overestimate disinfection efficacy or microbial quality of disinfected samples, since they are relatively susceptible to common disinfectants (chlorine and UV) and they have a higher die-off rate than other microorganisms.

2. "Dark" (non-photochemical) repair following UV irradiation may play an important role relative to the re-growth potential of UV disinfectant microbial samples. Similarly, "dark" repair mechanisms may also play a role in the fate of chlorinated microbial samples.
3. Based on the long-term trends in FC and total bacterial concentrations, wastewater effluents respond more favorably to UV irradiation than to chlorination/dechlorination.

4.5.2 Protozoa Disinfection Efficiency

Cryptosporidium was not recognized as an important human waterborne pathogen until the mid-1980s, and wastewater regulations have not incorporated removal or inactivation of oocysts in wastewater effluent standards (Clancy, et al. 2004). Animals and humans are reservoirs of this parasite, and it enters the environment through shedding of fecal material. Dozens of species harbor *Cryptosporidium* oocysts, including mammals (e.g. cattle, horses, rodents, deer, dogs, cats, kangaroos), birds, reptiles, and fish. As such, there are many routes for this parasite to enter the environment, including natural runoff (non-point sources), runoff from agriculture, effluents from industries such as meat processors, wastewater effluents, and combined sewer overflows (CSOs) (Clancy, et al., 2004).

Cryptosporidium parvum appears to lack host specificity, and has been shown to be able to cross-infect rodents, ruminants, and humans (Finch et al., 1993). *Cryptosporidium* is a significant concern to water suppliers worldwide, as this protozoan parasite forms highly-resistant oocysts that can survive in most environments for extended periods. In addition, oocysts are difficult to remove in water treatment by filtration due to their small size (4 to 6 μm) (Clancy, 2004).

Cryptosporidium oocysts can typically occur in all wastewater matrices, from raw sewage to tertiary effluents. The percentage of sanitary wastewater samples positive

for oocysts is relatively high. A fifteen-month *Cryptosporidium* study was conducted at wastewater facilities located in Alabama, California, Colorado, North Carolina, Pennsylvania and Vermont. The percent of samples positive for *Cryptosporidium* were as follows: 30% of raw sewage (95 samples total); 46% of primary effluent (84 samples total); 59% for secondary effluent (94 samples total); and 19% for tertiary effluent (16 samples total) (Clancy, 2004). While occurrence is common, a critical question for risk assessment is whether or not the oocysts recovered are able to cause infection in humans or animals.

Chlorine has been shown to have limited success inactivating protozoa. The resistance of *Giardia* cysts has been reported to be two orders of magnitude higher than that of enteroviruses and more than three orders of magnitude higher than the enteric bacteria. CT requirements for *Giardia* cyst inactivation when using chlorine as a disinfectant has been determined for various pH and temperature conditions. These CT values increase at low temperatures and high pH (EPA, 1999).

Cryptosporidium and *Giardia* in wastewater can be physically removed by the coagulation/filtration process. *Cryptosporidium* oocysts are resistant to chlorine-based disinfectants at the concentrations and contact times practiced for water treatment (Clancy, 2004). Chlorine has little impact on the viability of *Cryptosporidium* oocysts when used at the relatively low doses encountered in water treatment (e.g., 5 mg/L). Approximately 40 percent removal (0.2 log) of *Cryptosporidium* were achieved at CT values of both 30 and 3,600 mg.min/L at pH 8, a temperature of 22°C, and contact times of 48 to 245 minutes. CT values ranging from 3,000 to 4,000 mg.min/L were required to achieve 1-log of *Cryptosporidium* inactivation at pH 6.0 and temperature of 22°C. One trial in which oocysts were exposed to 80 mg/L of free chlorine for 120 minutes was found to produce greater than 3-logs of inactivation (EPA, 1999).

Cryptosporidium oocysts are generally more resistant to water treatment processes and disinfection practices than other ubiquitous waterborne microorganisms. Because of chlorine's extremely high virus inactivation efficiency, CT values are almost always governed by protozoa inactivation. For example, the CT values required to achieve the recommended disinfection efficiency for conventional filtration systems (i.e., 0.5-log *Giardia* cysts and 2-log virus inactivation level) are 23 and 3 mg min/L, respectively (EPA, 1999).

Protozoan cysts, specifically *Giardia* and *Cryptosporidium*, and bacteria spores are more resistant to ozone than bacteria and viruses, although moderate degrees of inactivation (see Table 4-6) have been demonstrated under realistic ozonation conditions. It has been reported that microorganism reactivation after ozonation is unlikely to occur (Paraskeva and Graham, 2002).

Giardia lamblia has sensitivity to ozone that is similar to the sporular forms of *Mycobacteria*. The CT product for 99 percent inactivation of *Giardia lamblia* at 5°C is 0.53 mg min/L. Data available for inactivation of *Cryptosporidium* oocysts suggest that compared to other protozoans, this microorganism is more resistant to ozone. *Cryptosporidium* oocysts are approximately 10 times more resistant to ozone than *Giardia*. Table 4-7 summarizes CT values obtained for 99% inactivation of *Cryptosporidium* oocysts. A wide range of CT values has been reported for the same inactivation level, primarily because of the different methods of *Cryptosporidium* measurement employed and pH, temperature, and ozonation conditions. As shown in Table 4-7, the CT requirements reported in the literature vary from study to study, which adds uncertainty to the design of CT requirements for specific applications and regulatory needs (EPA, 1999).

The performance of ozone with other microorganisms and parasites in wastewater effluent is presently unclear because of the lack of sufficient studies. Some studies

have shown that in tests with tertiary-treated municipal effluents, ozone was very effective towards *Pseudomonas aeruginosa*, moderately effective toward *Giardia lamblia*, and substantially ineffective toward *Cryptosporidium parvum* (see Table 4-8). The low numbers of *Cryptosporidium parvum* in the untreated effluent probably made the results uncertain.

UV has been used for drinking water treatment in Europe since the early 1900's, but until the mid-1990's it was not considered to be an effective treatment for protozoan pathogens such as *Cryptosporidium* (Clancy et al., 2004). Several recent studies have shown that UV is highly effective at relatively low UV doses (10 mJ/cm^2) for control of *Cryptosporidium*. The results of recent research indicate that both low and medium pressure UV irradiation are very effective for inactivation of *Cryptosporidium parvum* spiked into wastewater effluent. Infectivity assays using cell culture indicated that inactivation levels greater than three log_{10} can be achieved in wastewater with a UV dose of only 3 mJ/cm^2. Inactivation of *Cryptosporidium* was most effective in the 250 to 270 nm range, which includes both the low and medium pressure output ranges. The studies found that UV inactivated *Cryptosporidium* oocysts are not able to restore their infectivity in cell culture hosts following exposure to either light (photoreactivation) or dark DNA repair protocols (Clancy et al., 2004).

According to *WERF* (2005), the natural occurrence of *Cryptosporidium* in wastewater is too low to allow for the determination of log inactivation from UV exposure. *Cryptosporidium* oocysts have been reported in secondary effluent at a concentration of 140 oocysts/100L, while *Giardia* cysts were found to range from 440 to 2297 cysts/100L. Therefore, in most pilot-scale results, it is necessary to spike *Cryptosporidium* into the wastewater effluent to test for levels of inactivation. However, this may not represent the true physical state of *Cryptosporidium parvum* in wastewater (*WERF*, 2005).

Chang et al. (1985) reported that the UV dose necessary to cause 99% inactivation of *Giardia lamblia* was within the operating range of many UV disinfection systems, but it was beyond the usual operating dose. Neither *E. coli* or fecal coliform can serve as a quantitative model for disinfection of protozoa or viruses. According to Chang et al. (1985), the extreme resistance of *Giardia lamblia* makes it unlikely that normal UV irradiation procedures would be sufficient to destroy the cysts.

Use of multiple disinfectants in series can be an effective strategy for inactivation of the wide range of pathogen types found in wastewater. An approach that utilizes UV disinfection followed by free chlorine dosing and subsequent formation of monochloramine (due to ammonia in the wastewater) along with a long CT should be capable of achieving significant inactivation of most microorganisms within a practical range of UV and free chlorine/monochloramine doses (Clancy, 2004). Extended CT with chlorine was also found to be effective in achieving inactivation of particle-associated coliform bacteria in wastewater. However, the formation of chlorinated by-products may be a concern (Clancy, 2004).

4.5.3 Virus Disinfection Efficiency

Although viruses cannot replicate outside their host's cells and, therefore, cannot multiply in the environment, they can survive for several months in fresh water and for shorter periods in marine water. Their survival in the environment is prolonged at low temperatures and in the presence of sediments, onto which they easily adsorb. Exposure to sunlight, higher temperatures, and high microbial activity will shorten the survival of enteric viruses. Low dose infectivity, long-term survival, and relatively low inactivation or removal efficiency by conventional wastewater treatment are some of their key disinfection characteristics (Lazarova and Savoye, 2004).

There are several important characteristics associated with virus disinfection (Thurston-Enriquez, et al., 2003; Thurston-Enriquez, et al., 2003a; Lazarova and Savoye, 2004):

1. There have been several studies dealing with viral inactivation. The inactivation of viruses has been shown to be a first-order type, and Chick's law type equations can be used to describe the viral inactivation.
2. Viruses are more resistant to chloramination than the coliform bacteria and are one of the most resistant targets of UV disinfection.
3. Viruses have a low infectious dose and represent a range of illnesses.
4. Viruses are used as a target organism for designing disinfection systems in some applications. For example, California Title 22 is focused on virus inactivation.
5. The dose-response function for rotaviruses has been used in drinking water risk assessment.
6. Adenoviruses are the most resistant to UV disinfection and are found in high concentrations in municipal wastewater.

Enteric viruses are extremely small microorganisms that multiply only in the gastrointestinal tract of humans and other animals. Enteric viruses cannot multiply in the environment, but they survive longer in water than most intestinal bacteria and are more infectious and resistant to disinfection than most other microorganisms. Wastewater treatment that does not include a disinfection step is relatively inefficient at removing viruses. In contaminated surface water, levels of 1–100 culturable enteric viruses per liter are common. In less polluted surface water, their numbers are closer to 1–10 per 100L (Health Canada, 2004).

Removal or inactivation of enteric viruses depends on two factors—their physical characteristics and their susceptibility to disinfection. The removal and inactivation of

some enteric viruses from raw water are complicated by their small size and relative resistance to commonly used disinfectants such as chloramines.

From pilot-scale experiments started in 1998 by the Monterey Regional Water Pollution Control Agency, it was found that a 5-log removal of enteric viruses was achieved, mostly during the chlorine disinfection step (Nelson, et al., undated). Table 4-9 presents a summary of CT values for the inactivation of selected viruses by various disinfectants at 5°C. Based on the results in Table 4-9, it is apparent that ozone, free chlorine and chlorine dioxide are much better disinfectants than chloramines. However, ozone may be unreliable when turbidity is high or variable, because viruses are protected in flocculated particles (Health Canada, 2004).

According to Thurston-Enriquez, et al. (2003a), dispersed adenoviruses and *Caliciviruses* would be inactivated by commonly used free chlorine concentrations of 1mg/L and contact times (60 to 237 min) applied for drinking water treatment in the United States. However, higher CT values may be required for viruses that are aggregated and associated with organic and inorganic matter in the environment. Inactivation rates of these viruses were reported in the range of 2 to 4 log.

Wastewater disinfection with chlorine, UV or ozone can significantly reduce the virus load (see Table 4-9). However, UV light disinfection is not as efficient at inactivating viruses as the more traditional chlorine-based disinfection processes (Health Canada, 2004).

Both *Caliciviruses* and enteric adenoviruses are on EPA's Drinking Contaminant Candidate List (CCL). These viruses are on the CCL for regulatory consideration since little to no information regarding health effects, nor analytical methods are currently available. Limited information regarding the effectiveness of UV radiation on the inactivation of *Caliciviruses* and enteric adenoviruses is available. Adenoviruses are

believed to occur in greater concentrations in wastewater than other enteric viruses. Adenoviruses are more resistant to UV light disinfection compared to enteric viruses or spore forming bacteria. Human adenovirus type 40 is the most UV light-resistant enteric virus reported to date. The greater resistance of adenoviruses is attributed to the fact that they contains double-stranded DNA and are able to use the host cell enzymes to repair damages in the DNA caused by UV irradiation. Double-stranded DNA viruses are likely the most resistant viruses to UV light disinfection. Consideration should be given to the resistance of adenoviruses to UV light disinfection when appropriate doses for the control of waterborne viruses are being determined (Gerba et al., 2002).

Research on the inactivation of adenovirus type 2 by UV light has been conducted with starting concentrations ranging from 2×10^7 to 1×10^6 per ml. The results indicate that for a 90, 99, 99.9, and 99.99% inactivation, the following UV exposure dosages were required: 40, 78, 119, and 160 mW/cm^2 (Gerba et al., 2002).

Adenoviruses are extremely resistant to UV disinfection, compared with other enteric viruses (Meng and Gerba, 1996). Analysis of human *Calicivirus* resistance to disinfection is hampered by the lack of animal or cell culture methods that can determine the viruses' infectivity. UV disinfection experiments were carried out in treated groundwater with Feline *Calicivirus* (FCV) and adenovirus type 40 (AD40). AD40 was more resistant than FCV. The doses of UV required to achieve 99% inactivation of AD40 and FCV were 109 and 16 mJ/cm^2, respectively. The reported doses needed to inactivate 90% of AD40 ranged from 30 to 50 mJ/cm^2. The reported dose needed to inactivate 99.99% of AD40 ranged from 124 to 203 (extrapolated value) mJ/cm^2. The results of this study show that, if FCV is an adequate surrogate for human *Caliciviruses*, then their inactivation by UV radiation is similar to those of other single-stranded RNA enteric viruses, such as poliovirus (Thurston-Enriquez et al., 2003). Meng and Gerba (1996) had reported 30 and 124 mJ/cm^2 UV dosages for 90 and 99% inactivation of AD40, respectively.

As a result of its high level of resistance to UV treatment, adenovirus is being considered by the U.S. EPA as the basis for establishing UV light inactivation requirements for enteric viruses (Gerba et al., 2002). A multi-disinfectant strategy involving UV light as the primary disinfectant followed by a secondary disinfectant (free chlorine) may prove to be most effective in controlling enteric viruses, as well as other microorganisms (Health Canada, 2004).

The UV doses commonly applied for water and wastewater treatment are between 30 and 40 mJ/cm^2, and the National Science Foundation (NSF) has increased UV water treatment standards for class A point-of-entry and point-of-use to 40 mJ/cm^2 (American National Standards Institute/NSF Standard 55). Under these standards, and as discussed above, FCV would be reduced by more than 99.99% in water supplies. Higher doses would be required to reduce AD40, since 40 mJ/cm^2 would not be adequate for even 90% reduction (Thurston-Enriquez, et al., 2003).

In a study involving five U.S. wastewater facilities, a coliphage (F specific and somatic) concentration estimate of 75.6 plaque forming units (PFU)/100L was used as an average value in a 12-month study of a full-scale facility's secondary effluent. This coliphage concentration was combined with experimentally measured log_{10} reductions achieved via UV disinfection and chlorination in bench-scale exposure studies of indigenous coliphage. Table 4-10 summarizes the results. Water quality characteristics in each facility likely impacted the coliphage inactivation. The inactivation was also dependent on the type of bacterial host used (*WERF*, 2005). In the case of UV disinfection, doses of 10 and 20 mJ/cm^2 are representative of UV exposure scenarios to be applied in municipal wastewater treatment facilities. Coliphage inactivation by disinfection ranged from 0.32 log_{10} to 3.61 log_{10} units and was generally greater when using UV than with chlorine. As shown in Table 4-10, facilities A, B, and D achieved

the greatest reductions via UV, while facilities C and E achieved greater or equivalent coliphage reductions by use of chlorine.

Little information is available regarding the effectiveness of ozone on the inactivation of *Caliciviruses* and enteric adenoviruses. CT values for a 4-log (99.99%) ozone inactivation at 5°C and pH 7, ranged from 0.07 to 0.60 mg/L min for AD40 and <0.01 to 0.03 mg/L min for FCV (Thorston-Enriquez et al., 2005). However, these experiments were carried out in buffered, disinfectant demand free water. These conditions may not be representative of treated wastewater.

4.6 Summary and Conclusions

Decisions regarding the need for effluent disinfection must be made on a site-specific basis. According to *WERF* (2005), disinfection is warranted in situations where direct human contact in the immediate vicinity of an outfall is possible or where effluent is discharged to areas involving the production of human food. Disinfection is warranted in situations where its application leads to a reduction in the risk of disease transmission. As illustrated by post-disinfection regrowth of bacteria, relatively poor virucidal behavior, and generation of persistent DBPs, it is not clear that wastewater disinfection always yields improved effluent or receiving water quality (*WERF*, 2005). The effectiveness of the following disinfection technologies were evaluated for the risk assessment study:

- UV
- Ozonation
- Chlorination/Dechlorination

The effectiveness of disinfection is a complex function of several variables including type and dose of disinfectant, type and concentration of microorganisms, contact time,

and water quality characteristics. In most cases pilot-studies and other considerations guide the selection process.

If available, published data regarding pathogen inactivation achieved by disinfection are typically used to estimate the concentration of pathogens in disinfected wastewater. A summary of disinfection efficiency data for chlorination/dechlorination, UV, and ozonation are presented in Table 4-11 for the microbial pathogens of this study. Based on the information presented in the previous sections, the following conclusions can be drawn about the disinfection effectiveness:

1. Fecal coliforms, when used as an indicator, may overestimate disinfection efficacy or microbial quality of disinfected samples, since they are relatively susceptible to common disinfectants and they have a higher die-off rate than other microorganisms.
2. To evaluate if disinfection is effective in reducing bacterial risk, it is necessary to consider re-growth and pathogen ratio.
3. Chlorine is an extremely effective disinfectant for inactivating bacteria.
4. UV irradiation and chlorination/dechlorination, when applied with the goal of complying with conventional effluent discharge regulations, are similar in terms of their ability to inactivate water-borne bacteria.
5. The conditions that are used to accomplish indicator bacteria inactivation based on chlorination/dechlorination are relatively ineffective for control of waterborne viruses.
6. Both pilot-plant studies and results from operating plants have shown that ozone effectively removes fecal and total coliforms, as well as enteric viruses from secondary effluents.
7. *E. coli* is one of the most sensitive types of bacteria to ozone disinfection and a 4 log reduction (99.99 percent removal) in *E. coli* can be achieved.

8. Significant differences in ozone disinfection efficiency have been found among *E.coli* and other pathogens such as *Salmonella*, which are all sensitive to ozone inactivation.

9. Sporular bacteria forms are always far more resistant to ozone disinfection than vegetative forms, but all are easily destroyed by relatively low levels of ozone.

10. An important factor affecting long-term disinfection efficacy is re-growth potential. After disinfection, some sub-lethally damaged bacteria may be able to repair disinfectant-induced damage. Together with organisms that retain viability following disinfection, it is possible for the microbial community to re-grow.

11. "Dark" (non-photochemical) repair following UV irradiation may play an important role relative to the re-growth potential of UV disinfected microbial samples. Similarly, "dark" repair mechanisms may also play a role in the fate of chlorinated microbial samples.

12. Chlorine has been shown to have limited success inactivating protozoa. The resistance of *Giardia* cysts has been reported to be two orders of magnitude higher than that of enteroviruses and more than three orders of magnitude higher than the enteric bacteria.

13. Chlorine has little impact on the viability of *Cryptosporidium* oocysts when used at the relatively low doses encountered in water treatment (e.g., 5 mg/L).

14. *Giardia* and *Cryptosporidium* are more resistant to ozone than bacteria and viruses, although moderate degrees of inactivation have been demonstrated under realistic ozonation conditions.

15. Reactivation of *Giardia* and *Cryptosporidium* after ozonation is unlikely to occur.

16. The performance of ozone with protozoa in wastewater effluents is unclear because of the lack of sufficient studies.

17. UV is highly effective for control of *Cryptosporidium*.

18. UV inactivated *Cryptosporidium* oocysts are not able to restore their infectivity in cell culture host following exposure to either light (photoreactivation) or dark DNA repair protocols.

19. Removal or inactivation of enteric viruses depends on two factors—their physical characteristics and their susceptibility to disinfection. The removal and inactivation of some enteric viruses from raw water are complicated by their small size and relative resistance to commonly used disinfectants such as chloramines.

20. Wastewater disinfection with chlorine, UV, or ozone can significantly reduce the virus load. However, UV light disinfection is not as efficient at inactivating viruses as the more traditional chlorine-based disinfection processes, especially adenoviruses. The inactivation of viruses depends on the UV dosage and whether they are dispersed or aggregated in the wastewater.

21. Limited information regarding the effectiveness of UV radiation on the inactivation of *Caliciviruses* and enteric adenoviruses is available.

22. Adenoviruses are believed to occur in greater concentrations in wastewater than other enteric viruses. Adenoviruses are more resistant to UV light disinfection compared to other enteric viruses or spore forming bacteria. Human adenovirus type 40 is the most UV light-resistant enteric virus reported. The greater resistance of adenoviruses type 40 was attributed to the fact that it contains double-stranded DNA and is able to use the host cell enzymes to repair damages in the DNA caused by UV irradiation. Consideration should be given to the resistance of

adenoviruses to UV light disinfection when appropriate doses for the control of waterborne viruses are being determined.

23. Adenoviruses are extremely resistant to UV disinfection, compared with other enteric viruses. As a result of its high level of resistance to UV treatment, adenovirus is being considered by the U.S. EPA as the basis for establishing UV light inactivation requirements for enteric viruses.

24. Analysis of human *Calicivirus* resistance to disinfection is hampered by the lack of animal or cell culture methods that can determine the viruses' infectivity. However, its resistance is believed to be similar to other single-stranded RNA viruses.

In summary, the information summarized above indicates great variability in the performance and uncertainty in the efficacy of disinfection. There are many unanswered questions with respect to disinfection efficiency data for microbial indicators and pathogens. Many of the studies cited in the previous sections were bench-scale or pilot-scale experiments and not full-scale operations. Therefore, it is uncertain if disinfection designed to remove indicators can be effective in the removal of pathogens and in the reduction of pathogen risks.

In applying any disinfectant, it is important to strike a balance between risks associated with microbial pathogens and those associated with DBPs. DBPs are persistent chemicals, some of which have relevant toxicological characteristics. The inventory of DBPs that have the potential to cause adverse health effects is large and highly variable among POTW effluents. Certain organic constituents in wastewater form chlorination by-products including chloroform, and chlorinated aliphatic and aromatic compounds. THMs, mainly $CHCl_3$, $CHBrCl_2$, $CHBr_2Cl$, and $CHBr_3$ account for the majority of by-products on a weight basis. Haloacetic acids are the next most significant fraction, accounting for about 25% of disinfection by-products; aldehydes account for about 7% of disinfection by-products (Viessman and Hammer, 1993;

EPA, 1999). By-products such as aldehydes, ketones, acids, and other species can be formed upon ozonation of wastewater. UV disinfection results in the formation of negligible DBPs.

Bisulfite is a common dechlorination reagent used. The reactions between bisulfite and free chlorine, or bisulfite (S[IV]) and inorganic combined chlorine are extremely rapid. However, less is known about the kinetics of reactions between bisulfite and organic combined chlorine. Studies have indicated that some organic chloramines are recalcitrant to S(IV)-based dechlorination and may cause toxicity in dechlorinated wastewater effluent.

The human health effects associated with chemical contaminants that are influenced or produced as a result of disinfection operations tend to be chronic in nature. Therefore, the development of a risk assessment for exposure to chemical constituents, including DBPs, is far more complex than the microbial risk assessment. Risk assessments of wastewater disinfection should consider microbial and chemical quality. The health effects of disinfectants are generally evaluated by epidemiological studies and/or toxicological studies using laboratory animals (*WERF*, 2005).

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SECTION 4

TABLES

Table 4-1. Summary of Disinfectant Characteristics (Adapted from EPA, 1999; Montgomery 1985)

Characteristics	Free Chlorine	Chloramines	Chloride Dioxide	Ozone	Ultraviolet Radiation
Disinfection	Excellent (as HOCl)	Moderate			
•Bacteria	Excellent (as HOCl)	Poor (good at low contact times)	Excellent	Excellent	Good
•Viruses			Excellent	Excellent	Good
pH influence	Efficiency decreases with increase in pH	Dichloramine predominates at pH 5 and below; monochloramine predominates at pH7 and above. Overall, relatively independent of pH.	Slightly more efficient at higher pH	Residuals last longer at low pH	Insensitive
Effluent Disinfectant Residual	Yes	Yes	Yes	Yes, but it degrades rapidly	No
By-products					
•THM Formation	Yes	Unlikely	Unlikely	Unlikely	Unlikely
•Other	Uncharacterized and oxidated intermediates; chloramines; chlorophenols	Unknown	Chlorinated aromatic compounds; chlorate chlorite	Aldehydes; aromatic carboxylic acids; phthalates	Unknown
Experience	Widespread use	Widespread use in the U.S.	Widespread use in Europe; limited use in the U.S.	Widespread use in Europe and Canada; limited in the U.S.	Use limited to small systems

Table 4-2. List of DBPs and Disinfection Residuals (EPA, 1999)

DISINFECTANT RESIDUALS	HALOGENATED ORGANIC BY-PRODUCTS
Free Chlorine	Trihalomethanes
Hypochlorous Acid	Chloroform
Hypochlorite Ion	Bromodichloromethane
Chloramines	Dibromochloromethane
Monochloramine	Bromoform
Dichloramine	Haloacetic Acids
Trichloramine	Monochloroacetic Acid
Chlorine Dioxide	Dichloroacetic Acid
INORGANIC BY-PRODUCTS	Trichloroacetic Acid
Chlorate Ion[a]	Monobromoacetic Acid
Chlorite Ion[a]	Dibromoacetic Acid
Bromate Ion[a, b]	Haloacetonitriles
Iodate Ion[a, b]	Dichloroacetronitrile
Hydrogen Peroxide[b]	Bromochloroacetonitrile
Ammonia[a]	Dibromoacetonitrile
ORGANIC OXIDATION BY-PRODUCTS	Trichloroacetonitrile
Aldehydes	Haloketones
Formaldehyde	1,1 -Dichloropropanone
Acetaldehyde	1,1,1 -Trichloropropanone
Glyoxal	Chlorophenols
Hexanal	2-Chlorophenol
Heptanal	2,4-Dichlorophenol
Carboxylic Acids	2,4,6-Trichlorophenol
Hexanoic Acid	Chloropicrin
Heptanoic Acid	Chloral Hydrate
Oxalic Acid	Cyanogen Chloride
Assimilable Organic Carbon	N-Organochloramines
	MX[c]

Notes:

a. DBP due to chlorine dioxide disinfection
b. DBP due to ozone disinfection
c. 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone

Table 4-3. Status of Health Information for Disinfectants and DBPs (EPA, 1999)

CONTAMINANT	CANCER CLASSIFICATION [1]
Chloroform	B2
Bromodichloromethane	B2
Dibromochloromethane	C
Bromoform	B2
Monochloroacetic Acid	--
Dichloroacetic Acid	B2
Trichloroacetic Acid	C
Dichloroacetonitrile	C
Bromochloroacetonitrile	--
Dibromoacetonitrile	C
Trichloroacetonitrile	--
1,1 –Dichloropropanone	--
1,1 ,1-Trichloropropanone	--
2-Chlorophenol	D
2,4-Dichlorophenol	D
2,4,6-Trichiorophenol	B2
Chloropicrin	--
Chloral Hydrate	C
Cyanogen Chloride	--
Formaldehyde	B1[2]
Chlorate	--
Chlorite	D
Bromate	B2
Ammonia	D
Hypochlorous Acid	--
Hypochlorite	--
Monochloramine	--
Chlorine Dioxide	D

[1] The scheme for categorizing chemical according to their carcinogenic potential is as follows:*

Group A: Human Carcinogen	Sufficient evidence in epidemiologic studies to support causal association between exposure and cancer.
Group B: Probable Human Carcinogen	Limited evidence in epidemiologic studies (Group B1) and/or sufficient evidence from animal studies (Group B2)
Group C: Possible Human Carcinogen	Limited evidence from animal studies and inadequate or no data in humans
Group D: Not Classifiable	Inadequate or no human and animal evidence of carcinogenicity
Group E: No Evidence of Carcinogenicity for Humans	No evidence of carcinogenicity in at least two adequate animal tests in different species or in adequate epidemiologic and animal studies.

* EPA is in the process of revising the Cancer Guidelines Source

[2] Based on inhalation exposure

Table 4-4. Principal Known By-products of Ozonation (Adapted from EPA, 1999)

DISINFECTANT BY-PRODUCTS	
Aldehydes	**Aldo- and Ketoacids**
Formaldehyde	Pyruvic acid
Acetaldehyde	**Brominated By-products***
Glyoxal	Bromate ion
Methyl Glyoxal	Bromoform
Acids	Brominated acetic acids
Oxalic acid	Bromopicrin
Succinic acid	Brominated acetonitriles
Formic acid	**Others**
Acetic acid	Hydrogen peroxide

*Brominated by-products are produced only in waters containing bromide ion

Table 4-5. Ozone Disinfection Studies Involving Indicator Bacteria
(Adapted from Paraskeva and Graham, 2002)

TYPE OF EFFLUENT	DISIN-FECTION O_3 DOSE (mg/L)	CONTACT TIME (min)	RESIDUAL OZONE (mg/L)	TYPE OF MICRO-ORGANISM	INITIAL CONCEN-TRATION (CFU/100 mL)	FINAL CONCEN-TRATION (CFU/100 mL)	LOG REDUC-TION
Secondary	7–14	5	0.05	FC	$5.2 \times 10^3 - 8.5 \times 10^5$	$0.32 \times 10^2 - 8.0 \times 10^2$	
				FS	$0.7 \times 10^3 - 5.0 \times 10^3$	$0 - 1.3 \times 10^2$	
				TC	$4.0 \times 10^5 - 9.0 \times 10^6$	$0.1 - 2.6 \times 10^3$	
Secondary	8–14	21	0.2–0.4	TC	$2.4 \times 10^5 - 9.3 \times 10^6$	$9.3 \times 10^2 - 1.5 \times 10^4$	
Raw	2–4	20	0.1–0.4	EC	$2.4 - 3.7 \times 10^6$	$0.1 - 1.0 \times 10^4$	
				FS	$0.2 - 4.0 \times 10^5$	$3.6 - 7.0 \times 10^2$	
Secondary	6–15	10–25	0.3–0.4	FC	n/a	n/a	3–4
				FS	n/a	n/a	2–3
Tertiary (sand filtration)	6–9	10–30	0.2–0.8	TC	$0.1–1.0 \times 10^7$	$0.1–1.0 \times 10^3$	4
Secondary Nitrified	1.5	>30 min	n/a[b]				
Secondary	6–12	15	0.1–0.5	FC	$\times 10^3 - 1.0 \times 10^8$	n/a	4
				FS	$\times 10^{2.5} - 1.0 \times 10^6$	n/a	3

Table 4-5. Ozone-Disinfection Studies Involving Indicator Bacteria-(cont.)
(Adapted from Paraskeva and Graham, 2002)

TYPE OF EFFLUENT	DISIN-FECTION O_3 DOSE (mg/L)	CONTACT TIME (min)	RESIDUAL OZONE (mg/L)	TYPE OF MICRO-ORGANISM	INITIAL CONCEN-TRATION (CFU/100 mL)	FINAL CONCEN-TRATION (CFU/100 mL)	LOG REDUC-TION
Secondary	3–10	n/a	n/a	EC	n/a	n/a	1.3–4.5
Storm drain water	10–20	n/a	0.1–1				
Secondary	12–15	n/a	n/a	FC	n/a	<200	n/a
Filtered nitrified	3–5	n/a	n/a	FC	n/a	<200	n/a
Secondary clarified	15	10	n/a	TC	1.4×10^6	1.1×10^3	
Filtered clarified	7	5	n/a	TC	0.8×10^6	0.9×10^2	
Secondary	4–6	1–10	0.2–0.8	EC	$3.0 \times 10^3 - 4.2 \times 10^5$	4.0 – 65.0	
				TC	$3.0 \times 10^4 - 2.5 \times 10^5$	$8.0 - 1.5 \times 10^3$	
Secondary	15	2	n/a	FC	$1 \times 10^{4.3} - 1 \times 10^{6.5}$	$<1.0 \times 10^3$	
Secondary	4	2	n/a	FC	$1 \times 10^{3.6} - x\ 10^{4.5}$	$<1.0 \times 10^3$	
Tertiary	2	2	n/a	EC	$1 \times 10^{2.7} - 1 \times 10^{4.5}$	$<1.0 \times 10^3$	

Note:

FC = fecal coliforms; FS = fecal streptococci; TC = total coliform, and EC = *E. coli* ; [b]n/a = not available

Table 4-6. Inactivation of Microorganisms by Pilot-Scale Ozonation
(Adapted from Paraskeva and Graham, 2002)

MICROORGANISM	TEMPERATURE (^{0}C)	pH	CT[a] (mg min/L)	LOG_{10} INACTIVATION RANGE
Bacillus subtilis endospores	22.7 ± 1.0	7.93 ± 0.32	0.70 – 18.35	0 – 2.17
Cryptosporidium parvum oocysts	24.5 ± 1.6	8.24 ± 0.20	2.55 – 7.15	0.57 – 2.67
Cryptosporidium muris oocysts	23.6 ± 1.6	8.40 ± 0.11	0.98 – 10.7	0.36 – 2.56
Giardia muris oocysts	25.2 ± 1.1	7.57 ± 0.29	0.28 – 1.04	1.52 – 2.70
Poliovirus 1	25.0 ± 1.0	8.05 ± 0.17	0.19 – 2.49	1.43 – 3.85

Note:

[a]Concentration x time (CT) product, based on integrated dissolved ozone concentration values (C) and theoretical residence time (t).

Table 4-7. Summary of Reported Ozonation Requirements For 99 Percent Inactivation of *Cryptosporidium parvum* Oocysts (Adapted from EPA, 1999)

Ozone Protocol	Ozone Residual (mg/L)	Contact time (min)	Temperature (°C)	CT (mg min/L)
Batch liquid/batch ozone	0.5	18	7	9
Batch liquid/batch ozone	0.5	7.8	22	3.9
Batch liquid/batch ozone	0.77	6	Room	4.6
Batch liquid/batch ozone	0.51	8		4
Batch liquid/continuous ozone	1.0	5 and 10	25	5-10
Flow through contactor/continuous ozone	Not available	Not available	22-25	5.5

Table 4-8. Reduction of Selected Pathogens by Ozone (15-Mg O3/L Dose, 10 Minutes) in Tertiary Municipal Effluents (Adapted from Paraskeva and Graham, 2002)

PATHOGEN	FEED CL[a]		FEED F[a]	
	INFLUENT	TREATED	INFLUENT	TREATED
Pseudomonas aeruginosa (CFU/100 mL)	1800	28	800	8
Giardia lamblia cysts (count/L)	213	92	33	10
Cryptosporidium parvum oocysts (count/L)	10	8	2	2

Note:

[a]CL = clarified and F = clarified and filtered

Table 4-9. Summary of CT Values For 99% (2-Log) Inactivation of Selected Viruses by Various Disinfectants At 5°C

(Adapted from Health Canada, 2004)

VIRUS	CT VALUES FOR 99% (2-LOG) INACTIVATION			
	FREE Cl_2 pH 6–7	NH_2Cl pH 8–9	ClO_2 pH 6–7	O_3 pH 6–7
Poliovirus 1	1.1–2.5	768-3740	0.2–6.7	0.1–0.2
Rotavirus	0.01–0.05	3806–6476	0.2–2.1	0.006–0.06
Bacteriophage-f2	0.08–0.18	ND[a]	ND	ND

[a]ND = not determined

Table 4-10. LOG_{10} Reductions Achieved for Coliphage During Disinfection of Secondary Effluent by UV Irradiation and Chlorination (Adapted from *WERF*, 2005)

FACILITY IDENTIFIER (PHAGE HOST)	LOG_{10} REDUCTIONS OF COLIPHAGE				
	UV Dose (mJ/cm^2)[a]			Chlorine contact time (min)[b]	
	5	10	20	20	40
E *(E.coli)*\ (F+amp)	0.47 0.68	0.94 1.37	1.88 2.74	1.81	3.61
B *(E.coli)*\ (F+amp)	0.88 0.59	1.75 1.19	3.51 2.38	0.25 0.13	0.5 0.26
C *(E.coli)*\ (F+amp)\	0.42	0.84	1.69	0.78	1.56
D *(E.coli)* (F+amp)	0.69	1.37	2.74	0.32 0.43	0.64 0.87
A *(E.coli)*\ (F+amp)	0.64 0.36	1.27 0.73	2.54 1.45	0.3 0.26	0.59 0.52
Mean *E.coli* **F+amp**	**0.61** **0.49**	**1.21** **0.99**	**2.42** **1.97**	**0.37** **0.25**	**0.75** **0.5**

Note:

[a]Exposure conducted in a well-mixed batch reactor under a collimated beam.

[b]Exposure conducted in a well-mixed batch reactor with an initial chlorine concentration of 2.0 mg/L (as Cl_2)

Table 4-11. Summary of Pathogen Disinfection Efficiencies

Pathogen	Ozonation	UV Disinfection	Chlorination/Dechlorination
E. coli	4 log (Note 1); 1.3 log-4.5 log (Note 2)	4 log (Note 8)	> 4 log (Note 8)
Pseudomonas aeruginosa	2 log (Note 2)	4 log (Note 8)	> 4 log (Note 8)
Salmonella	4 log (Note 1)	3-4 log (Note 10)	Not Available
Enterococci	Not Available	Not Available	More resistant than *E. coli* (Note 8)
Cryptosporidium	0.57 log-2.67 log (Note 2)	3 log (Note 3)	0.2 log-3log (Note 1)
Giardia	1.57 log-2.7 log (Note 2)	2 log (Note 10)	0.5 log (Note 1)
Total Enteric Viruses	5 log (Note 2)	0.32 log-3.61 log (Note 8)	5 log (Note 4)
Calicivirus	2 log (Note 5)	4 log (Note 7)	2 log (Note 5)
Adenovirus	4 log (Note 9)	1 log- 4 log (Note 6)	2-4 log (Note 11)

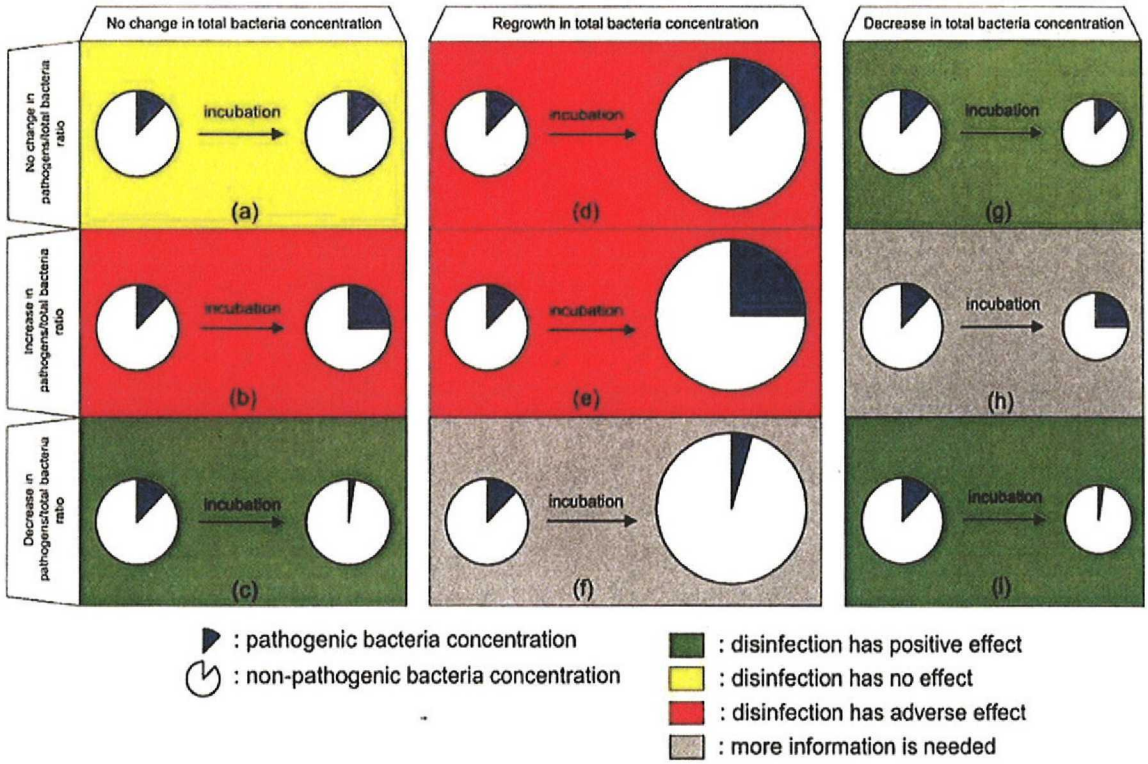
Notes:

(1) EPA (1999)
(2) Paraskeva and Graham (2002)
(3) Clancy (2004)
(4) Nelson et al. (undated)
(5) Health Canada (2004)
(6) Gerba et al. (2002)
(7) Thurston-Enriquez et al. (2003)
(8) *WERF* (2005)
(9) Thurston-Enriquez et al. (2005); results obtained in buffered disinfectant demand free water at 5°C and pH 7. These conditions may not be representative of wastewater.
(10) Chang et al. (1985)
(11) Thurston-Enriquez et al. (2003a)

SECTION 4

FIGURES

Figure 4-1. Conceptual Representation of the Possible Fates of Bacteria Disinfectant Exposure



Note:

Disinfection is considered to be antibacterially "effective" when the risk of human exposure to bacteria is reduced. Moving from left to right, the columns represent circumstances of no regrowth, regrowth, and decline in the total bacterial population, respectively. Moving from top to bottom, the rows represent circumstances in which the fraction of the bacterial population comprised of pathogenic bacteria does not change, increases, and decreases, respectively. Together, these two attributes (regrowth of the total bacterial population and changes in the fraction of pathogenic bacteria) determine the effectiveness of disinfection relative to human exposure to bacteria (adapted from *WERF*, 2005).

5.0 MICROBIAL RISK ASSESSEMENT

Quantitative microbial risk assessment (QMRA) was initially employed to assess the risks from microorganisms in drinking water (Haas, 1983; Regli *et al.* 1991). These methods were later adopted by the EPA to assess the safety of water supplies and establish criteria (based on *Giardia*) for finished water protective of human health. Other researchers have used QMRA methodology to assess microbial risks for a variety of activities and organisms (Haas *et al.*, 1996; Haas *et al.*, 1999; Gerba *et al.*, 1996; Crabtree *et al.*, 1997; Pouillot *et al.*, 2004). Microbial risk assessment techniques were used to quantitatively assess the health risks for the use of recreational waters that receive effluent discharges (Soller *et al.*, 2003) and were incorporated in the World Health Organization (WHO) Guidelines for Safe Recreational Waters (WHO, 2003).

The process of risk assessment is typically divided into four steps (EPA, 1989; NRC, 1994):

- *Hazard identification*, in which the human health effects of the particular hazard are described;
- *Exposure assessment*, which determines the relevant pathways and nature of the exposed population along with quantitative estimates on the levels of exposure;
- *Dose-response assessment*, which characterizes the relationship between administered dose and incidence of health effects; and
- *Risk characterization*, which integrates the information from the previous steps in order to estimate the magnitude of risks and to evaluate variability and uncertainty.

These four steps in the risk assessment are discussed in more detail in the following sections as they relate to the microbial risk assessment of the CWS.

5.1 Hazard Identification

Recreational use of the CWS may expose individuals through incidental ingestion, dermal, and inhalation pathways to disease-causing bacteria, viruses and protozoa within the waters. The health effects of microbial pathogen exposure to recreational water are

varied. Pathogens may infect the gastrointestinal tract, lungs, skin, eyes, central nervous system or liver (WHO, 2003). The most common illness is gastrointestinal upset (nausea, vomiting and diarrhea), usually of moderate intensity and short duration. However, in susceptible individuals such as infants, the elderly and the immunocompromised, the effects may be more severe, chronic (e.g., kidney damage) or even fatal (Hoxie *et al.*, 1997).

Exposure to microbial contaminated water may result in both gastrointestinal and non-gastrointestinal illness. However, gastrointestinal illness is the principal adverse outcome associated with exposure to microbially contaminated water. Most of the pathogens of concern cause gastrointestinal illness. Since there is a certain degree of correlation between different pathogens, indications of unacceptable levels of gastrointestinal illness may indicate a potential for other effects. Therefore, the risk of gastrointestinal illness was selected as the sentinel effect for conducting the quantitative risk assessment. Note that *Pseudomonas* is a bacterium that causes folliculitis and ear infections but not gastroenteritis (Asperen *et al.*, 1995). Risks from *Pseudomonas* are evaluated qualitatively to ensure that these risks are not overlooked in the assessment. The qualitative comparisons are provided by comparison of *Pseudomonas* levels under wet and dry weather conditions. Some adenovirus strains are primarily associated with respiratory illness (Gerba, 2007). However, fecal-oral transmission associated with gastrointestinal illness is the primary effect evaluated in this study. As a conservative assumption all detected adenovirus was assumed to contribute to gastrointestinal illness.

5.2 Exposure Assessment

Exposure assessment evaluates the duration, frequency and magnitude of pathogen exposure by one or more pathways. The assessment is dependent on adequate methods for detection, quantification, specificity, virulence and viability of the microorganisms in question and is often dependent on studies and models of transport and fate in the environment. Exposure assessment uses an array of information sources and techniques. Typically, data are not available for all aspects of the exposure assessment and those data that are available may sometimes be of questionable or unknown quality. In these

situations, qualified assumptions must be made. These are based on professional judgments and inferences based on analogy with similar microorganisms or processes. The end result is based on a number of inputs with varying degrees of uncertainty.

Potential receptor groups are identified in the exposure assessment and estimates of exposure are calculated based on assumptions regarding exposure pathways and exposure parameter inputs. For this assessment, CWS specific information was used whenever possible to characterize the population that may be potentially exposed to disease causing organisms in the CWS. The focus of the assessment was on the incidental ingestion pathway as discussed in more detail below. The subsequent sections discuss in more detail the types of receptor groups and waterway use evaluated in this assessment and the exposure inputs used.

Exposure to pathogens through recreational activities can occur through different pathways. The most important is via incidental ingestion but other routes can also be important for some microorganisms, like exposure via inhalation, eye or dermal contact (Haas *et al.*, 1999). Since the endpoint of this evaluation is gastrointestinal illness, exposure pathways that contribute to this effect were investigated. An initial evaluation of the contribution to total intake by several pathways (incidental water ingestion, inhalation and dermal contact) was conducted to determine the relative contribution of each pathway to total exposure to microbiological organisms in surface water while recreating. Dermal contact was assumed to not contribute to exposure that would lead to gastrointestinal illness. Inhalation exposure of spray or droplets containing pathogens which are subsequently swallowed may contribute to the total dose. The total ingestion dose was adjusted to account for this pathway. However, it is unlikely that users engaged in non-immersion activities would be subject to levels of inhaled mists or sprays that will lead to a substantially increased ingested dose. Based on this assessment, exposure from inhalation and dermal pathways were considered insignificant to the contribution to the risk of gastrointestinal illness or can be accounted for through the incidental ingestion term. An intake parameter for incidental direct ingestion of surface water was developed that incorporates minor contributions from inhalation while engaging in recreational activities along the waterways.

5.2.1 Waterway Use Summary and Receptor Group Categorization

Several sources of information were reviewed to estimate recreational use and exposure to the CWS (CDM, 2004; USACOE, 1994; EPA 2006). Each of these studies provides insight on the types and frequency of recreational exposure expected in the waterway. For quantitative risk analysis, the UAA study was used as the primary source for exposure use data for the CWS. The purpose of the UAA is to "evaluate existing conditions, including waterway use practices and anticipated future uses to determine if use classification revisions are warranted". As a part of the UAA, the CWS was divided into three major waterway segments each associated with a single WRP. A CWS map with the waterway segment divisions, WRP outfalls, and sampling locations is provided in Figure 5-1.

The UAA surveys were conducted to evaluate the types of recreational use that are currently being exhibited on each of the waterway segments. Based on the UAA, several recreational exposure scenarios were selected for evaluation in the risk assessment. The exposure categories listed in the UAA were divided into three groups based on the assumptions of varying exposure intensity. Immersion activities like swimming, skiing, and wading were not included in the risk assessment as these are not designated use activities allowed in the CWS. Jetski use is typically thought to involve immersion and thereby would not be allowed under the use conditions on the waterways. However, larger jetski boats would be allowed. The UAA report did not distinguish between these two types of watercraft. Receptors reported as using jetskis were grouped with the highest exposure classification (i.e. canoeing) for the purposes of deriving receptor user statistics for the risk assessment. However, it should be noted that the resulting risk estimates do not account for jetski use that involves immersion. In addition, the UAA waterway segments were grouped as appropriate to reflect the portion of the CWS that would be relevant for evaluating the three WRPs.

The receptor use categories are described below:

Canoeing

- Frequent contact with wet items (paddles, boat deck, equipment)

- Close proximity to water surface
- Occasional direct contact with water (hand immersion)

Fishing[2]

- Occasional contact with wet items (tackle, boat deck, equipment)
- Infrequent direct contact with water

Pleasure Boating

- Infrequent contact with wet items (boat deck, equipment)
- No direct water contact

The observation data from the UAA survey was grouped according to general activity categories as presented in Table 5-1. Based on the receptor use grouping and UAA reported activity levels, the proportion of users in each of the three exposure groups was calculated within each waterway (see Table 5-2).

To evaluate secondary attack rates (see Section 5.4.2), the number of family members that may be potentially exposed from a person infected while recreating on the CWS was needed. Family sizes for the Chicago area were derived from the 2004 America Community Survey conducted by the U.S. Census Bureau. Data for Cook County, the county in which the waterway segments traverse, were used to calculate percentages of households within a given size category. A household was defined by the Survey as including all of the people who occupy a housing unit as their usual place of residence. Approximately 9% of individuals live alone. The data indicated the percentages of household sizes for households in which more than one person resided (U.S. Census, 2005) as shown in Table 5-3.

[2] Exposure scenarios evaluated in this study are limited to water contact only and do not include potential food borne pathogen transfer (i.e. from consumption of inadequately prepared microbially contaminated fish).

5.2.2 Exposure Inputs

Several exposure parameters are required as inputs to the exposure model. These parameters include incidental ingestion rates and exposure duration (i.e., time someone may be in the CWS). This section discusses the exposure inputs and the rationale for their selection.

A probabilistic approach was selected to evaluate risks of gastrointestinal illness for recreational users of the CWS. Probabilistic risk assessment utilizes input distributions, rather than point estimates, to better represent the variability and uncertainty that exists for each input parameter (EPA, 1997). Thus, instead of using one value for exposure inputs such as exposure duration or incidental ingestion, a range of possible values (or more correctly, a probability density function) was used. These probability density functions are presented in the following subsections for each exposure input and receptor category.

Incidental Water Ingestion Rates

One of the primary exposure inputs in the analysis is the amount of water one may incidentally ingest when recreating on the CWS. Incidental ingestion may occur through secondary contact of surface water contaminated surfaces, hand-to-mouth activity, or direct ingestion if accidentally submerged. Ingestion rates for these pathways are expected to vary widely dependent on the recreational activity and chance occurrence of high exposure events. Incidental ingestion of surface water may also occur through inhalation and entrapment of mists and droplets in the nose and mouth with subsequent swallowing. The intake through this mechanism is likely dependant on proximity to the water surface, generation of mists during recreational activity and length of time exposed.

There are no direct studies that have quantified the amount of water that participants in low-contact water sports such as canoeing and boating may ingest. However, studies have reported observed illnesses in canoeists and kayakers boating in water with measured microbial contamination (Fewtrell, 1992; 1994). Fewtrell (1994) reports that studies of rowing and marathon canoeists showed approximately 8% of canoeists at freshwater sites reported capsizing and approximately 16% of rowers reported ingesting

some water. These studies indicate that these activities are likely to involve some degree of incidental water ingestion.

The exposure assessment literature was reviewed to identify recreational water ingestion rates that may be relevant to the types of low-contact use observed during the UAA. Water ingestion rates found in the literature were primarily from full contact swimming studies and ranged from 30 mL/hr (Crabtree *et al.*, 1997; Van Heerden *et al.*, 2005) to 50 mL/event (EPA, 1989, Steyn, *et al.*, 2004). These values are based on a swimming scenario which would result in ingesting significantly more water than one might ingest through low contact boating. Only for instances in which a canoeist might capsize could water be ingested at an appreciable rate. Other incidental water ingestion values were identified in the literature. A value of 10 mL/event was reported for accidental gulping of water during activities such as cleaning laundry, fishing and agricultural/horticulture irrigation (Genthe and Rodda, 1999 and Medema *et al.*, 2001).

To account for the reduced water ingestion rates associated with low contact use of the CWS, input ingestion rates were developed using a time-dependent ingestion rate to account for background intakes associated with inhalation, coupled with a variable term developed from a lognormal distribution. Lognormal distributions arise from a multiplicative process and tend to provide good representations of exposure parameters based on natural phenomenon (Ott, 1995).

For canoeists the lognormal distribution had a mean of 5 and standard deviation of 5 [LN(5,5)]. The fixed intake term was 4 mL/hr. In this case the median (50^{th} percentile) water ingestion rate was 7.52 mL/hr and the maximum (100^{th} percentile) was 34 mL/hr, within the range reported for full contact swimming. For the 90^{th} to 100^{th} percentile, ingestion rates ranged from 14 to 34 mL/hr, which implies that 10% of the population may be exposed to water ingestion rates approaching those observed in swimming or accidental gulping. This is consistent with the observation in the Fewtrell (1994) study in which 8% of canoeists reported capsizing, an event that may result in ingestion rates similar to gulping or swimming.

Even less water could be ingested by people fishing and boating as compared to canoeists. Therefore the input ingestion rates for these two categories were adjusted downward using professional judgment. Incidental ingestion rates for fisherman was assumed to follow a lognormal distribution mean with a mean of 3 and standard deviation of 2 [LN(3, 2)]. The incidental ingestion rate for a pleasure boater was assumed to follow a lognormal distribution with a mean of 1 and standard deviation of 0.5 [LN(1, 0.5)]. A fixed intake term of 1 mL/hr was added to the lognormal intake rate for both boaters and fisherman to account for background intake associated with proximity to the water. A graphical depiction of the lognormal portion of the distribution assumed for canoeists is presented in Figure 5-2 to show what a probability density function would look like based on the tabular information in Table 5-4.

Exposure Duration

To develop a distribution for exposure duration, assumptions regarding the length of time an individual might be on the waterway are required. Activity based assumptions were developed for this exposure input based on waterway specific information (where available) and professional judgment guided by literature refences.

For the canoeist scenario, canoeing event information from the Friends of the Chicago River was reviewed. Canoes can be launched at several locations along the waterway with several launch points along the North Side and the south Chicago River near downtown. A major event that occurs each year on the waterway is called the Flatwater Classic in which canoeists traverse approximately 7 miles of the CWS from the North Side to the Chinatown area. Race times in 2005 ranged from approximately 1 hour to 3.5 hours with the majority of times between 1.5 and 2.5 hours. In non-race situations a canoeist could take longer. Boat launch statistics are available but do not provide information on trip duration (EPA, 2007). Based on this information and professional judgment, a triangular distribution was assigned to this input with the minimum time a canoeist would be in the water of 1 hour and the likeliest time in the water of 2 hours. Triangular distributions are often useful inputs in situations where the extremes of a distribution are understood and a most likely value can be estimated. A graphical depiction of the triangular distribution is presented in Figure 5-3.

For Pleasure Boating and Fishing it was assumed that the likeliest time on the water would be for approximately 3 to 4 hours. For boaters it was assumed the maximum time on the water would be an 8 hour day. For fishing the maximum time was assumed to be somewhat shorter at 6 hours.

5.3 Dose-Response Assessment

Dose-response assessment defines the mathematical relationship between the dose of a pathogenic organism and the probability of infection or illness in exposed persons. Dose-response data are typically derived from either controlled human feeding studies or reconstruction of doses from outbreak incidences. In human feeding trials volunteers are fed pathogens in different doses and the percentage of subjects experiencing the effect (either illness or infection) are calculated. While feeding trials can provide useful dose-response analysis data, studies are usually performed in healthy individuals given high levels of a single strain. Epidemiological outbreak studies provide responses on a larger cross-section of the population but dose reconstruction is often problematic.

In most studies, the doses of pathogens encountered are high enough that a large percentage of the exposed population (often >50%) are affected. However, risk assessment is often interested in the response rates at doses where 1 per 1000 or fewer exposed individuals respond. To estimate the dose-response at lower doses requires modeling the available data and extrapolating to low dose. Different mathematical dose-response models have been proposed to fit experimental data (Crockett *et al.*, 1996; Teunis *et al.*, 1996). Biologically plausible dose-response models must account for two conditional probabilities: the probability that an organism is ingested and the probability that once ingested an organism survives to infect the host (Haas, *et al.*, 1999).

Dose-response models assume that even a single organism has a finite probability of initiating infection with an increasing number of pathogens resulting in an increasing probability. The most common models used in quantitative microbial risk assessment are the exponential and beta-Poisson dose-response models. In the exponential model it is assumed that all of the ingested organisms have the same probability, 1/k, of causing an infection. The dose ingested is assumed to be Poisson distributed with a mean of D

organisms per portion (Haas *et al.*, 1999). The probability of infection given a dose (D) is:

$$P(D) = 1 - \exp(-1/k * D) \qquad (5\text{-}1)$$

where $P(D)$ is the probability of infection, and $1/k$ is the parameter of the exponential relationship.

The median infectious dose (N_{50}; dose of an organism resulting in a 50% probability of infection) for an exponential dose-response relationship is derived from equation 5-1 and given by:

$$N_{50} = \ln(0.5)/(-k) \qquad (5\text{-}2)$$

In the beta-Poisson model, heterogeneity in the organism/host interaction is introduced and k is assumed to follow a beta-Poisson distribution (Haas *et al.*, 1999). The resulting model is more complex but can be approximated under the assumption that β is much larger than both α and 1 so that the probability of infection given a dose (D) is:

$$P(D) = 1 - \left(1 + \left(\frac{D}{\beta}\right)\right)^{-\alpha} \qquad (5\text{-}3)$$

where P(D) is the probability of infection, D is the dose ingested and α and β are the dose-response parameters for the beta-Poisson model. This model is the current state-of-the-science for characterizing dose-response relationships where the probability of host-pathogen survival is governed by a probability distribution (Haas, 1999; Teunis *et al.*, 1996).

The median infectious dose (N_{50}) under a beta-Poisson model is derived from equation 5-3 and given by:

$$N_{50} = \frac{\beta}{\left(\left(2^{(1/\alpha)}\right) - 1\right)} \qquad (5\text{-}4)$$

Published dose-response studies are available for some of the pathogens of concern for this assessment. Other pathogens lack specific dose-response studies but share sufficient pathogenicity with known organisms that surrogate dose-response relationships can be developed. The following section provides a brief overview of the pathogens of concern along with a description of the dose-response data available and the selected dose-response parameters used in this analysis. A summary of the dose-response parameters used in this analysis is provided in Table 5-5.

5.3.1 Enteric viruses

Viruses that grow and multiply in the gastrointestinal tract are termed 'enteric' viruses. Many different enteric viruses are associated with human waterborne illness. These include adenovirus, norovirus, hepatitis virus (A [HAV] and E [HEV]), rotavirus and enterovirus (poliovirus, coxsackievirus A and B, echovirus and four ungrouped enteroviruses). Enteric viruses often find a limited host range, but some can infect both humans and animals. For example, while humans are the only natural reservoir for hepatitis A virus, norovirus, enterovirus, rotavirus, and hepatitis E virus can be transmitted from animals-to-humans with animals serving as a natural reservoir (AWWA, 1999).

Enteric viruses are excreted in large numbers in the feces of infected persons and animals (both symptomatic and asymptomatic). They are easily disseminated in the environment through feces and are transmissible to other individuals via the fecal–oral route. Infected individuals can excrete over one billion (10^9) viruses per gram of feces. The level of viruses in a population is variable and reflects current epidemic and endemic conditions, with numbers in raw sewage ranging from 100 to over 10,000 infectious units per liter (Aulicino *et al.*, 1996; Rao and Melnick, 1986; Fields *et al.*, 1996). Numbers of enteric viruses tend to peak in autumn/winter (Goddard *et al.*, 1981).

Although viruses cannot replicate outside their host's cells and therefore cannot multiply in the environment, they can survive for several months in fresh water. Their survival in the environment is prolonged at low temperatures and in the presence of sediments, to

which they easily adsorb. Exposure to sunlight, higher temperatures and high microbial activity will shorten the survival of enteric viruses.

Dose-response

Development of a quantitative dose-response relationship for gastrointestinal illness caused by total enteric viruses is problematic. Methods for growth and detection of viruses are costly and inefficient, making exposure estimates difficult. The causative viral pathogen in gastrointestinal outbreaks where enteric viruses are suspected is typically not known, making specific dose-response estimation from outbreak studies difficult.

The EPA has proposed using rotavirus as a conservative surrogate enteric virus for gastrointestinal illness risk assessment. However, rotavirus is among the most infectious waterborne viruses. Because several different viruses are evaluated separately in the present analysis, including *Calicivirus* (norovirus), the use of the most infectious agent as a surrogate will over-estimate the true risks.

Of the enteric viruses, dose-response information is available for poliovirus I, echovirus 12, and coxsackie virus (Haas *et al.*, 1999). Each of these viruses fit an exponential dose-response model with exponential parameters (k) in a narrow range from 69.1 to 109.9 (Haas *et al.*, 1999). The dose-response for echovirus 12 (k = 78.3) was selected as a surrogate for total enteric viruses with an infectivity in the middle of this range. The selected value is within the range of values used in the WERF (2004) biosolids study. Table 5-5 provides a summary of dose-response parameters used in the risk assessment.

Secondary transmission is common for enteric viruses. It has been estimated that for every child with a waterborne viral disease, an additional 0.35 people will become ill (EPA, 2000). One study showed a household transmission of viral gastroenteritis by norovirus of 20% (Gotz *et al.*, 2002). Perry *et al.* (2005), conducted a prospective study of families in northern California and found an overall secondary transmission of 9%, with children having a much higher attack rate than adults. WERF (2004) reported a secondary attack rate of 4.2%. For the purposes of the risk assessment, a conservative

secondary attack rate of 25% was used for all the enteric viruses. This value accounts for both the highly infectious norovirus and the less virulent enteric viruses.

5.3.2 *Calicivirus*

The *Caliciviruses* are small (27 to 35 nm) RNA viruses with a distinctive spherical capsid surface with cup-shaped depressions. *Caliciviruses* are often named after the location of the outbreak from which they are derived (Norwalk, Ohio; Hawaii; Snow Mountain, Colorado; Taunton and Southampton, England; Otofuke and Sapporo, Japan). *Caliciviruses* are leading causes of gastroenteritis in the U.S., with dissemination predominately by the fecal-oral route (Greenberg and Matsui, 1992; Schaub and Oshiro, 2000). They produce gastrointestinal and respiratory infections in several animal species, including humans, swine, and cats. The *Calicivirus* most associated with human disease is norovirus (also called Norwalk virus), which is a major cause of epidemics of self-limited diarrhea and vomiting in school children and adults. Although most adults have serum antibodies to norovirus, the antibodies do not protect them from the disease. In fact, they may serve as a marker for increased sensitivity to illness (Johnson *et al.*, 1990).

Caliciviruses are endemic and commonly found in raw sewage at levels related to the viral activity in the community. Use of recreational water that may be contaminated with sewage or high bathing loads is associated with outbreaks of *Calicivirus* gastroenteritis (Hoebe *et al.*, 2004; Maunula *et al.* 2004; Levy *et al.*, 1998). It is likely that some portion of the nationwide incidence of acute gastrointestinal illness associated with swimming is caused by *Calicivirus*.

Dose-response

No human studies are available to derive a dose-response relationship for *Caliciviruses*. The EPA has suggested the use of rotavirus as a surrogate for dose-response relationships with other enteric viruses. A similar approach was used by WERF (2004) to assign dose-response parameters. Based on rotavirus dose–response experiments in human volunteers, the dose-response model for rotavirus fits a beta-Poisson model (Ward *et al.*, 1986). The median infectious dose (N_{50}) from that study was 6.17 with an α value of

0.2531. Like other viruses, the secondary attack rates for *Caliciviruses* can be quite high (Ethelberg *et al.*, 2004). One study suggests secondary spread within a family is approximately 86% (Gerba, 2005). Other studies show the household transmission of viral gastroenteritis by norovirus at lower levels (Gotz *et al.*, 2002). WERF (2004) utilized a much lower secondary attack rate of 7.6%. The higher secondary attack rate for norovirus of 86% (Gerba, 2005) was selected to match the norovirus for the primary dose-response parameters.

5.3.3 Adenovirus

Adenoviruses are 90- to 100-nm non-enveloped icosahedral viruses containing double-stranded DNA. Adenoviruses are a common cause of gastroenteritis and viral diarrhea, second in prevalence behind rotavirus. Incidence rates for gastroenteritis caused by adenovirus range from 1.55 to 12 percent (Shinozaki *et al.*, 1991; Wadell *et al.*, 1994). Infections occur year-round, with a slight increase in summer. Although diarrhea can occur during infection with any type of adenovirus, Ad40 and Ad41 are the subtypes most often associated with gastroenteritis and diarrhea. Other adenoviruses cause nose, eye, and respiratory infections. Contact with recreational water has been associated with adenovirus outbreaks (D'Angelo, 1979).

Humans are the primary reservoir for pathogenic adenovirus. High titers of virus are excreted during active infection and can continue to be excreted for months or even years after disease symptoms have ceased, with as many as 20% of asymptomatic healthy people shedding viruses (Foy, 1997). Adenoviruses are very environmentally stable, allowing for prolonged survival outside of the host. Like most viruses, they survive primary effluent treatment systems and are more resistant to disinfection systems than bacteria.

Dose-response

Several dose-response relationships are reported for adenovirus but none of these are specifically for Ad40 or Ad41, subtypes primarily associated with gastrointestinal illness. For example, an exponential model has been proposed for the respiratory subtype Ad4

with a k value of 2.397 (Haas *et al.*, 1999). This would suggest a highly infectious pathogen and could be used as a surrogate for the risk assessment. However, only a portion of the measured adenovirus corresponds to subtypes responsible for gastroenteritis. This will lead to an overestimate of the true risks for gastrointestinal illness. Therefore, the dose-response for echovirus 12 (k = 78.3) was selected as a surrogate for total enteric viruses with an infectivity in the middle of this range.

Studies have estimated the secondary attack rate for adenovirus in adults at 19% and in children at 67% (Fox *et al.*, 1977). A prospective study of children enrolled in day-care centers in Texas generated data elucidating the role of enteric adenoviruses in group settings (Van *et al.*, 1992). Children six to 24 months-old were monitored over five years. Ten outbreaks affecting 249 children were associated with enteric adenoviruses. The infection rate during the 10 outbreaks ranged from 20 to 60 percent (mean 38 percent), and 46 percent of the infected children remained asymptomatic. Based on these studies a composite secondary attack rate for both adult and children of 38% was used in the present analysis.

5.3.4 *Escherichia coli*

Escherichia coli are gram negative rods normally harbored as harmless organisms in the intestinal tracts of warm-blooded animals (Maier *et al.*, 2000). Several strains, however, are pathogenic and cause gastrointestinal illness in humans. These strains include enteroinvasive or enterohemorrhagic strains (e.g., O157:H7, O124, O143), enterotoxigenic strains (e.g., O6:H16, O148:H28), and enteropathogenic strains (e.g., O78:H11, O111, O55). There are an estimated 200,000 cases of infection and 400 deaths attributed to pathogenic forms of *E. coli* in the U.S. annually (Bennett *et al.*, 1987). A number of these cases are related to recreational use of contaminated water including several cases associated with *E. coli* O157 involving illnesses and deaths (Ackman *et al.*, 1997; Swerdlow *et al.*, 1989). The O157 strain is highly infectious, causing a severe dysentery-like illness that may lead to serious hemorrhagic or hemolytic uraemic syndromes associated with significant mortality and morbidity (Haas *et al.*, 1999).

Gastrointestinal illness is associated with the fecal-oral route of transmission for pathogenic *E. coli*. Enterotoxigenic strains (responsible for most cases of traveler's diarrhea) are species specific and indicate contamination with human feces (Maier *et al.*, 2000). However, humans, pigs, and cattle can harbor enteropathogenic and enterohemorrhagic strains. The environmental source for most O157 strains is livestock rearing. In recreational waters impacted by livestock excreta, there is a potential risk of transmission to humans. Up to 15% of cattle in the United Kingdom harbor O157 and higher rates have been reported in the U.S. (Jones, 1999).

In fresh surface waters, *E. coli* have a half-life of approximately 24 hours (Maier *et al.*, 2000). The half-life is shortened with elevated UV radiation and increased temperature. *E. coli* are effectively killed by disinfection techniques such as UV irradiation, chlorination, and ozonation.

Dose-response

Most *E. coli* measured in the waterway are not pathogens; therefore, an assumption was required to adjust the reported *E. coli* concentration to account for the fraction of pathogenic organisms. Limited data exists to estimate the proportion of pathogenic *E. coli* in recreational waters. Frequency of detection of the enterohemorrhagic strain O157:H7 in cattle hides or feces have been reported to vary between 0.2% to 30% (O'Brien *et al.*, 2005; Galland *et al.*, 2001). However, the absolute proportion of this pathogenic stain compared to all *E. coli*, even within cattle, is unknown. A survey of *E. coli* strains in the Calumet River is perhaps the best resource for establishing a proportion of pathogenic *E. coli* in the CWS (Peruski, 2005). This study was conducted in both wet and dry weather conditions. Results of the study found that 2.7% of the *E. coli* were pathogenic strains while 0.5% of the total *E. coli* were human pathogenic strains. Similar results were observed in both dry and wet weather events. As a conservative estimate a factor of 2.7% was selected for the fraction of pathogenic *E. coli*. This value likely over-estimates the true fraction of human pathogenic organisms; therefore, a single dose-response parameter that excludes the more infectious and less frequently encountered strains was employed to develop risk estimates.

The dose-response relationships for *E. coli* strains can be divided into two groups; 1) the enterohemorrhagic strains, and 2) the enterotoxigenic and enteropathogenic strains. The enterohemorrhagic strains are more virulent due to the presence of *Shigella*-like toxins enabling the bacteria to adhere to the intestinal lining and initiate disease. Because of the similarity in mechanism between enterohemorrhagic *E. coli* and *Shigella*, the *Shigella* dose-response relationship has been proposed as a suitable surrogate (Haas *et al.*, 1999). Risks associated with the remaining *E. coli* strains are best described by a beta-Poisson dose-response relationship. Several dose-response parameters have been suggested as appropriate for assessing risk for pathogenic strains of *E.coli* (Haas *et al.*, 1999; WERF, 2004). Parameters for a composite best-fit dose-response model were developed from using maximum likelihood methods (Haas *et al.*, 1999). Based on this analysis the median infectious dose (N_{50}) for enteropathogenic strains was 2.55E+06 with an α value of 0.1748. This dose-response parameter was selected as a conservative mixed strain model to account for potential pathogenic *E. coli* strains encountered in the CWS.

There is little data to support a pathogen specific secondary attack rate for pathogenic *E. coli*. One study has estimated secondary attack rates at ~15% based on illness spread within families (Parry and Salmon, 1998). However this study was not inclusive of all strains of pathogenic organisms. WERF (2004) reported a secondary attack rate of 2.7% for the highly virulent O157:H7 strain. A secondary attack rate of 25% was used for this risk assessment (Gerba, 2005). Again, this value is a conservative estimate and will tend to over-estimate risks for this pathogen.

5.3.5 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative, rod-shaped bacterium that can cause infection in a variety of organisms including plants, insects, birds, and mammals including humans (Maier *et al.*, 2000). In humans, it is known to cause skin rashes, eye infections, and is the primary organism associated with external ear infections (Kush and Hoadley 1980). Ear infections (otitis externia) have been associated with *Pseudomonas aeruginosa* after immersion activities in recreational water but these organisms do not

seem to produce gastrointestinal effects (Ontario Ministry of the Environment, 1984; Seyfried, 1984; Cabelli *et al.*, 1979).

P. aeruginosa is ubiquitous in U.S. waters with both fecal and non-fecal sources. Approximately 10 per cent of the healthy North American adults are intestinal carriers of *P. aeruginosa,* resulting in concentrations in raw domestic sewage ranging from 10^5 to 10^6 CFU/100 mL (Canadian Ministry of National Health and Welfare, 1992). Another study measured *P. aeruginosa* in raw sewage at a level of 1,800 CFU/mL, wastewater treatment effluent at 140 CFU/mL, and canal and lake water at 10 CFU/mL (Dutka and Kwan, 1977). In addition, *P. aeruginosa* levels in excess of 100 organisms/100 mL can be measured in waters receiving surface drainage from urban areas (Ontario Ministry of the Environment, 1984). *P. aeruginosa* survives longer in waters than do coliforms (Lanyi *et al.* 1966) and has the ability to multiply in waters with low nutrient content (Canadian Ministry of National Health and Welfare, 1992).

Dose-response

No quantitative dose-response studies are available for this pathogen. *P. aeruginosa* is not a significant cause of gastrointestinal illness in humans. However, the presence of this pathogen in recreational water may pose a significant risk for folliculitis and otitis (Asperen *et al.*, 1995). A quantitative exposure assessment for the dermal risks posed by this organism is problematic (Hardalo and Edberg, 1997). For example, folliculits requires a prior skin cut, open sore or abrasion to allow infection. The prevalence of this condition in the exposed population is unknown. Data from a 4-year study were used to develop a relationship between the concentration of *P. aeruginosa* in the bathing waters and the risk of ear infection (Ontario Ministry of the Environment, 1984). From this study it was estimated that when levels of *P. aeruginosa* exceed 10 CFU/100 mL in at least 25 per cent of the seasonal samples, otitis externa may be expected to occur.

No quantitative estimates of risks for non-gastrointestinal illness associated with *P. aeruginosa* are derived. Epidemiological evidence suggests that gastrointestinal illness is unlikely. A qualitative evaluation of the non-gastrointestinal (dermal) risks is discussed below as a comparison between the dry and wet weather data.

5.3.6 *Salmonella*

Salmonella are Gram-negative rod shaped bacteria. More than 2000 *Salmonella* serotypes are known to exist, with the number of non-typhoid salmonellosis cases in the United States per year estimated to be between 2 million and 5 million. *Salmonella* is one of the most common intestinal infections in the U.S. *Salmonella typhi* and *paratyphi* are strictly human pathogens and domestic animals play no role in the epidemiology of these infections. All of the other "non-typhoid" *Salmonella spp.* (e.g., *Salmonella enterica*) are ubiquitous in the environment and reside in the gastrointestinal tracts of animals (Haas *et al.*, 1999). The vast majority of human cases of salmonellosis are acquired by ingestion of fecal contaminated foods or water, with cases more common in the warmer months of the year (Maier *et al.*, 2000). Person-to-person transmission of *Salmonella* occurs when a carrier's feces, unwashed from his or her hands, contaminates food during preparation or through direct contact with another person.

Dose-response

Dose-response data were obtained from human feeding studies conducted by McCullough and Eisele (1951), who investigated the pathogenicity of five *Salmonella* species isolated from eggs and egg products. The analysis concluded that the lognormal and beta-Poisson model fit the majority of the data. The parameters of the beta-Poisson dose-response model for non-typhi *Salmonella* in general were reported as $\alpha = 0.3126$ and a median infective dose $N_{50} = 2.36 \times 10^4$ (Haas *et al.*, 1999). This value is within the range of those reported in WERF (2004). Limited information is available on the secondary attack rates for *Salmonella*. A secondary attack rate of 0.3% was used by WERF (2004) to develop risk for exposure to biosolids. A conservative secondary attack rate of 25% was used in this study (Gerba, 2005).

5.3.7 *Cryptosporidium*

The host ranges of different types of *Cryptosporidium* vary. Infections of *Cryptosporidium* in humans are caused by *C. hominis*, previously classified as *C. parvum* genotype 1, or by the animal genotype 2, *C. parvum* (Xiao *et al.*, 2004). The protozoa

cause self-limiting diarrhea, however cryptosporidiosis can be life threatening in immunocompromised people. *C. parvum* is very common among newborn calves that can excrete oocysts in high numbers, but is also frequently found in adult livestock and other ruminants. The oocysts are extremely resistant to chlorination and have been involved in many waterborne outbreaks (see Milwaukee outbreak review by MacKenzie *et al.*, 1994; Hayes *et al.*, 1989).

Cryptosporidium are shed by livestock and other mammals and acquired by humans through ingestion of drinking water or incidental ingestion of recreational water (Gallaher *et al.*, 1989). *Cryptosporidium* are responsible for major waterborne outbreaks in the U.S. and elsewhere in the world in recent years. Harvest and post-harvest uses of contaminated water are of immediate concern, although the link between livestock grazing or dairy operations and potential for infection from produce consumption is very uncertain. *C. parvum* oocysts were detected in 40 to 90% of the surface waters tested between 1988 and 1993. *C. parvum* is shed by humans, cattle, sheep, goats, pigs, horses, deer, raccoons, opossums, mice, brown rats, feral pigs, and rabbits. Chickens and turkeys do not appear to be hosts. Shedding is usually limited to livestock under 6 months of age at concentrations of up to 10 million oocysts per gram and 10 billion oocysts per day, typically for 3 to 12 days. Twenty-two percent (22%) of U.S. dairy calves tested positive for *Cryptosporidium parvum.* Contamination of waterways by direct defecation, runoff from grazed pasture, contamination of old or poorly constructed wells, and subsurface flow are all documented routes of pathogen infestation of water sources. More than 5,000 oocysts per liter were detected in irrigation water passing through cattle pastures. In addition to livestock and wildlife, recent studies have traced the source of groundwater contamination to poorly designed septic systems and adjacent old wells that are no longer properly sealed (Moore *et al.*, 1993; Kramer *et al.*, 1996; Levy *et al.*, 1998; Barwick *et al.*, 2000).

Oocysts apparently die following drying; however, the lack of direct and definitive infectivity assays limits the strength of proof in any viability-based assessment. Oocysts are very resistant to chlorination, but are inactivated by properly designed ozone injection or UV disinfection systems. Oocysts were viable for more than one month in cold river

water. Oocysts were non-viable after exposure to 64°C for at least two minutes (Haas *et al.*, 1999).

Dose-response

The *Cryptosporidium* dose-response relationship is well characterized by use of an exponential model. Outbreak and human feeding studies suggest that this organism is highly infectious with an exponential dose-response parameter (k) of 238 (Haas *et al.*, 1999).

Cryptosporidium parvum is highly transmissible and infective in the family setting, with transmission rates similar to other highly infectious enteric pathogens such as *Shigella* species. In a community study of the infectivity of *Cryptosporidium* in families living under crowded urban conditions in Brazil, secondary attack rates were calculated at 19% (Newman *et al.*, 1994). High secondary attack rates are supported by reports from United States daycare centers experiencing cryptosporidial diarrhea episodes (Current and Garcia, 1991; Driscoll *et al.*, 1988). WERF (2004) reports a secondary attack rate of 3.7% to derive risk for transmission from biosolids. A more conservative secondary attack rate of 19% was used in this study.

5.3.8 *Giardia*

The flagellated protozoa *Giardia* has been found in a variety of animals. The species *Giardia lamblia* is known to infect the gastrointestinal tract of humans. *Giardia*sis is the most common protozoan infection of the human intestine worldwide. It occurs throughout temperate and tropical locations, with its prevalence varying between 2 and 5% in the industrialized countries and up to 20 to 30% in developing countries (Fraser, 1994; Kappus *et al.*, 1994). The symptoms usually manifest themselves about seven to ten days after the organism is ingested. *Giardia*sis may be chronic in some patients, lasting for more than one year.

Giardia is an opportunistic organism and infects a wide range of hosts including wild and domestic animals, birds, and humans. The CDC (1999) estimates that approximately 2 million Americans contract *Giardia*sis every year. Infection from *Giardia* can occur

from consuming contaminated food or water. It can also be transferred from animal or human feces. Although infection manifests itself with severe diarrhea and abdominal cramps, many infections may be asymptomatic and these individuals may still serve as a carrier of the disease. *Giardia* infection is a concern for people camping in the wilderness or swimming in contaminated streams or lakes, especially the artificial lakes formed by beaver dams. *Giardia* can survive out of water for an extended period of time in cool moist conditions.

Dose-response

Outbreak and human feeding studies suggest that *Giardia* infectivity fits an exponential model with a dose-response parameter (k) of 50.5 (Rose *et al.*, 1991). Household transmission of infectious gastroenteritis caused by *Giardia* is likely to account for a substantial portion of community incidence. With the exception of a few prospective studies (Dingle *et al.*, 1964; Koopman *et al.*, 1989), studies of household transmission of gastroenteritis have typically reported on community outbreaks of individual pathogens followed up in the home (Pickering *et al.*, 1981; Gotz *et al.*, 2002; Kaplan *et al.*, 1982; Morens 1979; Parry *et al.*, 1998). Pickering *et al.* (1981) reported an overall secondary attack rate of 11% among family members of children involved in daycare outbreaks. WERF (2004) reports a secondary attack rate of 0.72%. A more conservative secondary attack rate of 25% was used in this study.

5.4 Risk Characterization

The main objective of the risk assessment was to use a probabilistic approach to develop risk distributions for GI illness associated with virus, bacteria and protozoa exposure over a recreational season including both dry and wet weather days. The second objective of the risk assessment was to estimate the change in risk if disinfection techniques were employed to reduce the influence of the WRP effluent on the waterway pathogen concentrations. Methods used in the probabilistic assessment are described below.

Daily average microorganism concentration data for discrete segments of the waterway were used with receptor use patterns and exposure assumptions in a probabilistic risk

assessment. Based on the exposure information and the dose-response information gathered from the primary literature, risk of illness for recreational users was calculated for each segment of the CWS. In addition, risk from secondary exposures was computed (see Disease Transmission Model below). Results are expressed as the number of illnesses per exposure event or exposure day, broken down by WRP segment, recreational activity, weather and microorganism. This analysis provides information on the expected number of illnesses associated with different recreational uses of the CWS, the microorganisms responsible, and the waterway segments that contribute the highest risks.

5.4.1 Probabilistic Analysis

A probabilistic approach was selected to evaluate risk of gastrointestinal illness for recreational users of the CWS. Probabilistic risk assessment utilizes input distributions, rather than point estimates, to better represent the variability and uncertainty that exists for each input parameter. Thus, instead of using one value for exposure duration, water consumption, or pathogen concentration, a range of possible values (or more correctly, a probability density function) is used. This is a more precise reflection of actual populations and results in a more accurate prediction of potential risk. The probabilistic approach (one-dimensional, based on both variability and uncertainty) selected for this risk impact analysis is Monte Carlo simulation using Crystal Ball © Pro software operating on a personal computer.

This system uses randomly selected numbers[3] from within defined distributions (e.g., exposure duration and ingestion rate) and selected equations to generate information in the form of risk distributions. Input distributions were sampled using Latin Hypercube sampling techniques to ensure equal representation of all parts of the input distributions. Using this process, the various possible outcomes (risk levels) and the likelihood of achieving each outcome (percentages of the population protected at each forecasted risk level) can be determined. From this, a projected risk distribution can be derived for each

[3] A fixed seed value was selected to begin the random number generation (123,457). By using the same seed value within the Monte Carlo software, the same sequence of random numbers can be replicated.

waterway segment where use and pathogen concentrations are defined (North Side, Stickney, and Calumet). The contribution of each pathogen to the total risk was also computed. The potential for secondary spread of gastrointestinal illness within the immediate family of recreational waterway users was estimated based on simulations taking into account the family size and characteristics of secondary illness transmission within families for each pathogen.

The following section presents the Monte Carlo Simulation terms and definitions.

Bootstrapping: Bootstrapping is a widely accepted and extensively used procedure in statistical analysis and represents a process of selecting a random input from a dataset. This technique is useful in Monte Carlo analysis when the exact distributional form of an input variable is either unknown or unable to be represented with a continuous distribution. Bootstrap samples are random selections from the empirical data with replacement. Bootstrap methods provide robust estimates of variability in Monte Carlo assessments as the probabilities associated with drawing extremes in the distribution is mimicked by the presence of extreme values in the empirical data.

Correlation, Correlation Analysis: Correlation analysis is an investigation of the measure of statistical association among random variables based on samples. Widely used measures include the *linear correlation coefficient* (also called the *product-moment correlation coefficient* or *Pearson's correlation coefficient*), and such non-parametric measures as *Spearman rank-order correlation coefficient*, and *Kendall's tau*. When the data are nonlinear, non-parametric correlation is generally considered to be more robust than linear correlation.

Cumulative Distribution Function (CDF): The CDF is alternatively referred to in the literature as the *distribution function*, *cumulative frequency function*, or the *cumulative probability function*. The cumulative distribution function, F(x), expresses the probability that the random variable X assumes a value less than or equal to some value x, F(x) = Prob (X x). For continuous random variables, the cumulative distribution function is obtained from the probability density function by integration, or by summation in the case of discrete random variables.

Latin Hypercube Sampling: In Monte Carlo analysis, one of two sampling schemes is generally employed: simple random sampling or Latin Hypercube sampling. Latin Hypercube sampling may be viewed as a stratified sampling scheme designed to ensure that the upper and lower ends of the distributions used in the analysis are well represented. Latin Hypercube sampling is considered to be more efficient than simple random sampling, that is, it requires fewer simulations to produce the same level of precision. Latin Hypercube sampling is generally recommended over simple random sampling when the model is complex or when time and resource constraints are an issue.

Monte Carlo Analysis, Monte Carlo Simulation: Monte Carlo analysis is a computer-based method of analysis developed in the 1940's that uses statistical sampling techniques to obtain a probabilistic approximation to the solution of a mathematical equation or model.

Parameter: Two distinct, but often confusing, definitions for parameter are used. In the first usage (preferred), parameter refers to the constants characterizing the probability density function or cumulative distribution function of a random variable. For example, if the random variable W is known to be normally distributed with mean μ and standard deviation σ, the characterizing constants μ and σ are called parameters. In the second usage, parameter is defined as the constants and independent variables which define a mathematical equation or model. For example, in the equation $Z = aX + bY$, the independent variables (X, Y) and the constants (a, b) are all parameters.

Probability Density Function (PDF): The PDF is alternatively referred to in the literature as the *probability function* or the *frequency function*. For continuous random variables, that is, the random variables which can assume any value within some defined range (either finite or infinite), the probability density function expresses the probability that the random variable falls within some very small interval. For discrete random variables, that is, random variables which can only assume certain isolated or fixed values, the term *probability mass function* (PMF) is preferred over the term probability density function. PMF expresses the probability that the random variable takes on a specific value.

Random Variable: A random variable is a quantity which can take on any number of values but whose exact value cannot be known before a direct observation is made. For example, the outcome of the toss of a pair of dice is a random variable, as is the height or weight of a person selected at random from the Chicago phone book.

Representativeness: Representativeness is the degree to which a sample is characteristic of the population for which the samples are being used to make inferences.

Sensitivity, Sensitivity Analysis: Sensitivity generally refers to the variation in output of a mathematical model with respect to changes in the values of the model's input. A sensitivity analysis attempts to provide a ranking of the model's input assumptions with respect to their contribution to model output variability or uncertainty. The difficulty of a sensitivity analysis increases when the underlying model is nonlinear, nonmonotonic or when the input parameters range over several orders of magnitude. Many measures of sensitivity have been proposed. For example, the partial rank correlation coefficient and standardized rank regression coefficient have been found to be useful. Scatter plots of the output against each of the model inputs can be a very effective tool for identifying sensitivities, especially when the relationships are nonlinear. For simple models or for screening purposes, the sensitivity index can be helpful. In a broader sense, sensitivity can refer to how conclusions may change if models, data, or assessment assumptions are changed.

Simulation: In the context of Monte Carlo analysis, simulation is the process of approximating the output of a model through repetitive random application of a model's algorithm.

Uncertainty: Uncertainty refers to *lack of knowledge* about specific factors, parameters, or models. For example, we may be uncertain about the mean concentration of a specific pathogen at a specific location or we may be uncertain about a specific measure of intake (e.g., incidental ingestion rate while canoeing). Uncertainty includes *parameter uncertainty* (measurement errors, sampling errors, systematic errors), *model uncertainty* (uncertainty due to necessary simplification of real-world processes, mis-specification of the model structure, model misuse, use of inappropriate surrogate variables), and

scenario uncertainty (descriptive errors, aggregation errors, errors in professional judgment, incomplete analysis).

Variability: Variability refers to observed differences attributable to *true heterogeneity* or diversity in a population or exposure parameter. Sources of variability are the result of natural random processes and stem from environmental, lifestyle, and genetic differences among humans. Examples include human physiological variation (e.g., natural variation in susceptibility), weather variability, variation in use patterns, and differences in pathogen concentrations in the environment. Variability is usually not reducible by further measurement or study (but can be better characterized).

5.4.2 Disease Transmission Model

A single exposure event can cause illness in both the initial receptor exposed to the waterway and secondary receptors that may later come into contact with the infected initial receptor. Because the magnitude of this secondary transmission varies depending on the microorganism, failing to account for secondary transmission may bias the impacts of highly communicable microorganisms. This bias is particularly problematic when evaluating effluent treatment options where variable microorganism killing and uneven contributions of microorganisms from WRP and other sources create selective microorganism concentrations within the waterway.

To account for secondary transmission, a dynamic risk model was developed that considers secondary exposure through contact with CWS recreational users. Estimates of the infectivity and transmission rate as inputs for the dynamic model were derived from the primary literature for each of the microorganisms of interest. Because the number of individuals exposed through recreation on the CWS is a relatively small proportion of the total population of the Chicago metropolitan area, population levels of acquired immunity and illness by secondary transmission were not impacted. Therefore, the proposed dynamic model considers a steady-state level of immunity and estimates disease incidence only in the recreational receptor population and their immediate family. This approach addresses the important dynamic aspects of disease transmission from CWS exposure in the population most at risk.

The probability of contracting gastrointestinal illness from contact with an infected individual is termed the secondary attack rate. Secondary attack rates for various organisms depend on the virulence of the organism in question, the amount of organisms an infected individual sheds, and the environmental stability of the organisms. Secondary attack rate data are available in the primary literature from studies on the spread of gastrointestinal illness within confined groups of people (e.g. families, cruise ship passengers, nursing home residents). More detailed information is provided in the dose-response section for each pathogen. Table 5-6 presents a summary of secondary attack rates used in this analysis.

5.4.3 Microbial Exposure Point Concentrations

Receptors utilizing the waterway may encounter variability in pathogen concentration over both time and space. Receptors traveling in watercraft may be exposed to pathogens over a large stretch of the CWS. Even receptors fishing from the bank may encounter waterway pathogen concentrations that vary over the course of the exposure duration. The pathogen concentration term used to estimate risk reflects the average pathogen concentrations encountered over the course of the exposure in the CWS.

The dry weather sampling results and risk characterization were developed by segregating data based on location relative to the WRPs (i.e. upstream and downstream). (See Section 2.2.1 for details). All upstream and downstream samples were collected from locations at 15 waterway widths (within two miles) from the WRP outfalls. Results from the dry weather risk assessment showed that risks were low from both upstream and downstream locations, with most pathogens having slightly higher downstream concentrations. However, the relative differences in concentration between upstream and downstream pathogen concentrations were small in comparison to concentration data between dry and wet weather conditions.

Wet weather samples were collected from locations both directly upstream and downstream and additionally along the entire length of each waterway segment downstream of the North Side, Stickney and Calumet WRPs (see Section 2.2.1 for details). In contrast to the dry weather conditions where the WRP effluents constitute the

major flow and pathogen input to the CWS (more than 70 percent of the flow), wet weather inputs (CSO overflows, pumping station discharge points, and stormwater discharges) are widely distributed along the waterway. The larger spatial coverage of the wet weather sampling reduces the uncertainty in the waterway pathogen concentration in areas distant from the WRP effluent discharge where recreational use is most likely to occur. In addition, recreational users may be exposed to pathogens over long stretches of the waterway through watercraft use. For this assessment recreational use is assumed to occur along the entire WRP waterway segment. The average pathogen concentration along the waterway is the best representation of the exposure that a receptor might encounter. Based on this analysis, the results for the combined upstream and downstream samples were deemed most appropriate for characterizing overall risks for the CAW. For each of these groups, the variability in pathogen concentration was captured by bootstrap sampling from the entire WRP waterway segment dataset. Outfall data was combined as the arithmetic average of all outfall samples for each WRP.

Typically dry weather periods allow any residual pathogens from CSOs or other wet weather inputs to attenuate. For this study the dry weather sampling data was reflective of the effects of WRP effluent on the pathogen concentrations in the waterway with as little impact as possible from residual wet weather effects. There were no samples collected in intervening period between the wet weather and dry weather sampling events. However, these days represent a large portion of the recreational year and estimates of the concentration in the waterway on days between wet and dry weather conditions are an important consideration in the risk assessment. Estimates of pathogen concentrations in the days following a wet weather event were estimated based on modeling the attenuation of pathogens from the wet weather data through the following two days.

The attenuation of pathogens through natural processes tends to follow an exponential decay curve (Haas et al., 1999). The general exponential decay function is described below.

$$Conc(x) = \exp(-t*\beta) * Conc(i)$$

Where:

Conc(x) = pathogen concentration at time period x

t = time period of interest

i = initial time period

β = decay constant per time period (assumed =1)

Selection of an exponential decay constant (β) was based on a parsimonious fit to the data for organisms detected in both wet and dry sampling events. Using a β=1 with the geometric mean of the wet weather sampling data tends to produce values at the 72 hr time frame that approximate the geometric mean of the concentrations seen in the dry weather sampling. While organism specific attenuation factors could be developed, the variability observed in the data suggests that the uncertainty in these values would be large. Therefore, a simple exponential decay was selected as the model to estimate the pathogen concentration at 24 and 48 hour intervals post wet weather events. A pseudo-dataset was constructed using each of the original wet weather data points to develop a 24 and 48 hour post-wet weather dataset.

Currently, there are no site-specific data available to determine the effectiveness of WRP effluent disinfection on CWS pathogen concentrations. An estimate of this effect, however, can be derived using the dry and wet weather sampling data along with the published technical literature on pathogen reduction rates under various disinfection techniques.

Dry weather waterway concentrations are largely the result of WRP effluent discharges. Under idealized dry weather conditions (no upstream microbial loads or residual wet weather effects), any disinfection technique applied to the WRP effluent would have a proportional effect on the dry weather waterway pathogen concentrations (i.e. a 100 fold decrease in the effluent would result in a 100 fold decrease in waterway concentrations). Pathogen concentrations measured during wet weather conditions result from the

combined contributions of WRP effluent and wet weather discharge (i.e. CSOs, pumping stations, stormwater runoff) microbial loads.

For the disinfection scenario, the waterway pathogen concentrations were estimated by combining the waterway concentrations associated with wet weather conditions with the estimated residual post-disinfection dry weather concentrations for the respective pathogens. Disinfection efficiencies used in this approach are discussed in detail in Section 3 and are summarized in Table 5-7. In the absence of site specific disinfection treatability results, this technique provides an approximation of the anticipated pathogen concentrations in the CWS if disinfection were to be implemented.

Giardia is reported as both viable and non-viable cysts. Only viable *Giardia* cysts are capable of causing illness. An estimate of the number of viable *Giardia* cysts is required for use in the risk assessment. Concentrations of *Giardia* across all samples were generally very low, as few as a couple, if any, detected cysts in each sample analyzed. The precision of the viability assay is diminished because of the low frequency of detection. For example, consider a sample with one cyst detected. In this case the *Giardia* is either viable or not (100% viable or 0% viable). If this one cyst analyzed is non-viable then the risk assessment may be biased low. If the one cyst analyzed is viable then the risk assessment may be biased high. To better estimate viability over a larger dataset, a WRP-wide viability value was generated and applied to the total number of *Giardia* cysts for each sample within that WRP segment. As discussed in Section 3.3.2 above, dry and wet weather viability values were generated by pooling the total viable and non-viable cysts in both instream and outfall samples from each WRP segment. The overall dry weather viability values used are 26%, 21% and 10% for the North Side, Stickney, and Calumet WRP, respectively. The overall wet weather viability values used are 49%, 47% and 10% for the North Side, Stickney, and Calumet WRP, respectively.

5.4.4 Weather

Waterway pathogen concentrations are highly dependent on the weather conditions which tend to influence the microbial loading rates to the waterway. On dry weather days the principal input (more than 70% of the flow) to the waterway are the WRPs effluent

discharges. On days with light rainfall, direct waterway inputs from minor tributaries and surface water runoff may influence pathogen concentrations. In addition, WRP effluent flow rates may increase as stormwater collects in area sewers and fills the Tunnel and Reservoir Plan (TARP, also known as "Deep Tunnel"). Higher rainfall levels increase sewer levels and may trigger CSO events to discharge to the CWS. As the TARP capacity is reached, the area pumping stations may discharge overflow water directly to the waterway.

To represent risks from recreational exposure across the entire recreational season, the input pathogen concentrations used in the risk assessment should account for the probability of encountering pathogen concentrations related to different weather conditions. The proportion of days under each weather condition in a recreational year (April through November) was developed from historical records of CSO and rainfall records. Data from the 2006 recreational year was selected as representative of rainfall and CSO patterns for the CWS. Data from the 2005 drought year recreational season was not used in the analysis as this data is not reflective of the general rainfall patterns characteristic of the Chicago area and use of the 2005 data may underestimate risks. Earlier data was also excluded as it fails to incorporate the effect of the stormwater and CSO management plans on CSO frequency. The input distribution used in the simulations for selecting weather specific pathogen concentrations is shown in Table 5-8.

A simplifying assumption in this analysis is that recreational use and weather conditions are not correlated. Common experience would suggest this is not the case as people tend to spend less time recreating during rain events. However, data on the numbers of recreational users under various weather conditions is lacking. Furthermore, recreational use may resume shortly after rain events when waterway concentrations are still strongly influenced by the preceding weather patterns.

5.4.5 Simulations

Exposure parameters and pathogen levels were combined in a probabilistic risk assessment to estimate primary and secondary illnesses associated with recreational use of the CWS. For each simulation, a hypothetical receptor was created based on the

underlying exposure distributions and the risks for this receptor were computed. The process was repeated 1,000,000 times (i.e., the probability for a recreator to become ill was examined by simulating 1,000,000 recreational encounters), and the results tracked for each simulation. The probability of developing illness was computed by comparing the ingested dose with the potential of each pathogen to produce illness at that dose. The probabilistic analysis proceeded using the following sequence:

1. Determine the weather-influenced waterway dataset for microbial concentration based on the frequency of that type of weather in the recreational season.
2. Bootstrap sample a representative microbial exposure point concentration from the appropriate dataset (select the pathogen concentration for the recreator on the day of exposure).
3. Select an individual's recreation type (canoeing, fishing, boating).
4. Select that individual's exposure duration (based on recreator type).
5. Select that individual's ingestion rate (based on recreator type).
6. Develop a dose for that individual (intake * time * concentration).
7. Determine that individual's infection/illness.
8. Determine if secondary exposure/illness results.

5.4.6 Risk Assessment Calculation Results and Conclusions

The estimated number of individuals developing illness was based on one million simulated recreational use events computed for each waterway using either dry weather, wet weather, or a combination of dry and wet weather data as described in section 5.4.3. Results for primary illness associated with each waterway are provided in Table 5-9. As expected, higher rates of illness are predicted during wet weather events, with the Stickney waterway segment having the highest and the Calumet waterway segment the lowest expected illness rates. For comparison purposes, the EPA guidelines for acceptable risks associated with various recreational activities and the density of sentinel microbial species is provided in Table 5-10. The results of this analysis demonstrate that the expected illness rates for receptors exposed to the combined wet and dry weather events were all below the 1986 EPA limit of 8 illnesses per 1000 exposure event for primary contact exposure in heavily used swimming areas and the proposed EPA limit of

14 illnesses per 1000 exposure events for freshwater recreational use including immersion/swimming activities.

For each waterway segment the risks associated with exposure to the wet weather concentrations were higher than those associated with dry weather concentrations. Under dry weather conditions, the exposure risks were of similar magnitude between the three waterway segments with the Stickney risks slightly higher than those from the North Side or Calumet waterway segments (see Table 5-9). Under wet weather or combined weather conditions the North Side waterway segment had higher levels of risk than either the Calumet or Stickney waterway segments. Overall risk levels are not solely correlated to pathogen concentrations in the waterway. This result is largely due to differences in exposure. For example, the exposure intensity for recreational users on the North Side segment (larger percentage of canoe use) is significantly higher, leading to the additional probability of illness.

Risks calculated above were developed for all users, in proportion to the frequency of use, for each waterway segment. Risks were also tabulated individually for each of the three different classes of recreational use that span the range of exposures reported in the UAA survey. The frequency that specific recreational users contribute to the expected illnesses is shown in Table 5-11. The recreational activity with the highest potential for exposure was fishing while that with the lowest exposure was pleasure boating. Which recreational activity results in the greatest number of affected users, however, depends on both the proportion of users engaged in that activity and the pathogen load in that waterway segment. For example, in the North Side segment, 33.7% of the illnesses are predicted to result from canoeing, but canoeing accounts for only 20% of the users of the North Side waterway. In the Stickney and Calumet segments, the predicted illnesses were predominantly from fishing and boating due to the low frequency of canoeists in these waterway segments. To further characterize the risk stratified by the recreational use activity, risk per 1000 exposure events were computed separately for canoeing, boating, and fishing recreational uses. Results are shown in Table 5-12. As expected, the highest risks were associated with recreational use by the highest exposure group (i.e. canoeing). However, for each waterway the risks associated with the highest exposure

use are below the proposed EPA limit of 14 illnesses per 1000 exposure events for freshwater recreational use including immersion/swimming activities.

Table 5-13 presents the risk estimates by the pathogen responsible for illness. For the North Side and Stickney waterway segments the majority of predicted illnesses were the result of concentrations of viruses, *E. coli* and *Giardia*. For the Calumet waterway the risks are generally lower with multiple organisms contributing to overall risk. Secondary transmission for these pathogens resulted in an approximately two fold increase in population illness associated with the primary recreational user illnesses. However, secondary transmission rates are higher for the North Side and Stickney waterway segments where the highly communicable *Calicivirus* is a dominant pathogen. Secondary transmission considers spread from individuals who may become infected but not ill, a common condition for a number of these pathogens.

The effects of various disinfection techniques on risk reduction were estimated for combined wet and dry weather days. Total primary illness results, both with and without disinfection, for each of the waterway segments is provided in Table 5-14. Similar effects were seen in all three WRPs. Under dry weather conditions using the assumption that all CWS pathogen loads results from effluent discharge, disinfection decreases the illness rates from low to essentially zero. However, the impact of disinfection under real world conditions (simulated wet and dry weather) is less clear cut. For example, ozonation would decrease illness rates at the Stickney waterway segment from 1.74 illnesses/1000 exposures to 1.64 illnesses/1000 exposures. These results suggest that disinfection of effluent has little impact on the overall illness rates from recreational use of the CWS.

Although *Pseudomonas aeruginosa* is not a pathogen that is linked to gastrointestinal illness, this pathogen has been linked to recreational illness outbreaks involving dermal (folliculitis), eye, and ear (otitis externia) infections. For this reason the levels of *Pseudomonas aeruginosa* were evaluated under the sampling program for this risk assessment. However, quantitative evaluation of the risk for this pathogen is problematic. There are no published dose-response relationships for *Pseudomonas*

aeruginosa. Without a clear dose-response relationship there is no way to establish the expected illness level associated with any particular waterway concentration. The dermal pathway for estimating exposure to *Pseudomonas aeruginosa* is also problematic. Ear and eye infections associated with contact by *Pseudomonas aeruginosa* contaminated water are typically associated with full immersion activities. Since these types of activities are not permitted or designated uses of the CAW the incidence of ear and eye exposures are expected to be low and as the result of accidental or intentional misuse of the waterway. *Pseudomonas* related folliculitis commonly requires a break in the skin from a preexisting cut, open sore or scrape as an entry point for infection. Immunocompetent individuals without skin abrasions rarely develop folliculitis by exposure to intact skin. For these reasons a quantitative evaluation of risks is not feasible.

A qualitative review of the wet and dry weather data, however, may provide some insight on the relative risk from *Pseudomonas* exposure. Comparison of the waterway level to the outfall levels may also provide an indication on the effectiveness that a disinfection step may have on *Pseudomonas* levels in the waterway.

Comparisons are provided for wet, dry and outfall *Pseudomonas* concentrations at the three WRP segments in Table 5-15. The mean dry weather *Pseudomonas* concentration represents the combined surface and 1 meter-depth samples at both upstream and downstream locations. Mean wet weather values include all samples taken along the WRP waterway segment. As shown in the table, the wet weather levels are higher than those in the dry weather conditions. Perhaps more importantly, the outfall samples show lower levels of *Pseudomonas* than the corresponding wet weather samples. This suggests that the major inputs for *Pseudomonas* in the waterways are sources other than the WRP effluent. Therefore, disinfection of the WRP effluent would have minor effects on the overall loading of *Pseudomonas* in the waterway and risks associated with recreational exposure to this pathogen.

The results presented herein indicate that the levels of pathogens in the waterway representing the spectrum of waterway conditions experienced in a recreational year are

low. These low pathogen levels correspond to a low probability of developing gastrointestinal illness, even for the most highly exposed recreational users in areas of the CWS in close proximity to the District's WRP non-disinfected effluents from Stickney, Calumet and North Side. For all designated recreational uses evaluated, the risks of developing illness were less than the the proposed EPA limit of 14 illnesses per 1000 exposure events for freshwater recreational use including immersion/swimming activities.

5.4.7 Sensitivity and Uncertainty Analysis

A sensitivity analysis was conducted in order to identify the contribution of each input distribution to the variance of the resulting risk estimates. Receptor pathogen dose levels from the combined wet and dry weather assessment were used as the basis for the sensitivity analysis. Results from the sensitivity analysis are present in Tables 5-16. The input assumptions that contribute the greatest to the variance differ depending on the waterway segment. Model input sensitivity seems to correlate with the input assumptions for the dominant recreational user class in each waterway. Incidental ingestion rates and weather are the largest contributors to the sensitivity analysis for the North Side waterway segment. Recreational user type (receptor type) followed by incidental ingestion rate, exposure duration and weather contributes the most to the variance for the Stickney and Calumet waterway segments.

An alternative sensitivity evaluation is shown in Table 5-17. Illness rates for the North Side waterway segment are presented in cases where the incidental ingestion rate and exposure duration inputs varied by either plus or minus 25%. Increasing the intake assumptions lead to 19% increase in estimated risk while decreasing the intake assumptions results in a 27% decrease in estimated risk. The effect of changing the weather type is also provided on the table. The effect of changing the recreational use assumption is provided in the stratified risk estimates on Table 5-12.

The probabilistic analysis conducted for this study was one-dimensional, focusing on variability. A probabilistic assessment of uncertainty combined with variability data could be used to create a two-dimensional probabilistic output. However, such

assessment was outside the scope of this study due to logistical constraints (i.e. boundary conditions).

Uncertainty in the risk estimates is an important part of the Risk Characterization. The following factors may lead to an overestimation or underestimation of risk:

- Exposure parameters may be biased high or low. In general, the exposure parameters were selected to provide a central tendency or 'best approximation' estimate for the risk assessment. Follow-up epidemiological studies that measure actual illness rates could be evaluated in terms of this risk assessment to allow model validation and fine-tuning of exposure parameters. Such an Epidemiological Study is currently being conducted for the CWS by the University of Illinois at Chicago, on behalf of the MWRDGC.
- Risks are calculated based on dose from ingestion, the predominant route of exposure, and may be biased low for receptors with significant inhalation exposure to water droplets from sprays or mists.
- Secondary transmission rates are generally at the high end of those reported in the technical literature. Therefore, the assumptions on secondary transmission are conservative and the resulting secondary illness rates may be biased high.
- For the purposes of this study, the population at risk from secondary transmission spread is limited to the immediate family of primary recreational users. The secondary transmission model is included to estimate the wider effect of recreational illness beyond those directly exposed to the waterway. In some cases the population at risk may include larger groups of individuals with secondary exposure to a primary recreator. Examples of these groups include infected individuals working with the public at larger institutions (schools, hospitals, daycare centers). Due to the small recreational population compared to the total metropolitan population and the endemic nature of the pathogens in the population, this potential underestimation of risk and the effect of recreational illness on the baseline population illness rate is likely very low.
- This study did not account for all pathogens that may be present in CWS recreational water. However, the pathogens that were selected for inclusion in the study include regulatory indicators and those that could be measured by EPA approved methods that were judged most likely to produce gastrointestinal illness (see Section 2.1 for a more complete rationale on pathogen selection).
- The measured pathogen concentrations under dry weather conditions are limited to sampling locations near the WRPs and they were used as representative concentrations of the entire waterway downstream of the WRP. Under dry weather conditions, these concentrations will be biased high relative to concentrations at locations more distant from the WRP.

- The measured concentrations of *E. coli* are assumed to represent the most virulent strain; the percentage of pathogenic *E.coli* was conservatively assumed to represent 2.7% of the total measured concentrations. For other organisms, such as adenovirus, all the organisms are assumed to represent the pathogenic strain leading to gastrointestinal illness. This assumption may overestimate the illness associated with exposure to these organisms.
- Virus concentrations measured by the assay systems may overestimate viral risk. Viral assay are not specific to the pathogenic virus in question and may detect less pathogenic viral strains.
- Recreational use may be inversely correlated with wet weather. CWS recreational use was assumed to occur randomly over the course of the recreational season. The majority of the illnesses were associated with wet weather events. If the frequency of exposure on wet weather days is lower than average then the resulting risk estimate may be biased high.
- Some receptors with frequent use of the CWS may have lower sensitivity to some pathogens due to acquired immunity. Repeated exposure to pathogens in water is known to produce tolerance in individuals through immune related mechanisms. Dose-response parameters used in the assessment are generally derived from "naive" individuals and represent upper-end estimates of infectivity for the general population. Since repeated exposure to the waterway is likely for a significant subset of the recreational population, the risk of illness for these individuals is probably over-estimated by this risk assessment.
- Risk calculations do not account explicitly for immersion activities. While canoeing incidental ingestion rates incorporate the occasional high ingestion event, direct immersion activities such as swimming and water skiing are not considered in the risk calculations. Swimming and water skiing are not designated uses of the waterway. To the extent these activities are undertaken, the risks for receptors in these categories are not accounted for in the results.
- No consideration is given to upsets or interruptions in WRP treatment or City infrastructure that might result in increased pathogen loads. Waterborne disease outbreaks are often associated with failures in equipment or processes that influence water quality. Estimating the frequency or magnitude of such events is difficult if not impossible. The risk evaluation presented here does not account for such low probability occurrences and assumes that the measured pathogen concentrations are representative of on-going conditions experienced in the waterway.
- Risks do not explicitly account for recreational activities associated with sediment or sand ingestion. Pathogen concentrations in environmental media along shorelines where recreational receptors might interface with the waterway are unknown.

- Aerosolization and drift of pathogens from the waterway to affect on-shore non-recreational receptors is not accounted for in the model. Exposure based on airborne transport of pathogens from the waterway is expected to be very small. Attenuation of pathogens in air occurs rapidly due to temperature, UV, and oxygen conditions. However, intimate exposure near areas that might produce considerable mists, such as aeration stations, may represent an additional risk not accounted for in this assessment.

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SECTION 5

TABLES

Table 5-1. UAA General Activity Groups and Risk Assessment Categories

UAA Activity Group	Risk Assessment Category
Canoe	Canoeing
Kayak	Canoeing
Sculling	Canoeing
Jetski	Canoeing
Power boat	Pleasure Boating
Water taxi / tour boat	Pleasure Boating
Fishing from boat	Fishing
Fishing	Fishing
Evidence of use[a]	Fishing
Passive Recreation	Fishing
Wading[b]	Not Included
Swimming[b]	Not Included

[a] UAA survey includes observations or evidence of recent use/fishing in results.

[b] UAA observations of these uses were not included in a risk assessment category

Table 5-2. Proportion of Users in Each Risk Assessment Activity Category by Waterway

	Waterway		
Risk Assessment Category	**North Side**	**Calumet**	**Stickney**
Canoeing	20.2%	1.2%	0.5%
Fishing	72.2%	28.4%	47%
Pleasure Boating	7.6%	70.4%	52.5%

Table 5-3. Household Size for Cook County, Illinois

Household Size	Percentile
2-person household	37.4%
3-person household	21.8%
4-person household	22.5%
5-person household	10.4%
6-person household	5.2%
7-or-more person household	2.7%

Table 5-4. Incidental Ingestion Rate Percentiles

Percentiles	Boating (mL/hr)	Fishing (mL/hr)	Canoeing (mL/hr)
10%	1.49	2.98	5.21
25%	1.65	3.30	6.02
50%	**1.90**	**3.79**	**7.52**
75%	2.23	4.47	10.15
90%	2.64	5.28	14.16
95%	2.95	5.89	17.84
97.5%	3.26	6.51	21.99
100%	7.43	22.13	34.00

Table 5-5. Summary of Dose-Response Parameters Used for Risk Assessment (Adapted from Haas, 1999; and Rose *et al.*, 1991)

Pathogen	Beta-Poisson		Exponential
	(α)	N_{50}	(k)
Total Enteric Viruses			78.3
Adenovirus			78.3
Calicivirus (norovirus)	0.2531	6.17	
Cryptosporidium			238
Giardia			50.5
Salmonella	0.3126	23600	
Escherichia coli	0.1748	2.55E+06	

Table 5-6. Summary of Secondary Attack Rates

Pathogen	Secondary Attack Rate
Total Enteric Viruses	25% (assumed)[1]
Adenovirus	67% child 19% adult (38% assumed)[2]
***Calicivirus* (Norovirus)**	86%[3]
Cryptosporidium	19%[4]
Giardia	8-10% (25% assumed)[5]
Salmonella	25% (assumed)[6]
Escherichia coli	25% (assumed)[7]

Notes:

1. A secondary attack rate of 25% was used (Gerba, 2005). Enteric virus estimates vary depending on organism. Virus independent estimates range from 9% (Perry *et al.*, 2005) to 35% (EPA, 2000).
2. Mean value from prospective studies in children (Van *et al.*, 1993) and within the range reported from other studies (Fox *et al.*, 1977).
3. Reported secondary infectivity for norovirus (Gerba, 2005).
4. Based on spread in urban families (Newman *et al.*, 1994).
5. A secondary attack rate of 25% was used (Gerba, 2005). .
6. A secondary attack rate of 25% was used (Gerba, 2005). Several studies report secondary infection (Parry et al., 1998; Kaplan et al., 1982). Family members with children ill from daycare report 11% attack rate (Pickering, 1981).
7. A secondary attack rate of 25% was used (Gerba, 2005). No general pathogenic strain secondary attack rate identified in the literature. General *E. coli* secondary spread estimated at 15% within families (Parry and Salmon, 1998).

Table 5-7. Fold Attenuation of Pathogen Concentration by Various Treatment Methods

Pathogen	Ozonation	UV Irradiation	Chlorination
E. coli (pathogenic)	10000	10000	10000
P. aeruginosa	100	10000	10000
Salmonella	10000	1000 [b]	1000 [b]
Enterococcus	100 [b]	100 [b]	100 [b]
Cryptosporidium	17.0 [a]	1000	5.9 [a]
Giardia	114.8 [a]	100	3.2 [a]
Enteric virus	100000	11.7 [a]	100000
Calicivirus	100	10000	100
Adenovirus	100 [b]	100 [a]	100 [b]

Notes:

[a] Geometric mean of data (range) reported in Table 4-11.

[b] Estimate based on professional judgment.

Table 5-8. Proportion of Weather Days in Recreational Year[a]

Weather Conditions	Proportion of Season
Wet Weather	
Wet/CSO events	0.40
24 hrs post wet weather	0.30
48 hrs post wet weather	0.15
Dry Weather	
>48 hr post wet weather	0.15

[a] Recreational year includes dates from April to November; Data used to construct proportions based on MWRDGC CSO and rain gauge records for the 2006 recreational year.

Table 5-9. Total Expected Illnesses per 1,000 Exposures Using Different Estimates of Pathogen Concentrations with No Effluent Disinfection[a]

Exposure Input[b]	Waterway		
	North Side	Stickney	Calumet
Dry Weather	0.36	1.28	0.10
Wet Weather	2.78	2.34	0.36
Combined Weather Samples	1.53	1.74	0.20

[a] Includes all primary gastrointestinal illnesses from *E. coli*, *Salmonella*, total enteric viruses, adenoviruses, *Giardia*, and *Cryptosporidium* expected from the waterway exposures.

[b] Waterway concentration inputs for the simulations were randomly selected (bootstrap sampled) from datasets that include the indicated sample sets

Table 5-10. Criteria for Indicators of Bacteriological Densities

Acceptable Swimming-Associated Gastroenteritis Rate per 1000 Swimmers[6]		Steady-State Geometric Mean Indicator Density	Single Sample Maximum Allowable Density[4,5] (counts per 100 mL)			
			Designated Beach Area (upper 75% C.I.)	Moderate Full Body Contact Recreation (upper 82% C.I.)	Lightly Used Full Body Contact Recreation (upper 90% C.I.)	Infrequently Used Full Body Contact Recreation (upper 95% C.I.)
Freshwater						
enterococci	**8**	33[1]	61	89	108	151
E. coli	**8**	126[2]	235	298	406	576
Marine Water						
enterococci	**19**	35[3]	104	158	276	500

Notes:

1. Calculated to nearest whole number using equation:
   (mean *enterococci* density) = antilog$_{10}$ [(illness rate/1000 people + 6.28)/9.40]

2. Calculated to nearest whole number using equation:
   (mean *E. coli* density) = antilog$_{10}$ [(illness rate/1000 people + 11.74)/9.40)]

3. Calculated to nearest whole number using equation:
   (mean *enterococci* density) = antilog$_{10}$ [(illness rate/1000 people + 0.20)/9.40

4. Single sample limit =
   antilog$_{10}$ [indicator geometric + (Factor determined from areas under the normal probability curve for the X assumed level of probability) x (log$_{10}$ standard deviation)]

   The appropriate factors for the indicated one-sided confidence levels are:
   75% C.I. - 0.675
   82% C.I. - 0.935
   90% C.I. – 1.28
   95% C.I. – 1.65

5. Based on the observed log standard deviations during the EPA studies: 0.4 for freshwater *E. coli* and *enterococci* and 0.7 for marine water *enterococci*. Each jurisdiction should establish its own standard deviation for its conditions, which would then vary the single-sample limit.

6. EPA proposed acceptable illness rates are 14 per 1000 swimmers for freshwater users (Implementation Guidance for Ambient Water Quality Criteria for Bacteria, May 2002 Draft. EPA-823-B-02-003).

7. Source: EPA, 1986. Ambient Water Quality Criteria for Bacteria.

Table 5-11. Proportion of Recreational User Type Contributing to Gastrointestinal Expected Illnesses with No Effluent Disinfection[a]

Recreational Use	Waterway		
	North Side	Stickney	Calumet
Canoeing	33.7%	8.33%	2.9%
Fishing	58.7%	53.1%	38.2%
Boating	7.6%	38.5%	58.8%

[a] Based on combined waterway samples (upsteam and downstream) over the entire recreational season.

Table 5-12. Stratified Risk Estimates – Estimated Illness Rates Assuming Single Recreational Use with No Effluent Disinfection

	Illnesses per 1,000 Exposures for Combined Wet and Dry Weather Samples		
Recreational Use	**North Side**	**Stickney**	**Calumet**
Canoeing	2.45	3.19	0.52
Fishing	1.42	1.90	0.31
Pleasure Boating	0.66	1.05	0.14

Table 5-13. Breakdown of Illnesses per 1,000 Exposures for Combined Wet and Dry Weather Samples with No Effluent Disinfection

Pathogen	Primary (Secondary) Illnesses		
	Waterway		
	North Side	Stickney	Calumet
E. coli (pathogenic)	0.18 (0.1)	0.35 (0.1)	0.06 (0.0)
Salmonella	0.001 (0.0)	0.001 (0.0)	0.001 (0.0)
Giardia	0.19 (0.0)	0.04 (0.0)	0.005 (0.0)
Cryptosporidium	0.05 (0.0)	0.001 (0.0)	0.001 (0.0)
Enteric virus	0.002 (0.0)	0.002 (0.0)	0.001 (0.0)
Adenovirus	0.41 (0.3)	0.18 (0.1)	0.12 (0.1)
Calicivirus	0.72 (2.2)	1.20 (3.7)	0.02 (0.1)
Illnesses Primary (Secondary)	**1.55 (2.6)**	**1.77 (3.9)**	**0.21 (0.2)**
Total Illnesses Including Secondary	**4.15**	**5.67**	**0.41**

Table 5-14. Total Expected Primary Illnesses per 1,000 Exposures under Combined Dry and Wet Weather Using Different Effluent Disinfection Techniques[1, 2]

	Waterway		
	North Side	**Stickney**	**Calumet**
No Disinfection	1.53	1.74	0.20
UV Irridation	1.32	1.48	0.17
Ozone	1.45	1.65	0.19
Chlorination	1.43	1.63	0.19

[1] Estimates based on geometric mean pathogen concentrations and central tendency estimates for exposure assumptions. Waterway pathogen concentrations were developed by the difference in wet and dry disinfected concentrations.

[2] Includes all primary gastrointestinal illnesses from *E. coli*, *Salmonella*, total enteric viruses, adenoviruses, *Giardia*, and *Cryptosporidium* expected from the waterway exposures.

Table 5-15. *Pseudomonas aeruginosa* Concentrations by WRP Waterway Segment and Sampling Category[1]

	Waterway		
Sampling Category	**North Side**	**Stickney**	**Calumet**
Dry	3670 ± 7005	232 ± 366	398 ± 692
Wet	5426 ± 1956	13507 ± 14732	8325 ± 9484
WRP Outfall[2]	1350 ± 1184	4680 ± 5379	3250 ± 5111

[1] Values are the arithmetic mean ± the standard deviation of all data within group.
[2] Both dry and wet weather concentrations

Table 5-16. Sensitivity Analysis for Risks of Illness in WRP Segments

	Contribution to Variance		
Input Assumptions	**North Side**	**Stickney**	**Calumet**
Receptor Type	0.018	0.443	0.380
Weather Type	0.045	0.153	0.053
Fishing Incidental Ingestion Rate	0.283	0.048	0.020
Fishing Exposure Duration	0.548	0.096	0.035
Canoeing Incidental Ingestion Rate	0.055	0.001	0.0001
Canoeing Exposure Duration	0.041	0.001	0.0001
Pleasure Boating Incidental Ingestion Rate	0.002	0.048	0.101
Pleasure Boating Exposure Duration	0.008	0.210	0.411

Table 5-17. Parameter Sensitivity Analysis for North Side (Illnesses per 1000 Recreational Users)

	Input Option		
Input Assumptions	**-25%**	**Baseline**	**+25%**
Ingestion Rate	1.11 (-28%)[1]	1.53	1.82 (+19%)
Exposure Duration	1.11 (-28%)	1.53	1.82 (+19%)
	DRY	**Baseline**	**WET**
Weather Type	0.06 (-96%)	1.53	2.78 (+82%)

[1] Relative percent increase or decrease from Baseline illness rate.

SECTION 5

FIGURES

Figure 5-1. CWS Microbial Risk Assessment Segments

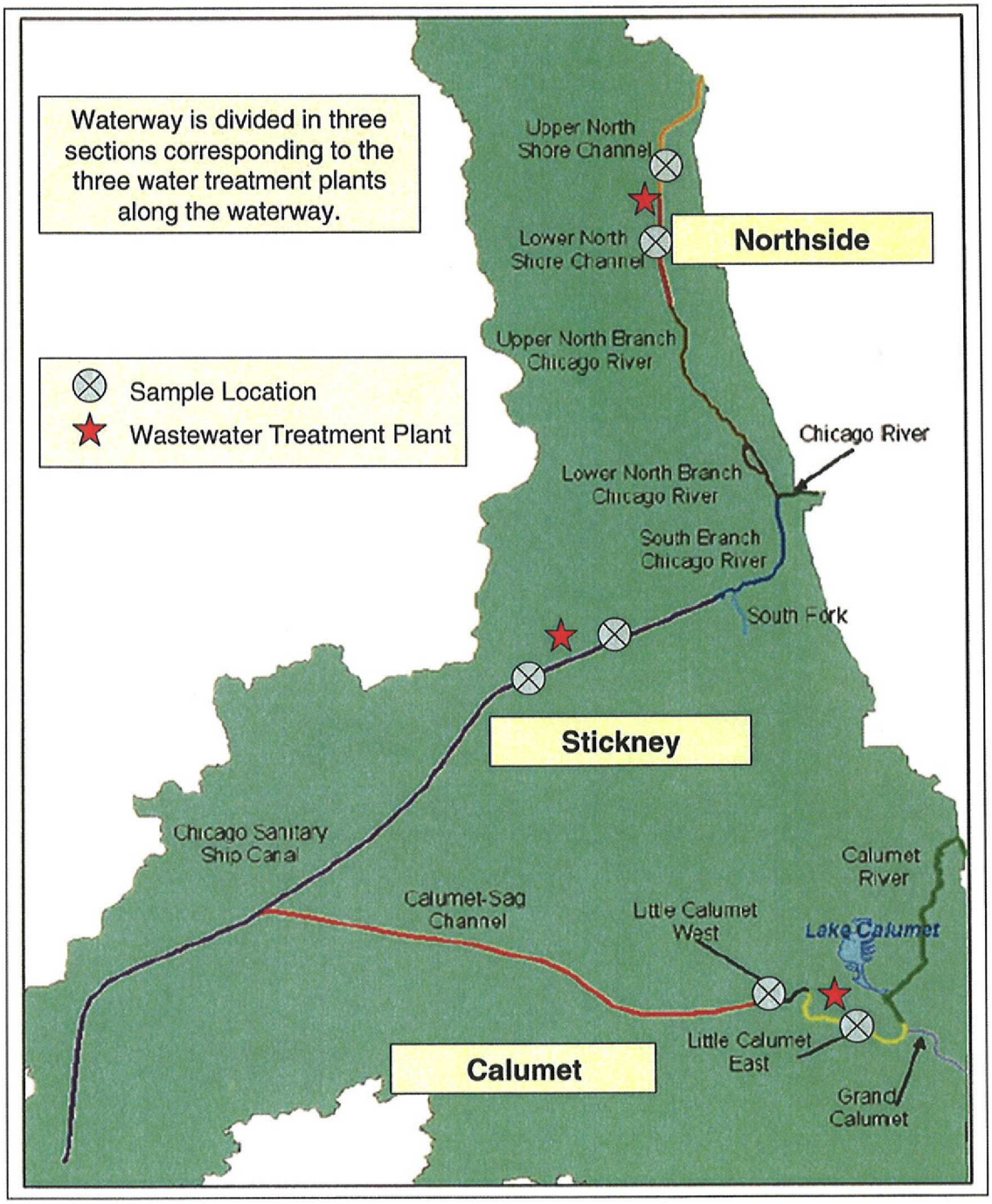
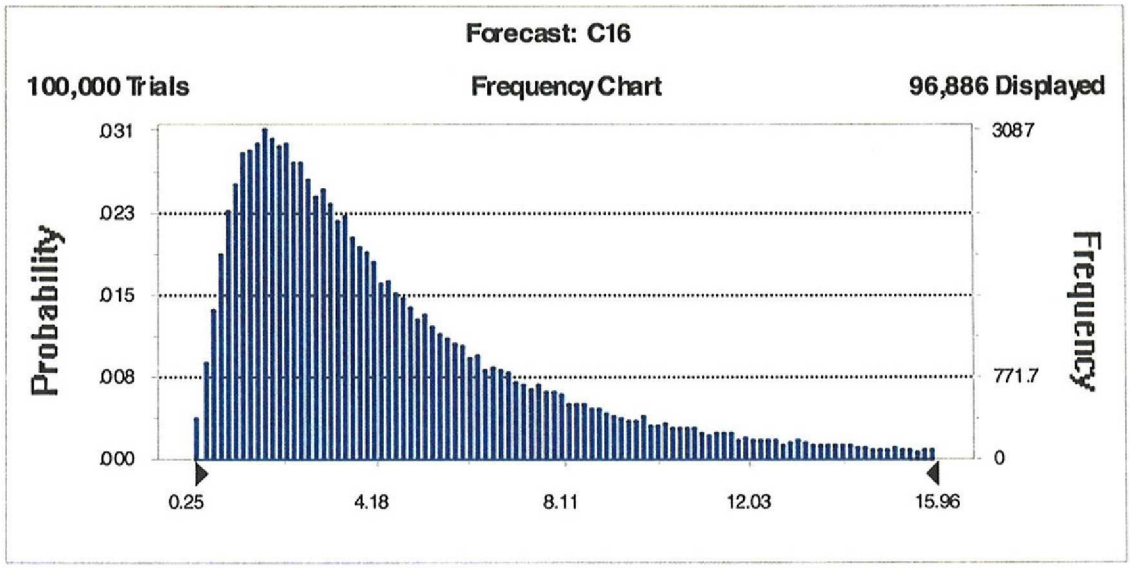


Figure 5-2. Incidental Ingestion Rate Distribution for Canoeists (mL/hr)



Intake Rate (mL/hr)

Note:

Range of values for variable ingestion input distribution is 0 to 30 mL/hr. Figure is truncated to better show the distribution shape.Total ingestion rate includes the variable portion shown in the Figure plus a fixed 4 mL/hr incidental ingestion.

Figure 5-3. Duration Distribution for Canoeists

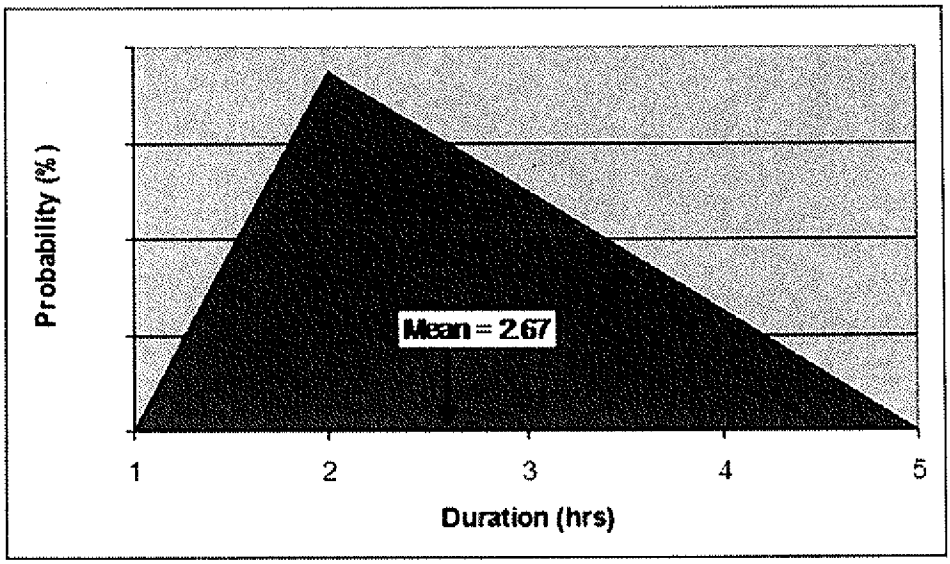
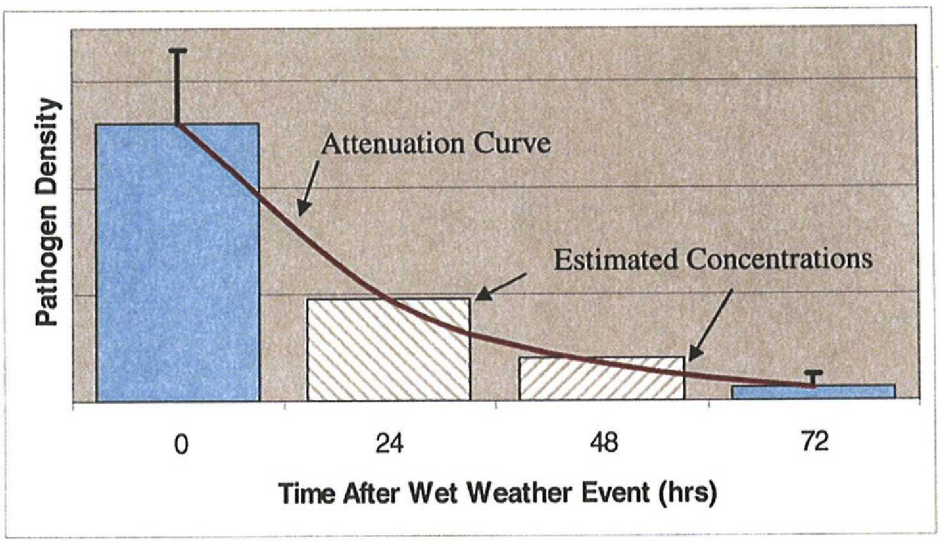


Figure 5-4. Estimated Pathogen Concentration between Wet and Dry Sampling Events



ATTACHMENT A

Dry And Wet Weather Bacteria Correlations In The Chicago Area Waterway System

LIST OF TABLES

LIST OF FIGURES

A. INTRODUCTION

Recent studies indicate that there is a poor correlation between bacteria indicator levels and levels of human pathogenic bacteria, viruses and protozoa (Noble *et al.*, 2006; Noble and Fuhrman *et al.*, 2001; Hardwood *et al.*, 2005; Jiang *et al.*, 2001, and Hörman *et al.*, 2004). The Geosyntec Team is not aware of any published results in the technical review literature that indicate statistically significant correlations between indicator bacteria and protozoa or virus pathogens.

Figure A-1 is a matrix plot of the dry weather bacteria results, which is a simple way of presenting a series of scatter plots. A matrix plot is used to visually discern correlations between multiple factors (or in this case, bacteria types). Each plot is to be read with the y-axis parameter shown on the right of each row and the x-axis parameter shown on the top of each column. For this correlation analysis, relationships between various bacteria parameters were investigated, with the initial hypothesis that various bacteria concentrations may be proportional to one another, as each is used as an indicator of magnitude of raw sewage contamination.

The matrix plots demonstrate that in dry weather samples there is a generally poor correlation between bacteria types, as evidenced by the low or negatively sloped trend lines (a relatively flat trend line would indicate random or unexplainable scatter), and the poor data fits to these trend lines. All instream results (i.e., "downstream" and "upstream" samples) are aggregated together here for the purpose of maximizing data robustness.

The objective of generating scatter plots is to identify relationships between fecal coliform and other pathogen concentrations. The reason for this is that there is a very large amount of historic District data for fecal coliform, and therefore if some clear and consistent trends or ratios – whether these are site specific or general in applicability – could be discerned, then the historic fecal coliform concentration data could perhaps be extrapolated to generate concentration statistics for other pathogens.

Given the modest correlations between *E. coli* and fecal coliform and *Enterococcus* and fecal coliform as identified in the matrix plots, the two scatter plots discussed below were generated to

further investigate these two relationships. Through the matrix plot analysis, all other bacteria combinations had insignificant correlations.

The first scatter plot (Figure A-2) shows approximately linear relationships between dry weather *E. coli* and fecal coliform and between *Enterococcus* and fecal coliform. The correlation between *E. coli* and fecal coliform has a better fit than the correlation between *Enterococcus* and fecal coliform as evidenced by the higher R^2 value (0.78 compared to 0.54).

Figure A-3 is a "marginal" scatter plot that further investigates the *E. coli* vs. fecal coliform relationship via scatter plot, but adds frequency histograms to demonstrate the probability distributions of the two datasets. Figure A-3 is in arithmetic space, in contrast to the scatter plot in Figure A-2, which is in log space. Figure A-3 shows a modest positive relationship between the two bacteria groups (*E. coli* and fecal coliform). Figure A-3 also demonstrates that both datasets are strongly left-skewed, implying distributions that may be lognormal.

To further investigate the relationship between dry weather *E. coli* and *Enterococcus* vs. fecal coliform, two correlation coefficients were computed: Spearman's and Pearson's. The Pearson's correlation coefficient is a parametric statistic, while the Spearman's rank correlation is a non-parametric statistic (Helsel and Hirsch, 2002). Both are used because each has its own advantages and disadvantages. The Spearman's correlation statistic is capable of indicating correlations even when the underlying relationship is non-linear. It can also be used in situations where the data is censored. Alternatively, the Pearson's correlation statistic is capable of indicating the strength of linear associations. A summary of these statistical values (for the log transformed dataset) by site, location, and bacteria combination is presented in Table A-1. Values above 0.7 are shown in bold, as they are considered indicative of reasonably good correlations (Helsel and Hirsch, 2002).

The results described above demonstrate a reasonable *E. coli* to fecal coliform (or "EC:FC") correlation at the North Side-upstream and Stickney-downstream location-site combinations. Also identified is the correlation at the Stickney-downstream location for *Enterococcus* vs. fecal coliform. Of these, the EC:FC correlation for the Stickney-downstream combination

demonstrated the best correlation. Calumet locations showed no correlations. It should be noted that all three correlations were consistently identified by both the Spearman's and Pearson's statistics. However, the reader should be cautioned that each of these site-location combination correlation statistics were developed based on only ten dry weather samples, and therefore don't represent particularly robust statistics.

The purpose of testing the correlation coefficients at each location is to determine if reliable EC: FC and EN:FC ratios could be determined. As described previously, such ratios could be useful for estimating *E. coli* or *Enterococcus* concentrations when only fecal coliform concentrations are available (or in this case, when fecal coliform datasets are more robust). However, based on the correlation checks by visual (using scatter plots) and statistical (using correlation statistics) approaches, there only appear to be a few bacteria-site-location combinations where these correlations may be strong enough to develop reliable ratios.

Figure A-4 is included to further investigate these site-specific EC:FC correlations. This scatter diagram shows dry weather *E. coli* to fecal coliform results for each site (WRP)-location (UPS, DNS, OUTFALL) combination. The slope of each trend line approximates the "average" EC:FC ratio.

The charts in Figure A-4 confirm the Spearman's and Pearson's correlation statistics shown in Table A-1 in that the Stickney-downstream and North Side-upstream site-location combinations in particular show the best correlations for EC:FC, with the Stickney-downstream site-location combination showing the best correlation for EN:FC.

Given the fundamental assumption of log-normality upon which this approach is based, the distribution must first be tested prior to proceeding with implementation of the method. Therefore, a test of normality was performed on the log-transformed ratios (i.e., *E. coli* concentrations divided by fecal coliform concentrations) dataset. The test results for all six site-location combinations are shown in Figure A-5. P-values near 1 (using the Anderson-Darling normality test), combined with observed linearity in the dataset; indicate normality. Tests on all

six site-location combinations confirm that the log (EC:FC) ratios are normally distributed, or that the raw EC:FC ratios are indeed log-normally distributed.

The mean values of the log-normally distributed ratio datasets were then determined for each site-location combination, with the results shown in Figure A-6. The results indicate that mean upstream ratios are consistently higher than corresponding downstream ratios. However, initial statistical test results indicate that the datasets are not robust enough to confirm significant difference between these upstream and downstream ratios (i.e., no rejection of null hypothesis).

A matrix plot of all wet weather results is shown on Figure A-7. The results indicate that there is a good correlation between fecal coliform and the other bacteria measured. The correlation of bacteria in wet weather samples is statistically better compared to the dry weather samples (see Table A-2).

When comparing the FC and EC geometric concentration under dry and wet weather (see Tables A-3 and A-4, respectively), it is revealed from the data that there is a higher FC concentration increase in the North Side and Stickney downstream segments of the waterway compared to EC under wet weather conditions. The ratio of the geometric mean (EC/FC) for these two sites is approximately 0.21 to 0.26 indicating that during wet weather condition only 21 to 26 percent of the fecal coliform is *E.coli*. During dry weather condition, about 43 to 52 percent of the fecal coliform is *E.coli*. In previous studies, the District estimated the EC/FC ratio to be between 0.84 and 0.97, indicating that 84 to 97 percent of the FC is *E.coli* in the District WRP final effluent (MWRDGC, 2004). The lower EC/FC estimates in wet weather condition could be attributed to non-point sources of the pollution not impacted by the outfall in the North Side and Stickney segments of the waterway.

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ATTACHMENT A

TABLES

Table A-1. Dry Weather Pearson's/Spearman's Correlations for *Enterococcus, E.coli* and Fecal coliform

Log Space		DNS		UPS	
Site	Correlation	EC vs FC	EN vs FC	EC vs FC	EN vs FC
North Side	Pearson's	0.46	-0.83	**0.75**	0.36
	Spearman's	0.28	-0.54	**0.71**	0.55
Stickney	Pearson's	**0.87**	**0.71**	0.34	0.39
	Spearman's	**0.81**	**0.78**	0.34	0.32
Calumet	Pearson's	0.12	-0.01	-0.33	-0.38
	Spearman's	0.17	0.16	-0.20	-0.29

Note:

EC= *E.coli*
EN=*Enterococcus*
FC=Fecal coliform

Table A-2. Wet and Dry Weather Pearson's Correlations for *Enterococcus, E.coli, Pseudomonas aeruginosa, Salmonella* and Fecal coliform

Wet Weather Bacteria Correlation

	EC	*EN*	*FC*	*PA*	*SA*
EC	1				
EN	0.85	1			
FC	0.73	0.76	1		
PA	0.73	0.84	0.65	1	
SA	-0.17	-0.15	-0.12	-0.17	1

Dry Weather Bacteria Correlation

	EC	*EN*	*FC*	*PA*	*SA*
EC	1				
EN	0.46	1			
FC	0.83	0.28	1		
PA	0.19	0.05	0.09	1	
SA	-0.12	-0.07	-0.14	-0.34	1

Note:

EC= *E.coli*
EN=*Enterococcus*
PA=*Pseudomonas aeruginosa*
SA= Salmonella
FC=Fecal coliform

Table A-3: Dry Weather Geometric Mean Concentrations for *E.coli* and Fecal Coliform (CFU/100mL)

Site	Location	Sampling Dates	*E.coli* (EC)	Fecal Coliform (FC)	Ratio EC/FC
North Side	UPS	7/28/05-9/01/05	273	713	0.383
	Outfall	7/28/05-9/01/05	26,413	42,411	0.623
	DNS	7/28/05-9/01/05	15,710	36,687	**0.428**
Stickney	UPS	8/01/05-8/31/05	254	1,061	0.239
	Outfall	8/01/05-8/31/05	29,042	56,391	0.515
	DNS	8/01/05-8/31/05	9,043	17,491	**0.517**
Calumet	UPS	7/26/05-8/30/05	71	170	0.418
	Outfall	7/26/05-8/30/05	13,917	56,287	0.247
	DNS	7/26/05-8/30/05	1,370	3,520	0.389

Notes:

UPS = Upstream

DNS = Downstream

Table A-4: Wet Weather Geometric Mean Concentrations for *E.coli* and Fecal Coliform (CFU/100mL)

Site	Location	Sampling Dates	*E.coli* (EC)	Fecal Coliform (FC)	Ratio EC/FC
North Side	UPS	6/26/06-09/23/06	27,106	100,962	0.268
	Outfall	6/26/06-09/23/06	20,952	22,026	**0.951**
	DNS	6/26/06-09/23/06	24,262	117,399	**0.207**
Stickney	UPS	6/10/06-10/11/06	54,176	231,345	0.234
	Outfall	6/10/06-10/11/06	14,045	38,949	0.361
	DNS	6/10/06-10/11/06	45,101	172,819	**0.261**
Calumet	UPS	8/24/06-10/17/06	6,073	19,165	0.317
	Outfall	8/24/06-10/17/06	11,309	25,168	0.449
	DNS	8/24/06-10/17/06	279	2,981	0.094

Notes:

UPS = Upstream

DNS = Downstream

ATTACHMENT A

FIGURES

Figure A-1. Matrix Plots of Dry Weather Instream (UPS and DNS) Bacteria Concentrations

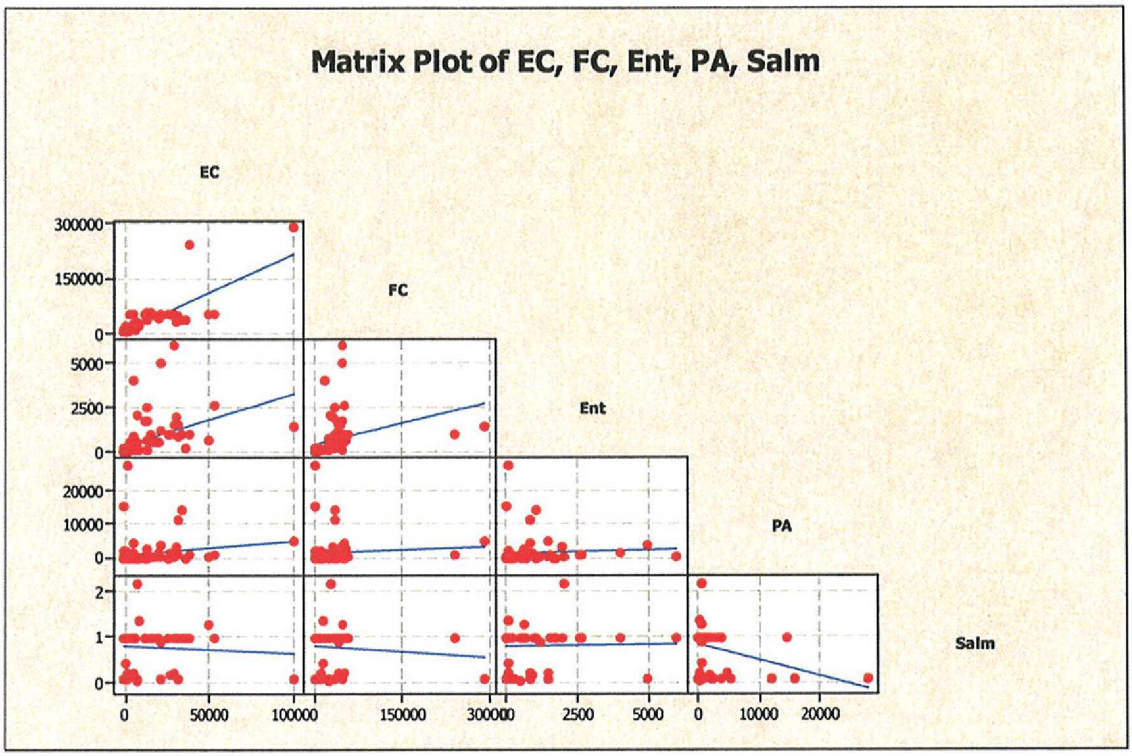
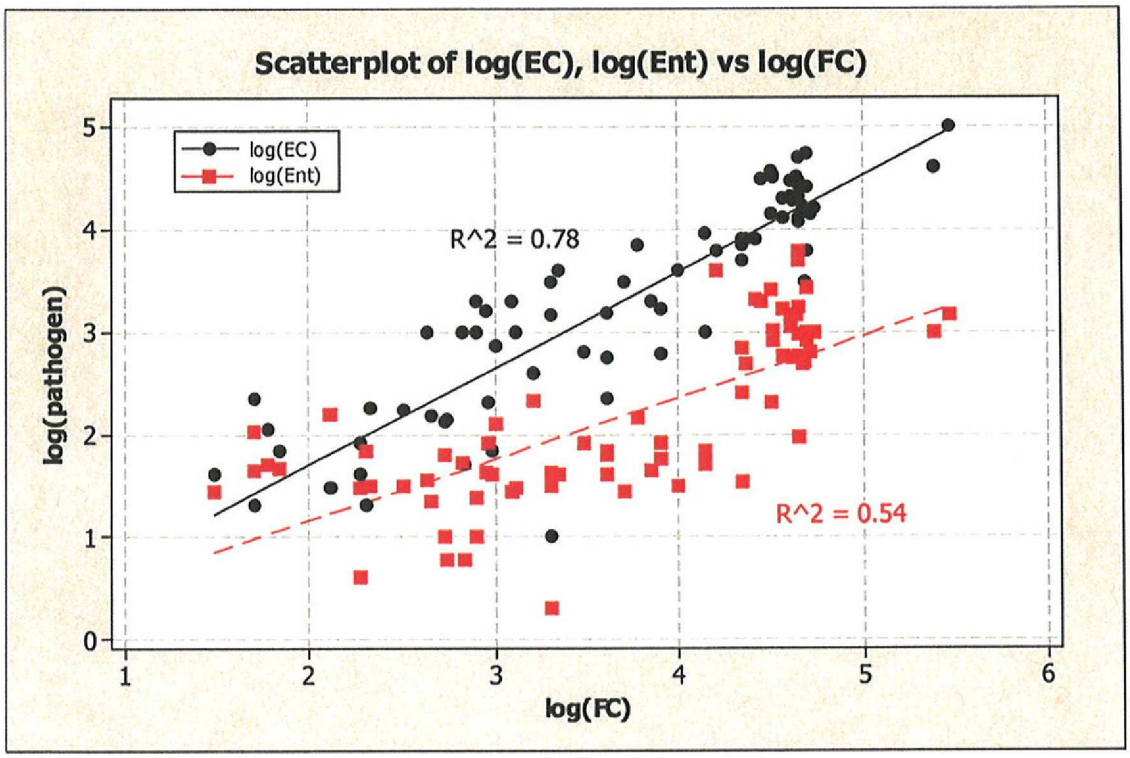


Figure A-2. Scatter Plot of Dry Weather Indicator Concentrations to Fecal coliform (In Log Space)



Note:

Enterococcus is not a pathogen; only certain strains of *E. coli* are pathogenic.

Figure A-3. Marginal Plot of Dry Weather *E. coli* vs Fecal coliform

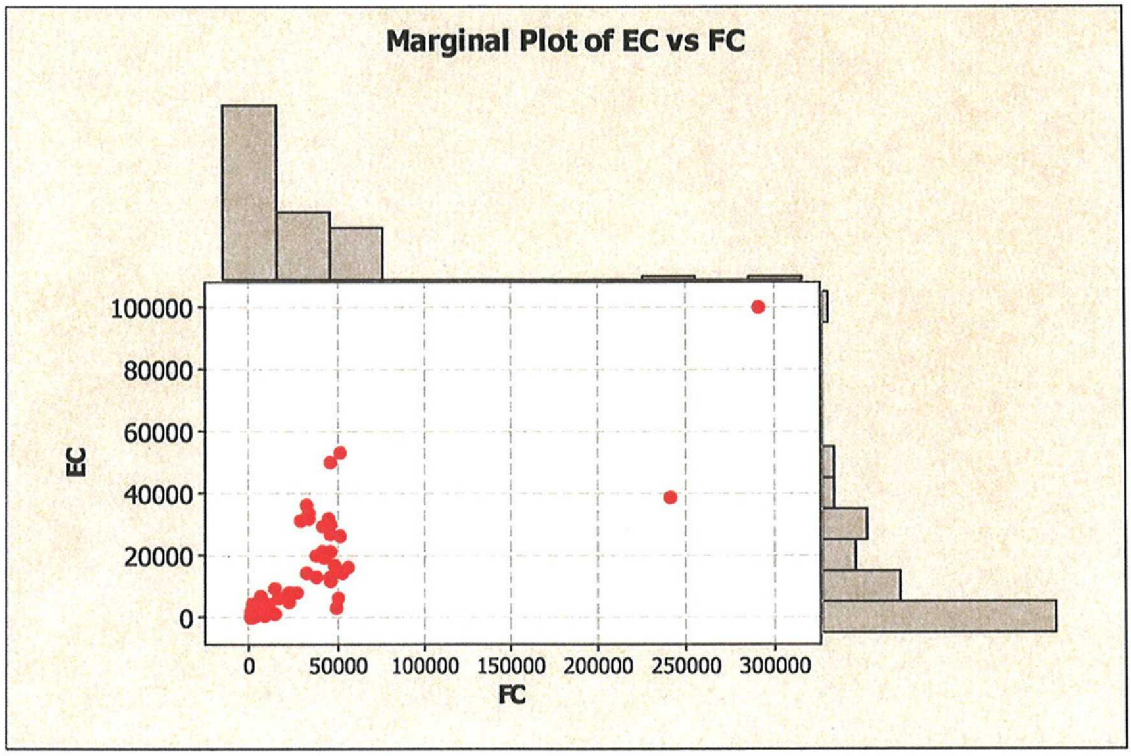


Figure A-4. Scatter Diagram of Dry Weather EC vs FC and EN vs FC, by Site and Location

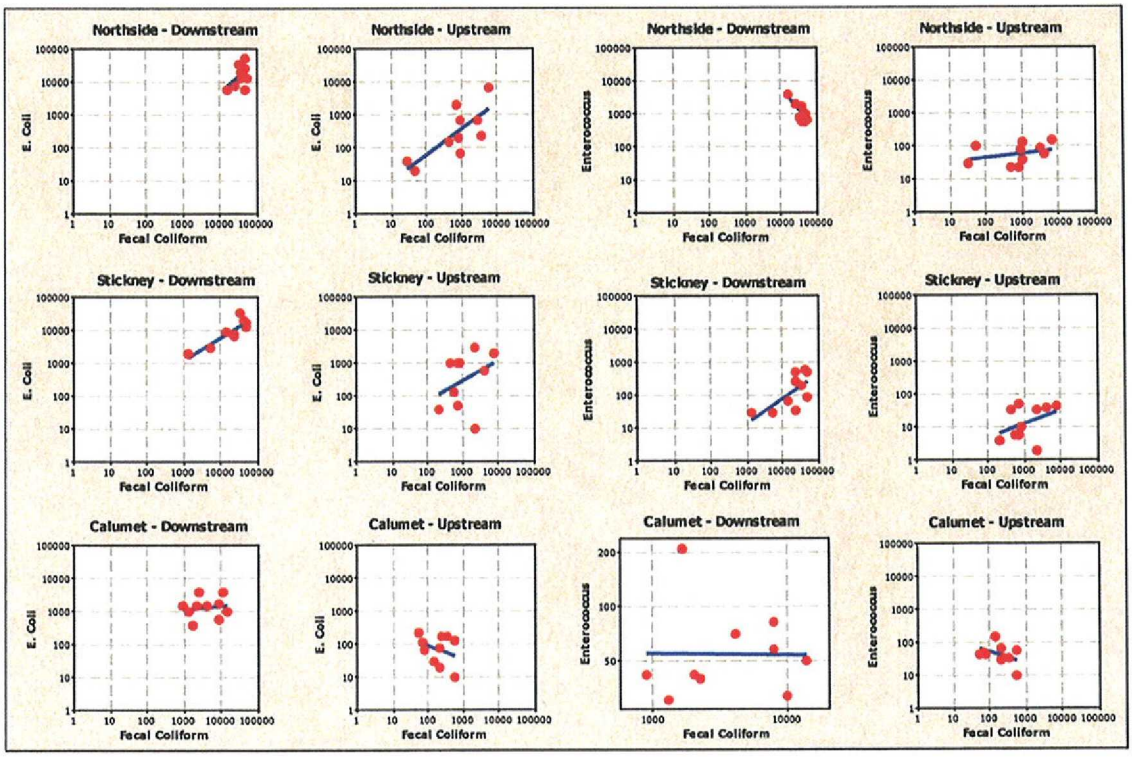


Figure A-5. Dry Weather Tests For Normality of [Log (EC/FC)] by Site and Location

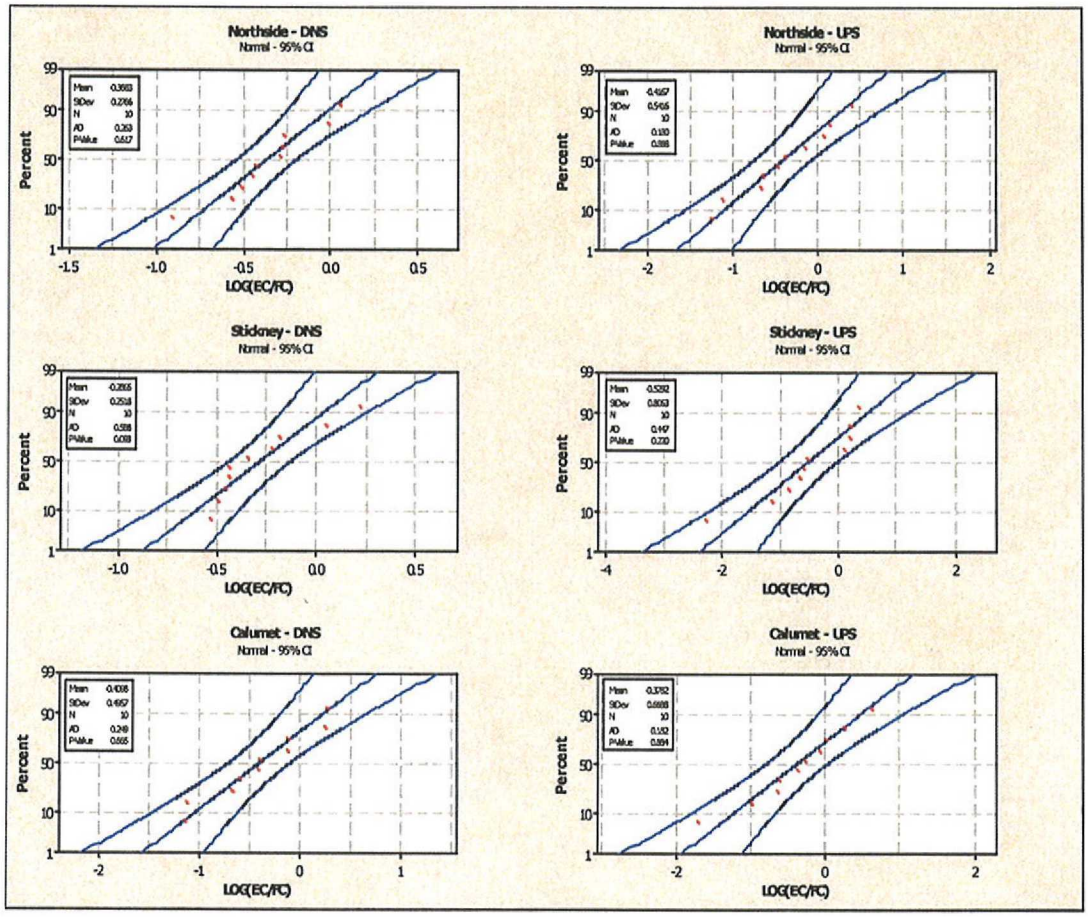


Figure A-6. *E. coli*:Fecal coliform (EC:FC) Dry Weather Ratio Estimates

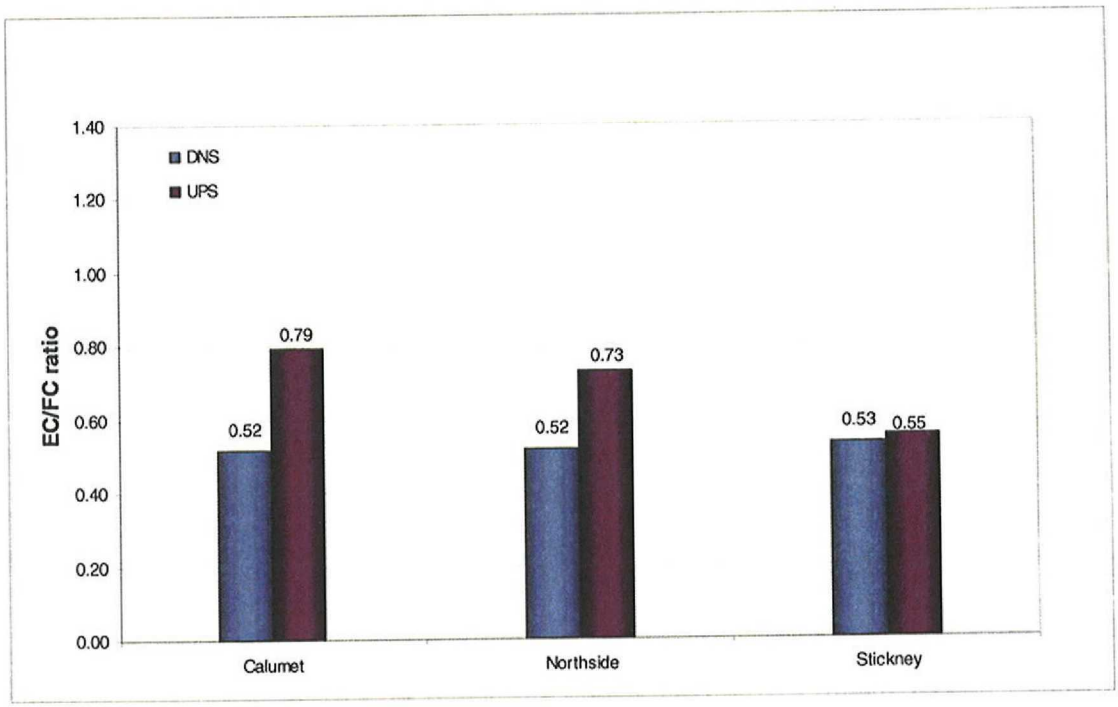


Figure A-7. Matrix Plots of Wet Weather Instream (UPS and DNS) and Outfall Bacteria Concentrations