



Microbial Source Tracking in Aquatic Ecosystems: The State of the Science and an Assessment of Needs

**NWRI Scientific
Assessment Report
Series No. 7**

and

**Linking Water Science
to Policy Workshop
Series Report**



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Microbial Source Tracking in Aquatic Ecosystems: The State of the Science and an Assessment of Needs

NWRI Scientific Assessment Report Series No. 7 and Linking Water Science to Policy Workshop Series Report

Workshop Proceedings

Council Chambers, Toronto Metro Hall
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For information on previous workshop themes, visit: <http://www.nwri.ca/sciencepolicy/ccmeworkshop-e.html>

Workshop Summary

Fecal pollution of aquatic ecosystems is a significant problem in many areas across Canada. This pollution can come from diverse sources such as municipal wastewater effluents, failing on-site sewage systems, livestock manure and wildlife droppings. Its impacts on human health, aquatic ecosystems and local economies can be significant through waterborne disease outbreaks, boil water advisories, contamination of irrigation waters, and beach and shellfish closures.

There is a growing need for a science-based approach to determine the source of fecal pollution in aquatic ecosystems. Identifying the correct fecal pollution source could help mediate conflicts between stakeholders in communities, and help target appropriate, cost-effective, corrective actions to prevent future pollution. Microbial source tracking (MST) is an emerging field that offers considerable promise for determining the source of fecal pollution contaminating aquatic ecosystems. In general, the MST approach is based on comparing the similarity of microorganisms collected from aquatic ecosystems to microorganisms collected from nearby fecal pollution sources in order to make inferences about the likely source of fecal contamination. However, the field is still under development. There are no standardized methods and there have been few field studies to test the reliability and accuracy of MST techniques. Nevertheless, there is considerable interest in this field of research, along with high expectations for tools to help resolve fecal pollution problems.

Consequently, a MST workshop was held March 7-8, 2005, in Toronto, Ontario, to review the state of MST science and its readiness for widespread application and to assess the fecal pollution source tracking needs in communities across Canada. The workshop was hosted by Environment Canada's National Water Research Institute and Ontario Region, the City of Toronto, the Ontario Ministry of the Environment, Health Canada, and Agriculture and Agri-Food Canada. Seventy participants attended from federal, provincial and municipal government agencies, non-government organizations, and from universities in Canada, the United States and Australia. Participants represented the scientific research community, and the water policy and program management perspectives relevant to MST in Canada. The workshop is another in the *Linking Water Science to Policy Workshop Series* (<http://www.nwri.ca/sciencepolicy/ccmeworkshop-e.html>) that brings together leading researchers with policy analysts and program managers to communicate recent science and to solicit feedback on research drivers and needs. The report is also another in the *NWRI Scientific Assessment Report Series* (<http://www.nwri.ca/publications/sars-e.html>) that publishes national scientific assessments of priority freshwater issues in Canada, serving as an authoritative review of current scientific knowledge, trends, and information and program needs to assist water science decision-makers, resource managers, and the research community in setting research priorities and in developing sound management policies and practices.

State of the Science

Presentations provided an overview of the state of MST science based upon recent scientific publications and case studies. Early MST studies in the late 1990s raised considerable expectations for MST tools to resolve problems in fecal pollution source tracking. More recent scientific publications have pointed to limitations in MST methods, particularly for studies of large watersheds with complex sources of fecal contamination. Library-based methods, such as those based upon *E. coli*, were seen to suffer from high misclassification rates and the need to have increasingly larger libraries to represent the diversity of potential *E. coli* isolates from fecal sources. Non-library-based methods, such as those based upon *Bacteroides* sp., require additional host-specificity evaluation and remain to be well tested in field studies.

While some of these recent MST studies were perceived to have placed a "wet blanket" on the field, cases of successful applications of MST in field studies continue to occur. In some cases, MST methods have identified unexpected fecal pollution sources, and

were instrumental in leading to effective remedial clean-up actions. In other cases, results from MST field studies have been consistent with other lines of evidence in identifying predominant fecal pollution sources. Additional presentations at the workshop showed that the field of MST is still evolving. Novel tools such as DNA microarrays and protozoan genotyping methods could help enlarge the existing MST toolbox. Other tools based on chemical tracers (e.g., coprostanol) or DNA markers for host animal cells sloughed off in feces may also prove useful for fecal pollution source tracking.

The Workshop recognized that while many methods exist in the current MST toolbox, there is no “silver bullet,” universally accepted best method. While some methods have achieved a level of maturity where they could be considered for standardization, others are still experimental or research-grade tools. Additional research is required to understand more fully the advantages and limitations of microbial source tracking methods, and wider application of the tools will require standard methods and careful consideration of appropriate experimental designs. Library-dependent MST methods require a library of an appropriate size and representation, which still remains to be determined. Library-independent MST methods require validation of host-specific markers in field studies. The Workshop placed emphasis on applying MST methods as part of multiple lines of evidence to resolve fecal pollution source tracking problems. While MST tools can be relevant to tackle such problems, it will be important to ensure there are realistic expectations among the end-user communities for MST studies in the near future.

MST Needs and Drivers/Policy and Program Relevance

With aging municipal wastewater infrastructure, urbanization and increasingly intensive livestock production capacity across Canada, the need for science-based tools like MST methods to resolve existing fecal pollution problems and prevent future ones can be anticipated to grow. Microbial source tracking has been slower to develop in Canada than in the United States. The U.S. Clean Water Act requirements for calculating Total Maximum Daily Loads for fecal contaminants in watersheds have been driving much of the application of MST studies in the United States. Canada does not have equivalent legislation, and applications of MST have been driven more by a “bottom-up” approach from local community concerns for beach and shellfish closures. MST studies may prove useful in helping to meet federal, provincial, territorial or municipal microbial water quality guidelines, objectives and regulations. In particular, identifying the source of fecal pollution is invaluable at better targeting cost-effective abatement options. Other drivers for conducting MST studies include the need to provide guidance for development of source water protection plans and to help target microbial pollution education and awareness initiatives at local levels. Ongoing MST research activities, including the Health Canada-Agriculture and Agri-Food Canada MST study under Canada’s Agricultural Policy Framework, will help raise awareness of the advantages and limitations of MST methods for water programs across Canada.

1. Introduction

Fecal pollution of aquatic ecosystems is a significant problem in many areas across Canada. This pollution can come from diverse sources such as municipal wastewater effluents, livestock manure and wildlife droppings. Its impacts on human health and local economies can be significant through waterborne disease outbreaks, boil water advisories, contamination of irrigation waters, and beach and shellfish closures.



There is a growing need for a science-based approach to determine the source of fecal pollution in aquatic ecosystems. Identifying the correct fecal pollution source could help mediate conflicts between stakeholders in communities, and lead towards appropriate, cost-effective corrective actions to prevent future pollution. Microbial source tracking (MST) is an emerging field that offers considerable promise for determining the source of fecal pollution contaminating aquatic ecosystems. However, the field is still under development. There are no standardized methods, and there have been few field studies to test the reliability and accuracy of MST techniques.

Although MST is still evolving, the potential benefits are significant enough for a national discussion on the state of the MST science and fecal pollution source tracking needs across Canada. With this in mind, a Microbial Source Tracking Workshop was held March 7-8, 2005, in Toronto, Ontario. The impetus for this workshop arose from a desire to clarify the state of MST readiness for practical application. On the one hand, there has been heightened interest in MST by policy and program managers at all levels of government to determine sources of fecal contamination in diverse aquatic ecosystems across Canada. On the other hand, while some practical MST case studies exist, the field is still largely under development and recent scientific publications evaluating MST methods have pointed to some limitations of the field (Griffith et al. 2003; Stoeckel et al. 2004). The timing seemed optimal to bring researchers together with policy and program managers to develop a realistic assessment of the state of the science, and get practical feedback from practitioners on their needs related to tracking sources of fecal contamination.

Workshop Objectives

Workshop objectives were to strengthen the linkage between Canadian water policy and program decision makers and MST research experts by providing:

1. MST Science Assessment to help municipal, provincial, federal and other decision makers obtain sufficient scientific knowledge about MST to shape their program, policy and regulatory needs. The science assessment would assess the state of MST science and its readiness for widespread application across Canada.
2. MST Needs Assessment to help the MST science and research community better understand Canadian water policy and program needs pertaining to fecal pollution. The needs assessment would characterize fecal pollution challenges across Canada.
3. Identification of MST Research Priorities in Canada.
4. MST Networking Forum to exchange MST information, network, make contacts, and identify opportunities to sustain dialogue between water policy decision makers and MST research experts.

Workshop Organization

The workshop was sponsored by Environment Canada's National Water Research Institute and Ontario Region (Great Lakes Sustainability Fund), the Ontario Ministry of the Environment and the City of Toronto, with organizational participation from Health Canada and Agriculture and Agri-Food Canada.

The Workshop was organized by a planning committee of the following individuals:

Dr. Tom Edge - National Water Research Institute, Environment Canada

Karl Schaefer - Science and Technology Liaison Division, Environment Canada

Dr. Todd Howell - Environmental Monitoring and Reporting Branch, Ontario Ministry of the Environment

Patrick Chessie - Water and Wastewater Services Division, City of Toronto

Will Robertson and Diane Medeiros - Water Quality and Health Bureau, Health Canada

Dr. Vic Gannon - Laboratory for Foodborne Zoonoses, Public Health Agency of Canada

Dr. Ed Topp - Soil and Environmental Sciences, Agriculture and Agri-Food Canada

The Workshop was held March 7-8, 2005, at the City of Toronto's Metro Hall, 55 John Street, Toronto, Ontario. It consisted of invited presentations with opportunities for questions, and a plenary discussion of MST drivers and needs (see Agenda in Appendix A). Participants were invited from the MST research community, water and wastewater managers in municipal/regional governments, point and non-point source pollution experts in watershed-based organizations (such as conservation authorities in Ontario), water regulatory officials and program/policy managers in provincial, territorial and federal governments, and in related water quality professional associations and organizations. Over 70 people participated in the Workshop (see List of Participants in Appendix B).

2. Microbial Source Tracking Overview

Microbial source tracking (MST) is an emerging field that seeks to identify the source of microbial contamination in the environment. The field has been developing rapidly to address growing needs to find the source of fecal pollution contaminating sources of drinking, shellfish and recreational waters. The general approach is based on comparing the similarity of microorganisms collected from aquatic ecosystems to microorganisms collected from known fecal pollution sources in order to make inferences about the likely source of fecal contamination.

Various methods have been developed for microbial source tracking (Simpson et al. 2002; Scott et al. 2002; U.S. EPA 2005). Since the field of MST is evolving rapidly, many new methods are also under investigation. The collection of methods for microbial source tracking has often been referred to as a toolbox, with some methods being more relevant to use than others in certain circumstances. This section provides an overview of some of the more commonly used MST methods.

MST methods can be classified as library-dependent methods or library-independent methods. To date, library-dependent methods have been more widely used in MST studies, although it can be very labour intensive and time consuming to develop the library for these methods. Library-independent methods are increasingly under investigation.

Library-Dependent MST Methods

Library-dependent methods are based upon choosing a fecal indicator microorganism (e.g., *Escherichia coli*) and establishing a reference library of characteristics of individual isolates of the selected microorganism obtained from known fecal pollution sources. For example, a library could be a database of DNA fingerprints of *E. coli* isolates obtained from fecal pollution sources such as animal feces, septic tanks or municipal wastewater effluents. The DNA fingerprints of *E. coli* isolates obtained from aquatic ecosystems ("unknowns") can then be compared to the DNA fingerprints in the library ("knowns") to make inferences about the source of the waterborne *E. coli* isolates. Sound taxonomic identification of the fecal and waterborne isolates is necessary to ensure similarity comparisons are warranted. A minimum size has not yet been established for libraries, although they need to be sufficiently large to reflect the diversity of isolates in the environment. A targeted sampling approach may be useful for focusing MST efforts (Kuntz et al. 2003).

The most common fecal indicator microorganisms used in library-dependent methods to date have been *Escherichia coli* and *Enterococcus* spp. These bacteria are common inhabitants of warm-blooded animal guts, are relatively easy to isolate and culture in the lab, and are widely used by water quality monitoring programs. The similarity between isolates of a fecal indicator microorganism can be measured by either phenotypic profiling or genotypic fingerprinting methods.



Phenotypic methods use cellular or physiological comparisons between the isolates based on features such as antibiotic resistance (Wiggins 1996; Hagedorn et al. 1999; Harwood et al. 2000) or carbon utilization (Hagedorn et al. 2003). Antibiotic resistance profiling has been the most commonly used phenotypic method to date. For this method, bacterial isolates can be inoculated onto the surface of many agar plates, with each plate containing a different antibiotic mixed into the agar. The isolates are incubated overnight on the agar plates, and their growth is compared to their growth on a control plate (i.e., the same agar without antibiotics). How well each isolate grows

on many different agar plates is used to develop a profile of its antibiotic resistance. An antibiotic resistance approach to MST is based on the assumption that bacteria in human and domestic animal guts are exposed to different antibiotics in medical and veterinary treatments, and that these gut bacteria will develop different resistance profiles. Since wildlife species do not receive direct antibiotic treatments, their gut bacteria are typically less resistant to antibiotics.

Genotypic methods use DNA sequence comparisons between the isolates based on approaches like DNA fingerprinting using rep-PCR (Dombek et al. 2000; Johnson et al. 2004), ribotyping (Carson et al. 2001), and amplified fragment length polymorphism (AFLP) (Guan et al. 2002; Leung et al. 2004). In these DNA fingerprinting methods, DNA is extracted from cells of an isolate and different DNA cutting or amplifying techniques can be used to obtain DNA fragments of different sizes. The different sized DNA fragments can then be separated on an electrophoresis gel into a ladder-like pattern of DNA bands that can be visualized and statistically analyzed as a unique DNA fingerprint. Ribotyping and rep-PCR have been the most commonly used DNA fingerprinting techniques to date.

Library-Independent MST Methods

Library-independent methods are based upon detecting host-specific markers to indicate the presence of fecal contamination from a specific human or animal host in the water. Most library-independent methods rely on the polymerase chain reaction (PCR) to detect host-specific markers. These methods do not generally require a cultivation step, although in some cases this is necessary to increase the numbers of microorganisms carrying a host-specific marker gene. For example, cultivation-based methods have been proposed for human-specific markers in *Enterococcus* (Scott et al. 2005), and cattle and swine markers in *E. coli* (Khatib et al. 2002, 2003).

Some of the most promising results to date for developing host-specific markers for fecal pollution source tracking involve 16S rDNA markers within the *Bacteroidetes* family. These anaerobic bacteria comprise a very large portion of the fecal flora in warmblooded animals. Bernhard and Field (2000a) found host-specific 16S rDNA sequences in *Bacteroides* sp. bacteria in human and cow fecal samples and used a method to track these sequences in coastal waters. Bernhard and Field (2000b) then developed *Bacteroides* 16S rDNA PCR assays specific to ruminants and humans as culture-independent MST methods. There is now an active research effort to validate and find new *Bacteroides* sp. host-specific markers (Field 2004). Additional host-specific markers have been proposed based on bacteriophages (Payan et al. 2005) and pathogenic microorganisms such as enteric viruses (Fong et al. 2005) and protozoa like *Cryptosporidium* sp. (Jiang et al. 2005).

Other Source Tracking Methods

While the field of microbial source tracking is growing rapidly, it should be noted that other methods can also be used to track fecal contamination in aquatic ecosystems. For example, chemical tracers have been used, most commonly to detect chemicals associated with human wastes. As the highest concentration of these chemicals is typically found in wastewater treatment plants, they have been proposed for tracking human fecal waste pollution. For example, fecal sterols and stanols, caffeine, detergents, laundry brighteners, fragrance materials and pharmaceuticals are among chemicals proposed as markers of fecal pollution (Elhmmali et al. 2002; Roser et al. 2003; Glassmeyer et al. 2005). Eukaryotic mitochondrial DNA markers have also been used to discriminate between fecal pollution sources in surface waters (Martellini et al. 2005) as outlined later in this Workshop Report.

3. Microbial Source Tracking Activities

Canada

This Toronto microbial source tracking workshop evolved from recent scientific publications and from a variety of microbial source tracking activities initiated across Canada in recent years. Dr. Tom Edge, Environment Canada, identified other MST workshops that have been held across Canada in response to concerns about the need to identify fecal pollution sources responsible for contaminating agricultural watersheds, and closing shellfish areas and beaches. A workshop sponsored by Health Canada and Agriculture and Agri-Food Canada was held June 19-20, 2003, in Ottawa, Ontario, to help design a large MST project under the Agricultural Policy Framework (Health Canada and Agriculture and Agri-Food Canada 2004). Another MST workshop was held April 5-6, 2004, in Guelph, Ontario (Goss and Dunfield 2004). Environment Canada, the National Programme of Action for the Protection of the Marine Environment from Land-based Activities (NPA), Acadia University and the Clean Annapolis River Project (CARP) supported an MST workshop April 14-15, 2004, in Wolfville, Nova Scotia (Sullivan 2004).



Microbial source tracking activities have been growing at a number of university, government and private-sector laboratories across Canada. Laboratory-based MST research programs were identified at institutions including the University of Victoria, Victoria, B.C. (Drs. Azit Mazumder and Rick Nordin); Environment Canada's Pacific Environmental Science Centre, Vancouver, B.C. (Heather Osachoff); Public Health Agency of Canada, Lethbridge, Alberta (Dr. Vic Gannon); Lakehead University, Thunder Bay, Ontario (Dr. Kam Leung); Agriculture and Agri-Food Canada, London, Ontario (Dr. Ed Topp); GAP Services Inc., London, Ontario (Gary

Palmateer); the University of Guelph, Guelph, Ontario (Drs. Carleton Gyles, Shu Chen and Michael Goss); Environment Canada's National Water Research Institute, Burlington, Ontario (Dr. Tom Edge); the Ontario Ministry of the Environment, Toronto, Ontario (Dr. Susan Weir); and Acadia University, Wolfville, Nova Scotia (Dr. Greg Bezanson). It is likely that other laboratories across Canada are also conducting MST studies.

Under Canada's Agricultural Policy Framework, Health Canada and Agriculture and Agri-Food Canada established the National Water Quality Surveillance Program. Dr. Ed Topp, Agriculture and Agri-Food Canada, described this program as aimed at gaining a snapshot of water quality on a small number of representative watersheds across Canada that vary in their land use. At the same time, a wide variety of biological and chemical endpoints are being evaluated with a view to testing and validating microbial source tracking technologies in the Canadian context. This presentation highlighted some of the research objectives of this federal initiative.

United States

An overview of microbial source tracking activities in the United States was presented by Dr. Valerie J. Harwood, University of South Florida (Dr. Jorge Santo Domingo, U.S. Environmental Protection Agency, was unable to attend). Protection from fecal microbial contamination is one of the most important goals listed in section 303(c) of the U.S. Clean Water Act for waters designated for recreation (primary and secondary contact), public water supplies and propagation of fish and shellfish. Detection of fecal bacteria and pathogens and tracking them to their sources are topics of intense interest in the United States in view of the current requirements for calculating Total Maximum Daily Loads (TMDLs). States, territories and tribes must comply with TMDL requirements within the next five to ten years.

A number of microbial source tracking methods are currently used in the United States to determine the origin of fecal pollution affecting natural water systems. MST is based on the assumption that, given the appropriate method and indicator organism, the source of microbial pollution can be identified. Many MST studies in the United States have relied on matching genomic "fingerprints" from bacterial strains isolated from a water system with those isolated from different hosts (e.g., humans, domesticated animals, wildlife). To successfully identify fecal sources, the most often used approaches rely on the development of comprehensive libraries (i.e., culture collections) of indicator bacteria (i.e., *E. coli* and fecal enterococci). While there are several laboratory studies supporting the use of library-dependent approaches for MST, their accuracy in field study situations has been questioned due to a number of problems associated with the target organisms, the level of complexity introduced by spatial and temporal vectors, the stability of the markers used, and poor sampling protocols. As a consequence, several library-independent approaches based on phylogenetic markers and virulence factors have been

developed recently to circumvent the limitations of culture-dependent approaches. However, a systematic evaluation of the latter methods against real world samples has not been conducted. The U.S. Environmental Protection Agency recently completed a scientific review of MST methods, and has developed a guide to assist water quality decision makers contemplating microbial source tracking studies (U.S. EPA 2005).

International

An overview of international MST activities was provided by Dr. Nicholas Ashbolt, University of New South Wales, Sydney, Australia. Managing the safety of drinking water systems has undergone a paradigm shift in the last few years. During the review and publication in 2004 of the WHO and Australian Drinking Water Guidelines, water agencies have been rethinking how to apply the proactive Water Safety Plan (WSP) approach rather than simply meeting end-of-pipe compliance levels for microbial indicators. A central theme within WSP is equivalent to the food industry's hazard analysis critical control point (HACCP) risk management approach. For water supplies, this means getting to know your key fecal sources and events that may lead to pathogen contamination of drinking waters – and fecal source tracking, along with sanitary surveys are pivotal tools to this end.

A range of microbial source tracking tools has been assessed during a recent EU project, with sorbitol-fermenting bifidobacteria, PCR identification of bifidobacteria, and phages infecting *Bacteroides thetaiotaomicron* being the most successful when applied to watershed samples. Yet, genotype II of F-specific RNA bacteriophages, and the ratio of coprostanol to coprostanol + epicoprostanol provided greater discriminating power with fecal samples, but low water concentrations limited their application with field samples. Recent Australian work utilizing a greater range of fecal sterols (8), however, has shown that via improved sample concentration and data analysis, and analyzing stanol/sterol and microbial ratios, the latter parameters can provide fecal source load estimations, currently not possible with most MST tools. A decision support system to aid in data analysis, called 'FaecalPrint' (Roser et al. 2003) has been prototyped and results were presented.

4. Microbial Source Tracking Science Assessment

The Workshop addressed the state of MST science through presentations and discussion about existing MST methods, emerging MST methods, and case studies of the application of MST methods to fecal pollution problems. Two recently published studies were presented at the Workshop as a basis for discussing the limitations of existing MST methods (Griffith et al. 2003; Stoeckel et al. 2004). Workshop discussion suggested that these two publications had placed a "wet blanket" over the field of MST. Library-based methods, such as those based upon *E. coli*, were seen to suffer from high misclassification rates and the need to have increasingly larger libraries to represent the diversity of potential *E. coli* isolates from fecal sources. Non-library-based methods, such as those based upon *Bacteroides* sp., require additional host-specificity evaluation and remain to be well tested in field studies. There was a growing recognition of the limitations of existing MST methods, and that initial optimistic expectations in the early days of MST needed to be tempered. This message also arose at a WERF MST workshop held February 16-18, 2005, in San Antonio, Texas. However, the Toronto Workshop also identified some promising new methods emerging in the MST toolbox, and case studies were presented showing that appropriately designed MST studies can be applied successfully to identify and resolve fecal pollution problems. Workshop discussion identified the need for further research to test and validate existing MST methods in field studies, and to continue to search for new host-specific fecal pollution markers.

Existing MST Methods

Dr. John Griffith, Southern California Coastal Water Research Project, Westminster, California, presented results from a recent comparative assessment of MST methods (Griffith et al. 2003). In this study, 22 researchers employing twelve different methods were provided sets of identically prepared blind water samples. Each sample contained one to three of five possible fecal sources (human, dog, cattle, seagull or sewage). Researchers were also provided portions of the fecal material used to inoculate the blind water samples for their use as library material. No MST method predicted the source material in the blind samples perfectly. Host-specific PCR performed best at differentiating between human and non-human sources, but primers are not yet available for differentiating among the non-human sources. Virus and F+ coliphage methods reliably identified sewage, but were not able to identify fecal contamination from individual humans. Library-based isolate methods were able to identify the dominant source in most samples, but had difficulty with false positives, identifying the presence of fecal sources that were not in the samples. Among the library-based methods, genotypic methods generally performed better than phenotypic methods.

Dr. Don Stoeckel, U.S. Geological Survey, Columbus, Ohio, presented results from another recent comparative assessment of MST methods (Stoeckel et al. 2004). In this study, seven protocols for microbial source tracking by typing *E. coli*, including two approaches for assigning *E. coli* to hosts, were evaluated. No tested protocol correctly assigned the majority of challenge isolates to the correct host of origin—those that attempted classification for only a few isolates tended to have high rates of correct classification, while those that attempted classification for most isolates had high rates of incorrect classification. Study results indicate that a library size of 1000 isolates was insufficient to conduct MST in the partial-county study area. The study area was on the order of 100 square miles (about 300 square kilometres). MST includes host specificity of fecal markers as its central hypothesis. Library-dependent MST requires either a limited number of host-specific types per source or within-host relatedness among the many types. Existence of cosmopolitan subtypes, high subtype diversity, and lack of relatedness among subtypes from the same host contributed to lack of accuracy in the study.

Dr. Paul Rochelle, Metropolitan Water District of Southern California, La Verne, California, provided an overview of results from a workshop on microbial source tracking, sponsored by the Water Environment Research Foundation and the Metropolitan Water District of Southern California, in San Antonio, Texas, in February 2005. The overarching objective for this workshop was to identify knowledge gaps and research needs for the application of microbial source tracking technologies by the wastewater and drinking water industries. It was generally agreed that there is a future for source tracking but more comparison and evaluation studies need to be conducted. There needs to be more interlaboratory comparisons of methods, adherence to standardized procedures, greater inclusion of QA/QC aspects into studies, and more thorough documentation of all study aspects in publications. It was recognized that some methods have achieved a level of maturity where they can be considered for standardization, whereas others are still experimental or research-grade tools. Analysis cost was a recurring theme and there was some debate of pragmatism (limited funding and timeline, human versus non-human discrimination) versus idealism (more isolates, bigger libraries, methods with increasing complexity, increased certainty, increased resolution). While there has been considerable debate in the past over which method is “the best,” a consensus was reached at this workshop that there is unlikely to be a single “silver bullet.” Rather, MST practitioners should utilize a toolbox approach, the individual components of which will vary depending on the type of watershed, potential sources of contamination, funding and time available, and the level of resolution required, among other considerations. Future comparison studies should evaluate different suites of methods to determine those that best meet accuracy, sensitivity and reproducibility criteria.

Emerging MST Methods

Dr. Roland Brousseau, Biotechnology Research Institute, National Research Council of Canada, Montreal, Quebec, provided a presentation on the application of DNA microarray technology to microbial source tracking. The parallel processing power of DNA microarrays, where hundreds of probes can be tested simultaneously by hybridization, makes them a logical tool to approach the difficult problem of MST. However, the sensitivity of microarrays is rather limited at the present time. Depending on methods and circumstance, a given bacterial species may have to be in excess of five percent of



a complex mixture before its presence can be detected by direct hybridization of total labeled DNA from an environmental sample. This limit can be brought down by an amplification step such as PCR before hybridization, at the expense of unknown and variable amplification bias. Despite these problems, microarray hybridization patterns from amplified DNA can be obtained that discriminate between different types of fecal matter. An alternative approach, using DNA extracted from isolated colonies, is currently being explored for *Escherichia coli* to ascertain whether the fine mapping of virulence and antibiotic resistance genes is amenable to MST.

Dr. Norman Neumann, Provincial Laboratory for Public Health (Microbiology), Calgary, Alberta, provided a presentation on the application of protozoan genotyping for microbial source tracking. Until recently it was assumed that both *Cryptosporidium parvum* and *Giardia lamblia* were capable of parasitizing a wide range of different mammalian hosts. However, recent data on the molecular epidemiology of these parasites suggests that different species and/or genotypes of *Cryptosporidium* and *Giardia* display host specificity, and this is changing perceptions regarding the zoonotic potential of these parasites and the waterborne risks they pose to humans. In studies assessing source water protection in two different watersheds (2003 and 2004), molecular forensic tools were applied to intensively study host-fecal sources impacting the South Nation River in Ontario and the Oldman River in Alberta. Sites within these watersheds were monitored weekly for *Cryptosporidium* and *Giardia*. Forensic profiling of *Cryptosporidium* parasites revealed a variety of host fecal impacts coming from wildlife (deer, fox, birds, skunks), agriculture and/or human sewage. This was based on the detection of *Cryptosporidium* oocysts that had molecular DNA sequence similarities to *Cryptosporidium* fox genotype (foxes), *Cryptosporidium* cervine genotype (deer), *Cryptosporidium baileyi* (birds), *Cryptosporidium* skunk genotype, *Cryptosporidium andersoni* (cattle), and *Cryptosporidium parvum* bovine genotype (cattle, ungulates and/or humans). In addition, at least three other unidentified genotypes of *Cryptosporidium* were detected, the host sources of which are unknown, but probably wildlife in origin. During a period of heavy precipitation within the South Nation River *Cryptosporidium andersoni* (host = cattle) was detected in a significant number of sites along the river, suggesting that an influx of agriculturally derived fecal material entered the river during this time period. Interestingly, wildlife genotypes were commonly detected throughout the studies, suggesting that wildlife accessing the river through their use of the riparian zone can significantly contribute to parasite contamination within the watershed. Molecular genotype analysis of waterborne parasites may be useful as a tool for microbial source-tracking, and for effective watershed and public health protection.

Dr. Pierre Payment, Institut national de la recherche scientifique (INRS), Laval, Quebec, presented results on a new molecular method developed to detect DNA from animal cells sloughed off into the intestine with feces (Martellini et al. 2005). It is based on

detection of mitochondrial DNA from various animal species to track the origin of surface water pollution, and to differentiate human and animal sources. Mitochondrial DNA sequences were used to design PCR primers specific for human, bovine, ovine and porcine DNA using single, multiplex and nested PCR protocols. The primers were tested with DNA extracted from untreated domestic sewage, agricultural soil runoff, swine farm effluents and water from two rivers with known pollution sources. At least one of the four species was detected in most of these samples. The limit of detection in wastewater was 10^3 to 10^4 cells per litre with a multiplex PCR protocol. This is the first report of a method using eukaryotic genetic DNA to detect and differentiate animal DNA from fecal sources in water. This innovative method is simple and could be used to differentiate sources of pollution in a watershed quickly.

MST Case Studies

Dr. Chuck Hagedorn, Department of Crop and Soil Environmental Sciences, Virginia Tech, Blacksburg, Virginia, presented several case studies to demonstrate how microbial source tracking methods can be employed at the watershed level. Profiles have been developed over the past ten years on thousands of *Enterococcus* and *E. coli* isolates from known sources based on pulsed-field gel electrophoresis, carbon-source utilization (with the semi-automated Biolog system) and antibiotic resistance analysis. Based on these profiles, it is possible to accurately and reliably separate *Enterococcus* and *E. coli* from human, avian, companion animals, domestic animals and wildlife sources, and then compare the profiles of isolates from environmental samples (unknown origin) to determine the sources of these isolates. Case studies in a variety of watersheds have demonstrated how well the application of *Enterococcus* and *E. coli* in MST appears to work, especially with regards to results from method comparison studies published recently. The Hagedorn lab approach is based on conducting thorough QA/QC on host origin libraries, and using multiple MST methods plus an alternative tracer. A novel approach involving detection of optical brighteners in detergents as a means of identifying human-derived sources of fecal pollution was also discussed.

Dr. Tom Edge, National Water Research Institute, Environment Canada, Burlington, Ontario, and Ted Bowering, City of Toronto, presented a case study on the application of MST to investigate the source of fecal contamination at two urban Toronto beaches on Lake Ontario. The study has used multiple lines of evidence for determining the source of fecal contamination at these beaches. Antibiotic resistance analysis and rep-PCR DNA fingerprinting were investigated in parallel using an *E. coli* library-based approach. Over 6000 *E. coli* were collected from beach waters, sand and nearby urban fecal pollution sources (e.g., wastewater, birds, pets). The MST results identified bird feces as an important source of fecal contamination at the beaches. These results were consistent with local knowledge of fecal pollution sources and observations of many gulls and geese (and their droppings) on the beaches. Results to date from multiple lines of evidence suggest bird droppings (gull and Canada geese) can be the prominent source of fecal pollution at times at these beaches.

Dr. Todd Howell, Ontario Ministry of the Environment, Toronto, Ontario, described fecal pollution problems at beaches along the Lake Huron shoreline (OMOE 2005). The southeast shoreline of Lake Huron, extending from the Ausable River to Point Clark, is the focus of a study of sources and pathways of fecal pollution. Recreational beaches are periodically posted as unsafe for swimming because of adverse water quality. Water quality appears to be highly variable among the beach areas monitored by the local Health Unit. The investigation of microbial pollution in this area extends back over 20 years. Land use in the tributary watersheds is dominated by agriculture with limited forest area and urban development. Elevated levels of fecal pollution indicators, namely *E. coli* and fecal coliforms, have been reported in numerous studies of tributary water quality. Previous studies have focussed on agriculture, faulty septic systems and sewage treatment plants as possible fecal pollution sources. Most of the tributaries that drain to the shoreline are small creeks that extend <10 km from the shoreline and are less than

5 km² in area. In contrast, there are a few large river systems that represent over 80% of the watershed area draining to the shoreline. The largest of these is the Maitland River which extends approximately 85 km from the shoreline and has a drainage area of almost 2600 km². Despite the undoubted importance of tributaries as sources of pollutants to the lake, there is limited understanding of the spatial extent or temporal frequency of the effects of these loadings on the occurrence of fecal pollution indicators in the nearshore. Seasonal and permanent homes relying on septic systems for waste disposal dot the shoreline of the lake in many areas. There has been limited investigation of the extent to which fecal pollution sources adjacent to shoreline beaches may be contributing to water quality problems. Wildlife, notably gulls and geese that occasionally frequent some beaches, has received little attention as a potential source in previous studies. Uncertainty remains as to the sources of fecal pollution to the lake that drive the adverse levels of fecal indicators periodically detected in the shoreline waters at these recreational beaches.

Dr. Vic Gannon, Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Lethbridge, Alberta, provided an MST case study for the Oldman River Basin in southern Alberta which is home to approximately 1.1 million head of cattle. The Little Bow River that runs through this basin is located 40 km downstream of the city of Lethbridge and passes through an area with many feedlots and high cattle density, where irrigation is used for field crops. This study sought to determine if there was a correlation between human and ruminant *Bacteroides-Prevotella* molecular markers and the presence of *E. coli* O157:H7 and *Salmonella* in the Little Bow River. Water samples from different sites were collected weekly through the summer months of 2002 and 2003 in the waters of the Little Bow River. The samples were cultured for *Salmonella* and *E. coli* O157:H7 and DNA extracted from sample filters was tested by PCR using the ruminant- and human-specific *Bacteroides-Prevotella* primer sets. The proportion of *E. coli* O157:H7 positive samples that tested positive for the ruminant markers was 90.9% (95% CI from 0.739 to 1.079, SE = 0.0867). In contrast, 44.8% (95% CI from 0.320 to 0.576, SE = 0.00426) of *Salmonella* positive samples tested positive for the ruminant markers. The odds of detecting the *Bacteroides-Prevotella* ruminant fecal markers when *E. coli* O157:H7 was present were 10 to 1 but was only 0.8 to 1 when *Salmonella* was present. The odds of detecting the human *Bacteroides-Prevotella* fecal markers when *E. coli* O157:H7 was present were 0 to 1 and 0.036 to 1 when *Salmonella* was present. Cattle and other ruminants are known to be reservoirs for *E. coli* O157:H7. As would be expected, the odds of detecting the ruminant marker when *E. coli* O157:H7 was present were high. In contrast, the *Bacteroides-Prevotella* host-specific markers used in the study did not predict the presence of *Salmonella*. This suggests a great diversity of host species sources for this pathogen in river water, e.g., many of the *Salmonella* serovars recovered suggest an avian origin and avian markers would have been missed using ruminant- and human-specific *Bacteroides-Prevotella* PCR assays.

5. Microbial Source Tracking Needs Assessment

Fecal contamination of aquatic ecosystems has resulted in adverse public health and economic consequences for many communities across Canada. This fecal contamination can originate from many possible sources, although municipal effluents, agricultural practices and wildlife populations represent the more common sources in many areas across Canada.

Municipal Effluents

Communities need to be able to track sources of municipal wastewater, combined sewer overflows and stormwater contamination quickly because of the relatively higher potential for the occurrence of waterborne pathogens of human health concern. This fecal pollution can come from inadequately treated effluents from sewage treatment plants,

or sewage treatment plant bypasses, and from stormwater and combined sewer overflows. Leaking septic tanks, and shipboard wastes or “grey water” can be other sources of human fecal contamination in aquatic ecosystems. One complication for microbial source tracking is that municipal wastewater may not contain microbial contaminants exclusively of human origin. Municipal wastewater can also contain fecal contamination from food processing activities, and from urban runoff sources like pets and urban wildlife.

Dr. Jiri Marsalek, National Water Research Institute, Environment Canada, Burlington, Ontario, provided a presentation on fecal pollution challenges in urban environments. Fecal contamination occurs frequently in urban waters as a result of discharges of various municipal effluents, among which wet-weather flows, stormwater and combined sewer overflows (CSOs) are particularly important. Both stormwater and CSO discharges can be highly contaminated with fecal bacteria and widely distributed throughout urban areas. As such, they need to be addressed in planning the protection of recreational waters. Stormwater characterization data indicate concentrations of *E. coli* or fecal coliforms in the range from 10^3 to 10^5 units per 100 mL. Such concentrations may be attenuated prior to discharge into open waters by stormwater management measures, or exceptionally by disinfection. The levels of indicator bacteria in CSOs are much higher than in stormwater, and can be as high as 10^6 *E. coli* per 100 mL. Consequently, the abatement of fecal contamination of CSOs is now considered in the design of CSO control and treatment, as stipulated in the Ontario Interim Directive F-5-5 for CSO abatement. In some cases (e.g., the Toronto Waterfront), the abatement of fecal contamination of receiving waters is among the primary drivers behind the often costly CSO abatement programs. CSO abatement options comprise combinations of storage and treatment, in which the CSO treatment train generally includes disinfection, particularly where CSO outfalls are located upstream of recreational waters. Indicator bacteria data from studies in the Upper Great Lakes Connecting Channels (Ontario) were used to demonstrate fecal contamination impacts of wet-weather flows.

Agriculture

While it is possible to treat livestock fecal wastes effectively, and apply manure to agricultural lands safely, poor farming practices, or storms and surface water runoff can result in fluxes of fecal pollution downstream into aquatic ecosystems. Communities need to be able to track sources of livestock fecal pollution quickly to prevent contamination of source waters used for drinking water, irrigation or recreation. Increasingly intensive rearing practices for livestock animals like cattle, hogs and poultry will present significant animal waste management challenges in the future. Management of aquatic ecosystems in agricultural watersheds will need to consider potential livestock fecal pollution sources (e.g., droppings on pastures, manure lagoons) and the timing of events like manure spreading when investigating potential fecal pollution sources.



Katrin Nagelschmitz, Strategic Policy Branch, Agriculture and Agri-Food Canada, provided a presentation outlining the diversity and numbers of livestock in agricultural areas across Canada. Livestock numbers have increased in Canada over the last decades. However, the impact of this trend differs across the country. The change in livestock densities and in manure production varies among regions. Technological and structural changes in the livestock sector are causes of the development. The trend is towards specialized larger farms employing a smaller immediate land base.

Wildlife

Wildlife can present an unpredictable and difficult fecal source tracking challenge, that is not so amenable to control and familiar waste treatment practices. Notable are the growing numbers of birds such as gulls and Canada geese in many areas across Canada. Where aquatic ecosystems occur near large wildlife populations (e.g., bird colonies), consideration needs to be given to monitoring wildlife populations, their fecal droppings, and their seasonal migrations or behaviour characteristics that could contribute to fecal contamination.

Dr. Tom Edge, National Water Research Institute, Environment Canada, Burlington, Ontario, provided a substitute presentation on wildlife fecal contamination sources. Fecal pollution from wildlife species has been shown to contribute to impairment of recreational waters in areas across Canada. For example, fecal droppings from birds along beaches or from birds roosting under bridges can lead to significant increases in waterborne fecal indicator bacteria. In some areas, efforts to enhance biodiversity habitat and establish buffer strips along streams may also facilitate increased loadings of fecal pollution from wildlife. MST studies need to evaluate wildlife species as possible sources of fecal pollution, and to consider the significance of local wildlife populations such as aquatic mammals or birds (e.g., gulls and geese), and the timing of wildlife movements and migrations.

6. MST “Drivers” in Canada

Microbial source tracking has been slower to develop in Canada than in the United States. The U.S. Clean Water Act requirements for calculating Total Maximum Daily Loads for fecal contaminants in watersheds (specifying that waters be “swimmable” and “fishable”) have been driving much of the application of MST methods in the United States, and have forced a “top-down” drive to perform MST studies. Similarly, risk-based initiatives in the European Union and in Australia provide much of the impetus for MST research. However, Canada does not have equivalent legislation or initiatives, and applications of MST have been driven more by a “bottom-up” approach stemming from local community concerns for fecal pollution problems like beach and shellfish closures. It is these types of environmental-related issues that provide the opportunity to further refine the science in Canada while meeting specific user needs. With aging municipal wastewater infrastructure and increasingly intensive urbanization and livestock production capacity across Canada, needs will grow for tools to prevent and resolve fecal pollution problems.



A major objective of the workshop was to get feedback from practitioners (policy and program managers) on their needs related to tracking sources of fecal contamination. Consequently, a group of potential MST “users” from all sectors formed a panel invited to offer their opinions on the relevance of MST. They were asked to outline their current issues, challenges and needs with respect to waterborne pathogen risk in their jurisdiction and the utility of MST. The comments below are a synthesis of key observations from panelists and the general discussion that followed among all workshop participants.

Implementation of Microbial Water Quality Guidelines, Objectives and Regulations

In Canada, all levels of government play a role to make sure water supplies are safe. Provincial and territorial governments are in charge of setting and enforcing standards to ensure drinking water safety. Local health authorities also monitor water quality, such as at public beaches. At the federal level, Health Canada works with other provincial and territorial agencies to develop and publish national guidelines such as the *Guidelines for Canadian Drinking Water Quality* and the *Guidelines for Canadian Recreational Water Quality*. These Guidelines are used by provinces and territories to help set safe microbial water quality standards to ensure that drinking and recreational waters do not present unacceptable health risks to the public. For example, the *Guidelines for Canadian Recreational Water Quality* recommend conducting an environmental health assessment or sanitary survey at the beginning of each bathing season, paying special attention to aspects like fecal pollution. In addition, Environment Canada works with other government agencies to implement the microbial water quality surveillance of the Canadian Shellfish Sanitation Program. In order to meet federal, provincial, territorial and municipal microbial water quality guidelines, objectives and regulations, MST studies may be required to help resolve specific fecal pollution problems.

Agricultural Policy Framework

Federal, provincial and territorial governments have been working with the agriculture and agri-food industry since 2001 to help strengthen and revitalize the sector through a new Agricultural Policy Framework (APF) for Canada. The APF is targeted at making Canada the world leader in food safety, innovation and environmentally responsible production. As part of the APF, a multi-year water quality surveillance initiative is being led by Health Canada and Agriculture and Agri-Food Canada to develop a better understanding of the impact of agriculture on the prevalence, levels and sources of microbiological (fecal) contamination at drinking water supply intake points and recreational beaches at selected sites across Canada. This initiative involves a large multi-year study evaluating different MST methods in agricultural watersheds across Canada, and was described earlier in the workshop by Dr. Ed Topp. Environment Canada and Agriculture and Agri-Food Canada are also working under the APF to establish standards for the occurrence of waterborne pathogens in agricultural watersheds under the National Agri-Environmental Standards Initiative (NAESI).

Canada-wide Municipal Wastewater Strategy

Under the Canadian Council of Ministers of the Environment, provinces, territories and the federal government are currently developing a Canada-wide Strategy for the Management of Municipal Wastewater Effluent. In addition to harmonizing the regulatory framework and developing risk management models, the Strategy will help co-ordinate the relevant science and research agenda. On this last item, the Strategy will review the state of knowledge on science and technology, assess the need for action on emerging issues and recommend approaches to fill information gaps. This initiative may provide some opportunity to elevate the issue and importance of microbial source tracking to help target the cost-effective abatement of fecal pollution from municipal wastewaters.

Local Community Management and Abatement of Fecal Pollution

Workshop panelists and participants suggested that perhaps the most compelling utility for MST is to target management and abatement options more effectively. For example, the Great Lakes Water Quality Agreement identifies Areas of Concern and calls on agencies to develop remedial action plans and contribute to delisting beneficial use impairments for aspects such as beach closures. For most municipalities there is simply insufficient funding for widespread abatement of fecal contamination problems on popular beaches. Major investments in wastewater and stormwater infrastructure are often predicated on their ability to reduce the number of summer beach closures, although in

practice this has not always happened. Clearly the management response could be swifter and the potential financial savings greater if one has a clearer picture of whether fecal contamination at a given beach originated in an upstream agricultural area, from municipal point sources, or from wildlife like gulls or geese on the beach. For many municipalities, simply knowing whether or not the source of fecal pollution is human or non-human can provide useful guidance. Similarly, conservation authorities in Ontario, for example, could better target agricultural non-point source abatement if they knew which animals were the primary source of a given fecal pollution problem. Although we may not yet be at the stage where MST can provide a reliable and practical “off-the-shelf” tool for broad application, it can be useful on a local basis for helping to target a fecal pollution management response, prioritize abatement options, and guide needs for any infrastructure investment.

MST Education and Awareness

MST is thought to have a role to play in helping educate citizens on the hazards of microbial pollution. This is often the case where source tracking data can be useful to help convince doubtful contributors of their role in fecal pollution. As noted above, MST can then help provide contributors with an indication of the tangible benefits of microbial hazard reduction associated with a range of best management practices. More broadly, MST can inform the discussion of new shoreline projects where habitat rehabilitation and bird re-colonization is planned in areas of high human beach use.

Guidance on Source Water Protection Planning

To help protect drinking water, many provincial governments are pursuing legislation and/or guidance on developing watershed-based source water protection plans. Many of these plans suggest that municipal source waters should be characterized microbiologically, in some cases delineating pathogen risk zones, in addition to specifying ways that BMPs can be used to reduce loading of pathogens. In the case of the Province of Ontario, it is anticipated that issue-specific guidance material would be provided to help municipalities develop these plans, and it is possible that MST studies could be a component of a pathogen-based guide document.

7. Conclusions

Early microbial source tracking studies in the late 1990s raised considerable expectations for MST tools to resolve complex fecal pollution source tracking problems. More recent laboratory studies, with carefully controlled experimental designs, have pointed to limitations in existing MST methods (Griffith et al. 2003; Stoeckel et al. 2004). These recent studies were perceived to have placed a “wet blanket” on microbial source tracking, particularly for studies of large watersheds with complex sources of fecal contamination. However, the field of microbial source tracking is evolving, and novel MST tools resulting from scientific advances in areas like genomics (e.g., DNA microarrays) may enlarge the MST toolbox in the future. In addition, cases where MST methods have been applied successfully on a local basis continue, and they were instrumental in leading to effective remedial clean-up actions. For example, a case study was presented at the workshop where antibiotic resistance analysis (ARA) found an unexpected human fecal pollution source, pulsed-field gel electrophoresis (PFGE) confirmed it, and fluorometry tracked it to its source.

The Workshop recognized that while many methods exist in the current MST toolbox, there is no “silver bullet,” universally accepted best method. While some methods have achieved a level of maturity where they could be considered for standardization, others are still experimental or research-grade tools. Additional research is required to understand better the advantages and limitations of microbial source tracking methods,



and wider application of the tools will require standard methods and careful consideration of appropriate experimental designs. Library-dependent MST methods require a library of an appropriate size and representation, which still remains to be determined. Library-independent MST methods require validation of host-specific markers in field studies.

Applications of MST in Canada have been driven more by a “bottom-up” approach from local community concerns for beach and shellfish closures, than by “top-down” regulatory needs such as in the United States. Although we are not yet at the

stage where there is an “off-the-shelf” MST tool for broad application, MST methods have been shown to be useful on a local basis for helping to target management responses, prioritize abatement options and guide investment. The Workshop placed emphasis on applying MST methods as part of multiple lines of evidence to resolve fecal pollution source tracking problems. While MST tools can be useful for addressing fecal pollution source tracking problems, it will be important to ensure realistic expectations among the end-user communities for MST studies in the near future.

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Appendix A - Workshop Agenda

Linking Water Science to Policy Workshop

Microbial Source Tracking in Aquatic Ecosystems: The State of the Science and an Assessment of Needs

Council Chambers, Toronto Metro Hall
55 John Street, Toronto, Ontario
March 7-8, 2005

DAY 1 – “MST Science Assessment”

8:30 Welcome

MST Overview

8:35 Workshop overview
Dr. Tom Edge, Environment Canada, National Water Research Institute, Burlington, Ontario

9:00 Towards the identification of fecal pollution sources
Dr. Jorge Santo Domingo, U.S. Environmental Protection Agency, Cincinnati, Ohio

9:30 FST needs for WHO Water Safety Plans: chemicals or microbials?
Dr. Nick Ashbolt, University of New South Wales, Sydney, Australia

10:00 Coffee

MST Science Assessment (state of science and readiness for widespread application)

10:30 National Water Quality Surveillance Program: A research platform for MST
Dr. Ed Topp, Agriculture and Agri-Food Canada, London, Ontario

11:00 2003 SCCWRP MST Method Evaluation Study: Validation or Calibration?
Dr. John Griffith, Southern California Coastal Research Project, Westminster, California

11:30 Evaluation of library-dependent, *Escherichia coli*-based microbial source tracking
Dr. Don Stoeckel, U.S. Geological Survey, Columbus, Ohio

12:00 Lunch

13:00 Use of microarrays for bacterial source tracking: problems and perspectives
Dr. Roland Brousseau, National Research Council, Montreal, Quebec

13:30 Using the Waterborne Parasites *Cryptosporidium* and *Giardia* as Microbial Source Tracking Agents
Dr. Norm Neumann, Provincial Lab, Calgary, Alberta

14:00 Source-tracking: moving away from microbes
Dr. Pierre Payment, Institut national de la recherche scientifique (INRS), Laval, Quebec

14:30 Coffee

MST Case Studies (evaluation of MST challenges and limitations)

14:45 Identifying, Confirming, and Mitigating Sources of Fecal Pollution in Water
Dr. Chuck Hagedorn, Virginia Tech, Blacksburg, Virginia

15:30 Beach closure case study (small-scale urban)
Ted Bowering, Toronto Water and Dr. Tom Edge, NWRI, Burlington, Ontario

15:50 Fecal Pollution of Shoreline Waters of Southeastern Lake Huron: The challenge of understanding where it comes from
Dr. Todd Howell, Ontario Ministry of the Environment, Toronto, Ontario

16:10 Association Between Zoonotic Bacterial Pathogens and *Bacteroides-Prevotella* Human and Ruminant Faecal Markers in the Oldman River Basin in Southern Alberta
Dr. Vic Gannon, Public Health Agency of Canada, Lethbridge, Alberta

16:30 – 17:00 Plenary discussion of case studies

DAY 2 – “MST Needs Assessment”

Summary of the State of the Science

- 8:30 Short summary of the state of the science (based on Day 1)
- 9:00 Results from the WERF MST workshop February 16-18, 2005
Dr. Paul Rochelle, Metropolitan Water District of Southern California, La Verne, California
Plenary discussion of the state of MST science
- 10:00 Coffee

Fecal Pollution Source Tracking Needs Assessment (trends, challenges, mitigation)

- 10:30 Assessment of urban wet-weather flow sources of fecal contamination: stormwater and combined sewer overflows (CSOs)
Dr. Jiri Marsalek, Environment Canada, National Water Research Institute, Burlington, Ontario
- 11:00 Distribution and Population Trends of Canadian Livestock
Katrin Nagelschmitz, Strategic Policy Branch, Agriculture and Agri-Food Canada
- 11:30 Wildlife source assessment – (TBC)
- 12:00 Lunch
- 13:00 **Panel on MST Water Quality “Drivers” in Canada**
Short (5 minute) presentations & discussion on fecal pollution source tracking needs, pressures, regulatory “drivers” and/or research needs from the municipal, conservation authority, provincial and federal government perspectives.

Panel Participants

- Will Robertson - Water Quality and Health Bureau, Health Canada
- TBC - Agriculture and Agri-Food Canada
- TBC - Environment Canada
- Renée Bowler - Drinking Water Management Division, OMOE
- Norm Neumann - Provincial Lab, Alberta
- Michael D’Andrea - Toronto Water, City of Toronto
- Tim Van Seters - Water Quality and Monitoring, TRCA

Next Steps

- 14:30 Workshop summary and identification of next steps
- 15:00 Workshop wrap-up

Appendix B - List of Workshop Participants

** indicates speaker/panelist; ** workshop organizing committee; *** workshop sponsor*

Provinces

Kevin Rieberger, B.C. Min. of Water, Land & Air Protection
Norm Neumann *, Prov. (Alberta) Lab. for Public Health
Wendy Ralley, Manitoba Water Stewardship
John Lynch, Ontario Ministry of the Environment
Susan Weir, Ontario Ministry of the Environment
Todd Howell * ** ***, Ontario Ministry of the Environment
Brian Whitehead, Ontario Ministry of the Environment
Ted Briggs, Ontario Ministry of the Environment
Renée Bowler *, Ontario Ministry of the Environment
Cecily Flemming, Ontario Ministry of the Environment
Ann Marie Weselan, Ontario Ministry of the Environment
Stewart Sweeney *, Ontario Ministry of Agriculture & Food
John Drage, Nova Scotia Environment & Labour
Martin Goebel, Newfoundland and Labrador Department of Environment and Conservation

Ontario Conservation Authorities

Mari Veliz, Ausable Bayfield Conservation Authority
Karen Maaskant, Upper Thames River Conservation Authority
Sandra Cooke, Grand River Conservation Authority
Tim Van Seters *, Toronto & Region Conservation Authority

Municipal/Regional Governments

Pat Chessie ** ***, City of Toronto
Ilze Andzans, City of Toronto
Ted Bowering *, City of Toronto
William Snodgrass, City of Toronto
Peter Gauthier, City of Toronto
Mahesh Patel, City of Toronto
Janis Cumin, City of Owen Sound
Jason Culp, City of St. Catharines

Federal Departments

Teresa Brooks, Health Canada
Will Robertson * **, Health Canada
Vic Gannon * **, Public Health Agency of Canada
Ed Topp * **, Agriculture and Agri-Food Canada
Katrin Nagelschmitz *, Agriculture and Agri-Food Canada
Roland Brousseau *, National Research Council of Canada
Tom Edge * ** ***, Environment Canada-NWRI
Karl Schaefer **, Environment Canada-S&T Liaison Division
Rob Phillips, Environment Canada-NWRI
Jim Maguire, Environment Canada-NWRI
Jiri Marsalek *, Environment Canada-NWRI

Allan Crowe, Environment Canada-NWRI
Jim Sherry, Environment Canada-NWRI
Heather Osachoff, Environment Canada-PYR
M.T. Grant, Environment Canada-AR
Sandra Kok ** ***, Environment Canada-OR-GLSF
Thomas Tseng, Environment Canada-OR
Janette Anderson, Environment Canada-OR
Lorraine Standing, Environment Canada-EPS
Elaine McKnight, Environment Canada-EPS

Professional Associations

Catherine Jefferson, Canadian Water & Wastewater Association
Cindy Toth, Water Environment Association of Ontario & Town of Oakville
Ian Mcilwham, Water Environment Association of Ontario & Region of Durham
Mary Jane Conboy, Ontario Federation of Agriculture
Sarah Winterton, Environmental Defence
Jeff Ridal, St. Lawrence River Institute of Environmental Sciences
Keith Sherman, Severn Sound Environmental Association

Academics

Greg Bezanson, Acadia University
Pierre Payment *, Institut national de la recherche scientifique (INRS)
Corinne Ong, University of British Columbia
Richard Nordin, University of Victoria
Shu Chen, University of Guelph
Kari Dunfield, University of Guelph
Michele Van Dyke, University of Waterloo

International

Don Stoeckel *, U.S. Geological Survey
John Griffith *, Southern California Coastal Water Research Project
Mike Jenkins *, U.S. Department of Agriculture
Cindy Nakatsu, Purdue University
Valerie J. Harwood *, University of South Florida
Charles Hagedorn *, Virginia Tech
Paul Rochelle *, Metropolitan Water District of Southern California
Nick Ashbolt *, The University of New South Wales