

# CHAPTER 1

## INTRODUCTION TO WORKSHOP

### RATIONALE

The primary reason for organizing this workshop was to address some of the issues related to evaluating and monitoring the quality of recreational waters in tropical locations based on U.S. Environmental Protection Agency water quality standards and guidelines. Over the years, studies conducted by researchers at universities in Hawaii, Guam, Puerto Rico, and south Florida have obtained evidence that the EPA-recommended recreational water quality standards may be ineffective in determining when tropical environmental waters are contaminated with sewage. Based on the published study results as well as discussions in various forums, EPA now recognizes that a potential problem may exist in assessing water quality in tropical locations of the United States. Concerns and future course of actions on this issue were published in the EPA Action Plan for Beaches and Recreational Waters (EPA/600/R-98/079) in 1999. The section describing the tropical indicator problem is reproduced below:

#### Tropical Indicators

Currently recommended fecal indicators may not be suitable for assessing human health risks in the tropics. Studies have suggested that at tropical locales such as Puerto Rico, Hawaii, and Guam, *E. coli* and enterococci can be detected in waters where there is no apparent warm-blooded animal source of contamination.

Whether or not current indicator bacteria proliferate naturally in soil and water under tropical conditions must be determined. If so, the range of conditions (such as nutrients, temperature, pH and salinity) under which the bacteria proliferate will be characterized and their geographical boundaries defined. If the phenomenon is widespread under tropical conditions, additional research will be conducted to modify approaches for monitoring, or to develop new tropics-specific indicators. Further evaluation of *Clostridium perfringens* and other microbial indicators (including coliphages) that do not flourish naturally in the tropics will be conducted to determine their usefulness as alternative indicators.

### ORGANIZATION

#### Funding

To address the need identified in the EPA action plan, Dr. Roger Fujioka of the Water Resources Research Center at the University of Hawaii applied for EPA funding to hold a workshop in Hawaii for the purpose of reviewing all of the existing data related to

application of the EPA recreational water quality standards in tropical areas. Initiation of this workshop was supported by the Department of Health, State of Hawaii. As a result, before this workshop was funded, considerable discussion took place among representatives of the University of Hawaii, Hawaii Department of Health, EPA Office of Science and Technology in Washington, DC, and EPA Region IX office in San Francisco. Primary funding for the workshop was provided by the U.S. Environmental Protection Agency, Office of Water. Matching funds were provided by the Department of Health of the State of Hawaii and by the Water Resources Research Center of the University of Hawaii. Fujioka served as principal coordinator for this project, and Mr. Rick Hoffmann of EPA served as the project program officer.

## **Formation of Planning Committee**

A planning committee was formed to establish the goals, format, and scope for the workshop. Based on previous experience, the ideal number of members for a planning committee for this type of project was determined to be five. Criteria for selection of members for this planning committee included key individuals involved in funding this study, scientists involved in conducting the key experiments in tropical locations, and those who could provide technical oversight. The seven people selected to serve on the planning committee are as follows:

1. Roger Fujioka, University of Hawaii, Honolulu, Hawaii
2. Alfred Dufour, EPA Research Division, Cincinnati, Ohio
3. Steve Schaub, EPA, Office of Water, Washington, DC
4. Rick Hoffmann, EPA, Office of Water, Washington, DC
5. Fred Genthner, EPA, Gulf Breeze Lab, Gulf Breeze, Florida
6. Gary Toranzos, University of Puerto Rico, San Juan, Puerto Rico
7. Eugene Akazawa, Hawaii Department of Health, Honolulu, Hawaii

## **Planning Guidelines**

The role of the planning committee was to establish the guidelines and agenda for the workshop. Most of the planning sessions were conducted through conference telephone calls, individual calls, and e-mail. The committee recommended the following guidelines:

1. The format should be a two-day workshop to be held in Hawaii. Thus, principal coordinator Fujioka scheduled the workshop for March 1–2, 2001 at the Waikiki Beach Marriott Resort in Honolulu.
2. The charge of this workshop would be to address scientific issues and data related to the problem of appropriate water quality standards for tropical locations. It was clarified that the impact of these findings on the regulations would be addressed but that questions from the regulatory point of view would not be pursued. Thus, the issues to be addressed would include an assessment of the environmental sources of fecal indicator bacteria as well as their persistence and multiplication in tropical areas.

3. Up to 20 experts would be invited to the workshop to allow a sufficient number of experts to effectively evaluate the proposed problem and still allow for effective discussions and interactions to reach consensus conclusions. The criteria for selection were based on the person's established professional reputation and expertise in water quality microbiology and on the person having some applicable working knowledge of water quality problems in tropical areas. The committee recommended that 18 national and international experts be invited. All 18 experts accepted the invitation. The name, address, and picture of each of the 18 invited experts are provided in Appendix A.
4. Recognizing that regulators and other members of the community would be interested in this workshop, a decision was made that a limited number (not to exceed the number of experts) of observers would be invited under agreed-upon guidelines. "Observers" were defined as those whose primary interest and experience represented a regulatory perspective or an environmental perspective. These observers would not be allowed to take part in the workshop deliberations but would be allowed to submit questions, which could be considered for discussion by the workshop experts. The name and address of each of the 14 observers who attended the workshop are provided in Appendix B.

## **IMPLEMENTATION**

### **Goal and Objectives**

The overall goal of this workshop was to address issues identified under the "Tropical Indicators" section of the EPA Action Plan for Beaches and Recreational Waters (EPA/600/R-98/079). To meet this goal, the following objectives were defined:

1. To critically evaluate published findings and monitoring data related to sources, persistence, and multiplication of EPA-approved fecal indicators (fecal coliforms, *E. coli*, enterococci) in tropical locations and the impact of such findings on the suitability of existing water quality criteria for these locations.
2. To critically evaluate published reports and other kinds of monitoring data related to use of alternative water quality indicators in tropical locations.
3. To reach conclusions on the identified problems and to provide suitable recommendations to address these problems. In this workshop, concluding statements were made as part of the final group discussion. All experts then voted to approve or not approve each concluding statement. After the voting was completed, the concluding statement was called the consensus statement. By definition, a consensus statement is a general agreement or collective agreement by the majority. The planning committee did not define what minimum percent approval (e.g., 75%) would constitute a consensus. At this workshop, the percentage of experts who approved the concluding statement was calculated, and this was taken as strength of consensus. The consensus statements represent general or collective opinion of workshop participants but not 100 percent agreement in most cases.

## Guidelines for Invited Experts

To help the experts prepare for this workshop, each was sent a packet of relevant research papers (see Appendix C) on current tropical water quality issues. This collection of research papers and reports contained data relevant to water quality issues to be discussed at the workshop. The invited experts were also sent a guidance document (see Appendix D) containing detailed information about the objectives and format of the workshop. The guidance document included five major questions as well as related questions of concern. These questions were formulated to guide the invited experts in their reading and evaluation of the documents sent to them. Listed below are the five major questions:

1. Are there sufficient experimental and monitoring data to conclude that the assumption used in interpreting water quality standards (there are no significant environmental sources of fecal coliforms, *E. coli*, and enterococci) is not applicable in tropical areas (Hawaii, Guam, Puerto Rico, south Florida) because these bacteria can be recovered in high concentrations from ambient environments (water, soil, plants) in these areas?
2. Are there sufficient experimental and monitoring data to conclude that the EPA criteria (*E. coli*, enterococci) used to assess the quality of environmental waters are not reliable in tropical locales (Hawaii, Guam, Puerto Rico, south Florida) because the selected fecal bacteria persist in these ambient environments and represent non-fecal contamination?
3. Are there sufficient experimental and monitoring data to conclude that the EPA-recommended recreational water quality standards are not suitable to assess the hygienic quality of environmental waters in Hawaii, Guam, Puerto Rico, and south Florida?
4. Are there sufficient experimental and monitoring data to conclude that fecal indicator bacteria (fecal coliforms, *E. coli*, enterococci) can multiply in tropical environments and that bacteria from these sources are indicative of lower health risk than those from fecal sources?
5. Are there sufficient experimental and monitoring data to conclude that the proposed alternative criteria and recreational water quality standards for Hawaii and Puerto Rico are more useful than the current EPA criteria and standards?

## Agenda and Format

The invited experts were initially sent the guidance document and relevant research papers/documents. Subsequently, the workshop agenda (see Appendix E) was sent to the invited experts as well as to the invited observers. Both the guidance document and the agenda contained the five major questions to be addressed at the workshop. These five questions were formulated to address specific problems that needed to be assessed and to be answered in some form of concluding statements.

The format for this two-day workshop was to convene sessions to address the five questions. Questions one, two, and three would be addressed during sessions on day one. These three questions were identified as basic questions, which needed to be answered first. Moreover, answers to these three questions were not expected to require complicated experimental data. Questions four and five would be addressed during sessions on day two. These last two questions were identified as more difficult because their answers were expected to require deliberation based on more complicated experimental data.

As session chair for both morning sessions, Fujioka initially reviewed the guidelines, objectives, and expectations for that day. He introduced each of the speakers of the respective morning sessions, which were devoted to the presentation and discussion of published data that addressed the objectives for that day. He also managed the question and answer sessions.

The focus of each afternoon session was to discuss the presented data and to deliberate on the questions assigned for that day. Since this part of the workshop required unbiased deliberations, acknowledged leaders whose place of residence was not in the tropical region were selected to chair the respective afternoon sessions. Dr. Charles Gerba was selected to chair the afternoon session for the first day and Dr. James Tiedje was selected for the second day. The afternoon session chair initially allowed all the experts to discuss the issues raised during the morning session for approximately 30 minutes. Following that discussion, the chair guided the experts to craft concluding statements and then to vote to agree or disagree with each concluding statement. The final statements, which were accepted by a large majority of the experts, were considered to be the consensus statements. The consensus statements represent general or collective opinion of workshop participants but not 100 percent agreement in most cases. During the last session of the workshop, recommendations and future courses of actions were developed. Staff members of the Water Resources Research Center tape-recorded the entire deliberations of the workshop, took notes, and recorded the final votes.

## **Final Report**

As coordinator of the workshop, Fujioka is responsible for writing the final report, the purpose of which is to accurately document the entire workshop proceedings and to highlight the consensus statements and recommendations made by the workshop experts. To expedite completion of the final report, the planning committee reviewed the first draft and made suggestions that were incorporated into the second draft. The second draft was then sent to all workshop experts for their review and subsequent input. The third draft was then submitted to EPA and to the Hawaii Department of Health for approval. The final report represents the fourth revision of the review process. Copies will be sent to the funding agencies (EPA, Hawaii Department of Health, and Water Resources Research Center); all experts who participated in the workshop; appropriate offices of EPA; water agencies responsible for managing the recreational waters in Hawaii, Guam, Puerto Rico, and Florida; and other relevant individuals and organizations.



# CHAPTER 2

## PRESENTATIONS BY INVITED EXPERTS: DAY ONE

### PROBLEM STATEMENTS

A guidance document (see Appendix D) sent to all experts to help them prepare for the workshop included questions that contained problem statements to be addressed during the workshop. The three basic questions addressed during the first day of the workshop are restated below:

1. Are there sufficient experimental and monitoring data to conclude that the assumption used in interpreting water quality standards (there are no significant environmental sources of fecal coliforms, *E. coli*, and enterococci) is not applicable in tropical areas (Hawaii, Guam, Puerto Rico, south Florida) because these bacteria can be recovered in high concentrations from ambient environments (water, soil, plants) in these areas?
2. Are there sufficient experimental and monitoring data to conclude that the EPA criteria (*E. coli*, enterococci) used to assess the quality of environmental waters are not reliable in tropical locales (Hawaii, Guam, Puerto Rico, south Florida) because the selected fecal bacteria persist in these ambient environments and represent non-fecal contamination?
3. Are there sufficient experimental and monitoring data to conclude that the EPA-recommended recreational water quality standards are not suitable to assess the hygienic quality of environmental waters in Hawaii, Guam, Puerto Rico, and south Florida?

### PRESENTATION BY DR. BRUCE ANDERSON

Department of Health, State of Hawaii

**Title:** Perspective of a regulator and policy maker

#### **Speaker information**

Dr. Bruce Anderson is director of Health for the State of Hawaii and, as such, is the top health official in the state. His responsibilities include supervision over four major programs headed by deputy directors of Health, Behavioral Health, Health Resources, and Environmental Health. Prior to being appointed director Anderson served for many years as

deputy director of Environmental Health and in that capacity, managed all of the state's water programs. He earned a Ph.D. in epidemiology from the School of Public Health, University of Hawaii. His professional training and his experience in Hawaii has enabled him to understand problems related to water quality in Hawaii as well the scientific basis by which EPA established water quality standards for the nation. Anderson is credited with establishing the current marine recreational water quality standard for Hawaii (7 CFU enterococci/100 mL), the most stringent standard in all U.S. states and territories. He is also credited with speaking directly with Mr. Chuck Fox of EPA to identify the need for EPA to support this Tropical Water Quality Indicator Workshop.

### **Presentation objectives**

Anderson gave the opening remarks and welcomed all participants to the workshop. He set the stage for this workshop by providing his perspective as a policy maker and regulator who must understand the scientific principles by which water quality standards are developed.

### **Major points**

1. Anderson thanked members of the planning committee for making this workshop a reality. He specifically acknowledged the many years of contributions made by Dr. Roger Fujioka in defining water quality issues in Hawaii and in identifying the need for reliable water quality standards for Hawaii. He acknowledged the useful role Fujioka has played in communicating Hawaii's concerns to EPA and in coordinating this workshop.
2. Anderson reviewed the basic problem of applying the EPA water quality standards in Hawaii, which he described as the detection of *E. coli* and enterococci in environmental waters where there is no evidence of fecal contamination by humans and warm-blooded animals. He pointed out that elevated counts of fecal indicator bacteria in the coastal waters of Hawaii correlated with rainfall and increased volumes of land-based discharges (streams, storm drains) into coastal waters.
3. Based on monitoring data in Hawaii, Anderson stated that the fecal indicators used to establish recreational water quality standards are not reliable indicators of risk.
4. Anderson agreed with the strategy of this workshop that scientists would be deliberating on the scientific data to establish recreational water quality standards for tropical areas and would not focus on the regulatory issues. However, he made a statement that "risk should be addressed by scientists, but management of risk should be addressed by enlightened policy-makers". He elaborated on this statement by pointing out that the scientists at this workshop are charged with addressing the risks as defined by use of indicators. As a policy maker and regulator, he is charged with managing the defined risk. He pointed out that these two activities or functions should not be entirely separated. He also emphasized that scientists should know the management issues so they can focus on addressing appropriate and relevant questions. Lastly, he emphasized that regulators need to know the limitations of science so they can appropriately judge the degree of risk in their decision-making.

## Conclusions and recommendations

1. Hawaii needs a more reliable water quality standard than the existing standard to accurately reflect the level of risk from swimming in marine waters contaminated with fecal material. He stated that results of monitoring environmental waters for *C. perfringens* indicate that this bacteria is a more reliable indicator of fecal contamination in Hawaii than *E. coli* or enterococci. He asked the experts to evaluate the usefulness of *C. perfringens* as an alternative indicator of water quality.
2. Hawaii needs a water quality standard that accurately reflects the risk of non-point source pollution. There is a need to identify the pathogens from non-point sources as well as their mode of transmission to swimmers of recreational waters.
3. Hawaii needs a standard that will apply to non-enteric bacteria, such as staphylococcus (*S. aureus*), in marine waters. Anderson stated that skin infections by staphylococcus represent the highest incidence of swimming-related illnesses in Hawaii. He stressed that there is no water quality standard for waterborne staphylococcus, no standardized method for measuring staphylococci and *S. aureus* in water, and no reliable surrogate test for this pathogen. He pointed out that many people who carry staphylococci on their skin and in their nasal cavity will release these bacteria into the water where they swim. Staphylococci bacteria are also known to survive well in marine waters. Based on these observations, Anderson stated that the risk of contracting staphylococcus skin infections related to swimming and other uses (surfing, paddling) of marine waters is too high to ignore. He stressed that reliable water quality standards are required to manage this significant problem in Hawaii.

## PRESENTATION BY DR. ALFRED DUFOUR

EPA, Cincinnati, Ohio

**Title:** Selection of fecal indicators and establishment of current recreational water quality standards based on measurable risk

### Speaker information

Dr. Alfred Dufour is an established and recognized expert microbiologist at EPA. He has been involved in the historical development of recreational water quality criteria by EPA. He and Dr. Victor Cabelli of EPA are credited with conducting the epidemiological and water quality studies that led to the current EPA recreational water quality standards.

### Presentation objectives

The objectives were to review how water quality standards were initially developed in the United States and then to explain the theory, experimental design, and data resulting in the development of the current EPA recreational water quality standards.

## Major points

1. The American Public Health Association adopted a bathing water classification scheme in 1943. At that time, total coliform counts ( $>1,000$  MPN/100 mL) were used to separate high-quality from poor-quality bathing waters. In the late 1940s, studies conducted at three sites by the United States Public Health Service determined that an excess of swimming-associated gastroenteritis occurred when the total coliform density approached or exceeded 2,300 MPN/100 mL.
2. In the 1960s, EPA recognized that methods to measure the total coliform group of bacteria resulted in very high and variable numbers. Moreover, some bacteria measured as coliforms were naturally present in soil or plants and therefore were not reliable indicators of fecal contamination. To eliminate the measurement of environmental sources of total coliform, a new monitoring test was developed to enumerate densities of coliforms, which multiply at an elevated incubation temperature of  $44.5^{\circ}\text{C}$ . Under this condition, many of the environmental (soil, plants) sources of coliform bacteria, which were enumerated by the total coliform assay at  $37^{\circ}\text{C}$ , were not enumerated at  $44.5^{\circ}\text{C}$ . The subgroup of coliform bacteria, which were enumerated at  $44.5^{\circ}\text{C}$ , was called “fecal coliforms” because this subgroup had growth characteristics similar to those multiplying in the intestine of warm-blooded animals and better represented coliform bacteria found in feces. EPA concluded that this new monitoring method, called the fecal coliform assay, was a better measurement of fecal contamination than the total coliform assay method. This led EPA to change the recreational water quality standards based on concentrations of total coliforms to fecal coliforms. Since the established ratio of fecal coliforms to coliforms in sewage is about 1:5 or 18%, EPA assumed that a health effect would be detected in recreational waters when the density of fecal coliforms exceeded 400 CFU/100 mL. Since water quality standards should be established below detectable health risk and should include some safety factor, the revised recreational water quality standard was established at geometric mean density of 200 CFU/100 mL. EPA implemented this new fecal coliform standard in 1976. However, subsequent studies showed that the source of some fecal coliforms was not feces and did not represent fecal contamination. This led to two developments. First, many scientists concluded that fecal coliforms is a misleading term and that thermotolerant coliforms would be a more accurate term. Second, additional studies were required to find better indicators of fecal contamination and risks to waterborne diseases.
3. Between 1972 and 1982, EPA conducted a series of studies examining the relationship between swimming-associated illnesses and water quality at several marine and freshwater sites impacted by point source (sewage) pollution. The study design of these studies was superior to those conducted previously. It was able to analyze the water for a large number of potential microbial indicators of water quality to determine which exhibited the best relationship to the rate of swimming-associated gastroenteritis. Correlation analyses were performed on the swimming-associated gastroenteritis rates with the corresponding geometric mean densities of each microbial indicator (obtained from multiple study sites). The results for most of the proposed indicator microorganisms monitored, including fecal coliforms, did

not indicate significant correlation between their concentrations in the water and the incidence rate of gastrointestinal diseases. Based on this, EPA concluded that measurements of fecal coliforms in recreational waters were not predictive of risks to diseases. However, concentrations of enterococci in water had a strong relationship with swimming-associated gastrointestinal illnesses in both fresh and marine waters, whereas *E. coli* concentrations showed a strong relationship to swimming-associated gastrointestinal diseases in freshwater but not in marine water. In 1986, EPA recommended that new recreational water quality standards be established at geometric mean densities of 126 CFU *E. coli*/100 mL or 33 CFU enterococci/100 mL for freshwater and 35 CFU enterococci/100 mL for marine water.

### **Conclusions and recommendations**

1. Many additional epidemiological and water quality studies have since been completed, and the study design of many of these studies was similar to that used by EPA. The results of most of these studies are similar to those of EPA, especially in showing a relationship between concentrations of enterococci or fecal streptococci in marine waters and incidence of gastrointestinal diseases among swimmers. The results of these additional studies support the use of recreational water quality standards as established by EPA.
2. The results of the EPA epidemiological studies did not show a correlation between *Clostridium perfringens* density in water and incidence of swimming-associated gastroenteritis. However, the results of an epidemiological study conducted in Hong Kong did show such a relationship.

### **Questions and answers**

1. *Were any EPA water quality studies conducted in tropical areas?*  
No; however, an epidemiological study of swimming-associated illnesses due to bathing-beach quality was conducted in the early 1990s in Hong Kong.
2. *In the EPA studies, was there sewage source input at the study sites?*  
Yes, sewage contamination was present in the studies from which the recreational water quality standards were developed.
3. *What is the impact on evolution of methodologies used in the EPA study design in the 1980s to those used more recently?*  
That is difficult to answer. It might be cheaper to determine contamination/risk using the current methodologies than using correlation coefficients. Perhaps the slope or points might scatter. Regardless of improvements in the methodologies, newer methods would probably measure the same health effects. Experimental data have shown that when two different methods are used to measure risk (health effects), two totally different relationships may be obtained. Therefore, to compare two different methods, a good mathematical statistical technique is required.
4. *In the EPA studies on swimming-associated illnesses, to what extent could the sampling procedures distinguish the sources of the organism or contamination?*  
Since the study sites were contaminated with sewage, the fecal bacteria recovered

from water were assumed to have come from the sewage. However, it was probable that, to some degree, the swimmers themselves shed fecal bacteria. A higher incidence of gastrointestinal illness (GI) sickness was noticed among swimmers compared to non-swimmers.

## **PRESENTATION BY DR. ROGER FUJIOKA**

University of Hawaii

**Title:** Environmental sources and persistence of fecal indicator bacteria in Hawaii and Guam

### **Speaker information**

Dr. Roger Fujioka is a researcher and professor at the University of Hawaii where he has been conducting research since 1972. One of his research programs involves the identification of environmental sources of fecal indicator bacteria, primarily in Hawaii, but also in Guam.

### **Presentation objectives**

The objectives were to review water quality data generated by Fujioka's laboratory and to assess the impact of these findings on the applicability of EPA standards to recreational waters in tropical locations.

### **Major points**

1. In Hawaii and Guam, most perennial freshwater streams consistently contain high concentrations of fecal coliforms, *E. coli*, and enterococci that are far in excess of the current recreational water quality standards. Based on EPA guidelines, these data indicate that all streams on Oahu are highly polluted with sewage. However, sewer lines are well established on Oahu, and sanitary surveys indicate that sewage is not a likely source of these fecal bacteria in streams.
2. A major environmental source of fecal indicator bacteria was determined to be the soil environments of Hawaii. Initially, soil samples from the banks of Manoa Stream and grassy areas on the University of Hawaii Manoa campus were reported to contain high numbers of fecal indicator bacteria (fecal coliforms, *E. coli*, enterococci) at the surface and even at depths of 36 cm. This observation was subsequently confirmed in samples of different soil types from numerous sites on the island of Oahu. Additional experiments showed that these soil-associated fecal bacteria could be easily washed off the soil by water.
3. Soil is the most likely source for the consistently high densities of fecal bacteria in the streams of Hawaii and Guam. This conclusion is based on findings that time after time, high numbers of fecal indicator bacteria have been recovered from soil as well as from stream water samples of both locations. In small islands, rainwater initially falls onto large areas of land and then excess water is channeled to form streams. This is the mechanism attributed for the transport of the soil-associated

fecal bacteria to streams, to storm drains, and eventually to receiving coastal waters.

4. Isolates of soil-associated *E. coli* and enterococci were identified and speciated using standard cultural and biochemical techniques. Using these traditional methods, the isolates from soil were determined to be of diverse strains and/or species. These results indicate that the soil environment was not selecting for specific strains of *E. coli* and enterococci that persist in that environment and further suggests that *E. coli* and enterococci are inherently capable of persisting and possibly multiplying in Hawaii's soil environment.
5. Soil moisture was found to be one of the major environmental factors affecting the survival and population dynamics of indicator bacteria. In this regard, as the soil moisture content dropped, culturable densities of *E. coli* in soil were reduced to a greater degree than populations of enterococci in the same soil sample.
6. EPA epidemiological studies to establish marine recreational water quality standards were conducted at three sites (New York City, Boston Harbor, and Lake Pontchartrain, New Orleans) where sewage discharge was documented. Thus, the concentrations of fecal indicator bacteria at these sites were most probably from the sewage (point source). In these studies, there was a correlation between the concentrations of *E. coli* and enterococci in recreational waters and disease incidences among swimmers. However, when EPA conducted another study in which the source of the fecal indicator bacteria in the recreational waters was not from sewage but from a non-point source, there was no correlation between the numbers of fecal indicator bacteria in the water and incidence of diseases among swimmers.

### **Conclusions and recommendations**

1. In the application and interpretation of recreational water quality standards, EPA relies on the assumption that there is no significant environmental or non-fecal source of fecal indicator bacteria. This assumption is not applicable to Hawaii and Guam where high concentrations of fecal indicator bacteria are naturally found in the soil environment and represent a significant environmental source of indicator bacteria. Moreover, rainfall was determined to be the mechanism by which the fecal bacteria in the soil are transported to streams. In summary, since the assumption used by EPA to interpret its water quality standards is not applicable to Hawaii and Guam, the same interpretation of water quality standards to establish risk to swimmers in these two tropical locations should not be applicable.
2. The situation in Hawaii and Guam is similar to that in the EPA study (Calderon et al., 1991) in which the fecal indicator bacteria in the streams came from a non-point source rather than from sewage. Based on that study, EPA concluded that the concentrations of fecal indicator bacteria in the streams did not correlate with disease incidences among swimmers. Thus, under ambient conditions in Hawaii and Guam, the high concentrations of fecal bacteria in streams and beaches represent pollution from non-point sources and hence do not represent the same

risk to swimmers as predicted in the application of the EPA-established recreational water quality standards.

3. EPA should recognize that differences in environmental conditions affect the expected quality of water. EPA should investigate these differences, especially when data are available to document their effect on water quality standards. If these environmental differences occur and interfere with the proper interpretation of EPA-recommended recreational water quality standards, then states should be supported to establish alternative water quality standards, i.e., standards which are more reliable in predicting risks to swimmers.

### Questions and answers

1. *Are there any data on the numbers of fecal indicator bacteria along Manoa Stream?*  
Yes, for most streams the fecal indicator bacteria numbers increase as the stream flows from the elevated mountain area (100 CFU/100 mL) to the lower urbanized areas (>1,000 CFU/100 mL). These results correlate to the fact that the soil drainage area for any stream is relatively small at upstream sites and increases as the stream flows through the downstream and urbanized areas. The larger soil drainage area represents larger sources of soil-associated fecal indicator bacteria that are transported to streams. However, urbanized areas contribute in other ways to increase the numbers of fecal indicator bacteria in streams. In urbanized areas, the soil is more enriched with nutrients that enhance multiplication of bacteria. Moreover, there are more opportunities for fecal contamination of the environment by fecal matter from humans, pets, and other animals in urbanized areas.
2. *Have the environmental isolates been characterized?*  
Yes, data of this kind will be presented on the second day of this workshop during the session on multiplication of indicator bacteria in the environment.
3. *Have other independent markers of fecal contamination, such as caffeine and coprostanol, been analyzed to determine the source of contamination?*  
No, those approaches have not been used in Hawaii. However, we would like to run those experiments in the future.
4. *Certain molecular methods such as bacterial fingerprinting are available to determine sources of contamination. Have any such methods been used to determine the sources of fecal contamination in Hawaii?*  
No, but we have plans to conduct these kinds of experiments in the future.
5. *Are there any cut-off points to determine whether fecal indicator bacteria are present in places like the volcano (Big Island) and other (less hospitable) areas?*  
No; however, it would be interesting to determine whether these fecal bacteria could persist under such conditions.

## **PRESENTATION BY MR. EUGENE AKAZAWA**

Clean Water Branch, Department of Health, State of Hawaii

**Title:** Evaluation of water quality monitoring data by Hawaii Department of Health

### **Speaker information**

Mr. Eugene Akazawa is the supervisor in charge of managing the water-monitoring program for the Department of Health, State of Hawaii. Having served in this capacity for many years, he has first-hand experience in evaluating environmental conditions at various sampling sites on all the islands throughout the state.

### **Presentation objectives**

The objectives were to review the monitoring program and to discuss the data obtained by the Hawaii Department of Health. An additional objective was to explain the state's strategy in using recreational water quality standards and guidelines for beach closures.

### **Major points**

1. Hawaii's Clean Water Branch has established a beach-monitoring program for all frequently used beaches of the major islands for the past 30 years. Most of these beach sites are monitored on a weekly basis, and the program is designed to inform the public of the health risks associated with swimming in the waters. For many years, the water quality standard of 200 CFU fecal coliform/100 mL was applied to both fresh and marine waters. Today, this standard is maintained for inland sites (freshwater sites). However, the emphasis has been to monitor marine water sites because these are the more popular sites. Instead of using the EPA-recommended standard of 35 CFU enterococci/100 mL, Hawaii has adopted the more stringent standard of 7 CFU enterococci/100 mL as the acceptable level of risk for swimmers. However, due to environmental sources of fecal coliforms and enterococci, recreational waters are also monitored for concentrations of *Clostridium perfringens* because in Hawaii, monitoring data for *C. perfringens* in environmental waters provide more reliable data to determine when sewage contamination has or has not occurred than monitoring data for fecal coliforms, *E. coli*, or enterococci.
2. Based on geometric mean concentrations of enterococci above and below the 7 CFU/100 mL standard, beaches are classified as very poor, poor, compromised, fair, good, and excellent. Under this classification system, a beach site having very poor water quality would still meet the EPA standard of 35 CFU enterococci/100 mL. Currently, the core numbers of beach sites are 11 on Kauai, 14 on Oahu, 10 on Maui, and 8 on Hawaii. New sites on each island are listed on a rotational basis. Data from monitoring activities are made available to the public and can be accessed through the internet.
3. A significant problem identified from extensive monitoring and surveillance studies is pollution from non-point sources (streams, drainage canals, storm drains). Inland streams have not been able to meet the state standard of 200 CFU fecal

coliforms/100 mL due to these non-point sources of pollution. Coastal marine beaches that receive runoff from streams or storm drains often contain elevated levels of enterococci, and these levels increase during heavy rain events. This phenomenon has been documented for all islands. The Department of Health recognizes that non-point source contamination (land runoff) is the primary factor for increased fecal indicator bacteria densities in all streams and some coastal recreational waters in Hawaii.

4. Due to contamination by non-point sources of fecal coliforms, *E. coli*, and enterococci, the yearly state monitoring data show that nearly all streams exceed the EPA-recommended recreational water quality standards. As a result, these standards are not useful from the standpoint of determining when streams in Hawaii are actually contaminated with sewage and when there is a creditable, increased risk for swimmers to contract gastrointestinal diseases. Despite high concentrations of fecal indicator bacteria in Hawaii's streams, incidences of increased gastrointestinal diseases due to swimming in freshwater streams have not been reported. As a practical solution to the naturally high concentrations of fecal bacteria in streams, the state now monitors streams and coastal waters impacted by stream and storm drain runoff for concentrations of *C. perfringens* because ambient concentrations of this species of fecal bacteria are usually low but increase when sewage contamination occurs. Based on routine monitoring of streams for *C. perfringens*, the state was alerted to a probable occurrence of sewage contamination at one stream site based on elevated numbers of *C. perfringens*. Monitoring data for traditional fecal indicator bacteria at this same site were not distinctive because of their naturally high concentrations at all stream monitoring sites. By measuring for increased concentrations of *C. perfringens* at upstream sites, the general area of contamination was determined to be in an area of Palolo Stream. Further investigation led to the documentation of a sewer line break in that area.

### **Conclusion and recommendations**

1. Inland streams cannot be expected to meet the EPA-recommended standards for recreational waters because of non-point sources of the EPA-recommended fecal indicator bacteria. Despite the naturally high concentrations of fecal indicator bacteria in the streams of Hawaii, reports of gastrointestinal diseases have not been associated with swimmers and users of these streams.
2. For coastal marine beaches, non-point sources of pollution from streams and storm drains are known contributors of fecal indicator bacteria that on occasion occur at densities which exceed the Hawaii standard of 7 CFU enterococci/100 mL. This has made it difficult for the Hawaii Department of Health to determine the true health risk to swimmers. As a result, the state does not close beaches when the concentrations of fecal indicators exceed the recreational water quality standards except when a known sewage contamination occurs.
3. Hawaii needs recreational water quality standards that measure true risk to sewage contamination and sewage-borne diseases because EPA-recommended standards and health-risk guidelines are not reliable for conditions in Hawaii. As a result, the

state relies on the proposed *C. perfringens* standards to determine when recreational waters are contaminated with sewage and when there is a creditable risk for sewage-borne disease transmission to swimmers.

### Questions and answers

1. *Using the beach classification system, can the sources (of contamination) be determined for sites where the water quality is found to be poor?*  
Yes, such sites receive inland flows from streams or storm drains.
2. *Were there mammals (mongoose, pigs) in the upper Palolo (on Oahu) area?*  
Yes, wild animals such as mongoose and pigs are usually found in that area. In addition, extended surveys revealed the presence of dogs in residential backyards.
3. *Were other measurements of water quality such as carbon, nitrogen, phosphorus, and biological oxygen demand (BOD) determined to indicate the degree of eutrophication when the water samples were turbid?*  
Yes, such parameters were measured; however, the data did not correlate well with the source of contamination.
5. *Was any sediment analysis of the streams conducted to indicate other sources of contamination?*  
No. Sediment samples were not collected in the monitoring studies.
6. *Did the microbiological analyses for Clostridium perfringens represent total cells or the spores alone?*  
They represented *Clostridium perfringens* spores.
7. *If E. coli and enterococci were proliferating in the soil, it would seem like the actual counts in the soil must be several orders of magnitude higher than that is actually recovered. Please comment upon this.*  
The first flush of soil by rain would probably account for a significant contribution of the soil-associated fecal indicator bacteria into streams. The concentrations of fecal indicator bacteria in streams, especially following a rain event, can range from  $10^3$  to  $10^4$  CFU/100 mL and most probably reflects the degree to which fecal indicator bacteria in soil and other environments have been transported to the stream.

## PRESENTATION BY DR. GARY TORANZOS

University of Puerto Rico

**Title:** Review: Environmental sources and persistence of fecal indicator bacteria in Puerto Rico

### Speaker information

Dr. Gary Toranzos is professor of Microbiology at the University of Puerto Rico. Puerto Rico is the other tropical site where substantial data have been collected to document

the presence of fecal indicator bacteria in environmental waters in the absence of fecal contamination. These data have been generated by the Department of Biology at the University of Puerto Rico, initially by Dr. Terry Hazen and then continued by Dr. Toranzos.

### **Presentation objectives**

The objectives were to review the 20 years of data collected in Puerto Rico and to identify the problem of stream water quality assessment due to environmental sources and persistence of fecal indicator bacteria.

### **Major points**

1. In Puerto Rico, studies have shown that densities of fecal indicator bacteria (fecal coliforms, *E. coli*, enterococci) in environmental waters often exceed recreational water quality standards, and these elevated densities have remained unchanged over the past 20 years.
2. Environmental sources of fecal indicator bacteria have been determined in the absence of fecal contamination. For example, water trapped by bromeliads entrenched in the trees of the tropical rain forest has been documented to contain *E. coli* in the absence of evidence of fecal contamination.
3. The data collected over the 20 years did not indicate a correlation between densities of fecal indicator bacteria in environmental waters and human health risk.
4. The data showed that *E. coli* can persist for long periods, remain metabolically active, and multiply under environmental conditions in Puerto Rico. These results indicate that concentration of fecal indicator bacteria in environmental waters do not reflect the degree of fecal contamination.
5. Coliphages are incapable of multiplying under environmental conditions. In Puerto Rico, their concentrations in streams are better correlated with sewage contamination than concentrations of EPA-approved fecal indicators.

### **Conclusion and recommendations**

1. Environmental sources of fecal indicator bacteria recorded from numerous sites in Puerto Rico were independent of sewage or fecal matter. This finding argues against the assumption used in the interpretation of water quality standards that there are no significant environmental sources of fecal indicator bacteria unrelated to sewage or fecal matter.
2. The ecology of microorganisms in the environment has been ignored by EPA despite its being probably the most important discipline to apply in understanding the fate and significance of high densities of fecal indicator bacteria in tropical environments. Bacteria categorized as coliform bacteria are believed to have been present on this planet before mammals. When mammals populated the planet, another environment was created for bacterial growth. Based on ecological principles, it can be reasonably argued that the environmental coliform bacteria adapted to grow in the intestinal environment of human and warm-blooded animals. If this is true, it is not surprising that intestinal sources of fecal indicator

bacteria such as *E. coli* can readily adapt to its original habitat (such as soil). Moreover, multiplying under environmental conditions (e.g., soil or leaf axillae of bromeliads) in a tropical climate is very likely to occur because environmental temperatures (e.g., 20° to 25°C) are well within the known growth range for fecal bacteria such as *E. coli* and enterococci.

3. Alternative fecal indicators, which have been shown to be more reliable in tropical areas, should be further evaluated. Rigidly controlled epidemiological studies should be conducted in Puerto Rico using current and alternative indicators such as coliphages for the purpose of determining which fecal indicator can best predict the actual health risk to swimmers.

### Questions and answers

1. *Is there evidence that these fecal bacteria (fecal coliform, E. coli) can survive in temperate soils?*  
Several studies conducted in temperate climates show that *E. coli* can survive for extended periods and could leach through soil columns.
2. *What were the survival conditions (temperature) used in the studies to determine multiplication of coliphages in the environment?*  
The temperature in the survival chambers ranged from 20° to 23°C. In other studies conducted in the laboratory, the maximum temperature used was 45°C.
3. *Were any pathogenic E. coli identified among the environmental isolates?*  
This was a general question addressed to the participants. Fujioka replied that a study to screen environmental *E. coli* isolates for toxic genes related to pathogenic *E. coli* classified as ETEC or EHEC was completed in Hawaii. The results indicated that the isolates of *E. coli* recovered from environmental waters (coastal waters) were not carriers of these toxic genes.
4. *Are there any data on molecular characterization of the environmental E. coli isolated in Puerto Rico?*  
Analyses of environmental *E. coli* isolates recovered from the bromeliads revealed that, phenotypically, the isolates were indistinguishable from the standard or prototype strains of *E. coli*. These environmental *E. coli* isolates were shown to have total DNA homology ranging from 35% to 78% with standard strains of *E. coli*.

## PRESENTATION BY DR. HELENA SOLO-GABRIELE

University of Miami

**Title:** Environmental sources, persistence, and multiplication of fecal indicator bacteria in Fort Lauderdale, Florida

## **Speaker information**

Dr. Helena Solo-Gabriele is an associate professor at the University of Miami. She initiated a program to determine the quality of inland and tidally influenced bodies of water in Fort Lauderdale, Florida. She is the first scientist to report the natural occurrence of fecal indicator bacteria in the subtropical soil environments of the continental United States.

## **Presentation objectives**

The objectives were to review the monitoring data collected from south Florida (Fort Lauderdale), which is characterized as subtropical climate, and to identify the water quality assessment problem due to persistence and multiplication of fecal indicator bacteria (*E. coli*, enterococci) in the soil environments along the riverbank.

## **Major points**

1. Soils along the riverbanks were confirmed to be the primary sources of *E. coli* in the river between storm events. Intensive grid sampling showed the highest densities of *E. coli* near the channel banks. Elevated counts in the water were also characterized by high numbers in the soil. Increased *E. coli* counts in water were found to correlate with cycles of high tides when water rises to drain the banks.
2. Soil moisture was found to play a critical role in the multiplication of *E. coli*. Upon soil drying, increasing numbers of *E. coli* was evident, which was probably mediated by death of its predator (protozoa) at lower moisture levels. Local conditions (i.e., warm humid climatic conditions and long, shaded river embankments characterized by a large surface area subjected to periodic wetting and drying cycles) appeared to influence the rate of *E. coli* multiplication in the soil.
3. Tidal simulation and soil wetting experiments confirmed that *E. coli* and enterococci can multiply in the soil under favorable conditions caused by drying and rewetting cycles. Addition of sterile soil to river water resulted in the multiplication of both *E. coli* and enterococci. Similarly, mixing natural soil and river water resulted in their multiplication. However, *C. perfringens* did not multiply to a noticeable degree in either soil or water.

## **Conclusion and recommendations**

1. Riverbanks along the North Fork of New River in Fort Lauderdale were found to be significant sources of *E. coli*. They contributed to the concentrations of this bacteria in the river, especially when tides and storm events flooded the soil in this area. These studies indicated that the warm, humid climatic conditions of south Florida were sufficiently favorable for the growth of *E. coli* in the natural environment (soil, water).
2. The use of *E. coli* as an indicator of fecal contamination is unreliable in the subtropical stream system of Fort Lauderdale.
3. Other alternative fecal indicators should be evaluated to identify better indicators of fecal contamination in this subtropical and other tropical areas of the world.

## Questions and answers

1. *The data do not seem to indicate actual multiplication of E. coli. The increase in numbers between 0 and 20 hours could be attributed to various factors (for instance, recovery of stressed organisms). Were measurements taken between 0 and 20 hours?*

In the experiment carried out to determine the growth of *E. coli* in sterile soil, no measurements for *E. coli* counts were taken between 0 and 20 hours. However, in the tidal simulation experiment, samples were taken every 6 hours.
2. *What was the contribution of Clostridium in the original soil samples?*

The counts were relatively high (>50 CFU/100 mL). *Clostridium* counts were also found to vary with tidal cycles.
3. *What was the source of Clostridium perfringens in the river water?*

The source was not specifically identified but was suspected to be the soil since *C. perfringens* was recovered in the (soil) samples collected along the riverbanks.
4. *In some estuarine environments, re-suspension of sediment-borne organisms to overlaying water (due to tidal effects) has been shown to be the primary reason for elevated counts of fecal bacteria. Was this aspect looked into in this study?*

No, sediment samples were not analyzed for densities of indicator bacteria in this study.

Note: The expert who raised this issue further proposed that it might be worthwhile to look at the relative numbers of indicator bacteria in the sediment samples.
5. *Were there any data on the relative distribution of fecal indicator bacteria in the river as a function of depth, and how far below the surface is the river sediment?*

In the intensive sampling efforts, samples were collected at depths of 30 and 90 cm below the surface. The sediment is at about the 150 cm depth.

## PRESENTATION BY DR. DAVID MORENS

National Institutes of Health

**Title:** Evaluation of an epidemiological/water quality in Hawaii

### Speaker information

Dr. David Morens is an epidemiologist with the National Institutes of Health. Previously he was a faculty member of the School of Public Health and the School of Medicine at the University of Hawaii. He was the principal investigator of the only epidemiological/water quality study conducted in Hawaii to determine health risks associated with swimming in marine beach waters.

## **Presentation objectives**

The objectives were to explain the design of the epidemiological/water quality study conducted at Kuhio Beach in Waikiki and to report the conclusions that were drawn from the study.

## **Major points**

1. A rolling cohort study design was used in this epidemiological investigation. People who visited the beach were enrolled during each study day, and each was tracked for a period of three days following beach exposure. Completed questionnaires were obtained from 3,721 persons at the beach. Follow-up interviews with 2,556 persons (68.8% completion rate) were conducted using 1.2 person-years of interview time. The breakdown of the participants was 51.4% males and 48.6% females, ranging in age from 2 to 85 years, with the majority in their 20s and 30s. About 2% of the subjects (51 of 2,556) experienced one or more of the gastrointestinal symptoms in the three days following their interview. A large number of persons (65) had experienced similar symptom (s) in the three days before the interview, indicating that the frequency of the gastrointestinal symptoms upon exposure (during swimming) had not increased. This observation was probably due to the small number of cases in this epidemiological study.
2. Although 41 persons (1.95 cases per person-year) reported one or more ophthalmic (eye/ear infection) complaints, there were no significant differences in the frequency of ophthalmic complaints as a result of variables such as beach visited or gender, age, ethnic background, and residence of the participant.
3. Analysis of the water quality data showed a lack of association between fecal indicator densities and risk of illness. It should be noted that most of the time, the concentrations of fecal bacteria in the water were well below those reported in the EPA study. Elevated counts of indicator bacteria occurred on some days due to storm events, but this did not result in increased incidence rates of gastrointestinal symptoms.

## **Conclusion and recommendations**

1. No evidence of any major health risk associated with swimming at Kuhio Beach was detected in the epidemiological study conducted within the Waikiki Beach area. The limitations of this study were (a) the concentrations of fecal bacteria in the water were consistently low and therefore disease incidences at higher levels of fecal bacteria in the water could not be determined and (b) the source of the fecal indicator bacteria was storm drain discharge and therefore from a non-point source rather than from sewage, as was the case in the EPA study from which the standards were developed.
2. Although substantial numbers of interviews were completed, the data set could not detect low levels of disease incidence below 10/1,000 people.
3. The epidemiological pilot study generated a realistic cost estimate for a larger future epidemiological study of this type.

## Questions and answers

1. *Why was Kuhio Beach selected as the site for the epidemiological study? The site selection is questioned because the source of fecal bacteria contamination was the nearby storm drain discharge. Since the contamination is from a non-point source rather than sewage, a correlation between fecal indicator bacteria in the water and excess incidences of illness among swimmers at this beach would not be predicted based on EPA studies.*

The Kuhio Beach site was selected for several reasons. First, it is one of the most popular beach sites in Hawaii, and it is especially suitable for children. Second, it is located in the Waikiki area, where concern for water quality is the greatest. Third, of the beach sites in the Waikiki area, the Kuhio Beach site is the one whose water most frequently contains elevated levels of enterococci. Fourth, because a storm drain discharge occurs nearby, the concern for health risk to swimmers is greatest at this site because concentrations of enterococci periodically exceed the state's recreational water quality standard of 7 CFU enterococci/100 mL.

2. *Was any effort made to ask the subjects whether they swam at other beaches three days before their interview, and if their response was positive, were these subjects eliminated from the study?*

Yes, the participants in the study were asked whether they swam at other beaches during the past three days. However, the subjects were interviewed and the data collected were stratified. Evaluation of this stratified data indicated no bias by this data.

3. *Were the small numbers of subjects who swallowed water a limitation of this study?*  
No. A fairly good number of people indicated that they swallowed water. However, there was no association between people swallowing water and their developing gastrointestinal illness.

4. *Can you elaborate on the demographic data?*

Of the 2,556 respondents, 1,334 (52.2%) were residents of Japan, 621 (24.3%) were residents of the United States other than Hawaii, 273 (10.7%) were residents of Oahu, 20 (0.8%) were residents of other Hawaiian islands, and the remaining 308 (12.1%) were from other countries, including Canada. The majority of the participants (57.2%) identified themselves as being of Asian ethnicity.

5. *Children are known to be sensitive to exposure. Are there any data to indicate this observation?*

Following stratification of the data, no significant differences were seen among this population.

6. *How do you compare the data when there are multiple variables such as different sources of indicator organisms, multiple beach sites, control beach site vs. site of interest, and swimmers vs. non-swimmers?*

This was only a feasibility study. Such multiple variables were not controlled, but the major limitation of this study was that it was not big enough. The study site was picked because of much public concern and other related issues.

7. *In an epidemiological study done in Santa Monica, there was a higher incidence of illness due to discharge of storm drain water into a marine beach. How far from Kuhio Beach is the ocean sewage outfall in Mamala Bay, and were there any dye studies to track the movement of the discharge from this sewage outfall?*

A previous study indicated that the Sand Island sewage outfall was a sufficient distance away and did not contribute to the fecal bacterial concentrations in waters at Waikiki beaches. No dye studies were conducted in this investigation. The storm drain in this study differed from the storm drain in the Santa Monica Bay study, which had evidence of sewage or fecal input.

8. *Did people shower before entering the water?*

That was not assessed in this study. The general observation was that people showered after swimming but not before entering the water.

## **PRESENTATION BY MS. CHRISTINE BULLOCK**

Institute of Marine Affairs, Trinidad

**Title:** Evaluation of a prospective epidemiological and water quality study in Trinidad and Tobago, West Indies

### **Speaker information**

Ms. Christine Bullock is a microbiologist with the Institute of Marine Affairs, Trinidad, West Indies. She recently completed an epidemiological/water quality study in the West Indies. It was the first large-scale epidemiological study completed in a truly tropical region of the world.

### **Presentation objectives**

The objective was to review the results of an epidemiological and water quality study conducted at Trinidad, West Indies.

### **Major points**

1. Six survey areas located at four beaches (Maracas Bay, Chagville Beach, Welcome Bay, and Macqueripe Bay) were selected for the epidemiological study. Water samples were collected on 11 Sundays (4 days during the dry season and 7 days during the wet season) and analyzed for physical (temperature, turbidity), chemical (pH, salinity, dissolved oxygen), and biological parameters (microbial indicators of water quality such as coliforms, fecal coliforms, *E. coli*, and enterococci). The target population consisted of about 8,000 persons (mostly family groups). The participants were categorized as swimmers, waders, and non-swimmers. They were interviewed at the beach site, usually on Sunday. Telephone follow-up interviews were conducted 3 days after the beach interview date. The morbidity symptoms investigated included respiratory symptoms, highly credible gastrointestinal symptoms, ear infections, skin infections, allergies, and other symptoms (fever and

headache). Potential sources of non-sampling bias were controlled. To establish a statistically significant association between the microbial quality of the water and morbidity rates of different symptoms among the exposed group, suitable statistical approaches were used.

2. Of the 10,204 interviews conducted at the survey areas, 7,509 yielded usable data. People in the age group of 21–40 years represented the highest percentage of the study participants. The ethnicity groups of the study population were separated as follows: (a) East Indian descent (35.5%), (b) African descent (27.3%), (c) mixed descent (25.2%), (d) Caucasian descent (6.1%), and (e) Chinese descent (2.9%). Approximately 85% of the participants were categorized as swimmers, based on interview responses which indicated that 98% got their head wet, 38.5% to 53.4% swallowed water, and 97% swam for 10 minutes or longer. Approximately 15% of the participants were categorized as non-swimmers, including approximately 3% categorized as waders. About 63% to 96% of the study participants consumed food or drink at the beach sites. Over the study period, the physical and chemical characteristics of the water were as follows: water temperature, 20° to 31.8°C; turbidity, 0.6 to 386 NTU; pH, 7.6 to 8.4; dissolved oxygen, 6.3 to 7.9 ppm; and salinity, 15.5 to 33.5 ppt.
3. Significant differences ( $p < 0.001$ ) among the number of persons reporting symptoms of respiratory illness, gastrointestinal illness, skin infections, and other illnesses were evident. During both dry and wet seasons, symptoms of respiratory illness were reported most frequently (dry season, 37.2 cases/1,000 persons and wet season, 40.3 cases/1,000 persons).
4. Over the study period, results obtained for the beach sites were significantly different ( $p < 0.001$ ) in terms of participants who came in contact with water and reported one or more symptoms. There was good correlation between elevated indicator density and incidence of illnesses at beach sites that did not meet EPA water quality standards. Lower incidences of diseases among swimmers were observed at beach sites that met the EPA water quality standards.

### **Conclusion and recommendations**

1. This epidemiological study followed the EPA design. Beaches not in compliance with EPA standards had mean enterococci levels of 48 to 151 CFU/100 mL; these beaches received sewage contamination. Beaches in compliance with EPA standards had mean enterococci levels of 4 to 33 CFU/100 mL. The overall results were similar to those reported by EPA.
2. The observed illness rate (per/1,000 people) over the study period is summarized as follows:
  - a. Respiratory illness 39.0
  - b. Gastrointestinal illness (GI) 14.4
  - c. Other illness 14.4
  - d. Skin infections 11.7
  - e. Allergies 9.9

f. Eye infections	8.9
g. Highly creditable GI (HCGI)	5.3
h. Ear infections	5.1

### **Questions and answers**

Since this presentation was made during the luncheon, which took place in a separate room, there is no record of the questions and answers.

# CHAPTER 3

## PRESENTATIONS BY INVITED EXPERTS: DAY TWO

### PROBLEM STATEMENTS

The problem statements addressed on the second day pertained to issues in question 4 (multiplication of fecal indicator in the environment) and question 5 (use of an alternative fecal indicator to establish more reliable recreational water quality standards). These last two questions are considered very important because in the interpretation of water quality standards and guidelines, EPA accepts the dogma that indicator bacteria will not multiply to any substantial level in natural (ambient) environments. Consequently, if it is shown that fecal indicator bacteria are able to multiply in ambient environments, the EPA criteria would be considered unreliable and a new, alternative fecal indicator and new criteria may be required for such conditions. Moreover, EPA has stated that any fecal indicator used to establish new water quality standards must be based on measurable risk. The two questions addressed during day two of this workshop are as follows:

1. Are there sufficient experimental and monitoring data to conclude that the assumption used in interpreting water quality standards (there are no significant environmental sources of fecal coliforms, *E. coli*, and enterococci) is not applicable in tropical areas (Hawaii, Guam, Puerto Rico, south Florida) because these bacteria can be recovered in high concentrations from ambient environments (water, soil, plants) in these areas?
2. Are there sufficient experimental and monitoring data to conclude that the EPA criteria (*E. coli*, enterococci) used to assess the quality of environmental waters are not reliable in tropical locales (Hawaii, Guam, Puerto Rico, south Florida) because the selected fecal bacteria persist in these ambient environments and represent non-fecal contamination?

Although the workshop agenda was fixed, adjustments were made since Dr. Nicholas Ashbolt of the University of New South Wales in Australia, Dr. Richard Whitman of the United States Geological Survey, and Dr. Joan Rose of the University of South Florida came prepared to make relevant presentations. They were placed on the agenda by using the discussion time scheduled for the lunch period to schedule a speaker. Moreover, Dr. Solo-Gabriele indicated that she presented most of her data during the first day of this workshop, so she released her time slot for the second day to allow others to make a presentation.

## PRESENTATION BY DR. ROGER FUJIOKA

University of Hawaii

**Title:** Evidence for multiplication of fecal bacteria in Hawaii's soil environment and evidence that *Clostridium perfringens* is a more reliable indicator of fecal contamination

### Speaker information

Dr. Roger Fujioka is a researcher and professor at the University of Hawaii. He has spent more than 25 years assessing the quality of waters in Hawaii based on the EPA recreational water quality standards.

### Presentation objectives

The objectives were to review the data from Fujioka's laboratory on the issues related to multiplication of EPA-recommended fecal indicator bacteria in the environment, their impact on the elevated levels of fecal indicator bacteria in Hawaii's environmental waters (streams, storm drains, coastal waters), and the use of *C. perfringens* as an alternative and more reliable indicator of fecal contamination.

### Major points

1. Evidence was presented to show that soil contains adequate nutrients to promote growth of *E. coli* and enterococci. This was accomplished by successfully growing pure cultures of *E. coli* and *E. faecalis* on soil extract agar. Growth on this medium was similar to that observed on commercially available media. Moreover, a typical growth curve for *E. coli* was established.
2. *E. coli* and enterococci readily multiplied in sterile soil. However, in natural soil, which is characterized by the presence of indigenous soil microflora, the population of fecal bacteria persisted for long periods but measurable multiplication of *E. coli* was not observed after several days at ambient temperature (25°C). When this same soil sample was supplemented with nutrients such as glucose or peptone, increases in the concentrations of *E. coli* and enterococci were readily detected in a few days. Based on these results, it was concluded that indigenous soil microorganisms are much more diverse, abundant, and effective in utilizing available nutrients (e.g., carbon and energy sources) in this soil environment, as compared to fecal indicator bacteria. Due to out-competition for nutrients, multiplication of fecal indicator bacteria in the soil environment is restricted. However, when nutrients become available (e.g., due to the addition of excess nutrients), *E. coli* and enterococci readily multiply in the soil environment.
3. To maintain a population in soil, fecal bacteria need not multiply rapidly (hours to days), as is observed under laboratory conditions where growth conditions are ideal. Under environmental conditions, multiplication of soil-associated microorganisms is known to occur sporadically and in response to changing conditions, which only occasionally provides suitable growth conditions. The available data indicate that the soil environment in Hawaii provides sufficient

levels of nutrients, moisture and temperature to enable fecal indicator bacteria to sustain their populations. Under *in-situ* conditions, these fecal indicator bacteria most likely multiply very slowly (days to weeks) and sporadically, but this type of population growth rate is sufficient for *E. coli* and enterococci to become established in soil as a minor population ( $10^2$  to  $10^4$  CFU/g of soil) among the more predominant and culturable ( $10^8$  to  $10^9$  CFU/g of soil) indigenous soil bacteria. The colonization of fecal indicator bacteria in soil represents a significant environmental source of fecal bacteria, which are readily transported to streams. Since the concentrations of fecal indicator bacteria routinely exceed established recreational water quality standards, the standards are not useful in determining when streams are really contaminated with sewage and when there is increased health risk.

4. Evidence was obtained that most strains of *E. coli* and enterococci are able to grow in the soil environment of Hawaii and that the soil environment is not selective for a subpopulation with distinct metabolic properties to multiply. This conclusion was based on the recovery of various metabolic groups of *E. coli* as well as various species of *Enterococcus* bacteria from soil samples.
5. Monitoring streams for other alternate fecal indicators (*Clostridium perfringens*, FRNA coliphages) provided more reliable data to indicate when these streams were contaminated with sewage. There are three advantages in the use of these two alternative fecal indicator microorganisms. First, they cannot multiply in ambient soil environments and their ambient concentrations in streams are low. Second, they are present in sewage and hence their presence in streams is a reliable marker of sewage contamination. Third, their expected survival in environmental waters is closer to that of pathogens (viruses) than that of fecal indicator bacteria. Based on extensive monitoring data, the densities of *C. perfringens* in streams and coastal waters under ambient conditions and under conditions of sewage contamination have been determined. Based on these monitoring data, the recreational water quality standards for Hawaii have been proposed based on geometric mean concentrations of 50 CFU/100 mL in streams and 5 CFU/100 mL in marine waters. Since the ambient levels of FRNA coliphages in Hawaii's streams have not been determined, water quality standards have not been proposed for this group of coliphages.

### **Conclusion and recommendations**

1. Several lines of evidence strongly indicate that *E. coli* and enterococci can multiply in the soil environment of Hawaii. Several kinds of experiments have shown that the soil environments of Hawaii provide suitable conditions (temperature, nutrients, moisture, pH, non-toxicity) for multiplication.
2. The experimental data show that multiplication of fecal bacteria in soil is slow compared to multiplication of indigenous soil bacteria but sufficient to allow them to become established as minor populations in soil. However, the densities of fecal bacteria in soil are sufficient to contribute to increased levels in streams, which often exceed recreational water quality standards.

3. Monitoring and experimental data indicate that the assumption used by EPA that these fecal bacteria cannot multiply in the environment does not apply to Hawaii, Guam, and probably other tropical areas. These results support the overall conclusion that the use of the EPA-recommended recreational water quality standards is unreliable in Hawaii, Guam, and probably other similar tropical locations.
4. Monitoring for *C. perfringens* is the simplest and most reliable means to determine when streams and other environmental waters are contaminated with sewage.
5. Based on ambient densities of *C. perfringens* in sewage and streams, the most reasonable and reliable recreational water quality standards for Hawaii are geometric mean concentration of 50 CFU/100 mL for streams and 5 CFU/100 mL for coastal marine beaches.

### Questions and answers

1. *What were the sources of fecal indicator bacteria used in the multiplication studies?*  
Several sources were used, and bacteria from each of these sources multiplied. For example, pure cultures of *E. coli* and *E. faecalis* recovered from soil, streams, and sewage were used. In one experiment, a strain of *E. coli* was genetically modified by the insertion of lux gene marker. This *E. coli* produced luminescent colonies, which can be easily counted and distinguished from other strains of *E. coli*. Using this strain of *E. coli*, evidence for multiplication was readily obtained in soil under laboratory conditions. Unfortunately, we were not allowed to use this *E. coli* under field conditions.
2. *In the metabolic characterization (as determined by the Biolog carbon source utilization patterns) of E. coli and enterococci isolates from different sources (soil, sewage), did the clusters correlate with the source of the isolates?*  
No, the clusters overlapped. For instance, some sewage isolates clustered with soil isolates and vice versa.
3. *What is the density of C. perfringens in the sewage?*  
We generally measure about  $10^3$  to  $10^4$  CFU/100 mL in raw and secondary treated sewage without chlorination. Chlorination generally results in only one log reduction of *C. perfringens*.
4. *Since the density of C. perfringens in the sewage is lower, can it be readily detected in the water? And, how close does it relate to pathogens?*  
The advantages of monitoring for *C. perfringens* are several. First, the method is relatively simple, making it possible for most laboratories to use this method. Second, the spores of *C. perfringens* are so stable that their concentrations are not reduced over time as compared to *E. coli* and enterococci. Third, larger volumes of water samples (500 to 1,000 mL) can be filtered to increase the sensitivity of this method. This is especially applicable to marine waters, which are generally very clear. Fourth, since *C. perfringens* is always found in sewage and will survive longer than pathogens, the *C. perfringens* standard is a very conservative indicator of fecal contamination and the possible presence of sewage-borne pathogens.

## PRESENTATION BY DR. GARY TORANZOS

University of Puerto Rico

**Title:** Evidence for survival and multiplication of fecal indicator bacteria in the environment of Puerto Rico and evidence that coliphages are a more reliable indicator of fecal contamination

### Speaker information

Dr. Gary Toranzos has been conducting water quality studies in Puerto Rico for approximately 15 years and has obtained substantial data on the microbial quality of water in that tropical area of the world.

### Presentation objectives

The objectives of Toranzos's presentation were to review the results of his studies in Puerto Rico that provided evidence that fecal bacteria can multiply under ambient environmental conditions and to review the results of other studies showing that coliphages are reliable indicators of fecal contamination of environmental waters in Puerto Rico.

### Major points

1. Experiments were designed to determine whether *E. coli* can survive in soil containing or lacking indigenous microorganisms. In the autoclaved soil, added *E. coli* did not survive for extended time (28 days), indicating that it probably required certain growth factors for multiplication and continued persistence. When *E. coli* was added to natural soil containing other background microorganisms, multiplication of *E. coli* as well as other microorganisms was detected. However, after 28 days, *E. coli* was not detected by viable assays.
2. Various studies conducted elsewhere have shown that fecal indicator bacteria such as *E. coli* can survive for extended periods in sediments and that sediments provide a better environment for their growth than the water environment. Other microcosm studies have shown prolonged survival of *E. coli* in the plant rhizosphere region, indicating that root exudates (e.g., photosynthate) may provide nutrients to enable *E. coli* to survive and grow under these conditions. However, more experiments are needed to provide definitive answers to these observations.
3. Based on available data on the widespread occurrence and persistence of coliforms in natural habitats in the tropics, it is apparent that these organisms play important ecological roles under these situations. However, their ecological roles have not yet been fully determined.
4. Extensive sampling efforts covering various natural habitats in Puerto Rico revealed the presence of coliforms and thermotolerant coliforms (*Klebsiella*, *E. coli*) in natural environments, including water trapped in bromeliads growing high in the tops of trees in a pristine rain forest. Molecular techniques were used to determine whether environmental strains of *E. coli* were different from those recovered from feces of humans and other warm-blooded animals. However, it was not possible to discriminate the isolates into distinct sources, regardless of the

methodologies used. Currently, various molecular techniques such as ribotyping, RAPD, as well as PCR-based methods (REP PCR, BOX PCR, ERIC PCR) can be designed for specific purposes. However, it should be remembered that these techniques cannot provide answers to all our questions. Under these circumstances, the best strategy would be to use several different techniques (time and resources permitting) to determine whether environmental strains are different from strains recovered from human and animal feces.

5. Coliphages were determined to be superior indicators of sewage contamination of streams in Puerto Rico because these bacterial viruses were shown to be consistently present in sewage yet incapable of multiplying under environmental conditions.

### **Conclusion and recommendations**

1. When *E. coli* is recovered from environmental waters in Puerto Rico, it is not clear whether it originated from sewage or from an environmental source such as bromeliad plants or soil. *E. coli* was shown to survive for extended periods (28 days) in soil. This clearly is evidence of its persistence; however, *in-situ* experiments to demonstrate whether *E. coli* can multiply in soil was not obtained and may be difficult to obtain. Currently, isolates are being characterized and their characteristics compared with that of their source. One of the major problems in the identification of environmental isolates is the need and unavailability of a large database for characterizing *E. coli* isolates from various sources. Therefore, it is necessary to build a large database of genetic characteristics of fecal indicator bacteria recovered from environmental and known fecal sources.
2. The current practice of identifying and discriminating isolates using genetic methods has generally been based on characterizing the differences in one or two genes. This approach may not be sufficient for discriminating *E. coli*, which can easily alter its genetic composition and which is known to be able to grow in drastically different intestinal environments of humans, animals, birds, and reptiles as well as in the ambient environment. This strategy may never be fruitful. A new strategy to target the presence of several distinct genes, such as those responsible for its survival in the environment, seems to be a better approach for distinguishing environmental isolates from isolates from other sources.

### **Questions and answers**

1. *Did the counts of E. coli in the soil drop to zero in the microcosm studies? How did enterococci survive in these studies?*  
Viable counts of *E. coli* could not be recovered after 28 days following inoculation. These studies did not focus on the survival and growth of enterococci in the soil.
2. *Soil moisture has been reported to be critical for the survival and multiplication of fecal bacteria. Was soil moisture maintained at an optimal level (for microbial growth) throughout the experimental period?*  
No, it was difficult to maintain the soil moisture content at an optimal level throughout the experiment. In these microcosm studies, the soil tended to dry out

much sooner than soil in the environment. As a result, under these settings, it was very difficult to mimic natural field conditions.

3. *Did other sensitive recovery techniques detect the presence/survival of E. coli in the soil after 28 days?*

None was detected by viable assay. For example, even when the most probable number method was used, viable counts of *E. coli* could not be detected in the soil sample. However, using the DNA amplification techniques by PCR, positive signals for *E. coli* could be detected after 28 days.

4. *Based on studies conducted in Puerto Rico, are there alternative indicators identified and proposed for monitoring water quality?*

Yes, coliphages have been proposed as alternative indicators to *E. coli*.

5. *Based on these microcosm and other studies, would it be possible to conclude that growth/multiplication of E. coli can occur in the environment?*

Although the microcosm studies provided no evidence of the multiplication of *E. coli*, it was hard to demonstrate multiplication (through increase in significant numbers) because the conditions in those studies did not quite represent soil conditions from which the organism (*E. coli*) was obtained. However, because these fecal bacteria have been consistently recovered from natural environments, we consider them to be part of the natural environmental microbiota. It would be very difficult to prove the growth/multiplication of microorganisms in natural environments. Moreover, it would be prudent to assume that the growth rate under natural conditions may not be measurable because it might be too slow and discontinuous. Therefore, the important measurement under these conditions is to demonstrate the persistence of the *E. coli* population in soil over time. Also, it should be remembered that under natural conditions, microorganisms derive nutrients from various sources (photosynthate, organic matter from other populations). The concentrations and fate of most of these potential nutrients are not known.

## **PRESENTATION BY DR. JOAN ROSE**

University of South Florida

**Title:** Extreme events and contamination in the Charlotte Harbor estuary and Tampa Bay

### **Speaker information**

Dr. Joan Rose is a professor of Water Microbiology at the University of South Florida. She has completed several studies in south Florida to determine the quality of water and to assess the best fecal indicator to predict fecal contamination.

## **Presentation objective**

The objective was to review the data from some of her recent projects, in which fecal bacteria recovered from Tampa Bay's environmental waters were further characterized by ribotyping methods and by multiple antibiotic resistant patterns in an attempt to determine whether the source of these fecal bacteria were from human or animal feces.

## **Major points**

1. The twelve stations chosen for sampling in the northern portions of Charlotte Harbor included areas devoted to recreation, shellfish harvesting and residential drainage. Monthly water samples and quarterly sediment samples were analyzed for fecal coliforms, enterococci, *C. perfringens*, and coliphages. The quarterly samples were also analyzed for the human enteric pathogens, *Cryptosporidium* spp., *Giardia* spp., and enteroviruses.
2. Bacterial indicators of fecal contamination were found in higher densities in the sediment than in the overlying water column. Concentrations of *C. perfringens* were five orders of magnitude higher in the sediment than in the overlying water column. Higher numbers of fecal indicator organisms were generally found in water of low salinity and areas with high densities of septic tanks. Fecal pollution was more widespread during wet weather.
3. Enteric protozoa were detected occasionally and were not related to seasonal influences. Enteroviruses were detected between December 1997 and February 1998 at 75% of the sampling stations. In subsequent months, they were not detected.
4. Fecal indicators and enteroviruses were significantly associated with rainfall, stream flow, and temperature. Regression models suggested that temperature and rainfall can predict the occurrence of enteroviruses in 93.7% of the cases.

## **Conclusion and recommendations**

1. Ribotyping and multiple antibiotic resistance patterns of *E. coli* were also evaluated to distinguish the sources of contamination impacting the bay. When antibiotic resistant patterns and ribotyping of *E. coli* isolates and enteroviruses recovery data were combined to determine an index of human impact, there was no consistent agreement among the three approaches. In general, antibiotic resistant patterns correlated better in terms of matching with the enterovirus detection data than ribotyping patterns.
2. In determining index of human impact, significant correlations were obtained with concentrations of fecal indicator bacteria (enterococci, fecal coliforms, *E. coli*) and coliphages in water samples. Concentrations of *C. perfringens* were useful in distinguishing between polluted and non-polluted beaches. Coliphage and enterococci were good predictors of pathogen transport.
3. Fecal pollution in the Charlotte Harbor estuary was localized in regions of low salinity, high freshwater input, and dense septic systems. Widespread contamination of the harbor was found to be seasonal in nature; wet weather events

were important contributing factors in the transport of human viruses far into the estuary. Based on the findings of this study, it was determined that factors such as precipitation, stream flow, and temperature would be useful in modeling and forecasting periods of poor coastal water quality.

### Questions and answers

1. *Since C. perfringens densities are about 100 times less than that of other indicators such as fecal coliforms, filtering 100 mL of water samples may not detect the organism. Were larger volumes of samples filtered to detect C. perfringens?*

In the studies conducted in Florida, *C. perfringens* was below the detection limit. Therefore, large volumes of samples had to be used for its analysis. This was the case near some sampling sites (Charlotte Harbor study) where river water was greatly diluted by seawater. However, for some of the inland waters that were murky/turbid, large volumes of water could not be filtered; as such, a different methodology was required for *C. perfringens* analysis.
2. *Studies conducted in Hawaii have shown that C. perfringens can be used as a marker of sewage. If enterococci is used a marker of sewage and pathogens, why not C. perfringens?*

The EPA studies conducted in the early 1980s did in fact show that *C. perfringens* counts were measurable in the water at the impacted sites. However, the relationship between *C. perfringens* counts and health effects was very poor.
3. *What type of coliphage was used in this study?*

Male-specific (F-specific) FRNA coliphages were used.
4. *What criteria were used to define pollution/contamination of the study sites?*

Different concentrations of various fecal indicator bacteria (fecal coliforms, enterococci, *C. perfringens*) as well as other parameters were used. Based on these data, the sites were classified into low-risk, medium-risk and high-risk categories.
5. *How does one interpret data on enteroviruses, given the fact that there are numerous types of these viruses coming from different sources and also given the fact that cultivation and methodologies to recover these viruses differ so much?*

Only certain enteroviruses were identified in this study. Some cell culture PCR was done to detect those non-cytopathogenic effect producing viruses. Clearly, the cultivation of viruses from water samples underestimates the potential for loading of enteroviruses to the environment. Adenoviruses were not investigated in this study. However, previous studies conducted in California showed that adenoviruses are more persistent in the environment than enteroviruses. The Florida studies basically compared the die-off rates of enteroviruses under natural conditions and varying salinities. Under these conditions, substantial inactivation of these viruses can be achieved.
6. *Were the ribotyping patterns used to compare the various sources of fecal indicators?*

No. The ribotyping patterns were used to compare only human versus animal sources. The multiple antibiotic resistant patterns were used to answer several

questions—for instance, what was the likelihood that the isolates came from human, cattle, dog, or other sources?

7. *Studies conducted in Australia have shown that adenoviruses were 2 to 3 orders of magnitude more numerous than other human viruses. Given the chance that the study is repeated again, would enteric viruses still be chosen for monitoring and for correlation with index of human impact?*

Yes; however, the new study should add adenoviruses to the list at the expense of ribotyping. Multiple antibiotic resistance patterns would be used as one of the ways to discriminate among the sources of fecal indicators.

## **PRESENTATION BY DR. RICHARD WHITMAN**

USGS, Porter, Indiana

**Title:** Characterization of *E. coli* at 63<sup>rd</sup> Street Beach (Chicago)

### **Speaker information**

Dr. Richard Whitman is chief aquatic ecologist with the United States Geological Survey at the Lake Michigan Ecological research Station in Porter, Indiana. He has been conducting water quality studies in the Chicago area and has reported high numbers of fecal bacteria in water at Chicago beaches, in the absence of sewage contamination. The results of Whitman's studies were generally not available when this workshop was being planned.

### **Presentation objectives**

The objective of Whitman's presentation was to review the results of his studies of high densities of *E. coli* in the water at 63<sup>rd</sup> Street Beach in Chicago, a temperate area.

### **Major points**

1. A six-month (April to September 2000) intensive study was undertaken to determine the potential sources and distribution of *E. coli* in the water and sand at 63<sup>rd</sup> Street Beach, Chicago. In April, water samples were collected at two depths (45 cm and 90 cm) along five transects three days each week. Onshore and submerged sand samples were collected at the same transects. Between May and September, additional sets of samples were collected in the afternoon at the same locations along the five transects. Field observations of a number of seagulls on the beach, wind speed, air and water temperatures, and wave height at the 45 cm depth were recorded. To determine the effect of sunlight on *E. coli* survival, an on-site experiment (8:00 a.m. to 3:00 p.m.) was conducted using clear and dark bags containing lake water. Molecular techniques using DNA analysis of gull droppings were used to determine potential sources of *E. coli* at the beach. Finally, water samples were analyzed for wastewater compounds in order to determine potential sources of contamination.

2. *E. coli* concentrations in water samples collected at both depths (45 and 90 cm) and times (morning and afternoon) were correlated with each other. Similarly, *E. coli* in sand samples at foreshore and submerged sites were correlated. *E. coli* densities were lower in the deeper water (90 cm) than in the shallow water and higher in morning samples than in afternoon samples.
3. The *E. coli* counts were considerably higher in the sand samples than in the water samples; they were highest in the sand samples collected near the highest density of seagulls on the beach.
4. Hourly sampling results indicated a dramatic decrease in *E. coli* counts over the course of the day. The counts exceeding the safe limit (standard) in the morning typically dropped off to concentrations below the safe limit in the afternoon. The densities decreased exponentially between 8:00 a.m. and 3:00 p.m. Results of sunlight (light/dark bag) experiments provided evidence of sunlight inactivation of fecal bacteria and supported the hourly sampling *E. coli* results.
5. Fingerprinting of seagull isolates indicated that *E. coli* and enterococci at the beach were partly derived from the resident seagull population. DNA analysis of *Salmonella* spp. showed a relatively close match among gull droppings, water, and sand samples. However, it was possible that some *Salmonella* spp. could have come from other birds. Isolates of *E. coli* and *Salmonella* spp. were highly susceptible to antibiotics, indicating a non-human source.
6. Efforts to model the occurrence and persistence of *E. coli* at the beach showed that the best predictors overall were rainfall, wave height, wind speed, air temperature, solar radiation, lake level, turbidity, and chlorophyll A concentration of the lake water.

### **Conclusion and recommendations**

1. The focus of this study was to determine the potential sources and distribution of *E. coli* at the 63<sup>rd</sup> Street Beach in Chicago. Although all sources of *E. coli* were not identified in the course of this study, it was determined that seagulls and beach sand were among the largest contributors of *E. coli* to the beach water.
2. Predictive models that were developed over the course of this study might alleviate shortfalls in sampling precision and timely reporting. The complexity of the 63<sup>rd</sup> Street Beach system and the interacting factors associated with a beach in a metropolitan area would make source determination difficult. However, with more information about other beaches and influences along the Lake Michigan shoreline, *E. coli* contamination may eventually be minimized.
3. The results of this study were very significant because this was probably the first major report on environmental persistence and possibly multiplication of indicator bacteria such as *E. coli* under temperate climatic conditions.

### **Questions and answers**

1. *What was the nature of the sediment samples analyzed in this study?*  
About 96% to 97% was sand, and the rest constituted silt and organic matter.

2. *How long did it take for re-establishment of E. coli in the new sand that was brought from elsewhere to prevent/minimize contamination?*  
It took approximately two weeks.

## PRESENTATION BY DR. NICHOLAS ASHBOLT

University of New South Wales

**Title:** Blooming *E. coli*, what do they mean?

### Speaker information

Dr. Nicholas Ashbolt is an associate professor of Water Engineering at the University of New South Wales, Sydney, Australia. He has conducted numerous studies on the use of indicator bacteria to assess sewage pollution. He has evaluated many projects in other parts of the world, including tropical areas of Australia and Hong Kong.

### Presentation objectives

The objective of Ashbolt's presentation was to review the findings of his own work and a recent publication, which has the same title as this presentation.

### Major points

1. Historically, blooms of *E. coli* and other coliform bacteria belonging to genera such as *Citrobacter* and *Enterobacter*, as well as other aquatic bacteria such as *Aeromonas* have occurred in Lake Burragorang, which is located near Sydney, Australia. This artificial lake was formed in 1961 when Warragamba Dam was completed. The lake, which is approximately 48 km in length and 0.6 km in width, supplies drinking water to about 75% of Sydney's 3.8 million inhabitants.
2. Monthly data collected over a period of 20 years (1972–1992) indicated that thermotolerant coliforms can be recovered in surface waters, as was done in an area some 300 m from Warragamba Dam, with an Austral summer peak in December and a smaller peak during autumn in April. The rainfall data collected during the same period showed a similar bimodal distribution with peaks preceding the thermotolerant coliforms by 1 to 2 months. The maximum counts occurred in the first few meters of the surface water, then the counts decreased with depth.
3. Blooms of thermotolerant coliforms appeared to commence during stratification when the surface water temperature was about 18°C.
4. Based on several years of monitoring data, it was concluded that sewage/feces was not the source of the *E. coli* in the lake. If that were the case, the 1978 *E. coli* bloom, which resulted in nearly 300,000 CFU/100 mL, should have occurred in the presence of about 8,000 CFU/100 mL of fecal streptococci (based on the ratio of thermotolerant coliforms to fecal streptococci in sewage). The absence of fecal streptococci in the lake water could only be explained based on the quantities of

fecal materials otherwise required to give the densities of indicators observed. For instance, during the 1981 *E. coli* bloom, it would have required at least 300 tons of human feces, 12,900 tons of cow feces or 296,000 tons of horse, pigeon, or sparrow feces input to account for the *E. coli* concentration in the water.

5. Selected thermotolerant coliform isolates were identified by the API 20E system. A majority of the isolates were typed as *E. coli*, and these environmental isolates showed some distinct phenotypic markers when compared with clinical isolates.
6. Molecular techniques such as the entire 16S rDNA sequencing, ribotyping and restriction fragment length polymorphism were used to determine if environmental *E. coli* isolates were different. The environmental isolates were found to be different based on ribotyping and on sequencing of the 16S rDNA regions that were not previously reported as being variable. Further studies are underway to identify a region in the rRNA operon in order to develop specific probes for identifying the environmental strains.
7. Coprostanol, a fecal sterol, comes from cholesterol in the diet. Microorganisms in the human gut convert the cholesterol into 5 $\beta$ -stanol (coprostanol), which is then excreted with feces. The 5 $\beta$ -stanol or coprostanol is fairly human-specific. Studies were undertaken in Australia to examine the source and specificity of fecal sterols and to detail the distinguishing characteristics of the fecal biomarkers from humans and other animals. The sterol profiles of the herbivores were mainly dominated by C<sub>29</sub> sterols with relatively low concentrations of 5 $\beta$ -stanols. Human feces contained ten times more coprostanol on a dry weight basis than feces from cats or pigs. The feces of other herbivores such as cows, sheep and horses contained some coprostanol, but 24-ethylcoprostanol, which can be used as a biomarker of fecal pollution by these animals, dominated their sterol profiles. These studies clearly demonstrated that based on the ratios of total sterols to coprostanol, it was possible to distinguish between human and non-human contamination; an index >0.4 suggested human contamination in this study.

## Conclusion and recommendations

Twenty years of data collected in Australia have shown that thermotolerant coliforms such as *Citrobacter freundii*, *Enterobacter cloacae*, and *E. coli* have the ability to not only persist in an aquatic environment (Lake Burragorang) but also to multiply in the water, producing extensive blooms under warmer conditions (18°C). These studies concur with those done in tropical locations of the United States (Hawaii, Puerto Rico), the results of which showed that fecal indicator bacteria have the potential to grow under environmental conditions.

## Questions and answers

1. *How many different animals were looked at while evaluating the efficacy of fecal sterols as an indicator of fecal contamination?*  
Initially, 20 different animal species were tested with 25 samples from each species. However, most of the assays were done with 15 species of animals and 25 samples from each source.

2. *How was the “control experiment” done to demonstrate the inactivation of coprostanol in the water?*  
Human sewage was placed in large bags and immersed in the river. The data showed that loss or inactivation of the chemical (coprostanol) was mediated by background microorganisms and was dependent on temperature. In general, the rate of degradation was very similar to that observed for enterococci under comparable conditions.
3. *What was the sensitivity of the coprostanol assay in those studies?*  
This assay method can detect as low as nanograms (ng)/L of coprostanol in water samples.
4. *Since the fecal sterols are biodegradable, how will their decay rate be influenced by temperature?*  
Most of the studies (involving both marine and fresh waters) in Australia were carried out at 12° to 14°C. It is possible that faster degradation rates can occur at higher temperatures. However, further studies are needed to demonstrate the rate of degradation of these compounds as a function of temperature.
5. *What is the dynamic range of this assay?*  
The range is approximately 5 orders of magnitude in terms of gram per feces.
6. *Did any of the E. coli isolates recovered from the environment test positive for toxin production?*  
We did not focus on this aspect in our studies. However, one interesting observation was that most of the *E. coli* isolates from the environment were serotyped as O8. This particular serogroup is very common in cattle.

# CHAPTER 4

## WORKSHOP CONCLUSIONS AND RECOMMENDATIONS

### TASKS AND PROCEDURES FOR AFTERNOON SESSIONS

The format of this two-day workshop was to assign different tasks for the morning sessions and for the afternoon sessions. The task for the morning session was to have selected speakers present the available data to address the problem statements for that day. The problem statements, initially stated as agenda questions assigned for each of the days, were included in the guidance document (see Appendix D) sent to all experts before the workshop. The agenda questions typically reflected the concerns stated by EPA in the Beach Action Plan of 1999. The proceedings of the first day's morning session are summarized in Chapter Two of this report while the proceedings of the second day's morning session are summarized in Chapter Three. The task for each of the two afternoon sessions was to have all experts discuss the results of the morning presentations and to reach consensus conclusions and recommendations. This chapter summarizes the proceedings of the afternoon sessions during day one and day two. To ensure that the task for the afternoon session would be met, a session leader for each of the afternoon session was selected from among the invited experts. The session leaders were selected based on their acknowledged leadership among the experts and based on their being someone who was not involved in monitoring the tropical environment. The specific task of the session leader was to lead the discussions among the experts to assess the presentations made during the morning session and to develop concluding statements to address the issues that were discussed. The degree of consensus for the concluding statements was determined by a hand vote as to how many experts agreed, disagreed and abstained from voting for the concluding statement. After the voting, the concluding statement was called consensus statement. It should be noted that the problems to be addressed for each day of the workshop were stated as agenda questions. However, since some of the wording and statements used in the agenda questions meant different things to different experts, the group decided to craft their own concluding statements followed by some recommendations for future courses of action.

### AFTERNOON SESSION ON DAY ONE: DISCUSSIONS AND CONCLUSIONS

**Session Leader:** Dr. Charles Gerba, professor of Microbiology at the University of Arizona, led the discussion for the afternoon session on day one.

## Agenda Questions

The following three questions were placed on the agenda as problem statements to be discussed during day one of the workshop.

1. Are there sufficient experimental and monitoring data to conclude that the assumption used in interpreting water quality standards (there are no significant environmental sources of fecal coliforms, *E. coli*, and enterococci) is not applicable in tropical areas (Hawaii, Guam, Puerto Rico, south Florida) because these bacteria can be recovered in high concentrations from ambient environments (water, soil, plants) in these areas?
2. Are there sufficient experimental and monitoring data to conclude that the EPA criteria (*E. coli*, enterococci) used to assess the quality of environmental waters are not reliable in tropical locales (Hawaii, Guam, Puerto Rico, south Florida) because the selected fecal bacteria persist in these ambient environments and represent non-fecal contamination?
3. Are there sufficient experimental and monitoring data to conclude that the EPA-recommended recreational water quality standards are not suitable to assess the hygienic quality of environmental waters in Hawaii, Guam, Puerto Rico, and south Florida?

## Discussion Points Leading to Consensus Statements

1. *Are fecal indicator bacteria consistently found in the environment of tropical areas?*  
Although there was a general agreement that fecal indicator bacteria are naturally present in the tropical environment, the reported environmental sources of fecal indicator from the four tropical sites were not necessarily the same. Extensive data was obtained to show that soil is the predominant environmental source throughout Hawaii. Based on limited data, evidence was obtained to show that soil is perhaps the major environmental source in Guam. In south Florida, the source of environmental fecal indicator bacteria was related to wet and dry soil sites next to an inland river. In Puerto Rico, experiments focused on bromeliads as the environmental source of fecal indicator bacteria. These results reflected different emphases and experimental designs conducted at the different tropical locales. To determine whether environmental sources of fecal bacteria are similar at the different tropical locales, the same kinds of experiments must be conducted at these sites. In summary, although results from the four tropical sites support each other, the specific results obtained at one site cannot be assumed to be applicable to all other tropical locations.
2. *How reliable are current enumeration methods for fecal indicator bacteria?*  
Current methods used to enumerate fecal indicator bacteria from environmental waters cannot determine whether the identified fecal bacteria actually came from a human, animal, or environment source. Thus, one expert stated that monitoring data itself would be difficult to interpret because it could contain bacteria from several

types of contaminating sources. Environmental survey data are required to better interpret monitoring data. Even when this is done, such as the reported monitoring data from the four tropical locations, it is still possible that some of the fecal indicator bacteria recovered originated from fecal sources (e.g., animal waste, septic tanks) in addition to the proposed environmental source.

3. *Can bird feces be the source of environmental fecal indicator bacteria?*

Birds are known sources of fecal indicator bacteria that may contaminate many natural environments. One expert pointed out that there was a shortcoming in trying to explain the primary source and persistence of fecal indicator bacteria in all tropical environments. Under these conditions, the contributions of fecal indicator bacteria from animals, especially bird droppings, have not been sufficiently documented. It might be expensive to look for such sources, but it would be worthwhile to look at such evidence. Another expert suggested that if bird droppings were the major sources of indicator bacteria, the effect would be more localized, resulting in greater recovery of such bacteria from the contaminated sites and water site. However, numbers of fecal indicator bacteria in Hawaii's streams are consistently elevated and exceed the levels established for setting recreational water quality standards. These results do not support the observation that increased counts in the water are caused by contamination by feces from wild animals and birds. Based on observations in Puerto Rico, one expert expressed similar views. He stated that only a few mammals and birds are found in the mountainous rain forests in Puerto Rico, and yet environmental waters exceed the fecal coliform standard, indicating that animal and bird populations are not significant sources of fecal indicator bacteria in this location. Finally, it was pointed out that due to the dense snake population in Guam, there are few birds, and yet the high ambient concentrations of fecal indicator bacteria in the streams of Guam are similar to concentrations observed in Hawaii's streams.

4. *What is the role of microbial ecology in assessing water quality?*

One expert pointed out that historical and ecological studies indicate that the original natural habitat of coliform-type bacteria was the ambient soil and plant environment. In the historical time frame, mammals arrived later. One ecological theory states that some of these environmental coliform bacteria subsequently adapted to and colonized in the gut of mammals. If this hypothesis is correct, it would be reasonable to assume that fecal bacteria, which grow in mammalian gut, have the genetic capacity to multiply in the environment. Moreover, multiplication would be much more likely in tropical locales because they provide environmental conditions such as warm temperature, sufficient moisture, and nutrients, all of which are well within the range of growth requirements for fecal bacteria.

5. *Should the term "fecal coliforms" be replaced by the term "thermotolerant coliforms"?*

Several experts suggested that the term "fecal coliforms" is misleading because the method used to recover coliform bacteria at elevated temperatures (44.5°C) was supposed to select for coliform bacteria of fecal origin. However, the selectivity of this method is only an approximate because the same method recovers some coliform bacteria (*Klebsiella pneumoniae*, *Citrobacter freundii*), which have

natural environmental sources such as soil and plants. Thus, when the fecal coliform assay is used, most of the experts agreed that it would be more correct to call the isolates thermotolerant coliforms rather than fecal coliforms. This concept becomes more significant if the sample source is environmental. This raised another issue. If bacteria from feces became established and multiplied in soil, should they still be called fecal bacteria? In summary, most experts agreed that the term fecal coliforms is technically misleading because the method to recover fecal coliforms is known to recover coliform bacteria of fecal origin (*E. coli*) as well as of environmental origin. Although the experts agreed that it would be preferable to change the term to thermotolerant coliform, they also agreed that many people in the water industry have used and understand the term “fecal coliforms” and would be confused with the term thermotolerant coliform. Thus more education is needed before any change in terminology can be implemented.

6. *How reliable are the EPA-recommended water quality criteria for recreational waters?*

As summarized by Dr. Alfred Dufour in his presentation in Chapter Two, the first recreational water quality standards used in the United States was based on concentrations (1,000 MPN/100 mL) of total coliforms. In the early 1970s EPA recommended that the recreational water quality standards be changed to use the fecal coliform (200 CFU/100 mL) standard. In 1986, EPA recommended that all states adopt newer standards based on concentrations of enterococci and *E. coli* because the newer standards were based on measured health risks whereas the previous standards were not. Today, some states continue to use previous standards based on total coliform, fecal coliform as well as the current standards.

Implementation of both kinds of water quality standards have been credited with reducing incidences of waterborne diseases. During the discussion, experts pointed out that results of recent and additional epidemiological studies conducted throughout the world have supported the results of EPA studies, which showed a correlation between increased levels of fecal indicator bacteria (*E. coli*, enterococci) in water and increased incidences of disease rates among swimmers. The study conducted in the West Indies by Ms. Christine Bullock (presented in Chapter Two) is an example of a more recent epidemiological study conducted in the tropics that supports the results and conclusions of the EPA study from which the current recreational water quality standards were developed. The West Indies epidemiological study was similar to the classical EPA studies in that the beach sites were contaminated with a sewage source. Therefore, the source of fecal indicator bacteria in the water was most likely from sewage (point source).

One expert raised the question on the reliability of the current recreational water quality standard when the source of fecal bacteria is not from sewage but from a non-point source, since EPA reported that when the fecal indicator bacteria is from a non-point source, their numbers in recreational waters cannot reliably be used to predict illness rates among swimmers. Thus, when streams, storm drain discharges, or drainage from a complex watershed are the sources, the numbers of fecal indicator bacteria in environmental waters may not predict illness rates among swimmers, as implied by the use of the current recreational water quality standards. Moreover, it was pointed out that if fecal bacteria multiplied in the tropical soil,

their numbers would not be expected to hold the same relationship to fecal-borne pathogens because many sewage-borne pathogens (viruses, protozoa) would not be able to multiply under environmental conditions. This led to the reasoning that the EPA water quality standards are less reliable or are not suitable in tropical regions. However, another expert pointed out that, to determine the health risk, the tropical region in question must determine from its water monitoring data, what percentage of fecal indicator bacteria represents sewage and non-sewage or non-point source contamination. That expert did not agree with the overall statement that EPA criteria (*E. coli* and enterococci) are not reliable in the tropics. That expert stated that the reliability of the EPA criteria in all locations should be viewed from the context of changes in relationship between fecal bacteria and pathogens based on the source of the recovered microbial indicator. The limitation cited was that the approved monitoring methods do not determine the source of the fecal indicator. It was concluded that methods must be developed to determine the source of the recovered fecal indicator bacteria so appropriate risk assessment can be made.

7. *Are EPA recreational water quality criteria always reliable in temperate climates of the world?*

Although the workshop focused on the reliability of recreational water quality standards in tropical locales, several reports indicated that the recreational water quality standards may not always be reliable, even in temperate locales. In this regard, Dr. Richard Whitman reported that his studies on beaches in the Chicago area represent the first major report on environmental persistence and possibly multiplication of indicator bacteria such as *E. coli* under temperate climatic conditions in the United States (see summary of presentation in Chapter Three). Dr. Nicholas Ashbolt presented evidence for persistence and growth of *E. coli* in a natural reservoir near Sydney, Australia, when water temperature exceeded 18°C (see summary of presentation in Chapter Three). Dr. Gillian Lewis reviewed the conclusions of her previously published study (paper submitted to all experts before the workshop) that fecal streptococci detected in high concentrations in decaying seaweed found on beach sand in New Zealand represented bacteria from an environmental source in a temperate climate that was not related to a sewage or fecal contamination event. However, one expert commented that based on the results of several epidemiological studies, the current fecal microbial indicators of water quality seem to work well in temperate climates. On the other hand, another expert commented that the current indicators might not necessarily work in all temperate climates. Thus, the reliability of *E. coli* and enterococci as indicators of risk to humans under all conditions, even in temperate areas of the world, is not always certain.

8. *Why did Hawaii establish a marine recreational water quality standard of 7 CFU enterococci/100 mL, which is so much more stringent than the EPA standard of 35 CFU/100 mL?*

Hawaii's standard is more stringent because state epidemiologists concluded that the expected incidence of disease (19/1,000 people), which is predicted by EPA to be related to 35 CFU enterococci/100 mL, is too high a risk. Using EPA data, Hawaii raised the standard to 7 CFU/100 mL because this represented an acceptable risk of 10 disease incidences per 1,000 people. Thus, state

epidemiologists accepted the predictable risk of diseases based on EPA's epidemiological studies conducted at beaches where sewage was the source of fecal indicator bacteria. However, epidemiologists and other officials in Hawaii also concluded that the high concentrations of fecal indicator bacteria in the streams and storm drains as well as coastal waters that receive land-based runoff are from non-point sources (ambient environment, soil, urban runoff) and not from point sources (i.e., sewage). This is why the state does not close streams and coastal beaches when the water quality exceeds current recreational water quality standards. The next question for discussion was whether changing Hawaii's water quality standards to current EPA standards would eliminate the problems related to meeting water quality standards in Hawaii. The experts from Hawaii responded that if the state adopted the EPA water quality standard, most marine beaches would meet the EPA standard based on geometric mean concentrations of 35 CFU enterococci/100 mL. However, the quality of Hawaii's freshwater streams would still not be able to meet the EPA water quality standards based on geometric mean concentrations of 33 CFU enterococci/100 mL or 126 CFU *E. coli*/100 mL. Thus, Hawaii is in need of a reliable monitoring method and appropriate recreational water quality standard to assess when streams are contaminated with sewage.

9. *How can we assess risk associated with non-point sources (soil, plants, animal feces, bird feces) of fecal indicator bacteria in environmental waters?*

The experts expressed several views. One proposal was to further characterize the isolated fecal indicator bacteria by identifying the source, and then the source can be used to determine relative risk. However, reliable and feasible methods to identify all sources of fecal indicator bacteria are not available. Many methods are still in developmental stages, and the results of many of these methods indicate that a large database may be required and that the database established for one region of the country may not be applicable to another region. The second proposal, which came from several experts, was to monitor for pathogens as an alternative strategy to determine health risk. However, another expert pointed out that a major limitation of monitoring for pathogens was that there were so many different kinds of pathogens that it would not be feasible or practical to monitor for all pathogens. Another expert pointed out that certain pathogens such as enteric viruses in infections in humans were seasonal (May to October) in temperate climates but more consistent throughout the year in tropical countries. This can affect the pathogen monitoring data from environmental waters. Another expert pointed out that monitoring for pathogens on a regular basis would be very expensive and would require specially trained people and expensive equipment. These required personnel and equipment are not available in most monitoring laboratories. In the final analysis, it was pointed out that routine monitoring for specific pathogens was not feasible for most laboratories and so those pathogens could not be used as an indicator of risk. However, some projects can monitor for pathogens and could provide enough data to be used to assess risk to swimmers.

10. *Could the soil environment in tropical locations be a significant source of fecal indicator bacteria?*

Gerba asked the experts whether they agreed that soils in tropical locations could be a significant environmental source of fecal indicator bacteria. The experts

generally agreed that based on available scientific data, soil should be considered as a source of fecal indicator bacteria and that it should be added to the list of external sources of such bacteria. However, the experts also cautioned that more experiments should be conducted to confirm that soil is a major environmental source of indicator bacteria in other tropical regions of the world. One expert inquired whether plants should be similarly considered as sources of fecal indicator bacteria based on evidence collected in Puerto Rico. However, since data was not available to show significant concentrations of fecal indicator in various types of plants, further discussions on this matter were not pursued.

11. *Do experts want more information to reach consensus statements?*

One expert commented that all experts should hear the presentations during the second day before reaching conclusions on the first day because the issue of persistence of fecal microorganisms in the environment (first day question) is closely related to the issue of multiplication of fecal microorganisms (second day questions). Most of the experts agreed. As a result, although considerable discussion took place during the afternoon of the first day, final discussion to reach consensus statements took place the following afternoon.

## **Development of Consensus Statements: Day One**

The presentations made during day one addressed the problem statements raised in Agenda Questions One, Two and Three (see the “Agenda Questions” section in this chapter). These three questions focused on persistence of fecal indicator bacteria in the environment of tropical locales and their impact on reliability of EPA-recommended recreational water quality standards. The problem was characterized by the finding of environmental sources of fecal indicator bacteria in tropical locales, which contributed to the elevated densities of fecal indicator bacteria in environmental waters. The major issue was whether densities of environmental sources of fecal bacteria in recreational waters are predictive of sewage contamination and therefore correlated with health risks as implied by recreational water quality standards. Gerba initially led the experts to discuss the major points raised during the first day of this workshop and then led the group to develop consensus statements. All experts were involved in crafting the words for acceptable consensus statements. Two concluding statements were developed. All experts then voted to accept or not accept them. After voting, the concluding statements were called consensus statements. The consensus statements represent general or collective opinion of workshop participants but not 100 percent agreement in most cases. The two consensus statements with their voting results are presented below:

### **Consensus statement one**

Soil, sediments, water, and plants may be significant indigenous sources of indicator bacteria in tropical waters.

This statement was crafted in response to Agenda Question One. All 18 (100%) of the experts voted in support of this consensus statement.

## Consensus statement two

The inherent environmental characteristics of the tropics affect the relationships between indicators of fecal contamination (*E. coli*, fecal coliforms, enterococci) and health effects observed in bathers, which may compromise the efficacy of EPA guidelines.

This statement was crafted in response to Agenda Questions Two and Three. Sixteen of eighteen experts (88.8%) voted to support this statement. Two experts abstained from voting.

## AFTERNOON SESSION ON DAY TWO: DISCUSSIONS AND CONCLUSIONS

**Session Leader:** James Tiedje, professor of Microbiology at Michigan State University, led the discussion for the afternoon session on day two.

### Agenda Questions

The following two questions were placed on the agenda for day two of the workshop:

1. Are there sufficient experimental and monitoring data to conclude that the assumption used in interpreting water quality standards (there are no significant environmental sources of fecal coliforms, *E. coli*, and enterococci) is not applicable in tropical areas (Hawaii, Guam, Puerto Rico, south Florida) because these bacteria can be recovered in high concentrations from ambient environments (water, soil, plants) in these areas?
2. Are there sufficient experimental and monitoring data to conclude that the EPA criteria (*E. coli*, enterococci) used to assess the quality of environmental waters are not reliable in tropical locales (Hawaii, Guam, Puerto Rico, south Florida) because the selected fecal bacteria persist in these ambient environments and represent non-fecal contamination?

### Tiedje's Review of Principles of Microbial Ecology

Tiedje stated that the presentation of data during day two of the workshop focused on the fate (survival, persistence, multiplication) of fecal indicator bacteria under environmental conditions. He stated that microorganisms under environmental conditions are controlled by major physical factors (temperature, moisture, salinity, radiation), which are known to affect the survival of these bacteria. However, he stated that ecological principles for microbial interactions with the environment also play an important role in the fate of microorganisms but that this concept was not sufficiently discussed. As a result, Tiedje provided a brief summary of current concepts in ecological principles for microbial interactions in the environment. He stated that these concepts should be considered in the interpretation of data

on persistence and regrowth (multiplication) of indicator bacteria in natural environments under tropical conditions. These ecological principles are outlined below:

1. Resident microbe (vs. visitor) growth and reproduction
  - a. Growth requirements
    - 1) Temperature
    - 2) Moisture
    - 3) Food
  - b. Competition  $\mu_{max}/K_S$
  - c. Spatial isolation
  - d. Carrying capacity
2. Population genetics
  - a. Species not absolutely defined
  - b. Patterns of variation due to
    - 1) Specific habitat (ecovars)
    - 2) Random mutation (genetic variants)

Tiedje briefly discussed each of these ecological characteristics and suggested how they could be used to explain the persistence and multiplication of fecal indicator bacteria in natural environments in the tropics. In the soil environment, microbial growth and reproduction are controlled by various physical and chemical factors (temperature, moisture, pH, nutrients). Consequently, the same factors must also control the persistence and growth of fecal indicator bacteria in the soil. He emphasized that there was enough data (from Hawaii and Florida) to show how physical parameters such as temperature and moisture can influence the persistence of indicator bacteria in the soil environment.

Tiedje pointed out a well-established fact that microbial growth in natural environments (soil, water) is often limited by the nutrient supply. Therefore, the same ecological constraint is also applicable to fecal indicator bacteria found in such environments. However, it is not surprising that under these conditions microorganisms can utilize various strategies to sustain their populations over time. More often than not, they are known to be in a resting stage with very little metabolic activity. Other important survival strategies include competition with other microbes for available food and other essential nutrients, ability to utilize very complex organic molecules, and spatial isolation. Tiedje further elaborated on the issue of spatial isolation and indicated how this ecological phenomenon can negate the effect of competition between microbial populations. Although some of the studies conducted in Hawaii seem to suggest that competition for available nutrients (carbon and energy sources) might restrict *E. coli* multiplication in the soil, it needs to be determined whether fecal indicator bacteria are spatially isolated in order to persist and become part of the soil microbiota. In reply to a question of whether competition exists among the indicator bacterial populations occupying the same niche, Tiedje was affirmative about this possibility.

Tiedje further elaborated on other ecological issues such as the carrying capacity, which is the largest population that a habitat can support, and how this particular issue could explain the relative abundance of indicator bacteria in the soil. He commented that the carrying capacity could not be fully explained without taking into account the other biological aspects such as competition among microorganisms and their spatial isolation.

Regarding the population genetics of the environmental indicator bacteria, Tiedje suggested that there was not enough data at the present time to indicate that the environmental isolates were distinctly different from those occurring in the gastrointestinal tract of humans and animals. He said that based on available information, it is speculated that *E. coli* and *Salmonella* divergence occurred approximately 100 million years ago. However, at the present time, there is no good explanation to answer the question of how the fecal coliforms (*E. coli*) inhabited the soil environment. Two possible scenarios can be proposed in this regard. First, the soil colonization could have occurred following the divergence of *E. coli* and *Salmonella* species. In this situation, certain strains of *E. coli* could have colonized the gut of warm-blooded animals while other strains inhabited the soil environment. Second, *E. coli* colonization of soil could have occurred independent of *E. coli* and *Salmonella* divergence. Since genetic and habitat-specific (ecovars) variations are not uncommon among microorganisms, to what extent such variations can explain the occurrence of *E. coli* and other fecal indicator bacteria in human and animals vs. other natural environments (plants, soil, water) needs to be further explored.

In regard to the source of inoculum, Tiedje indicated that there was no definite answer to this question. He agreed that fecal matter from various sources (human, animals, birds) could serve as the primary source of inoculum for soil colonization of fecal indicator bacteria.

## Comments From Experts

Several comments came from experts while Tiedje was discussing the ecological issues to explain the persistence and multiplication of fecal indicator bacteria in natural environments. These comments are summarized below.

1. *Spatial isolation of microorganisms and competition*  
Competition would not be a major factor if microorganisms were spatially isolated. However, if closely related microorganisms occupy the same niche, competition between these organisms will affect their ability to survive.
2. *Microbial growth rate under natural conditions*  
Experiments designed under laboratory conditions to determine growth rate of microorganisms in various environments (e.g., soil) usually use simple substrates such as glucose. However, an expert pointed out that under natural conditions a mixture of complex substrates is often the source of nutrients. As a result, microbial growth under these conditions can be expected to differ from that under laboratory conditions.
3. *Persistence of fecal indicator bacteria in the soil environments and source of inoculum*  
Tiedje stated that deposition of fecal matter into the environment by animals as well as humans is a common occurrence and this is a likely source of inoculum for *E. coli* to colonize soil environments. However, another expert pointed out an alternative theory that *E. coli* had initially established itself as part of the

indigenous microflora of the soil environment and this source served as the inoculum for its establishment in the gut environment of all mammals.

4. *Recovery of fecal bacteria from various types of soil on island of Oahu*  
The ability of *E. coli* to colonize soil was inferred based on the recovery of fecal bacteria from different types of soil (9 soil orders). Although these results address the issue of prevalence in the environment, it was pointed out that the criteria used to classify soil might not be directly related to the ability of that soil to have a better carrying capacity for the colonization of *E. coli*. Other factors such as moisture content, available nutrients, and temperature may play more important roles.
5. *Reliability of molecular methods*  
Using molecular techniques, a small portion of the genetic material (such as the ribosomal DNA) is currently used to identify and characterize bacteria. Tiedje stated there is a limitation to this approach because the changes measured are a minor part of the genome and may provide misleading results. He stated that the better approach is to look for changes in larger portions of the whole genome instead of selecting only a small portion of it.

### **Discussion Points Leading to Consensus Statements**

1. *Based on available data, is there consensus agreement to conclude that indicator bacteria such as E. coli have the potential to multiply in the environment?*  
Most of the experts agreed that *E. coli* has the potential to multiply in natural environments such as soil and water. This conclusion was supported by widespread persistence of *E. coli* in environmental waters, primarily in tropical areas. Additional data was presented that this could be happening in some temperate areas as well. For example, evidence for multiplication of *E. coli* was reported in Chicago, Illinois, and in Sydney, Australia. Although growth of *E. coli* in soil was demonstrable, the experimental conditions did not reflect entirely natural conditions. Most experts agreed that definitive experimental data to prove that *E. coli* populations were growing under environmental conditions were not presented. In this regard, it was acknowledged that it would not be easy to obtain definitive data under entirely natural conditions to demonstrate regrowth of natural populations of *E. coli* or enterococci in soil. Finally, several experts stated there was less evidence to conclude that *E. coli* can multiply on plants.
2. *Is it reasonable to assume that human pathogens do not multiply in the environment?*  
Based on known growth requirements of most sewage-borne viruses and protozoa, most epidemiological studies previously conducted have assumed that human pathogens such as human enteric viruses and protozoa (*Giardia*, *Cryptosporidium*) cannot be expected to multiply under environmental conditions. In contrast bacteria have the capacity to multiply in the environment, and there have been some reports that some enteric bacterial pathogens are capable of multiplying in some environments. However, it was concluded that better experiments should be

conducted to determine the environmental conditions that would allow these bacterial pathogens to multiply.

3. *Are environmental sources of indicator bacteria indicative of lower health risk than those from fecal sources?*

There were mixed opinions regarding this statement. The major argument in support of this statement is that the source of serious waterborne pathogens (human enteric viruses, *Giardia*, *Cryptosporidium*) is sewage or feces and since they cannot multiply under ambient conditions, fecal bacteria from environmental sources would not represent these pathogens. Moreover, since most human enteric viruses are shed by human feces but not by animal feces, animal feces would pose a lower risk of human infection than human feces. However, animal feces is often associated with pathogenic bacteria (*Salmonella*, *E. coli* 0157) as well as *Giardia* and *Cryptosporidium* that contamination of water by animal feces represents a risk for transmission of diseases to humans. In summary, if indicator bacteria multiply in the environment, it would be reasonable to conclude that the increase in numbers measured in that environment (soil, water) would not be correlated to the degree of fecal contamination and predictable levels of fecal-borne pathogens. Under these conditions, measurements of environmental sources of fecal indicator bacteria in water are indicative of lower health risk as compared to measurements of fecal indicator bacteria from sewage or feces from humans and animals. The experts acknowledged that determining health risks associated with concentrations of *E. coli* or enterococci from environmental waters requires knowledge of the source of that fecal indicator bacteria. The experts also acknowledged that current methods used to enumerate fecal indicator bacteria in environmental water samples do not determine the source of such bacteria or whether the bacteria had multiplied in that environment.

Some practical and potential problems related to the current method not being able to identify the source of fecal bacteria recovered from recreational water samples were also discussed. The first identified problem is that even though tropical soil in Hawaii has been documented to contribute significant numbers of fecal bacteria (low risk) to streams, it should not be assumed that these water samples do not contain fecal indicator bacteria from human sewage (high risk) or animal feces (medium risk). A second identified potential problem is the possibility that some bacterial pathogens such as *Salmonella*, *Shigella*, or *E. coli* 0157 may also be able to multiply under the same environmental conditions as those in which environmental sources of fecal indicator bacteria have been reported. This possibility is reasonable because these bacterial pathogens are genetically similar to *E. coli*. The multiplication of bacterial pathogens in the environment may constitute a serious problem. Since data were not available to determine whether this represents a real problem, this issue was not further discussed. However, if enteric pathogens such as *Salmonella* are also able to multiply in the environment, the detection of environmental sources of *E. coli* would be a reliable indicator of environmental sources of fecal enteric bacterial pathogens. A third identified problem is the practice that when recreational waters are monitored for and determined to exceed water quality standards for fecal indicator bacteria, it is assumed that the bacterial source is sewage or feces and therefore this recreational

water represents a high risk for human use. The general guideline is that under these conditions, the recreational water site should be posted as being contaminated and not suitable for swimming. However, scientists from tropical regions such as Hawaii, Guam, Puerto Rico, and south Florida have stated that the assumption that fecal indicator bacteria recovered from environmental waters comes from a fecal source should not be accepted as a foregone conclusion because environmental sources of these fecal indicator bacteria rather than direct fecal inputs have been documented in these areas.

4. *If the EPA water quality criteria are not reliable in tropical areas, are there alternative, more reliable water quality criteria?*

The monitoring data from Hawaii, Guam, Puerto Rico, and south Florida indicated that applying the EPA-recommended recreational water quality standards to determine the hygienic quality of water does not provide reliable results because in these areas, there are significant environmental sources of these same fecal indicator bacteria. It was pointed out that in the application of recreational water quality standards, EPA guidelines assume that there are no significant environmental sources of the fecal indicator bacteria. When currently used criteria or water quality standards are not reliable, the general solution is to seek an alternative (a different indicator) or supplemental criterion or requirement so new water quality standards which provide more reliable data can be used. In accordance with EPA guidelines, scientists from Hawaii and Puerto Rico initiated studies to determine whether monitoring for alternative or other fecal indicators in these tropical regions could provide more reliable data to measure for the absence and presence of sewage contamination. In this regard, monitoring for *C. perfringens* in Hawaii and coliphages in Puerto Rico have been reported to provide more reliable information as to when environmental waters are contaminated with sewage. Both of these alternative fecal indicators are consistently present in sewage and will not multiply under environmental conditions. However, establishing water quality standards based on concentrations of these alternative fecal indicators is a difficult process. Thus, Puerto Rico has not established any proposed guidelines or water quality standards to interpret the significance of coliphages in environmental waters. In Hawaii, alternative recreational water quality standards have been proposed based on numbers of *C. perfringens* in streams (50 CFU/100 mL) and coastal marine beaches (5 CFU/100 mL). These standards were developed based on establishing ambient concentrations of *C. perfringens* and establishing a concentration of *C. perfringens* in environmental waters that would reliably signal contamination with sewage. These proposed *C. perfringens* water quality standards have been used in Hawaii as supplemental standards to assist in making decisions as to when environmental waters are contaminated with sewage.

One recognized limitation of the proposed *C. perfringens* standards is that they do not fulfill the EPA guidelines that water quality standards should be based on measurable health risk, which require an epidemiological study to demonstrate a correlation between numbers of the alternative fecal indicator in water and an increase in disease incidences among swimmers. An epidemiological study was conducted in Hawaii, but the concentrations of *C. perfringens* in coastal waters were too low to show an association with human health risks. Moreover, the source

of contamination was discharge from a storm drain rather than sewage. Although an epidemiological study conducted in Hong Kong reported a correlation in the numbers of *C. perfringens* in water and incidence of gastroenteritis, conditions at that study site were not comparable to conditions in Hawaii. Since the proposed *C. perfringens* standards for Hawaii were not based on measurable health risks, most experts felt they could not evaluate the validity of these proposed alternative standards and declined to discuss the merits of their use in Hawaii. However, the workshop experts encouraged continued use and further evaluation of the use of alternative fecal indicators such as *C. perfringens* and coliphages in tropical locales because these indicators can provide valuable information on the presence or absence of fecal contamination that cannot be reliably determined when monitoring is conducted using only EPA-recommended indicators.

5. *Are the results of these studies applicable to other tropical sites?*

Persistence and multiplication of fecal indicator bacteria (fecal coliform, *E. coli*, enterococci) in natural environments have been reported from limited tropical (Guam, Puerto Rico) and subtropical sites (Hawaii, south Florida). The explanation for these findings is that these environments provide for the three basic requirements (warm temperature, moisture, nutrients) for persistence and growth of fecal bacteria. Since these requirements are more consistently fulfilled in the tropical region of the world, a hypothesis was proposed that the findings for these limited sites may be applicable to other warm and humid tropical regions of the world. The tropical region of the world for purposes of this discussion was considered the region between the Tropic of Cancer (23.5°N) and the Tropic of Capricorn (23.5°S) where the environment is characterized by warmer temperature and higher humidity as compared with the rest (temperate) region of the world. In this regard, the map of the tropical region of the world in Chang and Lau (1993) is especially useful because it (see Appendix F) divides the tropics into the following four climatic subregions: (a) humid tropics, (b) subhumid tropics, (c) wet-dry tropics, and (d) dry tropics. Chang and Lau (1993) define humid tropics as regions where the mean 24-hour temperature of the coldest month is above 18°C and the wet season exceeds 4.5 months. According to the map in Chang and Lau (1993), a large area of the world categorized as humid tropics, subhumid tropics, and wet-dry tropics would most likely provide environmental conditions similar to that in Hawaii where fecal indicator bacteria to persist and multiply in the environment. Thus, there is a potential that the results discussed at this workshop will be applicable to a large area of the world where alternative or supplemental water quality standards may need to be developed. However, as pointed out by one expert, data must be obtained from these other tropical sites to define the extent of the problem of environmental sources of fecal indicator bacteria in other tropical sites around the world.

6. *Is there evidence for persistence and multiplication of fecal indicator bacteria in temperate climates?*

Although the focus of this workshop was the persistence and multiplication of fecal indicator bacteria in tropical climates, data that this same phenomenon may occur in temperate regions was also presented at the workshop. For instance, Ashbolt presented evidence for multiplication of *E. coli* in natural reservoirs near Sydney,

Australia; Whitman presented evidence for persistence of *E. coli* in beach sand in Chicago; and Lewis reported on her study of multiplication of enterococci on seaweeds in New Zealand (Anderson et al., 1997). These reports by competent scientists indicate that persistence and multiplication of *E. coli* and enterococci may occur under some conditions in temperate climates as well. These kinds of evidence should alert scientists in temperate climates of the world to assess the possibility of long-term persistence and multiplication of fecal indicator bacteria under environmental conditions as an explanation for their detection in elevated concentrations in environmental samples.

## Development of Consensus Statements: Day Two

The presentations made during day two of the workshop addressed the problem that fecal indicator bacteria may be multiplying in the environment of tropical locales (Agenda Question Four) and as a result alternative fecal indicators and standards should be used (Agenda Question Five). Tiedje discussed these two problems with the experts and together they crafted the wording to complete three concluding statements. All experts then voted to accept or not accept the concluding statements. After the voting, the concluding statements were called consensus statements. The consensus statements represent general or collective opinion of workshop participants but not 100 percent agreement in most cases.

### Consensus statement three

Fecal indicator bacteria (fecal coliforms, *E. coli*, enterococci) can multiply and persist in soil, sediment, and water in some tropical/subtropical environments (Hawaii, Guam, Puerto Rico, south Florida).

This statement was crafted to address the issue of multiplication of fecal indicator bacteria in tropical environments (Agenda Question Four). Seventeen of eighteen (94.4%) experts voted to support this statement. One expert abstained from voting.

### Consensus statement four: The preferred version

Recreational water quality guidelines for the tropics/subtropics should be supplemented with additional alternative indicators (*C. perfringens*, coliphages) for watershed assessment (or sanitary survey).

This statement was crafted in response to the question of the usefulness of alternative fecal indicators for tropical environments (Agenda Question Five). Thirteen of eighteen (72.2%) experts preferred this statement, while five of eighteen (27.7%) accepted this statement but preferred an alternate statement.

#### **Consensus statement four: The alternate version**

In the absence of a predominant point source pollution, recreational water quality guidelines for the tropics/subtropics should be supplemented with additional alternative indicators (*C. perfringens*, coliphages) for watershed assessment (or sanitary survey).

Some experts wished to express this consensus statement differently. As a result, this alternate version was crafted. Five of eighteen (27.7%) experts preferred this version, while thirteen of eighteen (72.2 %) found this statement to be acceptable.

Two reasons were offered as to why some preferred the first version while others preferred the second. Those who voted for the first version indicated that this statement is a more protective and conservative approach. This statement recommends the necessity for using alternative indicators to supplement the traditional methods used to measure recreational water quality in tropical/subtropical regions. Those who preferred the alternate version indicated that the statement is applicable in a situation where some preliminary studies have already determined the absence of a predominant point source of pollution and the ineffectiveness of the standard fecal indicators. Under these conditions, this statement supports the use of these alternative fecal indicators to determine recreational water quality in tropical/subtropical regions where considerable monitoring data have already been collected and analyzed.

### **WORKSHOP RECOMMENDATIONS AND RESEARCH NEEDS**

Workshop recommendations and research needs were developed during the last discussion session moderated by Tiedje. During the course of the workshop, it was recognized that additional research was needed in several areas to properly address and find solutions to existing water quality issues in the tropical and subtropical locations of the United States and its territories. Some of the recommendations and research needs identified are summarized below. An effort was made to compile the research needs identified during this session under appropriate headings.

#### **1. Conduct more controlled and *in-situ* studies to measure the survival and growth of indicator bacteria under ambient and different climatic conditions**

During the workshop, several reports on the multiplication of fecal indicator bacteria under tropical environmental conditions were discussed in depth. The rationale for this phenomenon was that tropical environments provide consistently suitable conditions for the growth of these bacteria. However, reports on multiplication of these same fecal bacteria under temperate conditions were also discussed. This includes bacteria found in seaweed in New Zealand, a natural water reservoir in Australia, and sand in Chicago. The results of these reports suggest that even short periods of warm weather (e.g., during summer months) can support the growth of fecal bacteria in the environment or that these bacteria can survive over

the winter months and multiply when conditions become favorable. The data from nearly all of these reports were site-specific because each site differed from the other. Moreover, experimental designs and the methodologies used at each of these locations were different. The identified need was to develop standardized experiments which can be conducted at various sites so the effect of environmental conditions on the fate of fecal bacteria can be compared from site to site. These kinds of experiments may lead to generalized conclusions as to the environmental conditions that support the growth of fecal indicator at any site. Some of the suggested experiments to address these issues are summarized below.

1. More studies need to be conducted under various defined natural conditions to determine the circumstances under which fecal bacteria can be expected to become established in the respective environments and to determine the extent to which the population can multiply.
2. Investigations should be made on growth response patterns for sewage and non-sewage (environmental) strains of fecal indicator bacteria to determine which of the strains grow better under natural conditions.
3. Research results from diverse tropical environments have shown that the conditions in these locations are within the permissive ranges to allow fecal indicator bacteria to persist and possibly grow. Studies should be conducted in various temperate climates to determine whether this phenomenon occurs, especially during the summer months. Moreover, studies should be conducted in many areas of the world and the continental United States where environmental conditions are not typically temperate and where tropical-like conditions occur for several months of the year.
4. Multiplication of fecal indicator bacteria has been predicted to occur under tropical environments. However, insufficient studies have been completed to determine if this prediction is true. Thus, studies to determine whether fecal indicator bacteria such as *E. coli* and enterococci can multiply in other tropical locations around the world should be encouraged.
5. Deep subsurface microbiology programs have revealed the presence of coliform bacteria and their phages, which are at least about 25,000 years old, at a depth of 1,700 feet. A number of microorganisms isolated during these studies were also found to be resistant to a number of antibiotics. These kinds of information should be further explored to understand the distribution, abundance and ecology of these organisms in various environments. The fecal indicator bacteria from these sources can serve as models to determine the growth capacity of these bacteria and to determine whether this capacity to grow under environmental conditions may have changed over time.

## **2. Model the transport of fecal indicator bacteria in the soil**

The contamination of groundwaters by chemicals being transported through the soil profile is relatively well understood from modeling studies. However, insufficient studies have been completed to document the movement/transport of microorganisms through the various soil profiles.

1. More studies are required to understand the mechanisms of microbial transport through soil profiles—more specifically, to understand how fecal indicator bacteria and pathogens survive and are transported through various types of soil to contaminate groundwater.
2. Studies are needed to model the effects of environmental conditions (moisture, sunlight, temperature, nutrients, salinity) on viability and measurable movements of fecal indicator bacteria and pathogens under surface water environments and under groundwater environments.

### **3. Determine the relationships of various fecal indicator microorganisms with pathogens to accurately determine health risks from monitoring data**

1. There is a need to explore the potential for growth of fecal indicator bacteria (*E. coli*, enterococci) in tropical soil and to compare the growth potential with other microbial pathogens (*Pseudomonas aeruginosa*, *Vibrio* spp., *Staphylococcus aureus*, *Salmonella*, *Shigella*, *Leptospira*, *Campylobacter*, *Helicobacter pylori*, *Mycobacterium avium* complex) detected in environmental waters. Can fecal bacteria be a useful indicator for the survival in the environment of fecally transmitted and non-fecally transmitted pathogens in recreational waters?
2. Monitoring of pathogens should be conducted to develop direct measurements for risk assessment using a range of enteric viruses, calicivirus, and adenovirus. The requirements as well as feasibility and costs to monitor for pathogens should also be determined.
3. New technologies should be developed for tracking and detecting the sources of indicator bacteria. This recommendation is in response to the conclusion made at this workshop that identifying sources of fecal indicator bacteria in environmental waters is important in determining the relative risk to human illness. Current monitoring methods cannot determine the source of the fecal indicator bacteria.
4. Current EPA regulations are based on determining acceptable risk. However, acceptable risk levels for water uses vary with time of year in the United States and are not directly applicable to all other countries. It is appropriate to establish acceptable risk levels for different communities. A desirable risk level described as zero or “no risk” is not realistic for implementation. EPA epidemiologists now prefer the term tolerable risk rather than acceptable risk.
5. *Staphylococcus aureus* infections in people have been associated with recreational use of water, and staphylococcus bacteria have been recovered from recreational waters. There is a need to improve methods to monitor recreational water to reliably recover *S. aureus* as a means to determine risk to this infection by swimmers.

#### **4. Meet the needs of a regulator for effective decision-making**

Regulators are charged with making decisions on risk to people who use recreational waters even when these risks have not been determined. Some of the needs of state regulators are listed below.

1. Reliable recreational water quality standards need to be developed for each environmental condition based on criteria that more accurately reflect the level of risk from swimming in marine waters contaminated with fecal material.
2. Reliable recreational water quality standards need to be developed based on criteria that accurately reflect the risk to swimmers from microbial pathogens of non-sewage origin characterized as non-point source pollution.
3. Reliable recreational water quality standards need to be developed based on criteria that accurately reflect the risk to contamination by pathogenic *S. aureus* and *Leptospira* spp. because both of these non-enteric bacteria have been shown to infect swimmers via the water route.

#### **5. Design sound epidemiological studies to understand the indicator-pathogen relationships**

Epidemiological studies are the only means to actually measure disease and risk to people who use recreational waters. Many of the early epidemiological studies had some flaws. More recent epidemiological studies have overcome the major flaws of earlier studies. However, scientists who have conducted recent epidemiological studies criticize other studies and reach different conclusions. Some of the reasons for these differences are attributed to different environmental conditions, use of different experimental design and use of different methods. Some recommendations for future epidemiological studies discussed at the workshop are presented below.

1. In the future, epidemiologists need to expend more effort to evaluate the experimental design before expensive and elaborate studies are undertaken.
2. Future studies should explore the use of molecular methods and molecular epidemiological approaches to clarify and identify the source of organisms in environmental waters, especially when results of cultural methods alone are limited or unreliable.
3. Future studies should be conducted at sites that are representative of all the major recreational beaches in the United States. Thus, sites should include beaches in which the primary source of elevated concentrations of fecal indicator bacteria is not from sewage but from some non-point source, because this represents the situation at many beaches, which is unlike the beach conditions used in the EPA study to establish the recreational water quality standards. Future studies should definitely include beaches at tropical island sites, as these environments have been shown to differ substantially from those on the continental United States.

## **6. Determine the usefulness of the uncertainty analysis approach in microbiological studies**

In programs to assess chemical pollution of water, uncertainty analysis is often used to catalogue those doubts that can affect the measurements of certain chemicals. A suggestion was made whether this kind of approach would be useful when used for microbial analysis of water.

## **7. Publish/disseminate research data for the benefit of both scientific and public communities**

It was agreed that all of the experts invited to this workshop are knowledgeable about the problems of water quality in tropical locales and should be contacted to review the final workshop report. Moreover, it was agreed that all experts should continue to discuss the issues raised during this workshop and to assist in implementing the conclusions and recommendations. Finally, it was agreed that all experts should participate in the presentation of the results of this workshop at a public forum and that the proceedings of the presentations made at this public forum as well as associated public discussions should be published for the benefit of both the scientific and public communities.