Stakeholder Webinar on EPA's Development of New or Revised Recreational Water Quality Criteria

October 12
1 – 4 pm (EDT)

Agenda

1:00-1:15	Welcome and Introduction of Speakers
1:15-2:00	Update on EPA's Research for the development of Recreational Water Quality Criteria
2:00-2:30	Research Update Q&A
2:30-3:15	EPA's Current Thinking on Elements of Criteria
3:15-3:45	EPA's Current Thinking Q&A
3:45-4:00	Wrap Up

Today's Presenters

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Update on EPA's Research for the Development of Recreational Water Quality Criteria

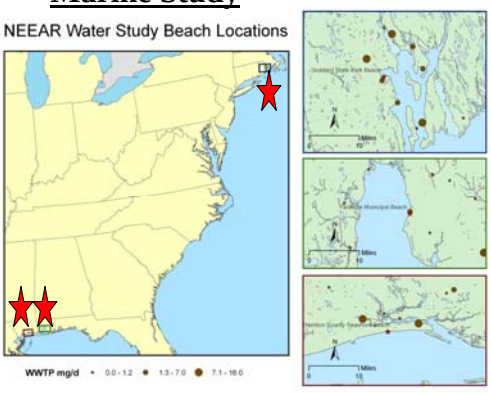
Epidemiology Studies

- 4 Freshwater studies conducted in Great Lakes (2003-2004)
- Studies conducted at 3 Marine locations (2005-2007)
- Study conducted at a marine beach impacted by urban runoff in a temperate region (2009)
- Study conducted in a tropical region (2009)

NEEAR Study Sites

Is there an association between illness and recreational water quality as measured by novel and rapid methods of determining water quality? (At sites impacted by treated sewage discharge)

Marine Study



Freshwater Study

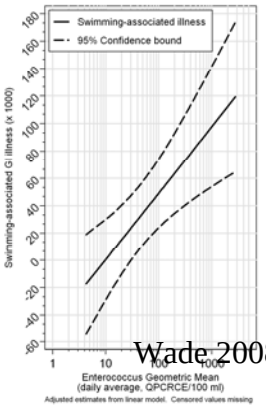
Health, Exposure and Population

- *Health outcomes:*
  - *Gastrointestinal illness (GI)*
    - **Diarrhea (3 or more loose stools in a 24 hour period); Vomiting; Nausea and stomach ache; Nausea or stomach ache and impact on activity**
  - *Upper respiratory illness (URI)*
    - **Any two: sore throat, cough, runny nose, cold, fever**
  - *Skin rash*
  - *Eye irritations (watery eye or eye infection)*
  - *Earache*
- *Swimming exposure:*
  - *Immersed body in water*
- *Subpopulations Assessed :*
  - *Children*
    - *Other subpopulations (elderly, pregnant…) did not have sufficient populations to assess*

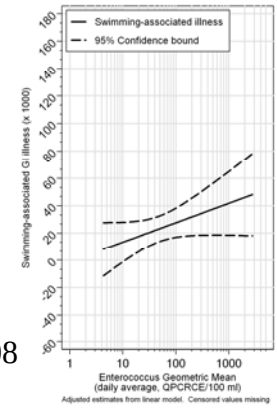
NEEAR Water Study: Results

- Seven beaches
  - Treated sewage impact
- *Enterococcus* qPCR CE
  - Associated with gastrointestinal illness among swimmers
- Some evidence of high "sensitivity" among children in freshwater study
  - Status: Marine Study Manuscript Submitted
  - *Preliminary Results: Enterococcus* qPCR CE, *Bacteroidales*, qPCR CE showed associations with GI illness

Age 10 and under



Age 11-54



Wade 2008

Urban Runoff Epi Study at Surfside, SC

- Study Data Collection (29 days)
  - 11,159 interviews from 5,205 households
  - 530 water samples collected (at beach)
- Water Quality (*Enterococcus*)
  - Good water quality
    - No days exceeded current criteria for marine water (not expected)
- Status: EPA report being externally peer reviewed
- Preliminary Results:
  - Positive but statistically insignificant associations with Enterococcus CFU, Entero qPCR CCE and GI illness

Figure 2e: Surfside Beach contaminated sample locations

Figure 7: Schematic of Surfside Beach

Tropical Waters Study at Boquerón, PR

- Study Data Collection (26 days)
  - 15,726 interviews from 6,611 households
  - 600 water samples collected (beach sites)
- Water Quality (*Enterococcus*)
  - Good water quality
    - High proportion of samples (~30%) showed problems with the internal positive control assay
    - *Currently collecting additional samples and investigating reasons for qPCR interference*
- Status: EPA report being externally peer reviewed
- Preliminary Results:
  - Good water quality, low exposure range, sample interference issue with qPCR
  - qPCR/health association-difficult to interpret due to the sample interference and low detection of indicator bacteria

Figure 2d: Boquerón Beach contaminated sampling locations (yellow pins)

Quantitative Microbial Risk Analysis (QMRA) Status (P4)

- ***Objective:*** obtain pathogen and fecal indicator measurements from animal source manure run-off for use in QMRA
- ***Approach:*** field study with simulated rainfall and controlled application of fecal material from cattle, poultry and swine
- ***Status/Results:*** results from two simulations will be used in QMRA; late September rains have now allowed for sample collection for the stream run-off studies

qPCR Signal Fate and Transport (P8) Status

Objective: To evaluate how well molecular and culture-based indicators perform as surrogates for the pathogens

Approach: measure pathogen and fecal indicator levels in untreated and treated wastewater and persistence in discharged effluents

Status/Results*

- Draft Report currently undergoing external peer review
- Enterococci qPCR measurements persisted more than culture through chlorine and UV disinfection.
- Both qPCR and culture measurements degraded at similar rates in discharged effluents.
- Pathogens were not found consistently or at sufficient levels to assess persistence

**subject to change, based on external peer review comments and revision*

Archived Sample Storage and Re-analysis Contingency (P16, P22)

- Reanalyze archived samples using new indicators and develop health relationship for new indicators (P22), **if** frozen archived samples have not degraded (P16)

Sample Storage Stability (P16)

- How long can refrigerated samples be held before they should be processed for analyses?
- How long can samples be stored frozen before they significantly degrade?
- Have the frozen samples, archived from previous EPA conducted epidemiology studies, significantly degraded?

P16 Approach

- ***Short-term holding time:*** fresh refrigerated samples were held and analyzed at 24 and 48 hours
- ***Long-term holding time:*** frozen samples were held and analyzed at 6, 12, and 24 months
- ***Archived sample stability:*** frozen samples from 2003-2007 epidemiology studies were analyzed

P16 Status/Results*

- Draft report undergoing peer review
- 24 and 48 hr results were limited, conflicting and difficult to interpret
- 12 and 24 month samples were degraded
- Reanalyzed archived samples were degraded
- ***Conclusion:*** Archived samples should not be used for establishing health relationships with new indicators (P22)
- No recommendation on holding time due to inconclusive results, but limited data suggests that refrigerated samples should not be held for 24 hours or longer [Note: 6 hours used in NEEAR studies].

* Subject to change, based upon peer review comments and revision

Microbial Source Tracking P19-P21

P19: Develop novel cattle and human microbial source tracking methods and complete performance assessment with other published methods

P20: Evaluate human PCR and qPCR-based assays with water samples impacted with different levels of fecal pollution from a wide geographic range

P21: Evaluate cattle PCR and qPCR-based assays with water samples impacted with different levels of fecal pollution to supplement site characterization and quantitative sanitary investigation

P19 Status

- 4 peer-reviewed manuscripts
- Shanks, O.C., C. Kelty, M. Sivaganesan, M. Varma, and R.A. Haugland. Quantitative PCR for genetic markers of human fecal pollution. (2009) Applied and Environmental Microbiology 75 (17):5507-5513.
- Shanks, O.C., K. White, C.A. Kelty, S. Hayes, M. Sivaganesan, M. Jenkins, M. Varma, R.A. Haugland (2010). Performance assessment of PCR-based assays targeting *Bacteroidales* genetic markers of bovine fecal pollution. Applied and Environmental Microbiology 76:1359-1366.
- Shanks, O.C., K. White, C.A. Kelty, M. Sivaganesan, J. Blannon, M. Meckes, M. Varma, and R.A. Haugland (2010). Performance of PCR-based assays targeting *Bacteroidales* genetic markers of human fecal pollution in sewage and fecal samples. Environmental Science & Technology 44:6281-6288.
- Haugland, R.A., M. Varma, M. Sivaganesan, C.A. Kelty, L. Peed, and O.C. Shanks (2010). Evaluation of genetic markers from the 16S rRNA gene V2 region for use in quantitative detection of selected *Bacateroidales* species and human fecal waste by qPCR. Systematic and Applied Microbiology: In Press.

P19 Key Findings

- **All assays:**
  - Most assays were not 100% specific
  - Different assays cross-reacted with different animal sources
  - Use of multiple assays required for full coverage
- **Bovine-associated assays:**
  - Large variability in performance between cattle populations
  - Animal management practices may influence performance
  - Must test local populations before use
  - Need for further method development
- **Human-associated assays:**
  - Many assays were highly specific
  - DNA targets widely distributed in untreated sewage
  - Ready for fate & transport and water quality case studies

P20 Status

- One manuscript submitted for review
- Green, H.C., O.C. Shanks, M. Sivaganesan, R.A. Haugland, and K.G. Field (2010). Extended survival of human fecal *Bacteroides* in marine water. Submitted to: Environmental Microbiology (August 2010).

P20 Key Findings

- Significant difference in decay of genetic markers in marine and freshwater systems.
- Significant difference in decay of *Enterococcus* genetic markers and cultivatable cells when exposed to sunlight.
- *Enterococcus* and *Bacteroides* genetic markers exhibit different decay profiles in same environment.
- General and host-associated *Bacteroides* genetic markers respond to the environment in a similar manner.
- Decay coefficients for genetic markers in manure amended soils available for QMRA modeling.

P21 Status

- One manuscript submitted for review
- Rogers, S.W., M. Donnelly, L. Peed, C.A. Kelty, S. Mondal, Z. Zhang, O.C. Shanks (2010). Decay of bacterial pathogens, fecal indicators, and real-time quantitative PCR genetic markers in manure amended soils. Submitted to: Applied and Environmental Microbiology (September 2010).

P21 Key Findings

- Predicted survival times of some fecal indicators and pathogens exceeded 1 year
- Concentrations of the pathogens and general fecal indicator genetic markers were correlated (r=0.528-0.745).
- Host-associated genetic markers decayed to non-detectable concentrations long before other fecal indicators and pathogens.
- Decay coefficients for genetic markers in manure amended soils available for QMRA modeling.

Beach Modeling Studies (P23-P25) Status

- ***Objectives:*** refine Virtual Beach model building prediction capabilities
- ***Approach:*** collect freshwater and marine fecal indicator and environmental data
- ***Status/Results:***
  - Builds models using culture and qPCR data
  - Report titled *"Predictive Modeling at Beaches-Volume II: Predictive Tools for Beach Notification,"* on results and Virtual Beach software, is undergoing external peer review

Avian Assays P39 Status

- **Identify** genetic sequences useful in chicken and seagull specific fecal source (P39-1)
  - o Identified genetic sequences for assays for chicken (3 sequences), seagull (1 sequence) and Canadian goose (1 sequence)
  - o Continuing to develop new markers for these and related waterfowl
- **Evaluate** chicken and seagull specific fecal source assays for specificity and sensitivity (P39-2)

P39-1 Status

- Lu, J., J. W. Santo Domingo, S. Hill, and T. A. Edge. 2009. Microbial Diversity and Host-specific Sequences of Canadian Goose Feces. Appl. Envir. Microbiol. 75(18):5919-26.
- Lu, J. and J.W. Santo Domingo. 2008. Turkey fecal microbial community structure and functional gene diversity revealed by 16S rRNA gene and metagenomic sequences. J. Microbiol. 46:469-477.
- Lu, J., J.W. Santo Domingo, R. Lamendella, T.Edge, and S.Hill. 2008. Phylogenetic diversity and molecular detection of gull feces. Appl. Environ. Microbiol. 74: 3969-3976.

P39-2 Key Activities

- Evaluation of the performance of avian markers in the detection of fecal sources in environmental waters
- Detection limits of avian-specific PCR assays against fecal and water DNA extracts
- Use of currently available avian markers in a quantitative manner
- Evaluation of the chicken markers in fate and transport studies
- Determine the correlation between waterfowl host-specific targeted populations with bacterial indicators (e.g., enterococci), and bacterial pathogens (i.e., *Campylobacter*) in fecal and water samples

Conduct Expert Scientific Workshop

- **Objective:** Obtain input on what future science and research might be conducted to further improve our understanding of potential human health risks from exposure to fecal contamination from avian and other wildlife in coastal recreational waters.
- **Date:** Fall, 2011
- **Location:** to be determined

Conduct statistical analysis of children data from epidemiologic studies - P29

- *Children assessed as a subpopulation in all epidemiological studies*
  - *Elevated risk in freshwater studies*
  - *Other subpopulations (elderly, pregnant…) did not have sufficient populations to assess*
- *Assessment for marine studies ongoing (under peer review)*
- *Several potential factors*
  - *Time spent in water, more likely to swallow water*

Quantitative Microbial Risk Assessment (QMRA) for Agricultural Animals

- EPA is conducting a QMRA (project P4 in CPSP) to estimate illness at a freshwater beach impacted by agricultural animal sources of fecal contamination.
- The risk assessment is based on microbes that are pathogenic to humans (e.g., *E. coli* O157:H7, *Cryptosporidium*, etc.) and come from ag. animal sources.

Quantitative Microbial Risk Assessment (QMRA) for Agricultural Animals [cont.]

- Evaluated the risk assessment approach in comparison to the NEEAR freshwater epi study results – “anchoring”
- Conducted field studies with simulated rainfall and controlled application of fecal material from cattle, poultry and swine
- Surveyed the scientific literature for information on zoonotic pathogen occurrence, distribution, prevalence, infectivity, and other parameters for use in the risk assessment.

Quantitative Microbial Risk Assessment (QMRA) for Agricultural Animals [cont.]

- Risk assessment is currently underway
- Incorporating the results from targeted field studies into the exposure assessment models
- Results should help inform on the relative nature of human health risks from various fecal sources

Enhanced Sanitary Investigation Tool

- Enhanced sanitary investigation instrument was developed that captures information sufficient to support the conduct of a QMRA
- The form was tested at EPA epi study sites in 2009.

Validation of Analytical Methods for Ambient Water Testing

- EPA completed a single-laboratory validation study of EPA's quantitative polymerase chain reaction (qPCR) procedures for the quantitative detection of enterococci (Method A) and Bacteroidales (Method B), in fresh and marine waters
- The purpose of the study was to:
  - optimize the methods (to further refine)
  - assess method performance in the single laboratory environment across multiple matrices
  - facilitate future modification of the two methods
  - provide a basis for a multi(ple) laboratory validation study
  - develop draft quantitative quality control (QC) acceptance criteria
- The draft methods are published (Method A: Enterococci EPA-821-R-10-004 and Method B: Bacterdoidales EPA-822-R-10-003) on the EPA web site at: http://www.epa.gov/waterscience/methods/method/biological/
- A multi-lab validation study for the two methods is underway for marine waters and the freshwater study will begin next spring

Evaluation of Indicator/Method Combinations

- EPA is evaluating indicator/method combinations for use in new or revised criteria
- Performance criteria for important features being evaluated include:
  - indicator/illness health relationship established
  - limit of detection
  - sensitivity
  - specificity
  - precision
  - percent false positives and false negatives
- Also evaluating qPCR and culture methods qualitatively to determine, for implementation purposes, appropriateness for each Clean Water Act program.

Indicators/Methods – Wastewater

- EPA has determined, based on the preliminary results of the studies in P8 and P18 that a new or revised wastewater test method is not necessary.
- The preliminary results from the P8 study indicate that the Enterococci qPCR method measures DNA levels in both wastewater and receiving ambient waters.
- The preliminary results from the P18 study indicate that existing culture methods approved for wastewater may be sufficient.
- Therefore, based on the results available at this time, a new wastewater method is not needed.

Other Indicator/Method Efforts

- Developing Approaches to Bring Additional Indicator/Methods into Criteria
  - Establish scientifically defensible “equivalency” of indicator/methods with an unknown health relationship to indicator/methods with an established health relationship.

Monitoring & Modeling

- EPA is preparing a report that describes the temporal and spatial variation associated with water sampling.
  - Considerations for developing sampling plans for beach monitoring
    - Inland vs. coastal
    - Where, when and how to sample
- Catalog and evaluate existing information on models and other tools to predict water quality at beaches and discuss protocols for model development.
- Predictive Models:
  - Provide results in a “timely manner”
  - Supplement to water sampling, not replacement

Inland Waters

- EPA is preparing a report to support its evaluation of applicability of NEEAR Great Lakes data to inland waters
  - Summarizes work of EPA and WERF related to assessing similarities and differences in inland waters, including:
    - Input from experts during February 2009 WERF Experts Inland Waters Workshop
    - Literature review of occurrence, persistence, fate & transport of indicators and pathogens in inland v. coastal settings
    - Comparison of Culture and qPCR methods
    - Method performance in inland fresh waters

Literature Reviews

- EPA conducted literature reviews to establish the state of the science in various areas related to criteria development.
- Two reviews have been published on EPA's recreational water quality criteria website
  - http://www.epa.gov/waterscience/criteria/recreation/
  - **"Review of Published Studies to Characterize Relative Risks from Different Sources of Fecal Contamination in Recreational Water",** EPA 822-R-09-001, CPSP P#30
  - **"Review of Zoonotic Pathogens in Ambient Waters",** EPA 822-R-09-002, CPSP P#31
- One other is in progress (i.e., incorporating the results of EPA's 2009 Boqueron, Puerto Rico efforts).
  - "**Review of Fecal Indicator Organisms Behavior in Ambient Waters and Alternative Indicators for Tropical Regions**" (P32)

EPA's Current Thinking on Elements of Criteria

EPA's Current Thinking Agenda

- No national-scale discounting for source anticipated
- Potential flexibilities for States
- Anticipated indicators and methods of measurement
- Human health risks from different fecal sources

No National-Scale Discounting For Source Anticipated

- Data supporting national-scale discounting for sources of fecal contamination have not been identified
- Criteria will likely depend upon epidemiology data from POTW-impacted beaches
- A combination of sanitary survey, QMRA and/or site-specific epidemiology studies may provide an option for states

Potential Flexibilities for States

- Consideration for incorporation of new technologies
  - Criteria recommendations will incorporate indicator/methods which have demonstrated a health relationship in epi studies
  - Molecular and other novel methods are evolving rapidly
  - Newer evolving methods may not have been linked to health through epi studies
  - States may want to consider the use of new technologies in water quality standards
- Consideration for Alternative Test Procedure (ATP)
  - States and vendors may want to demonstrate “comparability” for alternative qPCR methods
- EPA would provide methodologies for use in demonstrating “equivalency” of new technologies and “comparability” of alternative test methods

Approaches for Incorporation of New Technologies

- Approaches Being Evaluated by EPA
  - o Comparison of results of side by side analyses to determine if there is a predictable relationship between the results
  - o States may incorporate procedures for developing criteria values, or develop site-specific alternatives to standards
  - o A description of a possible approach to demonstrating method equivalence will be available in parallel with the final criteria

Alternative Test Procedure (ATP)

- The ATP protocol describes a series of lab tests with which one determines comparability between a Reference Method and a Candidate Method.
  - o Establishes a definition for determining comparability of a laboratory method result for a **genetic method** ATP for microbes.
  - o Analytical microbiological methods are comparable if
    - Same measurement technique
    - Measure the same analyte
- EPA is currently developing the genetic method ATP (Final version Dec. 2013)

Alternative Test Procedure (ATP) [cont]

- Examples of **comparable tests**:
  - two membrane filtration (MF) culture methods for Enterococci
  - two MTF culture methods for Enterococci
  - two qPCR methods for Enterococci that use different platforms (instruments)
- Examples of tests that are **not comparable**
  - a MF and a MTF culture method for Enterococci (different determinative technique)
  - a qPCR method and a RFLP method for Enterococci (both are genetic methods, but are different determinative techniques)
  - a MF method for Enterococci and a MF method for E. coli (different analyte)
  - a qPCR method and a culture method for Enterococci (different determinative technique)
  - a qPCR method for Enterococci that uses a different DNA standard

Tools for Development of Site-Specific Criteria

- EPA is working to identify and develop tools to assist with development of site-specific criteria by states
- Would allow for development of alternative criteria value as long as same level of risk is achieved
- May include:
  - Sanitary survey investigation and epi study
  - Sanitary survey investigation and QMRA

Anticipated Indicators and Methods of Measurement

- Plan to provide a combination of methods based upon qPCR and culture
  - o Enterococcus qPCR and culture
  - o *E. coli* culture
- Intended to
  - o Provide implementation flexibility to states
  - o Align costs and sampling needs with purpose of monitoring

Human Health Risks from Different Sources

Human Health Risks from Different Sources

- Recreational water epidemiology studies provide information on the:
  - Nature and extent of health effects (e.g., GI illness)
  - Relative magnitude of risks (swimmer vs. non-swimmer)
  - Association between the degree of fecal contamination (as measured by FIB levels) and levels of observed illnesses in swimmers
- These studies have not provided substantial information about the specific microbial agents that are responsible for the observed illnesses.
  - This information is important to understand risks for a wide variety of waterbody types and contamination sources

Goals of QMRA Activities Related to Sources of Fecal Contamination

- Examine the human health risks from exposure to recreational waters impacted by different sources of fecal contamination
- Understand which pathogens could cause illnesses to swimmers in waters impacted by specific sources of fecal contamination
- Evaluate the extent to which recreational water risks vary with the sources of contamination

Understanding Human Health Risks in POTW-impacted freshwaters

- We examined the reported epidemiologic results from studies conducted on the Great Lakes (i.e., beaches impacted by treated and disinfected effluent) in the US during 2003 and 2004 to estimate pathogens that could have caused the observed illnesses using QMRA.
- Soller et al. 2010. *Estimating the primary etiologic agents in recreational freshwaters impacted by human sources of faecal contamination*. Water Research. 44:4736-4747

Results from QMRA in POTW-impacted freshwaters

- Relatively few pathogens appear to cause the vast majority of illnesses.
- Human enteric viruses, and in particular norovirus, could have caused the vast majority of the observed swimming associated GI illnesses during the 2003/2004 epidemiology studies.
- The reverse QMRA is leading to a better understanding of freshwater POTW impacted waters.

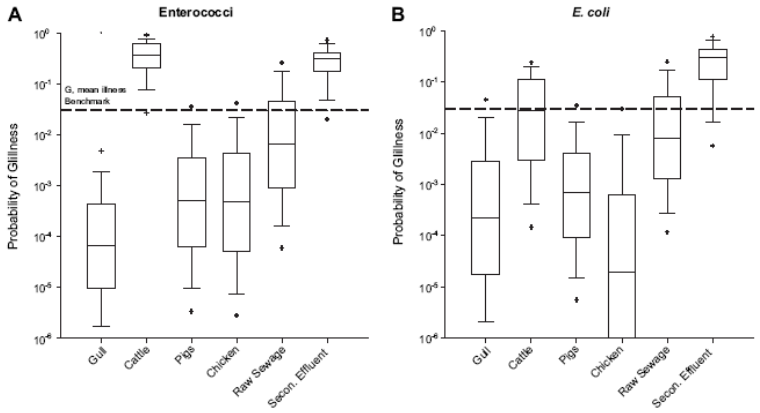
Relative Risks from Various Animal Sources

- Objective:
  - Predict relative risks of illness from ingestion of recreational water that is assumed to be contaminated with feces from a range of animals (human, cattle, pigs, poultry and gulls).
- Main question:
  - Are the relative risks from recreation in waters impacted by gulls, poultry, pigs, and/or cattle substantially different than those associated with recreational waters impacted by human sources?

Relative Risks from Various Animal Sources

- Evaluated “End of Pipe” scenarios for human, cattle, pigs, poultry and gulls.
  - For human, this meant using literature reported levels of pathogens and indicators found in treated, disinfected effluent and raw sewage
  - For cattle, pigs, poultry and gulls, this meant a ‘direct deposition’ event using literature reported data for pathogen and indicator levels found in their respective feces.
- Compared predicted illness rates for human impacted to non-human impacted waters at “fixed” FIB levels

Relative Risks from Various Animal Sources – Results



Soller et al. 2010. *Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination.* Water Research 44:4674-4691

Relative Risks from Various Animal Sources – Conclusions

- This is an initial step to understand the relative risks from exposure to recreational waters impacted by gulls, poultry, swine, and/or cattle and to compare them with POTW effluent impacted waters
- The illness risk associated with non-sewage impacted beaches appears to depend on the source of contamination, i.e. some animals show relatively lower risks than others, which could account for the conflicting epidemiology findings

Limitations

- No national level non-human source exclusion from the criteria due to limitations of available data
- EPA will not have sufficient data to develop different criteria values for different fecal sources
- Uncertainty involved with determining the proportion of human fecal material that may represent the dominant risk in a mixed-source situation
- Uncertainty involved with evaluating the differential disease endpoints between some zoonotic and human pathogens (e.g., *E. coli* O157:H7 versus norovirus).

Resources

- rec_criteria@epa.gov
- http://water.epa.gov/scitech/swguidance/waterquality/standards/criteria/health/recreation/index.cfm
- http://water.epa.gov/scitech/swguidance/waterquality/standards/criteria/health/recreation/oct2010_index.cfm


Clean waters are the goal.
Sound science takes us there.

THANK YOU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