Expert Report of Dr. Jennifer A. Jay

The development of revised Ambient Water Quality Criteria (AWQC) requires an understanding of health risks due to recreational exposure across a range of water body types (marine systems versus freshwater), differing source types, and geographical and climatic conditions. To accomplish this, appropriate indicators, methods, including rapid methods, and models for assessing water quality at widely varying sites must be investigated. Section I of this document lists critical science needs for the development of new criteria, while Section II outlines areas where these research needs are not fully addressed by two EPA documents: the Critical Path Science Plan for the Development of New or Revised Recreational Water Quality Criteria and the Criteria Development Plan and Schedule (the plan presented in these two documents will be referred to here as the Science Plan).

I. Summary of opinions: Near-term science requirements for water quality criteria development

The rationale for research needs listed in this section will be discussed in the following section.

A. Number and diversity of epidemiological studies required to better understand risk of recreational exposure under a range of geographical/source conditions

Epidemiological studies currently being used for criteria development are: Goddard, RI (marine, Publicly Owned Treatment Works (POTW) influenced), Fairhope, AL (marine, POTW influenced), Avalon, CA (marine, poorly treated human sewage) and Great Lakes (freshwater, POTW influenced). In addition to the epidemiological studies EPA is currently conducting as detailed in the Science Plan, studies should include:

- at least one additional urban runoff study in marine waters
- at least one urban runoff study in freshwater
- at least one agricultural runoff study in freshwater
- a study in a tropical region

B. Design of epidemiological studies
All epidemiological studies should include:

- o An investigation of non-gastrointestinal illnesses as well as gastrointestinal illnesses
- o Consideration of especially susceptible populations
    - ▪ Separation of children into age groups

        - Collection of data for analysis of the elderly, pregnant women, and immunocompromised individuals
    - **All** standard indicators (total coliform, enterococci, and *Escherichia coli*) by traditional and new methods, as well as *Clostridium perfringens, Bacteroides,* and F+ RNA coliphages
    - At least one rapid method
    - Analysis of sand
    - Evaluation of bather load as a nonpoint source
    - Improved exposure assessment
    - Inclusion of pristine reference beaches
    - Some investigation of disease etiology
    - Concurrent environmental data collection for model development

C. Other factors in criteria development
Criteria development should:

- Consider subpopulations with multiple exposures
- Include investigation of background illness rate
- Provide equal protection in various water types and regions, and a level of protection deemed appropriate by the public
- Validate a rapid method for use
- Take into consideration all high quality epidemiological studies post 1986
- Not eliminate *E. coli* as a freshwater indicator unless new research indicates that it is not a valid approach
- Involve an investigation of the applicability of water quality models

II. Basis for opinions: Research needs not sufficiently addressed by the Science Plan

While the Science Plan does address many significant research questions, it fails to adequately focus on several very important research needs that were identified in the Report of the Experts Scientific Workshop on Critical Research Needs for the Development of New or Revised Recreational Water Quality Criteria (referred to here as the Airlie Report). In general, in the Science Plan there is a great deal of focus given to the sanitary survey approach to criteria development. However, the more critical research need for criteria development is additional epidemiology studies.

A. Rationale: Number and diversity of epidemiological studies required to understand risk of recreational exposure

Need to investigate both animal and human sources in epidemiological studies

It is often stated that human feces likely pose a larger risk to humans than animal feces. This is partially because viruses are almost always host-specific; thus, viral pathogens in animal hosts would pose low risk to humans. However, a review of illness outbreaks due

to recreational water found that fully three-fourths of outbreaks, and incidences of disease, had either bacterial or protozoan etiology; only 7% of outbreaks and illnesses had known viral cause (Craun et al., 2005). In addition, recent research indicates that animal-human transmission of illness occurs more frequently than had been believed, and often with more significant health outcomes.

There are numerous bacterial and protozoan pathogens with animal hosts that do pose a serious health threat to humans, such as enterohemorrhagic *E. coli* (EHEC). And importantly, animal waste is often discharged to receiving water without treatment. *Leptospira* species, bacterial pathogens, are frequently found in wild animal urine, and can cause illness in humans through contact, ingestion, or inhalation of contaminated water, especially in tropical areas (Narita et al., 2005). Animal urine was the likely source of contamination leading to outbreaks of leptospirosis associated with recreational water use in Japan (Narita et al., 2005) and among participants in an adventure race in Guam (Lee et al., 2002). While some species of the pathogen protozoan *Cryptosporidium* are host-specific, and many outbreaks are clearly from human fecal contamination, other species are truly zoonotic (Dillingham et al., 2002; King and Monis, 2007). Runoff contaminated by cattle is the likely source for outbreaks of cryptosporidiosis in Indiana and Nebraska (Levy et al., 1998).

Health risk from swimming at animal-impacted sites is impossible to assess accurately until the data gap surrounding health impacts due to animal fecal sources is addressed. Determination of the particular pathogens of concern at animal-influenced sites is an important future research need; however, epidemiological studies are the preferred approach to risk determination at this time. The Airlie Report states "…there remains a paucity of data on the risk of illness for swimmers at beaches exclusively (or primarily) impacted by feces from animals," and recommends multiple epidemiological studies at locations near differing types of livestock in order to better understand the risk of animal feces to humans.

Epidemiological studies necessary in both freshwater and marine systems

Survival of both pathogens and pathogen indicators varies greatly depending on the salinity of the receiving water. For example, *Cryptosporidium* oocysts can survive months in freshwater (King and Monis, 2007) but just two weeks in marine water at 20 °C (Fayer et al., 1998) and a month in 4 °C marine water (Robertson et al., 1992). Similarly, *Giardia* cysts are quite sensitive to salinity, persisting for up to 28-56 days in freshwater, and for less than a day in marine (Johnson et al., 1997). However, poliovirus and *Salmonella* have been shown to persist longer in marine microcosms (Johnson et al., 1997). Additionally, work in my laboratory is indicating that microbial detection methods based on quantification of adenosine triphosphate (ATP), the energy currency in the cell, are appropriate only in freshwater, as the marine environment leaves the cells viable (and thus measurable by growth based methods such as membrane filtration and proprietary defined substrate technology by IDEXX) but with possibly irregular amounts of ATP per cell. This would be just one example of a currently unexplored mechanism for differential survival in marine versus freshwater, in addition to many known factors.

Salinity/source combinations. Table 1 shows what salinity/source combinations have already or are currently being investigated with epidemiological studies. Clearly there is a need to study non-human sources in both fresh and marine water.

Table 1. Current and planned USEPA and USEPA-supported epidemiological studies to be considered in criteria development

	Freshwater	Marine
Primarily influenced by POTW or poorly treated sewage	Great Lakes (previous)	Goddard, RI (current) Fairhope, AL (current) Avalon, CA (current)
Primarily runoff (agricultural or urban)	None	None

The Science Plan mentions that risks due to nonhuman sources may be addressed by comparing results from the 2007 study at Avalon, where the source is likely poorly treated human sewage, with those from 1) a recent epidemiological study at Doheny Beach, and 2) a study at Malibu Beach planned for next summer. However, statistical power at Doheny was low for the 2007 season (the study was curtailed early in July and moved to Avalon), and both have unknown sources, including septic systems at Malibu.

Need for epidemiological studies (as opposed to QMRA on its own)

EPA makes an insufficient commitment to the additional epidemiological studies recommended by the Airlie Report as necessary to understanding risks of swimming across a wide range of waterbodies and source types. For both runoff and animal sources, two high priority factors identified in the Airlie Report, the Science Plan states that to investigate health effects due to exposure, EPA will conduct an epidemiological study "and/or" quantitative microbial risk assessment (QMRA). While QMRA would be a valuable endeavor as an enhancement to an epidemiology study, QMRA is not by itself appropriate for complex mixtures of fecal sources, as the pathogens of concern are not known.

The first step in a QMRA is identification of the organism(s) of interest, followed by an exposure assessment, dose-response assessment, and finally, risk characterization. Because there are many different types of animal sources, each associated with varying sets of pathogens, it seems a QMRA approach for recreational water based on a few specific pathogens would be unlikely to capture true health risks associated with animal fecal sources. An epidemiological study, possibly combined with a QMRA approach, at a site with primarily or exclusively animal sources is a necessary step to understanding the critical question of relative risks between animal and human sources of fecal pollution. At this point, a QMRA approach should only be used for pathogens that are rare, but cause serious outcomes, such as *E. coli* O157. The reason for this is that particularly rare outcomes are not likely to be observed with a frequency allowing statistical analysis in an epidemiology study; however, the seriousness of some illnesses

warrants consideration in spite of this, and QMRA would then be a very appropriate approach.

Importantly, unlike with fecal indicator bacteria (FIB), there is not much information on how pathogens vary in density over time at beaches or in pollution sources such as storm drains and agricultural runoff. Monitoring data and numerous studies have demonstrated that FIB densities vary dramatically over time (Boehm et al., 2002; Taggart, 2002), so one could expect high variability for other microbes as well. High variability and the lack of accurate exposure assessment makes the use of QMRA as a stand alone tool an extremely uncertain method to protect the health of swimmers.

Need for epidemiological study in a tropical region

The Airlie Report states as a key near-term research need to: "Identify and develop indicators and corresponding methods that are appropriate for use in tropical and subtropical recreational water. Conduct epidemiological studies to link those indicators with illness at tropical and subtropical locations." (p. ES-4) In the Science Plan, EPA gave several reasons why this topic has been deferred for future research: 1) traditional indicators typically used in temperate and subtropical waters may not be effective in tropical regions, and 2) additional preparatory steps would be required, including identification of appropriate indicators, selection of a site, and establishment of a partnership with the municipality of the site.

While bacterial regrowth in soil (Fujioka et al., 1999; Desmarais et al., 2002; Byappanahalli and Fujioka, 2004; Shibata et al., 2004) calls into question the usefulness of traditional indicators, the assumption that traditional indicators are not valid in tropical regions has not been tested. In fact, some previous work at Mediterranean beaches does indicate a relationship between enterococci and gastrointestinal illness in children (Fattal et al., 1987). In addition, there has been significant work in the development of alternate indicators in tropical regions. There are several alternate indicators for which regrowth is not an issue, and these have been tested in tropical areas. A quantitative polymerase chain reaction (qPCR) assay targeting human-specific *Bacteroides* was a dependable indicator for sewage contamination in both beaches and streams in Hawaii, even though a qPCR assay using general primers for *Bacteroides* was not useful in indicating pollution (Betancourt and Fujioka, 2006). Other studies in Hawaii show that FRNA coliphage (Luther and Fujioka, 2004) and *Clostridium perfringens* (Roll and Fujioka, 1997) are reliable indicators for sewage pollution in streams. In Florida, work has shown that bacteriophage that infect *Bacteroides fragilis* may be a useful alternate indicator for sewage contamination; these phage were present in all sewage influents tested (and in some treated effluents) and were shown to persist much longer than coliphage in seawater (McLaughlin and Rose, 2006). A recent study in Uganda indicated that regrowth of *E. coli* was probably not significant (based on infrequent detection in pristine sites, and a lack of correlation between temperature and metabolic activity), and that *E. coli* and *C. perfringens* may be reliable for monitoring water quality in this tropical country (Byamukama et al., 2005).

Fecal sterols have also been investigated as possible alternate indicators of fecal contamination in tropical waters. Percent coprostanol was strongly correlated with *E. coli* in Malaysia ($R^2 = 0.85$) and Vietnam ($R^2 = 0.80$) (Isobe et al., 2002), in both coastal and inland sites. A follow up study of the influence of environmental factors on the relationship between coprostanol and FIB in tropical and temperate freshwater suggested concurrent monitoring of *E. coli* and coprostanol (Isobe et al., 2004).

Based on the Airlie report, the only way to demonstrate the effectiveness of a new indicator (that has been tested for adequate performance) is to show its relationship to health risks through epidemiological studies (it is not sufficient to show equivalency to an existing indicator.) Alternative indicators are currently being tested for relationship to health risks at Avalon, CA. Results of this study as well as existing literature could be used to inform the choice of indicators to test in tropical regions.

B. Rationale: Design of epidemiological studies

Non-GI illnesses need to be considered.

The Airlie Report states clearly that non-GI illness needs to be considered as a high priority in criteria development. Swimming in proximity to storm drains in Santa Monica Bay has been associated with increased relative risk of a wide range of upper respiratory as well as gastrointestinal symptoms (Haile et al., 1999), and skin rash as well as diarrhea were increased in swimmers relative to non swimmers at Mission Bay, another Southern California beach subject to nonpoint source pollution (Colford et al., 2007). A randomized trial involving 1273 adult volunteers showed an association between fecal streptococci and acute febrile respiratory illness; in addition, eye ailments were higher among swimmers, though a relationship with indicators was not evident (Fleisher et al., 1996).

The current Science Plan does not adequately address this issue. The planned epidemiological studies were not designed to investigate occurrence of any non-GI illness. While the questionnaires include questions on various non-GI illnesses, there is not currently a commitment to analyze the data to look for trends in non-GI illnesses. In addition, the pathogens thought to cause these outcomes are not always measured.

Need to consider additional susceptible populations

While the Science Plan does specify that data from epidemiological studies will be analyzed to determine how risks for children differ from those for adults, there is no mention of the elderly, pregnant women, or other immunocompromised individuals.

The Airlie Report distinguishes between two categories of vulnerability: 1) people at especially susceptible life stages (acknowledging that all humans vary in their susceptibility to disease at differing life stages), and 2) people with suppressed immune function. Of the people at susceptible life stages (children, pregnant women, and the elderly), children are thought to be most at risk due to both immature immune systems,

and their behavior (Nwachuku and Gerba, 2004). Reduced stomach acid and pepsin secretion may also increase the susceptibility of children to pathogens. Consideration of childhood as a life stage rather than a subpopulation bolsters the argument for considering children explicitly when developing AWQC.

EPA has committed to analyzing epidemiological data for children separately. The best way to do this would be to bin data relating to children into discrete age categories, due to the great changes that occur both physically and behaviorally as children grow up. For example, children in the 0 to 4 years old category have the least developed immune system, and are most likely to ingest sand, while children in the 5 to 10 years old category are most likely to swallow water, as most children in this age group are able to swim. This approach has been taken in epidemiological studies showing increased risk for children from tap water (Payment et al., 1991; Payment et al., 1997) and recreational water exposure (D'Alessio et al., 1981).

In addition to children, the elderly, and pregnant women comprise a life stage rather than a subpopulation; therefore, water quality standards should be adequately protective of these individuals. Data on age are already collected, so analyzing data for the elderly separately should be straightforward. Questions regarding pregnancy status should be included in surveys, thus allowing for separate analysis, with the understanding that very early pregnancies may not be reported.

While the Airlie Report states that some immunocompromised individuals may be considered as a subpopulation that may handle their risks on a more individual basis (through conversations with their doctors), it would be helpful if data on immune status were collected, even though the statistical power would be low at this point, so that at some time in the future, this important question could be addressed through a comparative analysis of data, once enough data has been collected. In addition, even if risk communication is still primarily through individual doctors, information from these epidemiological studies can allow doctors to better characterize risks.

All standard indicators must be included

EPA should include analysis of all traditional indicators, in addition to alternate indicators, in all epidemiology studies. *E. coli* was not analyzed in the Great Lakes study, even though this indicator has been used for decades to assess water quality. It should not be neglected unless there is strong evidence indicating that it is not an appropriate indicator at a particular site.

At least one rapid method must be tested

Current frequently used methods for analyzing FIB densities in recreational waters take 18 to 48 hours. Studies at Southern California beaches have demonstrated that these results are not predictive of water quality at the beach on the day sample results are obtained (Boehm et al., 2002). For example, a sample collected Monday morning will not provide water quality results until Tuesday. By then, the water quality may be

completely different than at the time of sampling. Rapid indicators associated with the incidence of adverse health outcomes will provide information to risk managers in a timeframe that will allow them to better protect public health by posting or closing the beach within hours of sample collection. Rapid methods currently under development include surface and whole cell recognition assays (such as immunoassays and use of probes), nucleic acid based techniques (PCR and qPCR) and enzyme/substrate methods (Noble and Weisberg, 2005). EPA should evaluate water quality with at least one rapid method in all epidemiology studies.

Need for assessing sand in planned epidemiological studies

While the case deals specifically with AWQC, it is becoming increasingly recognized that sand can be an important reservoir for FIB (Oshiro and Fujioka, 1995; Whitman et al., 2003; Alm et al., 2006; Lee et al., 2006; He et al., 2007; Yamahara et al., 2007). Sand can serve as a source of FIB to the water column in marine (Boehm and Weisberg, 2005; Boehm, 2006; Yamahara et al., 2007) and freshwater (Whitman and Nevers, 2003; Ishii et al., 2007), as well as an exposure route, as young children typically have very close contact with sand. Notably, in a Shigellosis outbreak in Pennsylvania, most confirmed cases occurred in children who were playing in sand at the water's edge (Levy et al., 1998).

In general, sediment may be more conducive to FIB and pathogen survival relative to the water column due to reduced sunlight inactivation (Sinton et al., 1999), protection from predators (Brettar and Holfe, 1992; Davies and Bavor, 2000), nutrient and organic carbon availability (Gerba and McLeod, 1976; LaLiberte and Grimes, 1982; Blumenroth and Wagner-Dobler, 1998; Craig et al., 2004), and the presence of a surface for the formation of biofilms (Brettar and Holfe, 1992; Davies et al., 1995; Decho, 2000).

On one hand, persistence and regrowth (Fujioka et al., 1999; Desmarais et al., 2002; Alm et al., 2006; Ishii et al., 2006; Lee et al., 2006) of non-pathogenic FIB in sediment may weaken the relationship between indicators and the pathogens they are meant to proxy. On the other hand, there is ample evidence sediments provide a favorable environment for pathogens, as they do for FIB, and may be an unexplored route of exposure. For example, decay rates for two species of *Salmonella* were shown to be higher in overlying water than in sediment, suggesting that sediment can act as a reservoir for these pathogens (Craig et al., 2003). The authors state that "sediments…should be considered when estimating environmental exposure." Similarly, viruses adhere strongly to sediments and show increased persistence when adsorbed (Gerba and Schaiberger, 1975; Ferguson et al., 1996; Gantzer et al., 1998; Meschke and Sobsey, 1998; Green and Lewis, 1999).

Addition of a sand component to an epidemiological study is straightforward. The questionnaire must include questions regarding duration and nature of contact with sand, and sand samples must be taken several times per day. Sand analysis is currently being included in the Avalon and Alabama epidemiological studies, and should be included in

further studies. Growth based FIB measurement can be conducted immediately, and DNA can be extracted and preserved for further analysis of specific pathogens.

Need to consider bather load as a nonpoint source

Bathers themselves can contribute substantial amounts of bacteria to the water during swimming (Cheung et al., 1991). A recent study showed that bathers released approximately 6×10^5 colony forming units of enterococci and 6×10^6 colony forming units of *Staphylococcus aureus* per person in the first 15 minutes of exposure (Elmir et al., 2007). Other work has shown associations between gastrointestinal illness and both bather density and *S. aureus* concentrations (Calderon et al., 1991; Charoenca and Fujioka, 1995). EPA should develop methods to consider this possibly important source.

Exposure assessment needs to be improved

Exposure assessment in epidemiological studies needs to be developed through further research. Individuals wading in water should not be included as "swimmers". Some previous studies have used the number of times the head is immersed in water as an exposure assessment variable. This should be continued, but the duration of the swim should also be included as a way to assess exposure. In addition, some estimate of volume of water ingested may prove useful. Protecting the populations most at risk should be kept in mind when developing strategies for exposure assessment.

Need for a reference beach for each epidemiological study

Reference beaches for each epidemiological study are not stated as a priority. However, a nearby, clean beach is necessary for determining whether unknown factors associated with a particular beach (undetected pathogens, other pollution sources such as creeks, rivers or stormdrains, etc.) are influencing the outcome. Also, it is well known that swimmers and non swimmers are very different populations; thus, comparing swimmers between clean and more contaminated beaches controls for confounding factors such as current illness history, food ingested, etc. EPA should make every effort to include nearby reference beaches for each epidemiological study.

Need for determining etiological agents of illness from recreational water exposure

The current causes of illness associated with recreational exposure to water are currently unknown. This information would of course be a critical input to a QMRA analysis, and the microbiological techniques allowing identification of specific pathogens from stool, saliva, and blood samples are available. The Airlie Report (p. 86) states that the current and planned epidemiological studies provide a "unique opportunity" to add components to the studies that would include specimen collection with the goal of identification of etiological agent. EPA should begin to address specific needs toward identifying the disease-causing agents in recreational water exposure include developing and evaluating collection techniques, and piloting approaches for determining etiological agent. In

addition, analysis for a greater number of pathogens in the epidemiology studies could inform an investigation into etiology.

C. Other factors in criteria development

Need to consider populations experiencing multiple exposures in criteria development

It is important to note that regular frequent swimmers or surfers, as well as other individuals experiencing multiple exposures during a short period of time, such as tourists, may be more susceptible to illness. Current and planned epidemiological studies do not include an analysis of how multiple exposures may affect risk. This is an important future research goal, as the necessary studies do not exist in the literature. In the meantime, the possibility for increased risk due to multiple exposures must be taken into account during final criteria development.

Need for investigation of background illness rates

There is currently no commitment in the Science Plan to conduct the necessary survey work to determine the background illness rate for gastrointestinal illness, upper respiratory illness, and skin rash in the general population. Cleary, an understanding of background illness rates is necessary for an informed evaluation of the appropriate risk level due to recreational exposure to ambient water.

Equal protection in marine and freshwater and appropriate risk level

The Airlie Report is very explicit about the need for equal protection in various types of water (freshwater/marine and temperate/tropical.) It states that there is no scientific rationale for differing risk level targets. However, the Science Plan is relatively silent here.

Based on the Airlie report, public involvement in the determination of "acceptable risk" levels is essential. The research required to determine the acceptable risk level to the public has not been conducted. EPA should conduct surveys to assess "acceptable risk" in the population.

Need to allow rapid indicators for monitoring/notification purposes

EPA needs to validate rapid methods for detecting beachwater contamination so that the public can obtain timely information about whether beachwaters are safe. Current culture-based methods require a long incubation period, producing results in 24 to 48 hours. This lag time between when pathogen contaminated waters are sampled and when the public is notified creates a dangerous window where swimmers can be infected. Rapid methods are now being used in some places that are based on the identification of genetic markers, which can be done in about 2 hours.

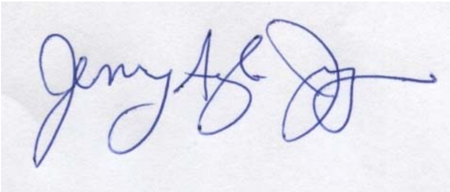
Need to consider other epidemiological studies in criteria development

All high quality epidemiological studies subsequent to 1986 should be considered in the development of the new criteria. Randomized control trials conducted in Europe (Kay et al., 1994; Fleisher et al., 1996; Wiedenmann et al., 2006) will yield useful information, and a prospective cohort study in California (Haile et al., 1999) is still the only epidemiological study as of yet with urban runoff as a source.

III. Timeline

The entire suite of studies necessary for the EPA to prepare revised AWQC, including the additional surveys and epidemiological studies recommended in this report, can be completed by 2010. Proposed studies P4 and P5 in the Science Plan involve "epidemiological study(ies) and/or QMRA" to characterize risks due to agricultural sources and urban runoff, respectively. If epidemiological approaches (including all of the elements recommended herein) were used for these projects, and if P6 were conducted in a tropical region, the number of necessary additional studies or surveys would be reduced. Epidemiological studies can be planned in a short timeframe. As an example of this, the Avalon study went from the planning stage to execution in one month.

I have not previously served as an expert witness. I have been compensated $150 per hour, to a maximum of $10,000.



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APPOINTMENTS

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AWARDS

Presidential Early Career Award in Science and Engineering (PECASE, 2004), Northrop Grumman Award for Excellence in Teaching (2007), Carnegie Foundation Faculty Fellow (2007-2008)

PROFESSIONAL AFFILIATIONS

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SELECTED PUBLICATIONS

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