New USEPA water quality criteria by 2012: GOMA concerns and recommendations

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ABSTRACT

The Gulf of Mexico Alliance (GOMA) was tasked by the five Gulf State Governors to identify major issues affecting the Gulf of Mexico (GoM) and to set priorities for ameliorating these problems. One priority identified by GOMA is the need to improve detection methods for water quality indicators, pathogens and microbial source tracking. The United States Environmental Protection Agency (USEPA) is tasked with revising water quality criteria by 2012; however, the locations traditionally studied by the USEPA are not representative of the GoM and this has raised concern about whether or not the new criteria will be appropriate. This paper outlines a number of concerns, including deadlines associated with the USEPA Consent Decree, which may prevent inclusion of research needed to produce a well-developed set of methods and criteria appropriate for all regulated waters. GOMA makes several recommendations including ensuring that criteria formulation use data that include GoM-specific conditions (e.g. lower bather density, nonpoint sources), that rapid-testing methods be feasible and adequately controlled, and that USEPA maintains investments in water quality research once the new criteria are promulgated in order to assure that outstanding scientific questions are addressed and that scientifically defensible criteria are achieved for the GoM and other regulated waterbodies.

Key words | EPA water quality criteria, epidemiology, fecal indicators, Gulf of Mexico, pathogens, recreational waters

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GULF OF MEXICO ALLIANCE

The Gulf of Mexico Alliance (GOMA) was formed by the Governors of Alabama, Florida, Louisiana, Mississippi and Texas, which are the five US states bordering the Gulf of Mexico (GoM). GOMA Priority Issue Teams include university and government researchers, federal and state agencies, and regulators and watershed managers. The Priority Issue Teams help identify high-priority problems pertaining to the GoM and its coast and establish goals to address those problems. These goals and the means to implement them are laid out in GOMA Governors' Action Plan as well as additional documents containing implementation details. The issues included in the Governors' Action Plan are those agreed on by all five states as the highest common priorities. GOMA completed over 95% of the actions identified in Action Plan I and is currently operating under Action Plan II, a five-year plan. One of the priority issues in Action Plan II is the need to improve microbial source tracking and pathogen detection methods for use under GoM conditions. Identification of goals, tasks and implementation activities surrounding these issues is spearheaded by the GOMA Pathogens Workgroup, a working group of the GOMA Water Quality Priority Issue Team. GOMA understands that establishing new criteria is a major undertaking for the US Environmental Protection Agency (USEPA) because they must encompass all types of waterbody across the entire country. All issues cannot be solved at once, but the purpose in this paper is to express the concerns of this group about new criteria suitability to waters of the GoM.

SETTLEMENT OF LAWSUIT PROMPTING DEVELOPMENT OF NEW RAPID METHODS

The Natural Resources Defense Council (NRDC) filed an action against the USEPA on the grounds that USEPA was not compliant with publishing of water quality criteria and completion of water quality studies as required by the Clean Water Act (CWA). The court ruled in favor of the NRDC and issued a Consent Decree (NRDC vs. Johnson 2008a) which established deadlines for the completion of these studies and publication of the water quality criteria. A Settlement Agreement set forth terms for certain matters not addressed in the Consent Decree (NRDC vs. Johnson 2008b). These items included detection of pathogens and fecal-indicator bacteria (FIB) in coastal waters and development of water quality criteria based on USEPA studies named in the Consent Decree. Terms defined in the Settlement Agreement include the 'Critical Path Science Plan' (USEPA 2007b) or 'CPSP', which refers to the document USEPA released on 31 August 2007. CPSP describes, among other things, the studies USEPA indicated it intended to conduct for the purpose of developing new or revised water quality criteria. Rapid test methods are described as methods that yield 'results as soon as practicable', which has been defined as two hours by the NRDC and as six hours by USEPA.

USEPA agreed to complete multiple recreational-water studies, including epidemiological studies at beaches affected by publicly operated treatment works (POTWs) (USEPA 2007b). As integral parts of these studies, USEPA included various parameters such as: (1) different sample handling techniques; (2) use of multiple FIB and method combinations to develop quantifiable relationships; (3) comparison of monitoring approaches that take into account spatial and temporal variability with sampling (USEPA 2007a); and (4) development, refinement, validation and publication of one or more new, rapid, ambient test methods. USEPA also agreed to evaluate the suitability of individual combinations of FIB and methods for different CWA programs, pilot beach notification and advisory/closure models, and statistically analyze data on children from epidemiological studies (USEPA 2007b). USEPA agreed to make study results known by December 2010. If studies cannot be completed, USEPA is to provide notification of its evaluations by July 2011. By October 2012, USEPA will validate and publish a rapid method for the new or revised water quality criteria. USEPA shall report the results of its evaluations to the parties of the Settlement Agreement by December 2012.

PRESENT STATUS OF USEPA RESEARCH

The Critical Path Science Plan (USEPA 2007b) identified the following four high-priority research areas: (1) human health impacts from different sources of fecal contamination; (2) measurement issues associated with climatic, geographic and temporal variability; (3) indicators and methods for measuring fecal contamination; and (4) determining risk level and subpopulations of concern. The high-priority research areas regarding climatic and geographical variability and fecal sources are particularly relevant to GOMA.

The USEPA has launched a commendable effort to reach the goal of finalizing new or revised water quality criteria in 2012, with completion notices for a number of research projects posted as of December 2010 (http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/index.cfm). For example, epidemiology studies including rapid methods (quantitative polymerase chain reaction, qPCR) and alternative indicators (e.g. *Bacteroides*) have been completed (Wade *et al.* 2006, 2008), with additional results becoming available recently for marine waters, including sites in the GoM (Wade *et al.* 2010a) and sites with urban runoff and tropical conditions (Wade *et al.* 2010b). In addition to studies directly conducted by USEPA, studies have been conducted at nonpoint source beaches in California by the Southern California Coastal Water Research Project (SCCWRP) (USEPA 2010) and on the Atlantic coast of Florida (Fleisher *et al.* 2010; Sinigalliano *et al.* 2010). USEPA work has been published finding increased risk of gastrointestinal (GI) illness for children 10 years and younger with exposure to *Enterococcus* at freshwater beaches polluted by sewage discharge (Wade *et al.* 2008); however, no such increased risk with children was found at marine beaches (including GoM sites) polluted by sewage discharge (Wade *et al.* 2010a). With regard to fecal sources, the EPA is:

> '… unable to evaluate method performance in different water body types based on the performance criteria … Additionally, method performance with respect to fecal source identification (i.e. human vs. animal) could not be evaluated because none of the current indicator/method combinations can distinguish between sources.' (USEPA 2010)

PRESENT STATUS OF PENDING REGULATORY CHANGES IN CONGRESS

The Clean Coastal Environment and Public Health Act (HR 2093, S878) amends the Federal Water Pollution Control Act (commonly known as the Clean Water Act [CWA]) to require USEPA to specify performance criteria for monitoring and assessing coastal recreational waters adjacent to beaches or 'similar points of interest (waters)' with available methods that are most likely to detect contamination by pathogens. This legislation would require coastal monitoring consistent with such criteria, public notification, source tracking, sanitary surveys and prevention efforts to address identified sources of contamination by fecal pathogens and FIB in such waters that are used by the public. The legislation includes language to use a rapid-testing method to detect levels of pathogens or FIB that are harmful to human health and for communicating any exceedance of applicable water quality standards for pathogens and FIB to specified officials within as little as two hours of the receipt of the results of a water sample, as well as initiating closures or advisories for beaches within two hours.

GULF OF MEXICO ALLIANCE CONCERNS ABOUT USEPA WATER QUALITY CRITERIA RESEARCH

Adequate incorporation of studies representing GoM conditions

The GoM has environmental areas and conditions that set it apart from other geographic areas that have been traditionally studied by USEPA; this raises concern about whether the results of the research informing the new water quality criteria will be suitable to address issues in the GoM. Specific areas of concern include the following: (1) low-use, low-population-density coastal areas (e.g. much of the northern Gulf coast and the Florida Big Bend region); (2) high-salinity areas (e.g. Laguna Madre, Texas); (3) areas of heavy rainfall; (4) subtropical latitudes and shallow areas where water temperatures can be higher than many other US coastal areas; (5) locations where waters contain a large amount of colloidal clay, organic detritus material, or colored dissolved organic matter (CDOM), which occurs as a result of tannins

being released from decaying detritus (e.g. Weeks Bay, Alabama; Suwannee River Sound, Florida); and (6) areas that predominately receive nonpoint sources of pollution rather than sewage-derived contamination. It is of interest to note that other coastal areas in the country (e.g. the North Carolina outer banks and the coasts of Oregon and Washington) share characteristics with the Gulf of Mexico; therefore, the concerns for the new criteria outlined here should apply to such areas, as well.

In general, past epidemiological data support a quantitative relationship between FIB and bather-related gastrointestinal illness for high-bather-density beaches influenced by POTWs. A USEPA report states that:

> 'numerous epidemiological investigations have been conducted since the 1950s to evaluate the association between the density of suitable FIB and the risk of illness to recreational freshwater users. Taken as a whole, the weight of evidence from these studies indicates that fecal bacteria (fecal streptococcus and *Enterococcus*, in particular) are able to predict GI and respiratory illnesses from exposure to recreational waters.' (USEPA 2009)

However, recent USEPA epidemiology studies are motivated by the question of whether it is 'scientifically valid to extrapolate results from studies conducted in the Great Lakes freshwater environments to other freshwater environments that may differ from the Great Lakes' (USEPA 2007b). GOMA feels that this concern about extrapolating results could apply to marine coastal waters from different geographic regions as well. In fact, a recent USEPA study (Wade *et al.* 2010a) stated that 'results may not be directly applicable to sites affected from other types of sources or sites with different climates'.

One reason site differences could be important is that the behavior of enterococci and *Escherichia coli* may vary in different climates and environments. Temperature influences the general persistence and survival of fecal-bacteria and specific pathogens in the environment (Anderson *et al.* 2005; Ishii *et al.* 2006; Walters & Field 2006). Therefore 'different FIB may be more appropriate for use in tropical or subtropical environments versus temperate ones' (USEPA 2007a). According to the EPA Experts Workshop (USEPA 2007a), 'appropriate indicators that correlate with recreational swimmer illness rates in tropical and subtropical climates are needed'.

GOMA notes that concerns regarding regulations based on traditional fecal-indicator bacteria (e.g. *E. coli*, enterococci and fecal coliform) are not limited to those regions with warm climates because a lack of correlation between pathogens and FIB has been cited by numerous researchers. For example, a USEPA-commissioned literature review (Bartrand *et al.* 2009) states that 'there is a near-universal lack of consistent co-occurrence or correlation of the waterborne pathogens investigated in this draft report with FIB, regardless of the setting (coastal, inland flowing, or inland non-flowing)'. In nearly all reported cases, pathogen occurrence differed temporally and spatially from that of FIB.

Differences between FIB and pathogen densities can arise from differences in loadings during discharge events such as ballast-water releases, fecal releases that can occur from wildlife or livestock (Wright *et al.* 2009), or perhaps from persistence in the environment. For example, a toxigenic *Vibrio cholerae* O1 biotype El Tor, serotype Inaba strain (in contrast to non-O1 strains, which are indigenous in warm estuarine waters and do not cause disease) was isolated in Mobile Bay and the Mississippi Sound. This strain was thought to have been released from ballast water (MMWR 1993; McCarthy & Khambaty 1994). Differences between FIB and pathogen densities may also occur through persistence and/or regrowth of FIB. FIB persistence is a concern for the development of water quality criteria because FIB densities may become uncoupled from the presence of pathogens, thus invalidating the fundamental assumptions upon which the water quality criteria are based. Persistence of FIB has been reported in tropical, subtropical and temperate areas of the country (summarized in USEPA 2007a), with beach sand and sediments (Alm *et al.* 2006; Lee *et al.* 2006; Hartz *et al.* 2010), beach vegetation (Whitman *et al.* 2003) and zooplankton (Signoretto *et al.* 2004, 2005) representing possible environmental reservoirs. The persistence of FIB in sediments can lead to an increase in their densities and a false indication of recent fecal pollution in adjacent water (Anderson *et al.* 2005). Also, differences in the fate and transport of pathogens relative to FIB can cause FIB and pathogen densities to become uncoupled. An example of concern for the GoM is the differential fate of organisms as they are transported from freshwater streams to seawater.

Furthermore, persistence of nucleic acids after FIB become non-culturable is a concern because of the possibility that nucleic acid detection (used in molecular methods) is uncoupled from the presence of viable organisms (Walters *et al.* 2009). In contrast, a USEPA study with POTW-impacted waters suggested that the persistence of *Enterococcus* spp. as measured by qPCR may confer a benefit to water quality analysis because the molecular signal persists longer than viable cells and thus may better map the fate and transport of pathogens. Wade *et al.* (2008) stated that 'the qPCR measure may be a truer representation of fecal contamination, because it measures all *Enterococcus* associated with feces, not just viable cells. The molecular measurement of *Enterococcus* DNA.…may mirror the dilution and dispersion of fecal material'. This appears to be a significant conclusion that warrants further investigation, particularly with waters of varying environmental conditions.

Applicability of criteria for areas predominated by nonpoint sources of pollution

GOMA is concerned that in the process of devising new criteria, nonpoint source conditions will not be adequately represented. Two epidemiological studies were performed at Fairhope Beach (Fairhope, Alabama) and Edgewater Beach (near Biloxi, Mississippi) (Heaney *et al.* 2009; Wade *et al.* 2010a). These beaches were chosen, in part, because they were impacted by POTWs. However, scientists generally have reservations about USEPA standards being based on studies of point-source locations instead of local recreational water conditions, where nonpoint sources such as animal feces, stormwater or other sources (e.g. leaking sewers) may be the major problem (Boehm *et al.* 2009). Scientists have also expressed concern about the use of fecal bacteria that are commonly associated with non-fecal sources, such as the epibiotic species of enterococci commonly found on aquatic plants, which are not distinguished by standard culture methods.

Calderon *et al.* (1991) noted that illness in swimmers was not statistically associated with densities of commonly used FIB in recreational water whose source was rainwater runoff from a forested watershed and stated that '*E. coli* and enterococci for fresh recreational waters are ineffective for predicting potential health effects associated with water contaminated by nonpoint sources of fecal pollution'. To protect all recreational water users, studies in different localities affected by various nonpoint sources, in different climates and water types, need to be conducted (Boehm *et al.* 2009). For instance, a principal source of microbial pollution of Mississippi coast beaches is freshwater creeks that flow into the estuaries (Flood *et al.* 2011). These creeks have no direct contact with sewage treatment plants or nearby domestic animal facilities but are subjected to the same quantitative guidelines as those that are affected by point sources. Indeed, the Experts Scientific Workshop (USEPA 2007a) pointed out that 'the need for additional epidemiological studies, especially at nonpoint source impacted beaches is essential to better define risk and guide criteria development'.

Recent publications (e.g. Wade *et al.* 2010a, b) to date have not quieted GOMA's concern about whether correlations established in POTW-affected waters hold in areas receiving nonpoint sources of fecal-indicator contamination. For example, studies at a nonpoint source beach on the Atlantic coastline of Florida did not find an association with GI or respiratory symptoms and FIB (Fleisher *et al.* 2010; Sinigalliano *et al.* 2010) nor did a USEPA study at a marine beach receiving urban runoff (Wade *et al.* 2010b). Previous studies in California's Mission Bay also failed to find positive associations with FIB levels (Colford *et al.* 2007). Results from more studies at nonpoint source beaches in California are pending (USEPA 2010).

Use of a single-criterion approach, rather than a 'toolbox' may result in reduced applicability in different environments

A single criterion is attractive for the sake of simplicity and coherence, but GOMA is concerned that this approach may not represent the varying conditions (and thus risks) of state waters. According to the Experts Scientific Workshop a 'one size fits all' criterion is inadequate for public health protection and 'differences in climatic regions and geographic areas throughout the country must be taken into consideration' (USEPA 2007a). Furthermore, there is concern that the scientific literature does not support revised water quality criteria that are based solely upon a single indicator, whether they are culture- or molecular-based methods

(USEPA 2007a). The National Research Council (2004) reports that no single FIB or pathogen is likely to reflect all exposure routes adequately, and other literature supports a suite of fecal indicators as being a better approach than single-species analysis (Harwood *et al*. 2005; Baums *et al*. 2007). Furthermore, Fleisher *et al*. (1996) reported that 'a single fecal indicator would not be sufficient to establish water quality standards aimed at protecting public health'.

A USEPA report states the following:

> '… it is not surprising that indicator organisms correlate well to some pathogens in some waters but correlate poorly in other cases … If new or revised water quality criteria are to quantitatively address the various sources of fecal contamination of potential interest in recreational waters covering diverse geographic regions, the approach will likely require a set or toolbox of indicators as measured by specific methods.'

To address concerns regarding the need for improved indicators and methods, a number of studies have included alternative methods (e.g. USEPA 2010; Wade *et al*. 2010a, b). For the study that included GoM sites (Wade *et al*. 2010a), the other methods tested (*Bacteroides* qPCR, *Clostridium*, and F+ coliphage) showed no clear advantage over *Enterococcus* spp. as measured by qPCR. However this study (and others; e.g., Wade *et al.* 2010b) also did not find a significant health risk associated with *Enterococcus* CFU as measured by culture (Wade *et al*. 2010a). Given that, in general, past studies supported a relationship between FIB and bather-related gastrointestinal illness for high bather-density beaches influenced by POTWs (USEPA 2009), this discrepancy suggests that enough questions remain to warrant continued investigation.

Use of a single FIB standard, even for waters primarily influenced by animal sources

GOMA is concerned that the application of a FIB water quality standard with no regard for the source of the indicators could result in criteria that overestimate the actual risk for bather-related illnesses, resulting in real and significant costs to the states. The potential for zoonotic pathogen outbreaks is a concern, but the risk of exposure to animal-contaminated water is unknown (USEPA 2009). There is little literature to draw upon to determine the extent to which fecal indicator levels (e.g. enterococci, *E. coli*, fecal coliform) predict the risk associated with zoonotic outbreaks. In contrast, there is ample literature documenting the near-ubiquitous nature of FIB, originating not only from humans and animals, but growing *ex situ* in water, sediment, soil, and on vegetation (e.g. Bermudez & Hazen 1988; Alm *et al*. 2006; Lee *et al*. 2006; Moore *et al*. 2008; Yamahara *et al.* 2009; Hartz *et al*. 2010). GOMA is concerned that a single FIB standard will not discriminate the relative risk associated with source differences.

The 1986 criteria (USEPA 1986) were not intended to be protective of zoonotic pathogen outbreaks, and USEPA does not have sufficient data to demonstrate that the 1986 criteria are protective of such outbreaks. According to a USEPA report, the 'recommended recreational water quality criteria do not differentiate between fecal sources of pathogens. Thus, USEPA's regulatory premise concerning recreational water quality has been that nonhuman-derived human pathogens in fecally contaminated waters are as hazardous as their human-derived counterparts' (USEPA 2009). This is in contrast to Wade *et al*. (2010b) which stated that beaches impacted by human sewage were used to derive indicator bacteria and health effects associations because 'it was expected that human-feces exposures pose a larger risk to human health'. Overall, USEPA has stated that 'method performance with respect to fecal source identification (i.e. human vs. animal) could not be evaluated' (USEPA 2010), thus it does not appear that the 2012 criteria will be able to demonstrate whether or not it is appropriately protective (either under or over) of zoonotic pathogen outbreaks. Perhaps future epidemiology studies will be able to include microbial source tracking to determine whether such an approach enhances illness risk estimates.

Although direct epidemiology data may not be available to assess the human health risks associated with exposure to recreational waters contaminated by animal sources, two recent quantitative microbial risk assessment (QMRA) studies estimated that seagull fecal contamination posed less risk than human fecal contamination (Schoen & Ashbolt 2010; Soller *et al*. 2010). Contamination by seagulls

leading to a geometric mean of 35 CFU of enterococci per 100 ml of recreational water yielded a QMRA estimated probability of illness that was less than the illness benchmark of 0.01 (USEPA 1986). Fecal contamination from other animals such as livestock, pets, wildlife and waterfowl (e.g. Wright *et al*. 2009; Ballesté *et al*. 2010) may be important to consider as well. In general, animal fecal contamination may be significant to the GoM and other US regions because of a preponderance of non-sewage fecal sources.

A 2009 USEPA report addresses the topic of animal waste in detail, and appears to support GOMA concerns by stating the following:

> 'In fact, the results of this literature review seem consistent with WHO's (2004) (Cotruvo *et al.* 2004) report on zoonoses, which indicated the following: Inadequate information exists on differentiating human versus animal strains of enteric pathogens, both in the field (e.g. pathogen typing and microbial source tracking) and analytically (e.g. relative infectivity and pathogenicity). Both of these areas are priorities for targeted research … Surveillance for waterborne disease in general and waterborne zoonoses in particular has failed to provide a meaningful indication of the associated burden of disease, even in countries with established surveillance systems … The risk of exposure to animal-contaminated water is unknown. Studies to define the risk associated with swimming in animal-contaminated water have not clearly indicated that this type of exposure results in an increased illness rate. The results of these studies do not support the premise that all fecally contaminated waters should be treated the same. New research to define the risk of illness posed by animal fecal wastes is needed.' (USEPA 2009)

Furthermore, a recent epidemiology study that included GoM sites (Wade *et al.* 2010a), also appears to support GOMA concern by stating that 'some human pathogens, particularly human enteric viruses, are unlikely to be associated with non-human sources of fecal indicator bacteria' and goes on to cite the lack of association between FIB concentrations and GI illness at a nonpoint source beach in Florida (Fleisher *et al*. 2010) as support for this limitation.

Current sampling procedures do not account for highly variable microbial density

Highly variable microbial populations indicate that single samples may not properly assess water quality, even if the results are obtained rapidly because samples can vary greatly (greater than the single-sample standard) over a very short time frame (1 to 10 min) (Boehm 2007). Boehm (2007) suggests that either collecting multiple samples or a spatially or temporally composited sample may better represent the true water quality (Boehm 2007). The implications of such findings are unclear with regard to whether routine sampling adequately protects public health and/or whether it results in unnecessary advisories. It is also unclear if public health protection can be improved via modification to sampling protocols, and, if so, whether such modifications are cost effective and/or logistically feasible. Finally, the implications of such findings should be considered in the context of rapid methods requirements. For example, requirements for rapid methods such as those mentioned in House and Senate bills HR2093 and S878 may address time frames for making results available to the public (2 h is currently mentioned) but not address the dilemma of whether the sampling regime adequately accounted for sample variability. In other words, if the sample was not representative, does it matter how promptly the results were delivered to the public? Overall, research by Boehm (2007) documenting the extreme variability in enterococci densities over very short time periods underscores the need for well-reasoned sampling approaches and perhaps a variety of fecal indicator/method combinations that address a range of contamination sources and geographic areas (USEPA 2009).

A promulgated approach without assessment of low-bather-density waters

Owing to USEPA's preference for the prospective cohort epidemiological study design, their studies are performed exclusively at high-density beaches where many participants can be enlisted. Fecal indicator to illness relationships developed from these studies may be quite dissimilar to low-density beaches, since the bathers themselves are a potentially significant source of fecal contamination at

high-bather-density beaches (Gerba 2000; Elmir *et al*. 2009). Research has shown that bather shedding can be a significant source of fecal indicators and pathogens (Gerba 2000; Elmir *et al*. 2007, 2009) and therefore can be a contributing factor in epidemiology studies. Bather density was considered in the principal components analysis in USEPA studies and did not reveal 'a high risk of illness resulting from other swimmers. However, bather density was rather crudely categorized … and may not have been adequately represented for the purposes of deriving health risks' (Wade *et al*. 2010b). Given that designated bathing beaches make up only a small fraction of the total recreational waters in GOMA states, and that the majority of the recreational waters receive low-density use, it is unclear how USEPA will account for this discrepancy when developing the revised water quality criteria or guidance (for example, whether guidelines may depend on a set of beach classifications in which bather number is considered).

GOMA CONCERNS REGARDING PENDING REGULATORY CHANGES

Regulations via Consent Decree rather than established science

USEPA stated that as a result of the Consent Decree and Settlement Agreement, research activities to support development of new or revised water quality criteria would be completed by December 2010. These research studies are complete, and the completion notices are shown at http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/index.cfm. However, ongoing research (e.g. by GOMA researchers) and analysis from recent EPA epidemiology studies (e.g. Wade *et al*. 2010a, b) pertinent to the development of the criteria may continue to come in after the deadline. Therefore, GOMA is concerned that the timeline dictated by the Consent Decree will force states to implement measures that are inadequately supported by science. Furthermore, although USEPA will be mandated to periodically review additional research after the revised criteria are promulgated, GOMA is concerned that once the new criteria are developed a lack of additional funding will limit the amount of new data available to ensure that the most defensible criteria are achieved.

Regulations that may have far-reaching Clean Water Act implications

GOMA is concerned that although the revised criteria affect all potential applications of the Clean Water Act (CWA), the timeline is driven by a lawsuit surrounding recreational waters and, more specifically, bathing beaches. For example, there was concern that rapid methods such as qPCR could be required for all water quality applications despite evidence that qPCR detection of FIB could be problematic for National Pollutant Discharge Elimination System (NPDES) (wastewater effluent) permitting (Boehm *et al*. 2009) because the molecular signal does not decrease post-chlorination in relation to viability (He & Jiang 2005; USEPA 2007a). One study (Noble *et al*. 2010) found that a low level of residual chlorine in secondary treated sewage either destroyed or rendered culturable enterococci non-culturable within minutes. However, when qPCR methods were used to enumerate enterococci in chlorine-disinfected wastewater, the enterococci concentrations remained relatively unchanged during 4 hours of exposure to the disinfectant. A recent USEPA study (Brenner *et al*. 2010) showed that the reduction of *Enterococcus* was similar during primary and secondary treatment, but differed significantly during disinfection with UV light or chlorine.

GOMA is hopeful that sufficient consideration will be made to how the criteria can best serve non-bathing-beach uses. Recently, USEPA recognized that the 'appropriateness of the method for the various CWA programs is important for state adoption and implementation of EPA's new or revised criteria recommendations' (USEPA 2010). This report recognized that 'time to results (rapid/results within 4 h of sample process/analysis)' was 'not important' to the NPDES, the total maximum daily load (TMDL), or the Monitoring and Assessment Program. The report goes on to state that 'rapid methods are suggested as a key component of the criteria for the Beach Program only'.

Although the recent EPA report (USEPA 2010) recognizes the importance of being able to maintain comparisons to historical data and states that rapid methods are suggested only for beach monitoring, the

report does not address the complication that many agencies implementing CWA programs use some level of coordination to promote efficiency. For example, the default designation of Florida waters is Class III ('Recreation, Propagation and Maintenance of a Healthy, Well-Balanced Population of Fish and Wildlife'). Such types of 'on-the-ground' consideration are key to how the new criteria will actually impact the states when implemented, and this is true for all states, not just the GoM. In addition, it is unclear whether the new criteria will take into account the overall monitoring burden of the states. For example, recreational and shellfish harvesting waters continue to be under separate regulatory guidance (USEPA vs. FDA), further complicating and increasing the states' burden. It is not clear whether in the course of devising the new criteria EPA may be able to work with FDA to develop more integrated standards.

Overall, GOMA is interested to know if and how the new criteria will affect other water quality responsibilities such as the Impaired Waters Programs (which generate total maximum daily load (TMDL) limits), NPDES permitting, stormwater programs, and shellfish-related regulations. How does USEPA envision that the new criteria will affect state regulations on fecal/total coliform monitoring? If the new criteria recommend new methods, will those be able to address requirements of multiple federal agencies?

Expectations for 'rapid' testing methods that are not generally feasible

Language in the Clean Coastal Environment and Public Health Act of 2009 (HR 2093, S878) refers to time frames as short as 2 h, not only for detection but also for notification and for closures or advisories. Studies (e.g. Haugland *et al.* 2005; Noble & Weisberg 2005) have shown that *analysis only* can be done in 3 h or less. However, the 3 h estimate does not include transportation time or the scale-up time necessary to achieve throughput of a large number of samples or the time necessary to extract nucleic acids and to incorporate controls and other quality assurance steps. Field sampling of multiple beaches usually consumes 4 to 5 h, so the first samples collected will not be delivered to the lab until the 6th hour; only then does the lab typically begin the process of sample filtration and DNA extraction.

It is unclear whether the studies being considered by USEPA include time assessments that are realistic to the water quality monitoring requirements of the states. Based on experience with the time it takes to fully process samples from collection to data output, several GoM researchers suggest that the time estimates proposed in bill language are not achievable. A pilot project performed by the Southern California Coastal Water Research Project (SCCWRP) during the summer of 2010 needed to choose field sites close to the laboratory and take samples separately from the normal samples in order to shorten the time from sample collection to results reporting (Griffith & Weisberg 2011). Current Act language implies that data can be recorded, quality controlled, and translated for notification and closure within a 2-h time frame, which appears unfeasible for monitoring a large number of sites. In isolated cases or for specific events (e.g. stormwater response, sewage overflow) where a single or few sites are visited, more rapid reporting may be feasible.

It should be noted that an analytical result in 2–4 h does indeed represent improved speed over current culture-based methods (overnight results). However, both methods require time, as outlined above, that are not included in this comparison. Any recommended methods must allow sufficient time to meet pre-analytical, analytical and post-analytical time requirements. Ideally, staffing realities should also be considered. For example, an afternoon qPCR run may be completed in only 3 h, but there are costs associated with after-hour staffing if results are to be made public before the next morning. If such operational realities are not considered, impacts to the states could be underestimated and the perceived benefit could be overestimated. For example, USEPA recently recognized that 'the sample costs associated with the use of (a) rapid method could become cost prohibitive or result in beaches being sampled less frequently or not-resampled to revise (an) advisory or closure decision during the day' (USEPA 2010). This potential for less sampling, as well as realistic operational costs and requirements, should be considered in an accurate assessment of the extent to which rapid methods may improve public protection. GOMA is concerned that inadequate consideration of these issues will lead to an under-funded mandate with ill-defined benefit.

Rapid methods that do not include all needed quality controls

A concern regarding promulgation of rules that require rapid (molecular) methods is the incorporation and standardization of adequate controls. Another analytical concern for rapid molecular methods is the error introduced by nucleic acid extraction techniques. If this first step is not reproducible, quantitative and sensitive (e.g. Baums *et al.* 2007; Goodwin & Litaker 2008), it is unclear whether quantitative molecular methods for regulatory use or methods to detect rare targets (e.g. pathogens) can be useful. Currently, each lab typically develops its own standards which are used only within that lab (e.g. extraction efficiency, PCR inhibition, etc.). Certified, reliable standards to which all labs can compare their quantitative results are needed (Haugland *et al.* 2005; Wade *et al.* 2010a).

USEPA may not continue the research needed for methods to have wider CWA utility

GOMA is concerned that USEPA may not get the financial support required to devote the level of effort needed to produce widely applicable methods and criteria after the deadline is reached. GOMA recognizes that USEPA must produce revised or new criteria by 2012 to meet the conditions of the Settlement Agreement, and GOMA recognizes that a substantial amount of work has been completed (e.g. USEPA 2010). However, as outlined above, not only is there ongoing work and analysis, there are remaining questions to be answered. GOMA strongly encourages USEPA to continue to support research efforts to produce more tools and criteria that meet all of the States' needs.

GULF OF MEXICO ALLIANCE PATHOGEN WORKGROUP RECOMMENDATIONS

Recommendation 1

> 'Conduct additional research specific to the Gulf of Mexico region and address pending scientific questions'

GOMA recommends that additional research studies be performed in order to address the concerns outlined above. GOMA specifically recommends that the USEPA conduct either an additional epidemiology study under nonpoint source conditions and/or 3–4 smaller projects to assess various fecal indicator/pathogen methods under GoM conditions to address issues such as temporal and spatial variability and persistence within warm, shallow coastal GoM waters. The risk associated with small beach-going populations typical of many GoM beaches should be better understood, as the level of exposure may be significantly lower compared with that estimated at high-attendance beaches, as outlined above. GOMA suggests that common seafood pathogens (norovirus, *Campylobacter*, *Salmonella* and *Vibrio*) and alternative fecal indicators and source tracking markers (e.g. *Bacteroidetes*, polyomavirus, *Methanobrevibacter smithii* [Harwood *et al.* 2009], male-specific coliphage, somatic coliphage, etc.) be evaluated in addition to the current water quality indicators during new studies.

GOMA suggests that researchers archive samples from all new studies. Study samples are critical region-specific ecosystem and public health material that should be available for future assessment with new methods. USEPA could encourage state laboratories to archive aliquots or filters from studies (frozen at $-80\ ^{\circ}C$) so that samples can be analyzed later as new technologies, targets and methods become available. Samples from epidemiological studies are especially valuable and need to be archived.

In general, GOMA recommends support for improving water quality criteria post-2012. As outlined above, a number of questions remain for priority issues so it is critical that USEPA continues to fund studies, request external investigators to develop projects, and team with other agencies (state and federal) to develop and optimize new and/or improved methods for quantification of fecal contamination and specific pathogens/markers. GOMA agrees with the observation made by the Experts Scientific Workshop (USEPA 2007a) that further development of new methods and tools should be proactively pursued to facilitate future enhancements to water quality criteria beyond 2012. Given that USEPA completed studies in December 2010 (USEPA 2007b; Wade *et al.* 2010b), it is critical that USEPA supports research that could lead to development

of supplemental water quality criteria. This needs to be an ongoing, long-term process, particularly as new technologies are developed.

Recommendation 2

> 'Apply rigorous, appropriate, and standardized QA/QC and validation procedures'

Currently there are two approaches to pathogen and pathogen indicator detection and enumeration. The first is the traditional culture-based technique, and the second is a newer, molecular-based method (qPCR). Current capabilities of molecular methods have 'increased regulators' and stakeholders' interest in seeing them applied to CWA activities to better protect public health' (USEPA 2007a). It is recognized that any new test method must provide a level of human health protection equal to or greater than that provided by the currently used tests (USEPA 2007a). GOMA assumes that any promulgated rules regarding analytical methods will include information about the method's sensitivity, specificity, precision and accuracy and will, in general, conform to USEPA's Alternate Test Procedure Protocols for Microbiological Methods (USEPA 2004).

USEPA researchers (Haugland *et al.* 2010; Wade *et al.* 2010a) have published descriptions of various QA/QC controls and strategies, although questions surrounding these methods appear to remain. For example, nucleic acid recovery and reaction efficiency is likely to be matrix and method dependent. Error thresholds for method failure, requiring re-running of samples, should be established for various points during the protocol. GOMA recommends that USEPA adequately identifies variable sample processing steps and structure appropriate controls around sources of sample variability. For example, it should be determined whether a whole-cell extraction control is necessary or if a purified nucleic acid extraction control such as salmon sperm DNA (e.g. Haugland *et al.* 2010; Wade *et al.* 2010a) is sufficient to determine DNA recovery. If clean-up methods for DNA/RNA purification are used, a standardized reference sample control is needed in order to determine the percentage nucleic acid loss during this step. If this is done by some type of calibrator whole cell, such physical reference material needs to be standardized and made readily available to the scientific community. It would be helpful if USEPA or a commercial, certified source could make controls available.

Controls that assess inhibition of PCR-based methods are needed to account for the fact that environmental samples may contain inhibitors that significantly alter PCR efficiencies measured under laboratory conditions. Reports describe different strategies to quantify targets and deal with inhibition (e.g. delta delta-CT versus delta-CT) (Wade *et al.* 2010a). However, issues appear to remain. For example, the USEPA study conducted in a tropical climate (Puerto Rico) was severely hampered by qPCR inhibition of unknown origin (Wade *et al.* 2010b). A similar USEPA report (Brenner *et al.* 2010) stated that a tropical epidemiological study was hampered because 'many qPCR assays could not be completed due to interfering or inhibitory substances in the water sample'. Studies should be conducted to determine the best controls and standardized methods to assess and correct for such inhibition. Needs include a defined reporting protocol if diluting the nucleic acid sample does not sufficiently correct for inhibition, ensuring that the detection limit was not violated upon sample dilution (e.g. using a matrix-appropriate reference sample such as a spike control of salmon sperm DNA), and determining the effect on quantification of using PCR additives to decrease inhibition while maintaining specificity (e.g. bovine serum albumin). In addition, the use of customized internal amplification controls (Haugland *et al.* 2010) and/or standardized controls (such as BioBall™) should be evaluated. Whatever controls are utilized, they should be standardized and made readily (and affordably) available to the regulatory and scientific community.

Recommendation 3

> 'All labs that perform fecal indicator/pathogen testing for public health purposes should be validated for sample analysis using the new 2012 standards'

Procedures to test laboratories for capability must be developed and a program implemented to certify laboratories for the new methods. This program must be in place before new methods/criteria are implemented for regulatory purposes. GOMA suggests that the generic method approval

and validation material forms that the Interstate Shellfish Sanitation Conference uses for single lab validation (http://www.issc.org/lmrforms.aspx) may provide a good model which could be adapted for use to help move methods from research to standard tools. Laboratories performing fecal indicator/pathogen testing for public health purposes need to perform inter-laboratory sample comparisons (e.g. round-robins) on blind spiked samples as part of the validation process (such as those performed by Harwood *et al.* 2009). Eventually, there should be standardized evaluations/certifications for these methods (e.g. through National Environmental Laboratory Accreditation Conference).

Recommendation 4

> 'Conduct studies to determine the relative persistence of fecal indicator organisms, marker signals and pathogens in the environment'

Environmental reservoirs of FIB are a major concern for the development of water quality criteria because of the possibility that FIB densities can become uncoupled from the presence of pathogens. GOMA recommends that USEPA and other agencies support studies to evaluate the persistence of cultivable and molecular targets. The relationship of molecular signal persistence should be understood in relationship to cell viability, particularly with respect to more persistent pathogens/indicators such as viruses and spores (e.g. *Clostridium perfringens*). The correlation of these parameters to health effects, including how those effects vary by source of FIB, should also be well understood. Studies are needed to either corroborate methods that improve the link between molecular signal and viability (e.g. Wagner *et al.* 2008) or to establish health risk criteria that are robust to the persistence of a molecular signal. It has been noted that 'environmental factors, such as sunlight, may affect differential persistence of the qPCR signal' (Wade *et al.* 2010a). The possibility that qPCR may better 'mirror the dilution and dispersion of fecal material' (Wade *et al.* 2008) warrants investigation and highlights the need to conduct additional studies to better elucidate how indicators measured by qPCR are affected by physical and environmental factors and under what conditions rapid fecal indicator methods can be applied (Wade *et al.* 2006).

Recommendation 5

> 'Assign differing criteria for different types of water to reflect differences in relative public health risk'

The 1986 USEPA criteria were developed on the basis of one source (incompletely treated municipal wastewater) in two types of water (fresh and marine). Boehm *et al.* (2009) recommend using separate criteria for three source types (urban runoff, animal feces and treated municipal effluent) in four types of water (temperate fresh/marine and tropical fresh/marine). USEPA should continue to pursue studies to determine whether this would be an appropriate approach.

Recommendation 6

> 'Conduct studies to determine whether testing for multiple markers and pathogens ('toolbox' approach) would be better for detection and source tracking of fecal indicator bacteria and pathogens and to help investigate illness risks at beach sites with a diverse range of fecal pollution inputs'

A single organism or marker method may not be applicable to all GoM environments or to all locations across the United States. USEPA should continue to fund studies and request external investigators and other agencies to conduct projects to develop and optimize new or improved methods for enumeration of fecal contamination and specific pathogens/markers that will result in methods and tools which are specific, rapid, and consistently support a consensus interpretation of contamination with a defined human health risk. The 'toolbox' approach may lead to an improved understanding of beach-specific risks of illness, unlike a one-size-fits-all criterion, particularly with regard to the risk posed by nonhuman fecal sources. Support to identify new fecal indicators, source markers and methods for pathogen detection should continue after 2012 in order to adequately protect public health. Such support is needed if states are to comply with current language, as well as the spirit, of the

Clean Coastal Environment and Public Health Act of 2009, in which both source tracking and pathogen detection are specifically mentioned.

CONCLUSIONS

One of the major goals of GOMA is to ensure healthy beaches and shellfish beds in GoM waters, thereby protecting human and environmental health. This will be accomplished by improving the understanding of waterborne fecal pathogens, including their sources and survival, so that state and federal agencies can make informed decisions that benefit public health and coastal economies. The new USEPA water quality criteria to be promulgated in 2012 will have a major effect on how GoM waters will be managed in the future. GOMA is concerned about the possibility of a single criterion for all fishable and swimmable waters throughout the country without adequately considering regional and geographic differences and scientific uncertainties. Specific areas of concern include the following: (1) low-use, low-population-density coastal areas; (2) high-salinity areas; (3) areas of heavy rainfall; (4) subtropical latitudes and shallow areas where water temperatures can be higher than most of the country's coasts; (5) locations where waters contain a large amount of organic detritus materials; and (6) areas that predominately receive non-point sources of pollution rather than sewage-derived contamination.

A major recommendation by GOMA is for USEPA to include data from a variety of climates, waterbody types and localities affected by various sources of contamination during development of the new water quality criteria. Other recommendations include the following:

1. Conduct additional research that is specific to the Gulf of Mexico Region, including epidemiology studies with non-point sources and investigations to develop fecal indicator and source tracking methods and criteria that are appropriate for the environmental conditions of the GoM, including those conditions that affect persistence of viable cells and molecular signals.
2. Apply rigorous, appropriate and standardized QA/QC and validation procedures to any methods being considered (e.g. molecular methods) for promulgation as the new standard(s) and incorporate standardized and accessible QA/QC procedures into the final criteria.
3. Labs performing water quality indicator or pathogen testing for public health purposes should be approved for sample analysis using the new 2012 standard(s) and appropriate time should be allotted to achieve this goal.
4. Conduct studies to determine the relative persistence of fecal indicator organisms, source marker signals and pathogens in the environment to ensure that studies to date (e.g. Wade *et al.* 2010a) well represent state waters.
5. Consider assigning differing criteria for different types of recreational water to reflect differences in relative public health risk and consider studying specific pathogens or new fecal indicators and source markers.
6. Conduct studies to determine whether testing for multiple markers and pathogens ('toolbox' approach) would be better for detection and source tracking of FIB and pathogens, and include source tracking in epidemiology studies to determine whether this enhances illness risk estimates.

GOMA believes that new USEPA criteria that: (a) apply to all CWA uses; (b) are based on a single fecal indicator organism and a molecular technique; and (c) require rapid tests for activities where they are unnecessary, are likely to place a heavy long-term financial burden on the states as a result of unnecessary beach and shellfish-bed closures and expensive qPCR reagents, on top of creating a short-term burden from the costs of new equipment and retraining of personnel. GOMA is encouraged that there is recognition that CWA applications differ (USEPA 2010); however, it remains unclear whether or not implementation of the new criteria will disrupt current monitoring synergies. In contrast, the hope is that the new criteria can facilitate monitoring efficiency. It would be beneficial if state labs could be allowed to mix and match culture- and molecular-based methods, depending upon the end purpose of the assays. In conclusion, GOMA understands that USEPA has been mandated to perform a near insurmountable task of developing new water quality criteria by 2012. This paper describes GOMA's concerns and recommendations related to the

process in order to help USEPA establish criteria that will be appropriately applicable to the spectrum of regulated waterbodies throughout the country and to the Gulf of Mexico, in particular.

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