Appendix A. Review of Epidemiological Studies for Fresh Waters

In 1986, the U.S. Environmental Protection Agency (EPA) recommended that *Escherichia coli(£. coli)* and enterococci bacteria replace fecal coliform bacteria as indicators of human health risks associated with recreational exposures to pathogens in marine and fresh "'ater (U.S. EPA, 1986). EPA based its 1986 criteria recommendation on a revie\-\ of epidemiological studies relating gastrointestinal illness to specific bacterial indicators. In *2002*, EPA reevaluated and upheld its 1986 criteria recommendation based on a review of epidemiological studies conducted after the initial review period (U.S. EPA, 2002).

The California State Water Board (State Water Board) has adopted criteria for marine waters that are consistent with EPA"s 1986 criteria recommendation, and is developing policy to update bacteria criteria for fresh waters. EPA (1986) recommends either *E. coli* or enterococci as bacterial indicators for fresh waters. This report reviews the freshwater epidemiological studies that EPA used in developing its 1986 criteria recommendation (U.S. EPA. 1986). and the reevaluation in which it upheld its 1986 findings (U.S. EPA, 2002), to assist the Board in selecting which of these indicators is most appropriate for inland waters. Also discussed are additional relevant fresh water studies, and a recent epidemiological stud:,. conducted in California recreational v.aters, although the California study is for marine water. Existing studies do not contain sufficient information to provide guidance on appropriate indicators for estuarine \Vaters that serve as boundaries bet\\een fresh and marine waters.

Section I is de,oted to those studies that provide evidence of illnesses resulting from recreational exposures to fecal polluted water. but do not relate the risk of illness to levels of a microbial indicator. Section *2* discusses studies that evaluate the relationship between the presence of microbial indicators and fecal pathogens in waters where exposures resulted in illness. The studies include both prospective studies, in which researchers enrolled the participants before the onset of disease and compared recorded exposures to disease rates, and retrospective studies. in which researchers identitied the participants by occurrence of the disease and reconstructed exposures from the patients' histories. Section 3 provides discussion and recommendations. The Attachment presents additional information on the studies reviewed.

A.1 Studies Associating **Illness with Exposure to Polluted Fresh Waters**

It has been corr.mon knowledge for many decades that swimming in fecally polluted "ater can result in illness, and this association has been documented in the literature. The literature includes studies in which the causative agent of disease is not identified. studies in which a bacterial pathogen is identified as the causative agent, and studies in which an enteric viral pathogen is identified as the causative agent of disease. However, \-\hile these studies provide justification for standard setting, they do not provide data useful for establishing a quantitative association bet\\een individual microbial indicators and illnesses following recreational exposures to fresh \vater.

A.1.1 Studies in which the Causative Agent of Illness Is Not Identified

Stevenson (1953) performed a prospective study of the association of illness to S\\-imming at freshwater recreational areas in Lake Michigan, Chicago, Illinois, in the Ohio River at Dayton, Kentucky, and in the Long Island Sound in New York state. Each location had two study sites, one having distinctly worse water quality than the other, as indicated by total coliform density [most probable number (MPN)]. Stevenson (1953) did not identify the sources of increased total coliform levels (e.g., runoff, wastewater effluent). The rate of illness was higher among swimmers than nonswimmers, although Stevenson did not calculate statistical significance.

Stevenson (1953) found no correlation between the rate of total illness; gastroenteritis: or skin, nose and throat, eye, or ear symptoms with the mean total coliform MPN during the study period. For example, at the Chicago nonh beach, with a total coliform MPN of 91/I00 mL, the ratio of illness incidence of swimmers to nonswimmers, or the risk ratio (RR), was *2.2* to 2.6 for all illness, 2.2 to 2.5 for nose and throat illness, 1.7 to 2.5 for gastroenteritis, and 2.-t to 3.1 for other illnesses. At the south beach, with a total coliform MPN of 190/100 mL, the RR was 1.2 to 1.5 for all illness, 1.2 to 1.4 for nose and throat illness, 0.7 to 1.0 for gastroenteritis, and 2.0 to 2.6 for other illnesses.

Fe\\<lrell eta!. (1992) conducted a prospective study ofthe risk of illness from exposure to river water during \vhite-\\ater canoeing by recruiting canoeists and spectators (controls) at each of t\vo venues: one in a lowland river that received sewage effluent and one in a pristine upland river. The authors did not specify the level of sewage treatment. Fe\vtrell et al. (1992) determined concentrations of fecal coliforms, fecal streptococci, total staphylococci, and enteroviruses at each location during each canoeing event, and identified the prevalence of illness through subjective responses to a telephone survey five to seven days after the event.

Fewtrell et al. (1992) found that the lowland site had higher counts than the upland site of fecal coliforms (p<O.OOI, ratio of counts 12.5), fecal streptococci (p<0.05, ratio of counts 1.1). and enteroviruses (p<O.OOI, 198 pfu peri 0 L v. 0). The lowland site also had higher reported rates of gastroenteritis (p<O.OI, relative risk 1.9) and skin symptoms (p<0.05. relative risk 2.7). The authors concluded that ingestion exposure to microbes occurs during white-water canoeing, causing an '"appreciable burden of illness" in the canoeists. They also concluded that enteroviruses might be better indicator organisms than fecal coliforms, although the relative ratios of illness and microbes at the two sites do not appear to substantiate that conclusion.

Lee et al. (1997) also studied the risk of gastrointestinal illness associated with white-water canoeing in a prospective study of canoeists at a river that received considerable volumes of treated sewage, and, at times of heavy rainfall, untreated sewage from storm overflows. They recruited canoeists for the study at the venue, and had them report medical and dietary history, activities, and any gastrointestinal symptoms occurring during the seven days following exposure. Lee et al. (1997) obtained hourly water samples during each event, v.hich were analyzed for turbidity, £. *coli*, enterococci (fecal streptococci), clostridia, and F-specific bacteriophage.

The authors concluded that gastroenteritis v.as positively associated with ingestion of water (RR = 1.5 for sA-allowing water once and RR = 1.9 for swallowing water two or more times). Gastroenteritis was positively correlated (RR = 1.6) with I to 6 uses of the course during the previous year and negatively correlated (RR = 0.3) \\<ith 7 or more uses of the course. They found no statistical significance for any other parameter except the concentration of F-specitic bacteriophage [RR 2.6 \\<hen F-specific bacteriophage concentration was >25/100 mL, with RR calculated relative to illness rates when the concentration was I to 3/mL]. The authors concluded that the negative association with the number of uses ofthe course was likely associated with the following: higher skill level ofthe frequent users, resulting in less ingestion of water; less frequent use by people who are naturally more susceptible to gastroenteritis; and possible immunity acquired by exposure the previous year.

Fe\vtrell et al. (1994) conducted a prospective study ofthe risk of gastrointestinal illness and other symptoms for participants and spectators in canoe races and rowing regattas on freshwater canals (Oxford, Staffordshire, and Worcester canals) and estuarine waters (the Torridge River). The authors measured concentrations of fecal coliform, fecal streptococci. total staphylococci, *Pseudomonas aeruginosa. Salmonella* spp., *Cr_lptosporidium* spp., and enterovirus during each boating event, and identified the incidence of iIIness from subjective responses to a telephone survey five to seven days after the event and a mail survey one to four weeks after the event.

Fewtrell et al. (199-l) found that the relative risk of gastrointestinal symptoms was higher for boaters 'v\ho reported ingesting some water during the event than for participates that reported not ingesting water (RR=2.20, p<O.OI). The authors also reported that the results were unaffected by stratification controlling for water type (fresh or estuarine). However, although this study includes an analysis of estuarine waters, the focus is on secondary contact recreation (e.g., boating) rather than primary contact recreation.

A.1.2 Studies in which Bacterial Pathogens Were Identified as the Causative Agent of Illness

Rosenberg et al. (1976) showed a statistical association between gastroenteritis caused by *Shigella sonnei* and swimming in the Mississippi River. They identified shigellosis by positive culture from patients in 29 families who were infected during a single outbreak. The researchers conducted a telephone survey of these families and control families with no history of illness during the outbreak to elicit information about possible routes of exposure. A comparison of activities bet\\<een the shigellosis patients and the controls

[1] It is not ckar from thc analysis if the group that did not ingt:$t \lat r includes spectators on I or boat.:rs \\ho did not report ingesting wat.:r during the event.

showed that swimming in the river was associated with shigellosis (p<O.OOO I).

Rosenberg eta!. (1976) also surveyed families who had stayed at a campground adjacent to the river to identify cases of gastroenteritis that had occurred during the outbreak. Among *20* cases identitied, swimming was associated with gastroenteritis (p<O.OOO I), but there was no association between illness and any other camping activity, including eating and drinking water at the campground. Infection appeared to depend on ingestion of water: among people v. ho reported intensity of exposure ranging from below the v.aist only to head under water with no water ingestion, the attack rate was 1.5%, whereas among those who ingested water while sv.imming the attack rate was 18%.

Water samples collected from the river shortly after the outbreak had fecal colifonn counts of up to -W0,000 per I 00 mL (mean of about 17,500) at the campground swimming area, and up to 5,000,000 per 100 mLjust downstream of the sewage treatment plant, which discharged chlorinated wastewater to the river after either primary and secondary treatment, or only primary treatment. However, public health workers could not identify the sewage treatment plant as the sole source of bacterial contamination in the river.

Rosenberg eta!."s (1976) study was the first demonstration that shigellosis can be caused by exposure \'.hen swimming in polluted water. However, the evidence is not adequate to show a quantitative association between any microbial indicator and the risk of shigellosis.

A.1.3 Studies in which Enteric Viruses Identified as the Causative Agent of Illness

Koopman eta!. (1982) showed a rise in titer of antibodies to Norwalk virus in a retrospective study of an outbreak of gastroenteritis among visitors to a recreational park. However, they did not measure any other microbial indicators. The authors associated gastroenteritis with submerging the head while swimming (p<O.OOO I), implying that ingestion of\,ater was the route of infection. The authors did not describe the type of water body (e.g., lake. pond) in which visitors swam.

Hawley et al. (1973) isolated coxsackie 85 virus from campers and the sv.imming area during an outbreak of viral illness at a boys' summer camp in Vermont. However, the authors could not detennine whether exposure during swimming was responsible for transmission of the virus. In particular, the fraction of campers who became ill was higher among the index patient"s cabin mates than in any other group of campers. Therefore, Hawley et al. (1973) inferred that direct person-to-person transfer contributed to the attack rate in addition to infection by ingestion of contaminated water.

D'Alessio et al. (1981) conducted a retrospective study of children with enterovirus-like illness during an outbreak in Madison, Wisconsin, in which they found that enterovirus could be isolated from about half the ill children. The authors grouped the results into three categories: ill children with enterovirus isolates (119), ill children without enterovirus isolates (I07), and well controls (679). Slightly over half of each group had been swimming at a city lake or a private swimming pooL D'Alessio eta!. (1981) showed an association between illness with virus isolation and sv.imming through odds ratios (p<0.005), especially with beach swimming exclusively (p<0.0005). The odds ratios for illness with no virus isolation and swimming were between I and 2, and they could not identify swimming as associated with illness (p>0.05).

Bryan et al. (197-t) conducted a retrospective survey after a cluster of hepatitis A infections among a Boy Scout troop to trace the origin of the disease. The study involved 25 boys and 5 adult leaders that camped on an island in an inland lake, and subsequently, contracted hepatitis A infections. Each patrol of scouts had camped, and had also taken part in activities separately from one another. Attack rates were 52% for the boys and 20% for the adult leaders. Attack rates among the patrols ranged from 0% for 2 patrols to I00% for one patrol. The campers had the same drinking *v.*ater source as users of a recreation area on the nearby mainland who had not become ill. Seven of the eight campers who drank the contaminated water or swallowed ..large quantities··of lake water while sv.imming became ill, v.hereas, of the ten campers that stated they did not swallow any lake v.ater, only two became ill. Therefore, the researchers attributed the infections to inadvertently drinking untreated lake water that was intended only for fire control. They showed that the association of illness with consumption of lake water was statistically significant using Fishcher's Exact Test (p=0.007). However, Bryan et al. (1974) made no attempt to correlate illness with any presumptive indicator bacteria.

A.2 Studies Addressing Selection of Indicator Organisms for Fresh Waters

A number of epidemiological studies have been performed to try to identify which microbial indicator organisms serve as reliable indicators ofthe risk of illness associated with recreational exposures. A lack of host-specificity contributes to difficulties in this task. Many coliforms present in the human intestine are not exclusive to humans, or even to the intestinal tract of warm blooded animals-- when ·fecal coliforms· are isolated, they include many organisms that have been demonstrated to be environmental rather than enteric in origin (LeClerc et al., 200 I). Furthermore, fecal microorganisms found in the environment do not necessarily come from persons v.ho have been ill. Therefore, the practical utility of monitoring indicator microorganisms in water has been to identify the presence and level of fecal pathogens versus fecal pollution. Exhibit A-1 provides a summary of some of the properties that make microbial indicators more or less useful as indicators of human fecal pollution.

Exhibit A-1 Properties of the Most Prominent Potential Indicators of Fecal Pollution

Indicator	Useful Properties	Confounding Properties
Total coliforms	Many species inhabit animal intestinal tract. Easily identifiable by growth conditions, microscopy, and simple metabolic tests.	Not all species inhabit animal intestinal tract exclusively; *Klebsiella* and, *Enterobacter* spp. may be found free living on plants. soils (SWCHMS, 2004).

Fecal collforms (thermotolerant subgroup of total coliforms)	Many species inhabit animal intestinal tract. Easily identifiable by growth conditions, microscopy, and simple metabolic tests.	Not all species inhabit animal intestinal tract exclusively; *Klebsiella* and, *Enterobacter* spp. may be found free living on plants, soils (SWCHMS, 2004).
E. coli	Specific for animal intestinal tract. Identifiable by growth conditions and an array of metabolic tests.	Not specific for humans.
Fecal streptococci	Many species inhabit animal intestinal tract. Easily identifiable by growth conditions, microscopy, and simple metabolic tests.	Not specific for humans.
Enterococci	Many species inhabit animal intestinal tract. Easily identifiable by growth conditions, microscopy, and simple metabolic tests.	Not specific for humans.
F-specific bacteriophage	Present in sewage. Specific for *E. coli*. Cannot reproduce in water.	Specific to F+ *E. coli* (a physiological subgroup) rather than all *E. coli*.
Human enteric viruses	Present in sewage. Specific indicator of human contamination. Cannot reproduce in water.	May not be readily cultured.

The generally accepted properties of an indicator microorganism include the following [adapted from Cabelli (1976) and Dufour(l98 a)]:

Normal resident of the human intestinal tract
Present in water when human pathogens are present
Cannot grow or multiply in aquatic environments
More resistant to disinfectants and antibiotics than pathogens
Easy to isolate and to determine the concentration unambiguously
Applicable to all kinds of water
Does not die off faster than pathogens
Concentrations in water are proportional to the likelihood of illness upon exposure to the water.

It is also important that the turnaround time for analysis of water samples for the indicator allows authorities to make public health decisions in a timely manner.

The assumption that there is some relatively constant rate of gastrointestinal illness in human populations, and therefore, a fairly constant risk of illness per dose of indicator organism is not well supported. Natural variability in the composition of sewage, and local and seasonal environmental influences on microbial populations in effluents, can vary the levels and ratios of microbial indicators and pathogens in recreational water that is influenced by treated sewage discharges. Nonpoint environmental sources of micr9bial pollutants (e.g., from agricultural runoff, bird droppings, or disturbed sediments) also contribute to variations in the indicator and pathogen populations in water.

The following sections present the results of epidemiological studies that evaluate the utility of various fecal and nonfecal microbial indicators in predicting illness associated with recreational water exposures.

A.2.1 Studies Relating Bacterial Indicators with Illness in Fresh Waters

Several studies evaluate the correlation of microbial indicator levels\\ith the risk of illness from recreational exposures to contaminated water. Ferley eta!. (I 989) studied 5,737 campers at 8 summer camps in the Ardeche River basin, in France. They sampled the river water twice per week at five beaches, analyzing it for total coliforms, fecal coliforms, fecal streptococci, *Pseudomonas aeruginosa,* and *Aeromonas* spp. The authors interviewed campers about their swimming activities and illnesses during the three previous days, and determined rates of: acute gastrointestinal disease; ..objective" acute gastrointestinal disease (involved vomiting and/or diarrhea): ear, nose, or throat disease; skin infections; eye disease; and pulmonary disease, for a total of 9,0I I person-days of camping\\ ithout swimming and I 8,9-lS person-days of camping with swimming. Rate ratios for swimmers compared to nonswimmers ranged from I.I for pulmonary disease through 2.3 for acute gastrointestinal disease, to 3.7 for skin infections.

Correlation coefficients \ ere highest between all cases and fecal coliforms (r = 0.51), acute gastrointestinal disease and fecal streptococci (**r** = 0.55), ..objective" acute gastrointestinal disease and fecal streptococci (r = 0.62), and skin disease and fecal coliforms (r = 0.67). Ferley eta!. (I 989) graphed the rates of disease as a function of bacterial concentration for s\\ immers , and plotted the incidence rate for all nonswimmers as a straight l ine because it does not vary with bacteria level s in the water. They assumed that the point at which the 95'h percentile lower confidence limit for swimmers intersected the 95'h percentile upper confidence limit for nonsv..-immers was the bacterial concentration that caused a significantly increased rate of illness. Significant concentrations included: 7 fecal streptococci/I 00 mL and 270 fecal coliforms/1 00 mL for total gastrointestinal disease; *20* fecal streptococci/1 00 mL and 800 fecal coliforms/I 00 mL for ..objective" acute gastrointestinal disease; and I 20 fecal coliforms/1 00 mL for skin infections.

In I 980, Seyfried et a!. (I 985) conducted a prospective study of I 0 Ontario beaches, showing an increased rate of illness among swimmers compared to nonswimmers (RR = 2.4 for all illness, 2.4 for respiratory, 3.9 for gastrointestinal, *2.2* for eye, ear and skin, 2.5 for allergies, and I .8 for other illness). The authors found that fewer than 25% of the illnesses were gastrointestinal. and the rate of respiratory illnesses was higher than for gastroenteritis. The authors reported that the rate of eye, ear, and skin infections was higher among swimmers\\ ho immersed their heads; however, respiratory and gastrointestinal symptoms were lower among swimmers who had immersed their heads. Seyfried et al. (1985) calculated odds ratios for total illnesses showing a dose-response relationship when dose was expressed as counts of fecal coliforms, fecal streptococci, or total staphylococci. The correlation oftotal illness with fecal coliforms (p<O.OOI) and total staphylococci (p<O.OO I) was better than with fecal streptococci (p = 0.0I6) or *Pseudomonas aeruginosa* in sediment (p = 0.36).

Dufour (1984a), using essentially the sam!! survey methods as St!yfrit!d et al. (1985), studied paired beaches at Keystone Lake, near Tulsa, Oklahoma, and at Presque Isle State Park, Erie, Pennsylvania. The author chose one beach at each location because of its proximity to sewage treatment outfalls and the other because it was expected to have lower levels of po!J ution. At Kt!ystone Lake in 1979 and 1980, Dufour (1984a) studied 8,180 swimmers and I ,325 nonswimmers at a beach about 3 miles from a sewage treatment discharge, and 6,002 swimmers and 856 nonswimmers at a beach about 5 miles from the discharge. In 1979, the sewage treatment plant discharged unchlorinated wastewater to tht! lake; in 1980, it disinfected half the wastewater prior to discharge. At Presque Isle, a pt!ninsula in Lake Erie, the author studied 8,857 swimmers and 4,247 nons immers at a beach less than I mile from a treated sewage outfall on the mainland side ofthe peninsula from 1980 through 1982, and 5,927 swimmers and 1,746 nonsv.immers in 1979 and 1980 at a beach on the shore across the peninsula from the discharge. The astewater was treated with activated sludge and chlorinated prior to being discharged into the lake. Sv.immers, detined as having immersed all upper body orifices in the water, reported symptoms during telephone interviews 8 to 10 days after sw1mmmg.

The author found that swimmers had significantly higher ($p < 0.05$) symptom rates than nonswimmers. especially for gastrointestinal symptoms, and that symptom rates were proportional to the logarithm of the bacterial densities for *E. coli* and enterococci. The author found that there was no relationship between symptom rates and the concentrations of fecal coli forms. Three measures of significance (slope of the response curve, standard error of the estimate, and correlation coefficient) v.ere very similar for *E. coli* and enterococci, leading the author to conclude that both could be used equally effectively as an indicator of potential illness. The author points out that the pattern of symptom rates for marine exposures is sufficiently different from the pattern in fresh water exposures. and that the same criteria cannot be used for both settings.

Dufour (1984a) presents graphs of bacterial densities as a function of the illness rate for highly credible gastroenteritis. For *E. coli*, the graphs show that rates of illness begin to increase when densities increase above approximately 20/100 mL. HO\\ever, these graphs are intended to guide the selection of numerical criteria, and the author pointed out that a local or state authority must detine acceptable risk.

Medema et al. (1995) conducted a prospective study comparing the frequencies of health complaints after a triathlon (sv..im-bike-run) and a control group in a run-bike-run event. Triathletes, 75% of *v.*hom ingested water during their *sv.*im, reported higher rates of illnt!ss during the week after tht! event as follows (95% confidence limits given in parentheses): gastroenteric, RR = 3.1 (0.75- 12.95); respiratory RR = 1.5 (0.44 – 4.9); skin/mucosal RR = 2.1 (0.26- 16.4); general RR = 2.9 (0.4- 21.9); and total RR = 2.0 (0.9- 4.5). The author measured concentrations ofthermotolerant coliforms (equivalent to fecal coli forms), *E. coli*, fecal streptococci, six other species of enteric bacteria, t\\o viruses, and F-spt!cific bacteriophage during the event. The geometric mean concentrations at which the authors measured the illness rates were 170/100 mL for *E.*

coli and 131100 mL for fecal streptococci. However, the study was intended to be a test of the study design rather than a comprehensive epidemiological study, and thus there was only one time point.

Van Asperen et al. (1998) extended the above study over two summers, studying 827 triathletes in 7 events and 773 participants in 15 run-bike-run events. The authors measured water quality all along the course during each triathlon, analyzing for thermotolerant coliforrns, *E. coli*, fecal streptococci, enteroviruses, and F-specitic bacteriophage. They collected data about illness during the 7 days folio" ing each event from questionnaires. The adjusted odds ratios for gastroenteritis in triathletes from the Netherlands, the United Kingdom, and the United States ranged from 1.6 to 2.3. The geometric mean concentrations of potential indicators during the triathlons (range in parentheses) ""ere: therrnotolerant coliforms, 781100 mL (0.6-650/100 mL); *E. coli*, 20-l/100 mL (II-2,600/100 mL); fecal streptococci, 16/100 mL (0.2-1,8001100 mL); enteroviruses, 0.04/L (0.007-17/L); and F-specific bacteriophage, 0.7/L (0.0I – 13.6/L).

The concentrations of therrnotolerant coliforms measured by van Asperen et al. (1998) \\ere below the European Union (EU) imperative level (95th percentile 2,000/100 mL); this concentration was clearly not protective. However, EU guidance levels of S I 00 thermotolerant coliforms/I 00 mL and S I 00 fecal streptococci/!00 mL appear to be protective. The authors concluded that attack rates for gastroenteritis \.\ere significantly increased at concentrations of 2: 220/100 mL thermotolerant coliforms and 2: 355/100 mL *E. coli*. They noted that the recovery of *E. coli* was higher by the method they used (growth at 4-lC after a 4-hour recovery period at 37C on tryptone bile agar overlaid with a thin layer oftryptone soy agar) than the recovery ofthermotolerant coliforms (on sodium Iaury! sulphate agar). of which *E. coli* is a member (note the ranges of concentrations given). They also stated that concentrations ofthermotolerant coliforms did not correlate well with illness rates, whereas concentrations of *E. coli* \.\ere much more closely correlated.

Calderon et al. (1991) and Colford et al. {2005) evaluated correlation of indicators with the risk of illness at closed or nearly closed water bodies that do not receive point source human pollution. Calderon et al. (1991) studied a pond that received runoff waste from animals but not from humans. The study population consisted of 10-l families ""ho had used the pond; swimming "as defined as submerging the entire head and body. The authors collected subjective responses to a questionnaire by mail; there \\as no medical confirmation of the nature and severity of illness. They observed an association between swimming and gastroenteritis (p<O.OOI). However, the associations of gastroenteritis with fecal coliforms, *E. coli*, and enterococci were not good (p = 0.16, p = 0.41, and p = 0.059, respectively). The best correlation was \.\ith the number of swimmers (p<O.OI), kading the authors to conclude that pathogens shed by the swimmers were the major cause of gastroenteritis illness when water was not polluted by human sewage.

Colford et al. (2005) identilicd recreationally exposed participants through a screening form administered by an interviewer at the Mission Bay, California, beach, and conducted a follow-up telephone intervie""; they did not medically verity the participants' subjective assessments of health outcomes. The authors observed statistically significant increases in illness rates for diarrhea and skin rash for any water contact, water on the face, or swallowing water. Cramps and eye irritation were associated with swallowing water but not with water on the face or any water contact. Results broken down by age showed a statistically significant increase in diarrhea predominantly in the >5 to 12 year age group. The authors concluded that no traditional fecal indicator organism was a good predictor of illness because the indicator organisms were predominantly from nonhuman (avian) sources, shown through the use ofribotyping and host-specific PCR (host origin could not be determined for only 10% of all the samples).[3]

Calderon et al. (1991) and Colford et at. (2005) showed that swimming in fresh and marine water, respectively, that is not polluted by human feces causes an increased incidence of illnes. but the increase is not related to any enteric indicator organism. Instead, Calderon et al. (199t) concluded that the source of infection in the fresh\.\ater pond was pathogens shed by the *sv.* immers themsetves. For other such waters, bacterial indicators may not to be useful predictors of risk of illness.

A.2.2 Reviews of Existing Studies

Pruss (1998) reviewed data from 22 epidemiological studies in recreational marine and fresh waters. and observed an overall causal relationship bet\'.een gastrointestinal symptoms and \\ater quality as measured by indicator bacteria concentrations. PrUss evaluated seven fresh water studies (Stevenson, 1953; Cabelli, 1982; Dufour, 198-la; Seyfried et at., 1985; Lightfoot, 1989; Ferky et at., 1989; Fewtrell et at., t992), and distinguished two types of studies: (I) studies comparing incidence rates for persons swimming in unpolluted water compared with nonswimmers; and (2) studies comparing incidence rates for persons swimming in polluted water and incidence rates of swimmers in unpolluted water. For gastrointestinal symptoms, the first category of studies reported relative risks between 1.0 and 2.5 for swimmers, with only I relative risk value being significantly different from 1.0. For the second categOl)' of studies, the relative risk ranged between 0.4 and 3.

With respect to indicator microorganisms that correlate "'ith these health outcomes. Pruss (t998) observed that enterococ;::i and fecal streptococci correlate welt with health outcomes reported in both marine and fresh water studies, and *E. coli* correlates well with health outcomes in fresh water studies. Increased risk of gastrointestinal i!Iness was associated with water quality ranging from "'only a few indicator counts/100 mL" to 30 indicator counts/100 mL, values that are low relative to water quality often observed in coastal recreational areas. Other microbial indicators for which correlations with health outcomes were observed included fecal coliforms and staphylococci, the latter being correlated to bather density and associated with ear, skin, respiratory, and enteric illness.

[1] When: bird densiti.:s ' en:: high. it is likd that skin rashes \\ere due to an alkrgic reaction from contact \\ith cercaria. the lanai forms of a'ian parasitic nat,,orms (sch•stosom.;s) (P.:rsonal communication "ith B. Olson. Uni\.:rsit) of California-Irvine. July 2005).

PrUss (1998) observed that the selection of indicator organisms as a measure of water quality is a main source of bias in the studies, that limited precision and seasonal versus daily measurements of the indicators causes increased inaccuracy , and that the indicator organisms used did not correlate well to the presence of viruses, v.hich may represent an important fraction of the etiological agents in the water.

Wade et al. (2003) further examined the relationship between specific recreational water quality indicators and gastrointestinal illness. They analyzed data from 17 marine studies and I0 fresh ""ater studies (Stevenson, 1953; Dufour, 198-lb; Seyfried et al., 1985; Lightfoot, 1989; Ferley et al., 1989; Calderon et al., 1991 ; Fewtrell et al., 1992; Medema et al., 1995; Lee et al., 1997; van Asperen et al., 1998) that had quantified the relationship between at least one v.ater quality indicator and gastrointestinal or other illness (excluding typhoid or polio) reported under endemic (not outbreak) conditions. The reviewers conducted separate analyses for each combination of water quality indicator, health outcome, and water type (fresh and marine).

For each study, they chose the median value of the reported indicator range as the exposure value, and v.here multiple health outcomes had been reported in a single study, selected the results associated with the highest exposure measure within each exposure category (Wade et aL 2003). The authors conducted a v.eighted regression for fecal bacterial indicators, modeling the indicator level (log base I0) as a continuous predictor of the natural log of the relative risk, and weighted the models by the inverse of the standard error of the natural log of the relative risk to account for study size. The model parameters are shown in **Exhibit** A-2. Wade et al. (2003) evaluated variability among the studies using a random effects meta-regression model (Thompson and Sharp (I 999), as cited in Wade et al. (2003)].

Exhibit A-2. Wade et al. (2003) Estimated Parameters for Fresh Water Regressions[1]

Indicatorl	Number of Effect Estimates	Coefficient	p-value	r
Fecal Coliform	11	0.0058	0.98	0 0083
Enterococci	8	0.0078	0.97	0016
E. coli	5	0.75	0 063	0.86

1. Represents model parameters from we1ghted linear regress1ons of the natural log relative nsks as a function of Indicator dens1ty (log base 10).
2. The authors had too few data po1nts to conduct the regress1on analysis for total cohforms, viral indicators (e.g., enterovirus. culturable enteric viruses, or bacteriophage) or nonfecal water quali:y indicators (e.g., *Staphylococcus* spp or *Pseudomonas* spp)

Wade et al. (2003) note that, based on the epidemiological studies reviewed, no single indicator could consistently predict illness in all environments at all times. Hov.ever, the

Ilan\OOd et al. (2005} came to a similar conclusion for \\aste,,at.:r n:clamation facilities. finding no strong correlation for an indicator-pathogen combination. and r.:commcnding that a suite of indicators may provide a bctta pn:diction ofth.:se pathogens in treated \\astC \\ater.

reviewers did conclude that *E. coli* was a more consistent predictor of gastrointestinal illness in fresh water than enterococci or other bacterial indicators, and that a log (base 10) unit increase in *E. coli* in fresh waters was associated with a 2.12 (0.925 -4.85) increase in the relative risk of endemic gastrointestinal illness. The researchers also found *E. coli* exposures below EPA (1986)-recommended levels for fresh water not to present a significant risk, while exposures above those levels were associated with an elevated, statistically significant risk of gastrointestinal illness.

A.3. Discussion

Epidemiological studies that have attempted to correlate fecal pollution and microbial indicator levels in fresh water with risk of illness from recreational exposure are limited in number. Also, some of the historical studies that laid the groundwork for current recreational water standards predate widespread implementation of secondary treatment, disinfection, and advanced treatment technologies by sewage treatment plants. When the early epidemiological studies reviewed by EPA were conducted, the efficacy of sanitary wastewater treatment was less than is required and commonly practiced today.

In these earlier studies, the main source of fecal pollution in recreational waters was most likely raw or poorly treated sewage discharges. More recently, fecal pollution is often attributed to a variety of other sources, including urban runoff (including runoff that carries pathogens from the feces of wild and domesticated animals) and bather inputs (swimmers· soiled bodies or fecal accidents). In addition, microorganisms entrained in sediments can be redistributed in the water column when those sediments are disturbed. However, in many areas, sanitary sewer overflows and combined sewer overflows still result in sewage inputs to recreational waters during wet weather. Thus, the potential for recreational waters to receive fecal pathogens originating from a variety of sources remains.

A.3.1 Summary of Research

Researchers conducting both retrospective and prospective epidemiological studies have sought to identify microbial indicators that are easy to isolate and enumerate, reliably predict the risk of illness, and support the setting of water quality standards. Studies specifically evaluating the link between levels of *E. coli* or enterococci and the incidence of illness resulting from exposure to fresh waters are summarized in Exhibit A-3.

Exhibit A-3 Summary of Select Epidemiological Study Results for Fresh Waters

Study	Indicators Evaluated	Results
Epidemiological Studies		
Colford et al. (2005)	Measured MPN of enterococci, fecal coliforms, and total coliforms; enterococci and bacteroides by PCR; enterococcus by enzyme activity; human enteroviruses; and bacteriophage	Showed no association between indicator densities and illness, even if water quality thresholds were exceeded.

Van Asperen et al. (1998)	Measured thermotolerant coliforms, *E. coli*, FS, and F-specific RNA bacteriophages.	Attack rates increased at levels above 220/100 ml for thermotolerant coliforms and 355/100 ml for *E. coli*. No exposure-response relationship for FS. enteroviruses, and bacteriophages.
Lee et al. (1997)	Measured *E. coli*, enterococci. suphite reducing clostridia, F-specific bactenophages, and enterovtruses	Showed a correlation with illness and *E. coli* and enterococci, however, not as strong as bacteriophage relationship.
Medema et al. (1995)	Measured thermotolerant cohforms, *E. coli*, FS, *Aeromonas* spp., salmonella, *Pseudomonas aeruginosa*, shigella, Campylobacter, staphylococci, enteroviruses, and reoviruses	Health risk for all symptoms was higher for "swimmers·. but was not statistically significant. Geometnc means during the study period for thermotolerant coliforms, *E. coli*, and FS were 110l100 ml, 170/100 ml, and 13/100 ml, respectively.
Calderon et al. (1991)	Measured *E. coli*, enterococCI, FC, Pseudomonas aerugt nosa, and staphylOCOCCI	Showed no assoctation between htgh fecal indicator bactena (e.g., FC, *E. coli*, and enterococci) and GI illness
Ferley et al (1989)	Measured TC, FC, enterococci, *Pseudomonas aeruginosa*, and *Aeromonas* spp.	Enterococci showed a much better relationship wtth AGIO than both TC and FC. Rtsk to swimmers exceeds risk to nonswimmers for AGIO when enterococci exceed 71100 ml, and 20/100 ml for "objective" AGIO.
Dufour (1984a)	Measured FC, *E coli*, and enterococci	FC densities are unrelated to GI. *E. coli* densities show an excellent relationship to GI. Enterococci results are very similar to those for *E. coli*.
Review of Epidemiological Studies		
Wade et al. (2003)	Evaluated 10 freshwater studies that measured indicator organisms, including *E. coli* and enterococci.	Found that *E. coli* was a more consistent predictor of illness than enterocoCCI or other bactenal indicators in fresh waters.
Pruss (1998)	Evaluated 7 freshwater studies that measured indicator organisms. including *E. coli* and enterococci.	Both *E. coli* and enterococci correlate wtth increased illness nsks in swimmers in fresh waters.

Acronyms·
AGI!J = acute gastrotntestinal disease
FC =fecalcoltforms
FS = fecal streptococci
GI=gastromtestinal
TC =totalcoliform

There is considerable heterogeneity in the results of these studies, even within similar studies. For example, van Asperen et al. (1998) found that attack rates for gastroenteritis increased when *E. coli* densities were above 335/100 mL, and Medema et al. (1995) found no statistically significant increase in illness rates at mean *E. coli* densities of

170/100 mL for triathletes with a median age of 33 years, whereas results of studies by Dufour (1984a) showed increasing rates of illness as densities increased above 20/100 mL for swimmers of all ages.

The correlation of indicator densities with illness rates was also variable. Dufour (1984a; 1984b) found that enterococci and *E. coli* were better correlated with illness than fecal coliforms, while Lightfoot (1989; as cited in Pruss (1998) and Wade et al. (2003)] and Ferley et al. (1989) reported that the relationship between indicator levels and illness are similar for fecal coliforms and enterococci and/or *E. coli*. Lee et al. (1997) found that F-specific bacteriophage densities correlated better with illness rates than did either *E. coli* or enterococci.

In their studies of closed water bodies with no known source of human fecal pollution, Calderon et al. (1991) and Colford (2005) found that illness was not correlated with the level of fecal coliforms, *E. coli*, or enterococci; rather there was a significant association between swimmer and staphylococci densities and illness. These results suggest that it may not be reasonable to enforce a fecal pollution indicator limit if there is no demonstrable source of human fecal pollution.

In some cases, microbial indicators that are correlated with illness may represent an association with a specific subpopulation of pathogens rather than with overall levels of fecal contamination. For example, Lee et al. (1997) reported on risk from exposure to river water in a white water canoe course. They found that F-specific coliphage were a better indicator of the risk of illness than *E. coli*, enterococci, clostridia, or enteroviruses.

Of the two reviews of the literature, Pruss (1998) found that, under many conditions, both *E. coli* and enterococci appear to be good microbial indicators of the risk of gastrointestinal illness in fresh water, whereas Wade et al. (2003) concluded that *E. coli* is a more consistent and reliable freshwater indicator than enterococci. Wade et al. (2003) stated that no single indicator will always predict illness accurately because of quantitative and qualitative variability in the pathogen population relative to total fecal indicators. variability in the sensitivity of exposed populations. and variability in the amount of exposure. However, they concluded that *E. coli* is superior to enterococci as an indicator of illness risk and that published results support EPA's current criterion (126 cfu/100 mL).

In their analysis of the published data, Wade et al. (2003) weighted those study results having small standard errors more heavily than results having high standard errors. They found that weighted risks correlated better with *E. coli* densities (r = 0.86) than with enterococci densities (r = 0.016), indicating a higher confidence in predictions of risk using *E. coli* than when using enterococci. Also, the slope of the regression line was greater for *E. coli* (0.75) than for enterococci (0.0078), indicating greater sensitivity of illness risk to *E. coli* densities. In addition, the relative risk for all exposures when enterococci densities were below the current criterion of 33 cfu/100 mL was higher rather than lower when densities were above the criterion (1.94 and 1.61, respectively). In contrast, the relative risk for all exposures when *E. coli* densities were below the current criterion of 126 cfu/100 mL was lower than the relative risk when densities \\ere above the criterion (1.20 and 1.81, respectively). Therefore, the authors concluded that *E. coli* is a sensitive and reliable indicator organism for fresh recreational \'tater.

Existing studies do not contain sufficient information to pro\ ide guidance on appropriate indicators for estuarine \-\aters that serve as boundaries bet\\een fresh and marine waters. Neither EPA's 1986 criteria recommendations not its draft bacteria implementation guidance (U.S. EPA, 2002) provide sufficient information for determining appropriate criteria for estuarine \-\aters. In addition, the State Water Board staff identified only one study for estuarine \\ aters (Fewtrell et al., 199-t), and the results are not sufficient to rt!commend an indicator organism nor provide a dose-response relationship bet\\een illness rates and levels of a given indicator.

A.3.2 Recommendation

Given EPA's recommended criteria for fresh waters and the literature to date, Wade et al. {2003) provides the most convincing evidence in the literature for the selection of *E. coli* over enterococci as the more appropriate indicator of health risk associated with recreational use of Califomia fresh waters.

However, future epidemiological research may identify more appropriate bacterial indicators (or suites of indicators) to protect recreational use of fresh waters, either as a \\hole or site-specifically. For example, the EPA and the Centers for Disease Control and Prevention's National Epidemiological and Environmental Assessment of Recreational (t\EEAR) Water Study, designed to evaluate rapid methods for evaluating \\ater quality at beaches concurrently with a health study, has shown that enterococcus measured using the quantitative polymerase chain reaction (QPCR) method are correlated with illness (Wade et al. 2006). The study may also provide new information for establishing new bacteria criteria for rapid analytical methods (e.g., QPCR) to assist in beach closures decisions.

Researchers \\Orking on future epidemiological studies should also make sure to control for the presence of C)-anotoxins. Some of the illness symptoms reported by participants in the epidemiological studies such as gastrointestinal illness and dermatitis, can result from exposure to blue-green algae toxins, also known as cyanotoxins. Outbreaks are usually associated \-\ith blooms. However, because cyanobacteria are not human gastric residents, indicator bacteria such as *E. coli* and enterococci are not likely to be predictive of their presence. Nevertheless, epidemiological studies should note, \\ here appropriate, the presence of such algae blooms.

Microbial source tracking studies are also adding to the body of knowledge that local agencies can use to interpret microbial indicator results in a specific watershed, although additional study is required to ensure that these methods are reliable. Augmenting traditional study methods with molecular genetic techniques to identify specific sources of pathogens and bacterial indicators may help clarify local and seasonal associations between water pollution and disease, and either confirm or rule out assumptions regarding the presence of microbial indicators and the risk of illness from recreational v.ater exposures. Further development of methods to identify sources of human pathogens v.ill help maximize recreational use of fresh water v.hile protecting public health.

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Attachment. Summary of Epidemiological Studies

Summary of Ep1dem10og1ca l Stud1es of Baelera l nd1cators

Reference	location	Method			Findings
		Water	Human Health	Risk Assessment	
Freshwater Studies EPA Used in 1986 Bacteria Criteria Recommendation (U.S. EPA, 1986)					
Ouiour (19B4a)	Keystone Lake. OK, Lake Ene. PA	• Measureo FC. E coil. and enterococo • Collected sample from beaches at intervals necessary to observe fluctuauons	• Measured Gl, resp1ra1ory DGI, HCGI, and otner (e g fever) symptoms • Conducled mmal beach 1nterv1ews 1n person and follow up phone 1nterv1ews 8-t 0 days later • Collected data on age sex. race. water actiVltieS, 1llness symptoms reasons for nol sv mmmg	• Manle-Haensel Ch1 Square test used to detemune nonrandom processes account for d1Herence 1n 1llness between SWimme!l; and nonsWimmers • Used regress1on analys1s to determ1ne 1f a d1rect rela11onsh1p between vanables ex1sts • Used corre1a11on coerf1C1ent. regress1on coeff1C1ent. and slanaara error of lhe est1mate to charac1enze strength of assoc1at1ons	• FC dens1t1es 1n fresh wa1er are unrelaied to Gl • E co/1 dens11ieS show an excellent relat1onsh1p to Gl • Slopes for symptom categones generated for enterOCOCCl are very S1m1ar 10 those for E cob

Stevenson (1953)	Lake M1ch1gan. Oh10 R1ver. KY L I. Sound. NY	• Measurec TC With MPN method • Collected sample from beaches at Intervals necessary to ooserve fluctuat1ons	• Measured *eye*, ear, nose. and throat a1lmen1s .gas1ro1ntesunal d1sturoances .and sk1n 1rntauons • Gave partiCipants calendar record forms to record SW1mm1ng and Illness expenence da1ly.follow up VISitS made 2-3 umes mnough SW1mm1ng season. final v1s1t to ensure complet1on and PlCk up of forms • Total of 22.164 from 3 areas part1c1pated (5 124 from Lake M1ch1gan. 7 520 fnom Oh10 R1ver. 9 520 from Longsland Sound) • Fam1lies from s1m1lar soc10econom1c levels and general geograpniC areas	• Used statiStiCal analys1s methods to determ1ne effect of water quality and SW1mm1ng expenence on prevalence and 1nc1dence of 1llness • Analyze 1llness over enure penod and days 1mmed1ately folloWing exposure	• In almost every mstance 1llness rate was h1gner 1n SWimmers than nonsWimmers • Lake M1ch1gan SWimm•ng 1n water with TC levels > 2,300/100 mL may cause more 1llness • Oh10 Rwer No S1gn1f1cant difference be en 1nc1dence of 1llness on days with greatest" and •teast" polluuon • L I Sound No s1gmficant d1fference 1n 1llness rates among sw1mmers 1n water of different warer quality
Freshwater Studies EPA Used in 2002 Reevaluation of the 1986 Bacteria Criteria (U.S.EPA, 2002)					
Fe eyet a1 (1969)	Ardeche RIVer. France	• Measured TC, FC. FS two pathogens • Samples taken IWICe a week at 5 oeacnes- 5 samples per :,eacn at a depth of 30 em • MF proceoure for TC and FC pour plate recnmque for FS Pnl-Xylose Amptcilltn agar a d ce1nm1de ana nahdxtc ac1d agar for two pathogens	• Caregones oflllness AGIO. skm and mucous membrane.ear nose. and throat eye and pulmonary • Interviewed 5 737 tounsts about previOus 7-day -.s1Vacllv111es (retrospective survey) • Collected data on water acuv1t1es meals ::onsumed annk.ng water consumptiOn age ana sex	• Only 1nterv1ewed each fam1ly once and only those 1nd1vlduals... mo swam at the sample beaches only (no where else) • Max1mum latency for the 1llness categones assumed to oe 3 days • Used we1ghted 1near regressiOn to relate gasuomtestmal mortlldlty tnctaence rates to dtfferenl of exposure	• Fecal streptOCOCCI show a much better retat1onsh1p With AGIO man both TC and FC • FC bestmd1cate general mortJid1ty and sk.n d1sease nsks • Level of FS thattne nsk to SWimmers exceeds nsk to nonSWimmers S 7/100 ml for AGIO and 20/100 mL for'ODJeCtlve AGIO levels • Greater sk1n d1sease nsk to SWimmers wnen FC levels> t20/100 ml

Seylned et *al* (1S85)	Ontano Canada	• Measureo FC.FS. coagulase-pos1hve and -negat1ve staphylocoCCI , Pseudomonas aerugmosa ana heterotrophiC bactena • Water and sed1ment samples taken at least tWice per day at depths of 50 em 1n locauons w•th h1gh SWimmer dens•ty	• Symploms spec1f•ed were sore throat, coiO, cough, runny nose. earache runny ears, eye IrTilatiOn. stomach acne, nausea. d1arrnea , vom11ing, boi S, skm rash. allergy , sunburn.and others • 6.166 people mterv1ewed (3.967 SWimmers and 2.105 nonsWimmers). lnlhal•nterv,ew 1n person follow up phone 1nterv ew 7t0 days later • Collected data on age.sex 1llness h1story ,SW1mm1ng aclivilleS , and tllness symptoms	• Stat•st1ca1 mooels developed to pred1ct probab1111y of SWimmers gelling 111 and determme how vanables (e g , water quality) affect pmbablhty • Used log1slic rejjress•on models to test speofiC organ1sm agamst e1ther total mortlldlty rate 1n SWimmers from all causes.or the rate for a speofiC symptom	• Morol(l1ty rates hKJher among sWimmers • Total staphylocoCCI. FC. and FS correlated best Wllh SWlffiffilng-aSSOC'ated mortJidlty • Total staphylocoCCI correlated With total Jllness and eye and sk1n d1sease • FC and FS also correlated With total 1llness but dose-response relatiOnShip not as strong as that for total staphylOCOCCI
Addtional Freshwater Epidemiological Studies					
N!::AR (2005)	Lke Mtcr.•gan . Lake Er:e	• ""ater samples collected at 2 depths. 3 lime per day • Samoles tested for enterccOCCI *ana* oac1er010es usmg QPCR	• tntervtewed 5.717 pai'11Cipants aoout SWlmm,ng and other aclivlt•es (resoonse rate of aoout 56%) • Contacted 10·12 days afier beach mterv1ew to ascertain health symptoms	• Evaluatea mean log,. of md1cators ano nsk of 1ilness • Calculated •ndw•dual aa11y averages for eaCh sne and average for all Sites together • Allcovanates m•ually 1ncluded 1n mcdel. then removed unulthose rema•n•ng resulted 10>5' change 1n the exposure to •llness ret at•onsn•p	• Log, 1ncrease 1n enterococcus was assoc•ated With a 1 37 1ncrease 1n the ados of Gl 1llness • Bacteroides were pos1t1vely assoc1ated W1tn •IIness at ake Ene, out trenos were borderl•ne stalist•cally s'gmficant and no assoc1at1on With 1llness at Lake M•ch•oan

Waae et al (2003)	Comp1la1ton of studoes	• Conducted an extens1ve literature search ana oden11fied 55 as relevant • 27 of the 55 fit selectiOn cntena for revoew • Evaluated 17 manne and 10 freshwater stucoes • Stud1es were tradotlonal prospectrve. prospec11ve dunng recreattonal events, randomtzed controlled tnals. and cross-sec11onal sludtes	• Separate analyses for each comb1natoon of 1no,cator heallh outcome . and water type • Calculated RRs as wetghted average u>mg random effects model • Conducted wetghted regresston to evaluate relallonsntp between tndocators and effects • Evaluated vanatll tty us1ng random-effects metaregresston model	• .AnaySIS supports use of enterOCOCCI on manne waters at EPA's 1986 levels • In fresh water found that *E coli* was a more conststent predoctor of GI tllness than enteroCOCCI or other bactenattndtcators
Pruss (19581	Comptlatlcn of StUdieS	• Identified 37 stuoll!s. ano revtew the 22 that a oressed assoc oattons of tnterest and fulfilled valtdoty cntena • Evaluated 7 freshwater ana 15 manne studoes	• Revtewed the results of eacn study • OostIngUished two types of studtes studtes comparmg tncooence rates for persons SWImmmg With nons"Mmmers and studtes companng modence rates for persons S'Mmmtng 1n polluted water and S'Mmmers 1n unpolluted water	• EnterOCOCCI correlates WIth illness for manne ana fresh waters • *E* coli correlates v.1th tllness for fresh waters • Observed that selecttonof onatcator orgamsms os a maon source of etas 1n studtes hmoted precos1on and seasonal versus datly measurements tncrease maccuracy.and *me* on01cators dtd not correlate well to the oresence of wuses

van Asperen et at (1598)	NetnManos	• Measured thermotolerant colhforms *E* colt. FS ana F-spec1f1c RNA bactenophages • Samples collected on exposure day du11ng recreat1on act1vl!y at depths rangmg from 0 to 30 em	• Measured gastroenten11s symptoms •nclud1ng nausea vom,ung . stomacn ache . d1arrhea and fever • Results based on postalsurvey of 827 tflathletes (sw1mmers) and 773 run-b1ke-runners (nonswtmmers) • Collected data on age . sex . current health,years pante1patmg 1n endurance spans .recent water actiVIties. and type of water gear worn 1n competitions	• Us d Manti-Haenszeltest for hnear trend to test s1gmficance of assoc•auons • Crude and aDjuSted odds rauos and 95"to confidence Intervals calcu'ate:j usmg log1st1c regress1on analySS to relate 11sk of gas!roentefltls symptoms and exposure • InteractiOn terms 1ncluded 1n models to :letermme 1f other vanables effectmg assooahons	• In wee• follbwmg exposure gastroenteritiS a!lack rate was htgher for tnathletes than run-b1ke-runners • Thresholdevels beyond whiCh mcreased a!laek rates were observed for thermotole rant cohforms was 220/100 ml and 355/100 ml forE *colr* • Exposure-response relationshiP not ooservea With FS enterovtruses. and bactenophages
Lee et at (1997)	No!!Ingnam . England	• Measured *E co!r* . enterococc., suphtte reducmg clostfldla. F-speof•c actencphages .and enterovtruses • Water samples collected at nourly Intervals du11ng sesstons	• Surveyeo 473 canoe•sts and rafters panakmg 1n events held at the Nat1onal Water Spons Centre • At least one study per month from March to November • Pan1c1pants surveyed on use of course medteal hiStory . food consumption, and Illness symptoms (resp11atory tract. Gl tract. ear and eye. sk1n and aeneral svmo:oms	• Geometnc means of mlcrOOIOioglca l data calculated fer each day • Log1sttC regressiOn analyses used With Gl1llness as dependent vanable	• Rate of Gl1lness correlated best With t:actenophages • Correlat1on v.th .!!ness ana *E coil* and enterOCOCCI however, not as strong as bactenophage relatiOnShip

AppendiX A

Medema et al (1995)	Netherlands	• Measured thermotolerant cohtorms, *E coli* FS. Aeromonas Pseudomonas aerugmosa . Campylobacter, salmonella, staphylococo. sh1gella. enteroVIfuses .and reov ruses • Samples taken at ume of tnathlon at 3 po1n1s along the course at a depth of 30 em	• Surveyed 314 tn3thletr.s and 81 run-b1ke-runner; at 1992 Ofymp1cs • Ouest10nna re mcluded quesuons on personal charactensucs. tra1n1ng expenences. ex;>osure to water ana occurrence of neattn effects dunng c•ent and 1n the week followinG lhf: event • Heallh effects 1ncluceo diarrhea, nausea,vomn•ng aooommal cramps sore throat . coughing, runny nose. sk1n. *eye,* ana ear problems fever and headache	• Used staust•ca l analyses to relate the occurrence or 111ness to water quality parameters • Looked at nsk ratiOS anc stat1St1cal s•gn1ficance of retat10nsh1ps	• Health nsk to rall symptoms was h1gher for tnathletes but was not SlatiSIICally Significant • The occurrence or symptoms m tnalhletes could nOl be related to length and mtens1ty of water exposure
Fel'o1rell et al (1994)	Unlled Kmgdom	• Measured FC. FS total staphyloCOCCI *Pseudomonas aerugmosa*, *Salmonella* spp , *Ctyprospond1um* spp .and emeroVIfuS • Samples taken from 2 freshwatar Sites (36 from Banbury. 50 from Ga1ley) and 2 estuanne s1tes (54 from Appleoore /Instow , 50 from Bodeford)	• Measured resporatory, ear.eye, GI skm.and otner • Surveyeo boaters and spectators 1n person pnor to event contacted by pnone 5-7 days later sent survey *rYf* maol 1-4 weeks later • Freshwater 558 pan1copan1s, estuanne 450 pan1c1pants • Collected data on age sex. d•etary haMs alcohol consumptoon, poe vem (3 weeks pnor) symptoms. ana place of residence	• Dtd not assess nsk relateo to bactena or wus counts • D1d not develop a dose-response relat1onsh1p	• Relallve nsk of GI symptoms h1gher tor boaters wno reponed 1ngesung water durmg the event than for non-mgesters (unclear of non-onges ters 1nclude boaters or only spectators) • Results are unaffected by stratofocatoon controlling for water type (fresh or estuanne)

Fewtrell et al (1992)	UOl!ed Kmgdom	• Measured FC FS IO!al stapnylococc•. enterov1rus • Collected 32 bactena and 10 v1rus samples at S1te A and 36 bactena and 9 v1rus samples at Sile B	• Measure:! flu Gl skm. resp•ratory. ear.eye otner • Surveyed cancers •n person prior to water contact ana after exposure.contacted by pnone 5-7 days later. sent a survey by ma1l 28 days later • 572 people 1n1t1ally 1nterv1ewed. 516 ccmpletec pho e 1nterv1ew 360 ma1led 28 oay survey • Cllected data on age. sex. occupation .place of res1dence. water act•v•hes.food consump11cn.and 1llness	• O.d not assess nsk relateo to bactena or v1rus counts or develop a dose-response relat10nsh•p	• Greater occurrence of symptoms m cancers from S1te A (rece1ves POTW effluents) tnan S1te B (pnst1ne source water) • Enterowuses .although most likely not causmg the symptoms .may be a betier md1ca1or of water quality than oactena
Calderon el al (1991)	Central ConnectiCut	• Measured E col1 enterocow . FC.Pseudomonas aerug1nosa. and staphylococc: • Samples taken from 2 SWlmm;ng Jreas 2-3 tur.es er day.knee deep • Also measured ramiall da1ly • No known numan fecal ource or pomt sources to the pond	• t04 fam1hes pan1c1pated 1n study - 1.310 SWimmmg exposure days and 8 356 nonsWimm,n g days • Sent :am111es self-completed queshonnaue on demographiC mformaliOn and da1ly health status and SWlmmmg ac!lv1lies • Measured Gt 1Uness symptoms such as vom1tmg .nausea, d1armea .stomacMcne and fever ana m1scellaneous symptoms such as neaoacne. backacne, earacne, I!ChyiWatery eyes. Sk1n rash. neez1ng and wneezmg	• Used Mantei-Haenzel test to evaluate d1fference 1n 1llness rates between SWimmers and nonsWlmmers • Calculated retat1ve nsk of SWimming-aSSOCiated illneSS • Used probability cnans 10 evaluate relatiOnSh ip between 1nd1cator dens,lies and 1llness rates	• E col1 i'C and enterOCOCCI densities vaned Wllh ramfall and correspondeo to one anotner • StaphyloCOCCI relatea to bather dens11y, not other fecal 1nd1Cators or ra1nfall • Gf 1llness strongly aSSOCiated Wllh SW1mm1ng • No assOCiation between h1gh fecal,ndiCator bac:ena or ramfall and Gl 1llness • S1Qn1ficant assoc1at10n between 1llness and staphylOCOCCI dens•t Cs

Koopman et at {1982)	Macomb County, MI	• Dta not sample '"ater quality	• Retrospective study of GIllness outbreak • Collected data from 2 groups VIStIlng same park on htstones regardtng occurrence of GI and resptratory symptoms and exposure to food, dunktng water, and swtmmtng water • 20 stool samples and 11 acute and conv alescent sera patrs were obtatned from 111 paltents • Stool samples exammeo for salmonella, sntgella *E co/J*, and campylabacter. Sera samples axamtned for Norwalk aoent	Stallsttcal methods to assess results not spectfteo	• 91% of those wtth pnmary tllness (onset wtthtn 60 hours) put the•r heads m the water • Levels of tndtcator orgamsms resulting 1n tllness were not examtned because no water quality samples were taken
Q,l.tessta et at {1981)	Maa.son. W,	• Ctd not sarr.p.e water qualtty on SpecifiC exposure days • Revtewed weeKly beac closure water quality sampling results	• From 6113177to 911177 surveyed cnttdren VISIItng pedtatnoans to obtatn aemograpntc tnfo. reasons lor clime vtsll. symptoms. swtmmtng acttvtUes 1n pnor 2 weeks, and locatton of swtmmtng • Collected pharyngeal and rectal swabs from 262 111 chtldren and 27 well control cnttdren to test for vtral, enterowus. and aaenowus ISOlates	• Cata categonzed by dtagnosttc groups. swtmmtng aclIvt ty sex. and age • log-ltnear mocel used to test for stattstlcat SIQntfiCance	• Old not evaluate matcatar levels that 1ncrease tllness nsk. Intendea to relate tllness rates to swtmmmg • Proponton of swtmmers 1n the enterowus ISOlate category was larger than 1n the well controls • Proponton of beach swtmmers tn enterowus ISOlate category twice as great as weil controls (no dtfference between pool swtmmers and well controls)

Rosenberg et at (1976)	Ououque IA	• Water samples from several MISSISSippi R potnt and well supp/ytng camptng parks tested for FC 10 August 1974 • Addthonal samples collected 4 5 m off shore at a depth of 15 em and tested for sntgella	• 45 members of 29 iamtlieS With culture positive shtgellosts between 7!9(!4 and 619(!4 surveyed • lnforma11on collected on age sex. exposure tO MtSSISSippt R, food consumplton at nver Sties datly activities. occurrence of dtarrnea. and contact wtlh ill persons	• 60 otner famtly groups surveyed to oeterrmne whether unreported cases of dtarmealtllness had occurred and whether such cases were assoctated wtth swtmmmg	• Of the 45 cases 32 were assoctated wtlh swtmmtng tn the nver • Water samples from 11 loca!lons had FC counts from 5,000 000/100 ml below the POTW to 400,000/1DO ml Shtgella was tsotated from a sample taken 9/4r74 • Does not re/ate level of FC or Shtgella With 1ncreases tn tllness nsk
Br;an et at (1974)	Lake WatP.ree SC	• Water samples not taKen m contunctton wtth study but lake was occasiOnally monttoreo	• 14 of 35 campers ana adults developed hepatitis-A • All campers and adults surveyed to gather data regard10g the posstble mode of exposure	• Used survey to determtne tf outbreak was due to a stngle source and what that source may be	• Determtne outbreak due to a common source • Lake water samp1es revealed gross contamtna!vn wtth cohforms on a number of occastons • Appeared that dnnktng water rather than swtmmmg tn tt resulted 10 qreater nsk of neoatttts-A

Hal'<1ey et al (1973)	Lake Champlam .VT	• 2 1-gallon samples of water obtamed from take earty 10 the mom1ng pnor to SW1mm1ng aCIIVIIIeS began • Samples taken 2 and 5 days aher first camper fell1ll	• 32 campers and counselors quesuoned about acute 1llness h1story and exammed for 1llness symptoms • Obta1ned 2 throat and 2 rectal swabs from each pamc1pant	• Used survey resulls aM water sampling to determme source of 1llness outbreak • Used swab test to determ1ne the type of tllness	• 21 0: 33 pan1opants had s1gns and symptoms of an acute wat1nfect10n • One of :he water samples y1elded wus • Allvtrus ISOlates (1ndud1ng water sample) typed as co1sack1ewus 85 except the first pauent • Pnnople mode of mfechon appears to be person-to-person. although 1nfect10n frorr. water cannot be ruled out
California-Specific Epidemiological Studies					

Colford e1 al (2005)	M1SS10n Bay, CA	• Measured TC.FC. and enterocoCCI us1ng MF,TC. FC and enteroCOCCI usmg CS methOd, enterocoCCI usmg QPCR method. Baclero,des. somauc coliphage male-speaflC cohpnage.adenov1rus ana Norwalk-like wus • Samples collecled a1 1of 3 temporal ,nlensltles depend1ng on 1nd1ca1or	• Categones of lllr.ess GI resp1ra1ory dermalolog'c and nonspecifiC symptoms (e g • fever ch1lls.ear and eye 1rntat1on) • 1n1erv1ewed 8.797 beach goers dunng the summer of 2003 • Conelucted InltlalnlefVIew ana survey on beach and follow up phone 1nterv ew 8-10 days late· • Collecteel da1a on poss1ble exposures pnor 1llness.race. 1ncome.educa11on level.wa1er aCIIVll:ES,acute heallh COnOIIOnS	• Evalua1eo wt.etner Slgr,lficant difference m 1llness ra1es between SWimmers and nonsWimmers • Exam1ned 1f relat1onsh1p ex1sts between 1llness nsk and wa1er qual:ty • Used un1vanate and mulhvanale models for analyses - mult1vana1e analyses ad1usted for confound,ng covanales suCh as age, genaer.and race	• Only sk1n rash ana d1armea were elevaled m SWimmers • No correlation between 1ncreased 1llness nsk and trad1 onalIndiCators • No correlaliOn between nsk of 1llness and levels of bacteroides.en1erococ.:us us1ng rapid methods. human pathogemc wus, or somaiiC phage • S1gn1fic3nt assoc1at10ns between levels of male-speafic coliphage and HCGI-1, HCGI-2.nausea. cough,and fever • Lack of relahonsh,ps *may* be due to faCI thai fecal sourc:es are predommantly nonhuman res10ence umes 1n bay are h1gh, and C1rculat1on IS reslncled

1 Caoe111 (1976, 1981), Dufour (1976), and Henderson (1968) are also cited 1n EPA (1985)

Acronyms

AGIO= acu1e gas1rom1es11nal d1sease
CS =chromogeniC subslra1e
DGI = d1sabhng gas1r01nteSt1nal
FC = fecal coliform
FS =fecal strep1ococc'
Gl = gastro,mesllnal
HCGI = h1gh cred1ble gasuo,n1es11nal
MF = membrane fdter
MPN = most probable number
POTW = pubhdy owned ueatment works
QPCR = quan lanve polymerase cna10 react1on
SRD SIQnfiCant resp ratory diSease
TC = total cokform