EXPOSURE TO HUMAN SOURCE-ASSOCIATED FECAL INDICATORS AND SELF-REPORTED ILLNESS AMONG SWIMMERS

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ABSTRACT

Melanie Denise Napier: Exposure To Human Source-Associated Fecal Indicators And Self-Reported Illness Among Swimmers
(Under the direction of Charles Poole and Timothy Wade)

Background: Current fecal indicator bacteria used to assess illness risks in recreational waters (*E. coli*, enterococci) cannot discriminate among sources of contamination. To address this limitation, human-associated *Bacteroides* and chemical markers have been proposed, but the risk of illness associated with human fecal indicators is unclear. We estimated associations between microbial and chemical markers of human fecal pollution and self-reported illness among body immersion swimmers at U.S. beaches during 2003 – 2007.

Methods: Participants were surveyed about beach activities and water exposure on the day of their beach visit and followed up 10 to 12 days later to document illness experienced since the beach visit. At 6 beaches, water was analyzed for the presence of human-associated *Bacteroides* markers: HF183, BsteriF1, BuniF2, HumM2. At 5 beaches, water was analyzed for 56 anthropomorphic chemicals. Adjusted standardized risk differences (RD) and 95% confidence intervals (CI) for the indicator-illness associations were estimated using model-based standardization. Human associated markers were assessed as modifiers of the association between general *Enterococcus* and illness using interaction contrast.

Results: Overall we observed little evidence of association between *Bacteroides* markers and illness, and between chemical markers and illness among body immersion swimmers. There was a pattern of increased risks of GI illness (RD=1.9%; 0.1%, 3.7%), diarrhea (RD=1.3%; -0.2%, 2.7%), and respiratory illness (RD=1.1%; -0.2%, 2.5%) associated with the BsteriF1 marker. There was no evidence that *Bacteroides* markers acted as modifiers of general *Enterococcus* and illness. Several chemicals also showed a pattern of increased risks, including bisphenol A-GI illness, cholesterol-GI illness, household wastewater products-respiratory illness, and tributyl phosphate-respiratory illness. Phenol exposure increased the magnitude of association between general *Enterococcus* dichotomized at policy-relevant cut-points and GI illness, eye ailments, and respiratory illness by 3-5%.

Conclusions: Human-associated *Bacteroides* and chemical markers were not consistently associated with swimming-associated illness, though patterns suggest possible increased risks. It is not clear that these findings are generalizable to beach sites impacted predominantly by animal sources, runoff, or sporadic sources of contamination. Additional research is needed to support the use of human-associated indicators in predicting illness risks from human fecal pollution of recreational water.

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LIST OF ABBREVIATIONS

95% CI	95% confidence interval
B. dorei	*Bacteroides dorei*
B. stericoris	*Bacteroides stericoris*
B. uniformis	*Bacteroides uniformis*
BEACH	Beaches Environmental Assessment and Coastal Health Act
C. perfringens	*Clostridium perfringens*
CCE	calibrator cell equivalents
CFU	colony forming units
CT	cycle threshold value
DAG	directed acyclic graph
DNQ	detected, not quantifiable
E.	*Enterococcus*
E. coli	*Escherichia coli*
EPA	U.S. Environmental Protection Agency
FIB	fecal indicator bacteria
FIO	fecal indicator organism
FST	fecal source tracking
GI	gastrointestinal illness
IC	interaction contrast
LOD	limit of detection
LOQ	limit of quantification
µg	microgram

NEEAR	National Epidemiological and Environmental Assessment of Recreational Water
P	P-value
qPCR	Quantitative polymerase chain reaction
RD	Risk difference
SD	Standard deviation
Spp.	Species
TSC	Target sequence concentration
URI	Upper respiratory illness
UTI	Urinary tract infection
UV	Ultraviolet
WWTP	Wastewater treatment plant

CHAPTER 1. INTRODUCTION

Monitoring recreational water quality using fecal indicator organisms (FIOs) has become standard practice in the US and other countries seeking to reduce the burden of swimming-related illnesses. An estimated 170 million respiratory and enteric illnesses worldwide are attributed to swimming in and consuming shellfish from polluted marine coastal waters each year (1). Exposure to water contaminated by human fecal sources is believed to pose a greater risk to human health than that from non-human sources (2,3) because they most likely contain human enteric pathogens. In particular, viruses are believed to cause a high proportion of swimming-associated gastrointestinal (GI) infections (e.g. hepatitis A, Norwalk virus, norovirus) (4-7) and enteric viral pathogens usually do not readily transmit infection to a host of a different species (2,3). However, enumerating conventional indicator bacteria – fecal and total coliforms, *Escherichia coli (E. coli)*, enterococci – cannot be used to discriminate between human and animal sources because of their widespread distribution in the feces of animals and humans. Previous epidemiologic research investigating the human health effects of water pollution often relied on proximity to sewage effluent from wastewater treatment plants as a proxy for human presence. Recently, source tracking methods that include microbial indicators capable of distinguishing human from animal fecal matter, as well as chemical markers of human presence are increasingly available, allowing the effect of human fecal pollution to be disentangled from that from nonhuman fecal pollution (8).

In this dissertation, I estimated associations between exposure to microbial indicators of human fecal contamination and chemical markers of human presence, and self-reported illness among swimmers 10-12 days after exposure. I explored whether the identification of human source strengthened the association between general *Enterococcus* and illness association. By investigating these associations, results from this study may help determine the utility of human-associated markers for source tracking of fecally-contaminated recreational waters.

CHAPTER 2. BACKGROUND AND SIGNIFICANCE

Use of fecal indicator organisms to track pathogens

Fecal contamination of water has always been a major human public health concern. Disease-causing pathogens can be present in sewage and transmitted via the fecal oral route (Table 2.1). For over 100 years, the quality and safety of recreational waters has depended on the enumeration of non-pathogenic microorganisms that normally inhabit the human and animal gastrointestinal tract and are shed in feces. As such, these FIOs signal the presence of fecal contamination, and are a convenient substitute for the costly and difficult task of directly measuring viral, bacterial, and protozoan pathogens. Measuring the pathogens directly would be ideal, but that approach is fraught with barriers. There are simply too many potential pathogens to practically monitor them all as a part of routine surveillance. Waterborne pathogens often occur at low concentrations and are unevenly distributed in the environment; their detection requires the collection of large volumes of water and assays specific to individual agents or classes of agents (9). In addition to being technically complicated and expensive to implement, these assays have not been optimized for every pathogen and methods to simultaneously enumerate multiple pathogens at once are still under development (9). It is much more feasible and cost-effective to measure and enumerate FIOs that are generally more abundant and easily measured.

Ideally, a FIO shares biological characteristics with the pathogens of interest so that measuring the FIO might give an "indication" of whether fecal contamination might be present. In addition to being non-pathogenic residents of the GI tract of warm-blooded animals, there is a general consensus that an indicator with the following characteristics would be most useful (2,10,11):

- Being present when pathogens are present and absent in uncontaminated samples
- Being present at densities correlated to the amount of pathogenic microorganisms
- Being present at densities correlated to a health hazard
- Being unable to grow in extra-intestinal environments
- Surviving as long or longer than the pathogen for which it is an indicator
- Being more resistant to environmental stress and disinfection than the pathogen for which it is an indicator
- Occurring in greater numbers than the pathogen to permit ease of detection
- Being rapidly detected and easily enumerated
- Being present in all types of water

No single indicator meets all the above characteristics, neither can any one indicator successfully identify or predict the presence or source of all classes of potential pathogens (3).

Conventional fecal indicators

Under the authority of the Clean Water Act and Beaches Environmental Assessment and Coastal Health Act (BEACH) of 2000, the U.S. Environmental Protection Agency (EPA) issues recommendations for indicator organism levels in recreational water settings. Beginning in 1976, the EPA recommended using fecal coliform bacteria as FIOs, and set a threshold of 200 fecal coliform colony forming units (CFU) per 100 ml (12) Based on research showing that *E. coli* and

enterococci were good predictors of GI illness, the 1986 criteria recommended using culturable *E. coli* in freshwater and culturable enterococci in marine and freshwater instead of fecal coliforms (13). In fresh waters, the geometric mean of ≥ five samples taken over a 30-day period should not exceed 33 CFU of *Enterococcus* per 100 ml or 126 CFU of *E. coli* per 100 ml. In marine waters, the threshold was 35 CFU of *Enterococcus* per 100 ml. Most recently, in 2012, the EPA revised the criteria; while *E. coli* and enterococci remained as the recommended indicators, they revised the threshold values to reflect different illness rates and research with non-GI illnesses (Table 2.2). Also, for the first time, the EPA provided thresholds for *Enterococcus* by qPCR (Table 2.3).

Since the EPA recommendations are intended as guidance, some states and jurisdictions choose to use coliforms or *Clostridium perfringens* to monitor their waters instead of, or in addition to *E. coli* and enterococci. To provide the foundation for further discussion of indicators that can distinguish source, a brief review of these conventional FIOs, their uses, and their limitations, follows.

Total and fecal coliforms

Coliform is the term for a group of bacteria that are gram-negative, catalase positive, non-spore-forming, aerobic and facultative anerobic rod-shaped that inhabit the GI tract of all vertebrates. Total coliforms (TC) are bacteria that ferment lactose to gas within 48 hours when incubated at 35°C. At this temperature, some members can be routinely found in the environment. Fecal coliforms (FC) are a subset of total coliforms that ferment lactose at the higher temperature of 44.5°C, which suppresses the growth and activity of environmental total coliform bacteria (14). While the FC group contains other genera, such as *Klebsiella*,

Enterobacter and *Citrobacter* (15), *E. coli* has been a strong indicator of fecal contamination because it is present in high numbers in feces and does not grow in the environment. Fecal source tracking studies have sought to use differences in the ratio of total coliforms to fecal coliforms as an indicator of host source, however, studies have concluded that the ratio is not able to distinguish between human and animal source (16).

E. coli

E. coli along with enterococci discussed below, are two microorganisms that have consistently performed well as indicators. *E. coli* is a common gram-negative facultative anaerobe found in numbers up to 10^9 per gram of mammalian feces (14). It can act as both an indicator and a pathogen. Non-pathogenic *E. coli* is termed commensal, and considered to be a beneficial, normal inhabitant of the gastrointestinal (GI) tract of warm-blooded mammals, although they can cause disease in immune-compromised hosts. Pathogenic *E. coli* cause disease either inside (diarrheagenic) or outside (extra-intestinal) the GI tract. *E. coli* has been widely used as an indicator of fecal contamination because of its property as a stable member of the intestinal community and abundance in feces. However studies have shown that *E. coli* is affected by environmental factors including temperature, UV radiation, and can survive in water 4 to 12 weeks, undermining its usefulness as an indicator of recent contamination (14).

Enterococci

Bacteria in the genus *Enterococcus* are gram-positive, catalase negative, facultative anaerobic diplococci that occur in the GI tract at densities ranging from 10^5 to 10^8 colony forming units per gram feces (17) and make up 1% of human intestinal flora (14). Enterococci

are found in soil, water, dairy products, food and plants, as well as feces. In humans, 90-95% of enterococcus is *E. faecalis* (which can act as a pathogen causing urinary tract infection, or an indicator) and 5-10% is *E. faecium* (which acts as an indicator). Enterococci are useful indicators because they are present in human and animal feces, have survival rates similar to waterborne pathogens, and are usually unable to multiply in the environment. Enterococcus surface protein may be a specific marker of human fecal contamination (18). Enterococcus is also the indicator recommended for marine recreational water quality monitoring by the WHO (19).

Clostridium perfringens

C. perfringens is a gram-positive, obligate anerobic, rod-shaped bacterium ubiquitous in soil, but also commonly associated with feces. It is prevalent in human feces (14) at concentrations of 10^5 to 10^7 CFU/g of feces (18,20), although concentrations vary between individuals. Some studies suggest *C. perfringens* is more prevalent in the feces of domesticated animals such as cats, dogs, pigs and poultry, than in cattle, sheep, and horses (18,21). Under the appropriate conditions, *C. perfringens* can cause a variety of diseases, including gangrene and food poisoning (22). *C. perfringens* may be a useful indicator of both past and present pollution because of its ability to occur in both vegetative and spore forms. Since it does not appear to grow in aquatic/soil environments, it is a useful fecal indicator for tropical environments where the regrowth of *E. coli* and enterococci in sand, sediment and water make them less useful (23).

Limitations of conventional indicators

Coliforms, enterococci, and *E. coli*, enumerated by culture-base methods, have documented limitations that undermine their usefulness as surrogates of fecal contamination and

support the identification of alternative indicators. A designated concentration of FIO is presumed to indicate fecal contamination, but studies in recent decades indicate these organisms may have an ability to persist and regrow in contaminated soils, sediments, marine waters (24), and other extra-intestinal environments (8,9,25-29), including areas removed from human activity (27). In addition, conventional FIOs have been criticized for being poor representatives of the fate, transport, and survival of human pathogens, particularly viruses and protozoa (8,27,28,30-32). They can be more susceptible to the disinfection process and inactivation by environmental stressors, like sunlight (33). Finally, none of the conventional FIO can be used to discriminate the source of fecal pollution in the water as human or non-human because these bacteria are found in various warm-blooded animals (8,9). Furthermore, standard culture-based methods of measuring FIO also do not distinguish between human and animal sources of pollution and limit their usefulness for water quality monitoring. Culture-based detection methods require growing FIO from filtered water samples to estimate their concentrations, a process that can take up to 24 hours for viable cells to be grown. Beach closings and advisories are then issued based on the previous day's indicator levels, which may differ widely from the present day's risk of fecal contamination due to weather, human events, or other factors. Understanding the dominant source of microbial contamination can inform the remediation of impaired water systems that support recreation.

Alternative indicators: Source tracking markers

Due to the limitations of conventional indicators, it is widely acknowledged that alternative indicators for assessing water quality are needed, but a clear consensus has not emerged. Fecal source tracking markers (FST) (such as members of the *Bacteroidales* order),

chemical source tracking markers, and rapid molecular-based measurement methods have been proposed that can discriminate the presence and source of fecal pollution and quantify the differential risk from various contamination sources.

Bacteroides *spp. as human-associated source tracking markers*

First proposed as fecal indicator bacteria in 1985 (34), members of the genus *Bacteroides* are one of the most promising library-independent FST markers currently available. *Bacteroides* spp. are gram-negative, obligate anaerobic bacilli that are commonly found in the GI tract of warm-blood animals. They are one of the most abundant bacteria in the intestinal tract of humans, found in up to 10^{11} CFU/g of feces (35), a concentration 1,000 fold greater than *E. coli*. *Bacteroides* spp. can account for up to 30% of the total fecal isolates (14). Since the 1990s, members of the genus *Bacteroides* have been suggested as FST markers because of their ability to indicate recent contamination and host specificity. Host-specific strains of *Bacteroides* (representing between 1 and 10% of the total *Bacteroides* fecal population (36)), particularly certain 16S rRNA genes, have been identified that may be strictly associated with human vs. animal feces (37). Although difficult to culture, quantitative polymerase chain reaction (qPCR) assays that measure gene copies of host-specific genetic markers of 16S rRNA are promising methods for source tracking of fecal contamination (37). It is unknown whether *Bacteroides* spp. performs as well as a FST marker in temperate vs. tropical zones of the world.

Advantages of *Bacteroides* spp. as FST markers of human fecal contamination include (1) presence in high concentrations in sewage; (2) inability to survive for long periods in the environment; (3) relatively high persistence through wastewater treatment plants, compared to conventional FIO like *E. coli* and fecal coliforms (coliphages may behave similarly to human

viruses during wastewater treatment); (4) molecular methods for detection of highly human-associated *Bacteroides* spp. has already been developed and has proven robust (37); and (5) specificity in the human vs. animal strains. Limitations of *Bacteroides* spp. as FST markers include limitations of the molecular assays used, which cannot distinguish between viable and nonviable cells; thus recent and past contamination events cannot be distinguished since DNA of selected pathogens can persist after cell death for up to three weeks (38).

Chemical compounds as human-associated source tracking markers

Certain chemical markers are also attractive as human source tracking markers because they typically require less time required for sample preparation and analysis than culture methods, they cannot regrow in the environment, and some may be more geographically or temporally stable (11). In comparison to many microbiological methods, chemicals have the advantage of low detection limits and relatively easy analysis. A wide range of chemical compounds has been investigated as potential tools for the identification of human fecal sources (2,11,39). These compounds fall into several classes:

- those that are produced and excreted by humans (e.g. Coprostanol);
- those that are ingested almost exclusively by humans (e.g. caffeine, nicotine, and certain pharmaceuticals like carbamazepine and diphenhydramine); and
- those that make it into the human waste stream (e.g. surfactants, fluorescent whitening agents).

Chemical compounds in the first two classes mentioned above that pass through the human digestive tract provide the most direct evidence that the fecal contamination is of human

origin. Compounds in the third class above may still indicate co-mingling with human sewage, but the compound may have originated from industrial sources or from the disposal of pharmaceuticals down the drain without passing through the human digestive tract or from surface runoff. Chemical compounds that appear to have the greatest potential include: (1) pharmaceuticals; (2) plant/animal fecal sterols; (3) household waste products, including personal care products, flame retardants and detergents; (4) industrial wastewater compounds; and (5) pesticides.

Pharmaceutical chemicals have been successfully detected in freshwater, seawater, estuaries, sediments, and wastewater effluents (11,40) and they have been examined as indicators of human wastewater pollution because of high water solubility and low levels in the background environment (41,42). Specific pharmaceuticals carbamazepine, codeine, dehydronifedipine, diltiazem, and fluoxetine have been found to indicate a uniquely fecal source because these pharmaceuticals are consumed and have no external uses and have been detected in 73 to 91% of wastewater treatment plant (WWTP) effluents at concentrations significantly greater than upstream locations (40).

The term "sterols" is a collective name for all sterols and stanols, and denotes a steroidal alcohol with some degree of unsaturation. *Fecal sterols* are commonly produced in the digestive tract of humans and other warm-blooded animals by microbial hydrogenation of cholesterol (42). The most commonly known fecal sterol, coprostanol (5β-cholestan-3β-ol), comprises 50-80% of total sterols found in human feces and was 10 times more abundant than cows, horses, sheep, hens, ducks, pigs, cats, dogs, and several other animals studied (11,42). Though largely of fecal origin, low-levels of coprostanol can be found in natural sediments because of re-isomerization of a α-configured form. To be used for identification of human waste pollution, both absolute

concentrations and ratios of various stereoisomers are needed. For example, a high relative amount of coprostanol-to-24-ethylcoprostanol is one useful ratio for identification of human contamination; a ratio of ≥1.5 indicates 100% human contamination (43).

Household and industrial waste products can contain endocrine-disrupting chemicals. Household wastes can include various personal care products such as d-limonene (fragrance in aerosols), acetophenone (fragrance in detergent, flavor in beverages), 1,4-dicholorobenzene (moth repellant, deoderant), triclosan (disinfectant), and DEET (*N,N*-diethyl-*meta*-toluamide, mosquito repellent). *Industrial* wastewater compounds encompass a broad range of chemicals that can be toxic to humans.

Optical brighteners, or *fluorescent whitening agents*, are compounds that emit light in the blue range (415-445 nanometers) and are added to 97% of laundry detergents in the US, used in toilet paper, and present in other home products (44). They are associated with human sewage in septic systems because household plumbing systems mix effluent from toilets and washing machines together. Optical brighteners are present in effluent regardless of how effective the treatment has been at inactivating pathogens, and so must be accompanied by counts of fecal indicator bacteria to be a useful indicator of human contamination. Advantages of the use of optical brighteners include rapid, simple, and low cost detection methods using fluorometry (11,45), and the abundance of optical brighteners in sewage. The limitations are dilution of the optical brighteners in large water bodies and potential interference from unknown compounds.

There is interest in *caffeine* as a potential human marker because of its high consumption levels in the US (210 mg/day) and high concentration in surface water. Both metabolized and un-metabolized caffeine in the form of coffee, tea, and caffeinated beverages may represent significant quantities in wastewater. Caffeine has been detected in septic tank effluent (46) and

wastewater effluent (47) and has been successfully isolated in freshwater, marine waters, and storm waters (11).

Lastly, certain *pesticides* may be useful as a human source marker because of their use in the controlling pests in a variety of settings, and release into the environment during production and formulation of pesticides. In a recent study of 110 chemicals, one insecticide, diazinon, was among the 35 chemical compounds found in >50% of the WWTP effluent samples associated with wastewater (40).

A tiered, "toolbox" approach to source tracking

A recurrent theme in the fecal source tracking literature is to use source-specific indicators like *Bacteroides* spp. and chemical markers as part of a tiered, "toolbox" approach incorporating multiple indicators or markers and analytical methods (e.g. qPCR, fluorometry, and antibody detection) to assess water quality and determine human and other fecal contamination sources (31,48-53). This can be done in many ways, but in one such approach, water quality assessment would begin by measuring conventional FIOs appropriate for a particular recreational water site, and then progress to more refined methods (e.g. molecular methods) and indicators that detect human, animal or environmental sources of fecal contamination (e.g. human-associated *Bacteroides* and chemical markers), if necessary (48). Human source markers can contribute additional confirmation of human source in situations where certainty about human source is critical or as a screening tool. For example, Coprostanol, caffeine, and pharmaceuticals carbamazepine and diphenhydramine are compounds highly specific to human sources that can be used for confirmation in the former case. In the latter case, using fluorometry to detect optical brighteners has been proposed as a low-cost initial screening

tool for detecting human fecal contamination that yields rapid results (11). By using multiple tools, investigators can utilize the strengths of each to ascertain and remediate poor water quality.

Illness risks associated with human fecal indicators

Numerous epidemiological studies have demonstrated an increased risk of GI, diarrhea, respiratory, skin, eye, and ear illnesses among swimmers exposed to elevated FIO levels in sewage-impacted waters (54-60). Findings from studies where non-point sources of pollution is the predominant contaminant have been more inconsistent, with some studies reporting an association between indicator and illness (59,61-63), while others do not (62,64,65). But even a non-point source-impacted water body may have a human source of fecal contamination nearby (59). Our study assessed the source of fecal contamination from human source indicators in the water, instead of relying on proximity to sewage as a proxy. In addition, this analysis provides additional evidence regarding indicator organism-illness relationships for skin, eye, and ear infections, which tend to be less commonly reported than GI and respiratory illness.

Studies estimating human health illness from exposure to human source indicators are rare. In 2007, Colford et al. (64) assessed for two human pathogenic viruses, adenovirus 40 and 41, and norovirus, as human-associated fecal indicators. In a cohort study of the health effects experienced by 8,797 swimmers at a nonpoint source beach in Mission Bay, California, the authors reported that both viruses were not associated with an increased risk of GI illness, respiratory symptoms, skin symptoms, fever, eye irritation, earache, or ear discharge. However, very low viral detection (adenovirus was detected in only one sample, and norovirus was not detected at all) casts doubt on the conclusion of no association (64,66). They also reported no elevated risk of illness from exposure to conventional indicators (fecal/total coliforms and

enterococcus) or alternative indicators (*Bacteroides* and somatic phage) (66). They *did* however find an increased risk of GI, nausea, cough and fever with male-specific coliphage, but few people were exposed.

Arnold et al. (67) and Colford et al. (65) used the qPCR assay Scorpion-2 for *Enterococcus*, which includes a primer-probe complex that amplified two common *Enterococcus* species found in human fecal contamination: *E. faecium* and *E. faecalis* (68). However, there is some doubt that this primer-probe design is exclusive to humans (69). In studies that examined marine beaches impacted by urban runoff, Arnold found that *Enterococcus* density was not consistently associated with swimmer illness (67), whereas Colford reported an association between $\log_{10}$ increase in *Enterococcus* density among swimmers who swallowed water on berm-open days and diarrhea (adjusted odds ratio (OR)=2.30 (1.46, 3.61)) and GI ((OR=1.70 (1.10, 2.63)) (65). (An open berm freely allowed an untreated creek to flow into the surf). However, *Bacteroides* species makes up a larger portion of the human intestinal bacteria (70) and is more abundant in feces than *Enterococcus (71)*. As a result, human-associated *Bacteroides* spp. markers may be more sensitive markers of swimming-associated illness risks. Very few studies have been conducted to evaluate *Bacteroides* spp. as predictive indicators of human illness risks from recreational use of water and in sites known to be impacted by human sources (e.g. sewage).

Sinigalliano et al. enumerated a suite of fecal indicators including 2 human *Bacteroides* markers by qPCR (HF8 (36,72) and UCD) during a prospective randomized exposure study in which each participant randomized to marine recreational water exposure sampled the water where they swam (62). The site of the study was a nonpoint source subtropical marine beach in Florida. Except for enterococci and skin illness, the authors found no significant relationships

between any of the indicator organisms and self-reported GI, diarrheal, respiratory, or skin illness 7 days after beach exposure. The strength of this report is in its randomized design, which may have avoided self-selection bias that non-swimmers are inherently different or less healthy than swimmers, and individual exposure samples. However, the limited size of the cohort prevented investigation of associations between specific alternative markers and specific diseases.

As stated earlier, a wide range of chemical compounds has been investigated as potential tools for the identification of human fecal sources. These studies demonstrate the feasibility of using chemical compounds to assess the human origin of pollution (9,40,73,74), or the relationship between chemicals and microbial FIO (11,75,76). However, the literature examining the relationship between the presence or concentration of chemicals and illnesses caused by human fecal pollution is even more limited than for microbial FIOs. To our knowledge, this research is the first study to examine the association between chemical indicators of human fecal contamination and illness risks due to contaminated recreational water.

Summary

Determining the source of fecal contamination in recreational environments is essential for estimating the illness risks associated with pollution and facilitating measures to remediate polluted waterways. Individually each fecal indicator is unlikely to give a complete picture of the source of fecal pollution and associated risks posed by fecal contamination. Together, microbial and chemical source tracking methods can be used to enable investigators to determine the sources of fecal pollution, but epidemiology studies are needed to investigate the utility of these source-tracking methods as indicators of fecal contamination. In this research, we aim to address

this gap in the literature using a prospective cohort. The human fecal indicators and associated detection assays considered for this research include some, but not all those that are proposed by the literature as showing promise for being host-specific (40). *Bacteroides* spp. is the most abundant inhabitant of the human gut and assays to detect host-specific species have been validated in different water types. The *Bacteroides* spp. microbial indicators considered – HF183, BsteriF1, BuniF2, and HumM2 – all use qPCR enumeration methods that produce results within 2-4 hours, allowing beach staff to make decisions about beach advisories and closures based on same-day sample collection. Chemical markers of human fecal contamination are under-studied. The 50 chemicals included in this study include those that are produced and excreted by humans, those ingested almost exclusively by humans (e.g. caffeine, nicotine, and certain pharmaceuticals like carbamazepine and diphenhydramine), and those that make it into the human waste stream (e.g. surfactants, fluorescent whitening agents).

Each of the indicators and assays discussed has limitations that may ultimately restrict their usefulness as a human source-specific marker. An important determinant of their usefulness is how well they correlate with human illness. To the best of our knowledge, this research is one of the first studies to investigate the association between the above *Bacteroides* markers and health outcomes; and the first study to examine the association between chemical indicators of human fecal contamination and illness risks due to contaminated recreational water in a large population-based prospective cohort.

Tables and Figures

Table 2.1. Select human pathogens associated with recreational water settings

Pathogen	Disease/ role	Symptoms	Incubation Period	Source
Bacteria				
Pathogenic *E. coli* (ETEC, EPEC, EAEC, EIEC, STEC)	Gastroenteritis (all), urinary tract infection (EIEC)	Diarrhea, bloody diarrhea	2-6 days	Animal/ Human feces
Campylobacter spp.	Acute enterocolitis, Guillain-Barré, infectious diarrhea	Diarrhea (occasionally bloody), cramping, abdominal pain, fever	2-5 days	Human feces, cow/bird feces
Salmonella spp.	Gastroenteritis, Typhoid fever	High fever, diarrhea, abdominal cramps	7-28 days	Human feces/ sewage
Shigella spp.	Shigellosis, bacillary dysentery	Fever, stomach cramps, bloody diarrhea	1-7 days	Human feces/ sewage
Vibrio spp.	Gastroenteritis, Cholera, Vibriosis, Necrotizing wound infections	Vomiting, diarrhea, abdominal pain, skin infections, fever, chills,	1- 6 days	Marine and estuarine environments
Enteric Viruses				
Norovirus	Gastroenteritis	Diarrhea, nausea, vomiting, abdominal pain and cramps	24-48 hours	Human feces/ sewage
Non-polio enterovirus	Gastroenteritis, heart anomalies, meningitis	Mild flu-like symptoms, skin rash, Paralytic disease, respiratory illness	3-14 days	Human feces
Adenovirus	Gastroenteritis, conjunctivitis, pharyngitis, pneumonia, appendicitis	Diarrhea, fever, vomiting, cough, sore throat, headache, eye infection	~10 days	Human feces, aquatic environments
Viral hepatitis – A and E	Infectious hepatitis	Jaundice, fever, anorexia, malaise	15-50 days	Human feces/ sewage
Rotavirus	Acute gastroenteritis	Gastroenteritis with nausea, vomiting	2-3 days	Human feces
Protozoa				
Entamoeba histolytica	Amoebiasis	Abdominal pain, bloody diarrhea	2-4 weeks	Human feces
Cryptosporidium spp.	Cryptosporidiosis	Watery diarrhea, stomach cramps, nausea, vomiting, mild fever	1-2 weeks	Human feces, animal feces
Giardia lamblia	Giardiasis	Acute diarrhea, dehydration, flatulence, abdominal cramps and nausea	5-25 days	Human feces, animal feces

Abbreviation: EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; STEC, shiga-toxin producing *E. coli*. Source: (3,77-92).

Table 2.2. 2012 EPA recreational water quality criteria for culture-based methods

Criteria Elements	Estimated Illness Rate (NGI): 36 per 1,000 primary contact recreators			Estimated Illness Rate (NGI): 32 per 1,000 primary contact recreators	
	Magnitude			Magnitude	
Indicator	GM (cfu/100 mL)[a]	STV (cfu/100 mL)[a]	OR	GM (cfu/100 mL)[a]	STV (cfu/100 mL)[a]
Enterococci – marine and fresh	35	130		30	110
OR					
E. coli – fresh	126	410		100	320
Duration and Frequency: The waterbody GM should not be greater than the selected GM magnitude in any 30-day interval. There should not be greater than a ten percent excursion frequency of the selected STV magnitude in the same 30-day interval.					

[a] EPA recommends using EPA Method 1600 (93) (or another equivalent method) to measure culturable enterococci and using EPA Method 1603 (94) (or another equivalent method) to measure culturable *E. coli.* Source: EPA 2012 (95)

Table 2.3. 2012 EPA recreational water quality criteria for qPCR-based methods

	Estimated Illness Rate (NGI): 36/1,000 primary contact recreators		OR	Estimated Illness Rate (NGI): 32/1,000 primary contact recreators	
	Magnitude			Magnitude	
Element	GM (cce per 100 mL)	STV (cce per 100 mL)		GM (cce per 100 mL)	STV (cce per 100 mL)
qPCR[a]	470	2,000		300	1,280
Duration and Frequency: The waterbody GM should not be greater than the selected GM magnitude in any 30-day interval. There should not be greater than a 10 percent excursion frequency of the selected STV magnitude in the same 30-day interval.					

[a] EPA *Enterococcus* spp. Method 1611 for qPCR (95) Source: EPA 2012 (95)

CHAPTER 3. SPECIFIC AIMS

Research Question 1: Are human-associated *Bacteroides* indicators associated with an increased risk of illness among swimmers in contact with water?

- **Specific Aim 1:** Estimate the association between the presence/absence of human-associated *Bacteroides* indicators of fecal contamination and the 10-12 day risk of seven self-reported symptoms and illnesses (gastrointestinal, diarrhea, respiratory, rash, eye ailment, earache, urinary tract infection) among swimmers. Objectives of this aim are to:
    a. Stratify by type of water (i.e. marine vs. fresh).
    b. Examine effect measure modification by level of swimming exposure (head immersion, body immersion, swallowing water) on the additive scale.
    c. Examine effect measure modification by general indicator total *Enterococcus* measured by qPCR.

Research Question 2: Are human-associated chemical markers associated with an increased risk of illness among swimmers in contact with water?

- **Specific Aim 2:** Estimate the association between chemical markers of human-associated fecal contamination and the 10-12 day risk of seven selected self-reported symptoms and illnesses among swimmers. This aim is identical to Aim 1 except it examines chemical markers. Objectives of this aim are to:

a. Stratify by type of water (i.e. marine vs. fresh).
b. Examine effect measure modification by level of swimming exposure (head immersion, body immersion, swallowing water) on the additive scale.
c. Examine effect measure modification by general indicator total *Enterococcus* measured by qPCR.

These aims were met through secondary analyses of the National Environmental and Epidemiologic Assessment of Recreational Water (NEEAR) study, an observational cohort of approximately 54,000 visitors to four United States (US) freshwater and five marine beaches during 2003-2009. For both Aims, the self-reported symptoms and illnesses included gastrointestinal illness, diarrhea, and several non-enteric illnesses: respiratory illness, rash, eye ailments, earache, and urinary tract infection. Through the use of this large cohort, I estimated whether including a human-associated marker improves the general indicator-illness associations published by Wade et al. 2008, 2010 (56,57). Results from this study may help to characterize illness risks specific to human sources of fecal pollution from point and non-point sources.

CHAPTER 4. RESEARCH DESIGN AND METHODS

Overview

We addressed the two aims using data from the National Epidemiological and Environmental Assessment of Recreational (NEEAR) Water study, a prospective cohort study of 50,000+ visitors to four US freshwater and five marine beaches during 2003-2009. The aims estimated the association between exposure to human-associated *Bacteroides* (Aim 1) and chemical (Aim 2) fecal indicators in recreational waters and 10-12 day risk of self-reported illnesses. In our examination of these aims, we assessed type of water, level of swimming exposure, and additive interaction by the general fecal indicator, *Enterococcus*. Since the investigation of these aims involved secondary de-identified data analysis of NEEAR participants, the UNC Public Health-Nursing institutional review board granted an exemption because it did not constitute human subjects research (13-2274).

Parent Study: National Epidemiological and Environmental Assessment of Recreational (NEEAR) Water Study

1. Study design and population

The NEEAR water study was a prospective cohort study that enrolled 54,250 men, women and children visiting four US freshwater and five marine beaches during 2003-2009 to

examine associations between swimming exposure, water quality and swimming-associated illnesses. The study also collected and analyzed numerous chemical and microbial fecal indicators of water quality. Study design, population, and data collection details have been previously published (56,57,96) but is summarized in detail below.

2. Beach descriptions

The NEEAR study focused on beaches impacted by nearby sewage effluents because such pollution is believed to contain potential human fecal contamination and cause the highest human illness risks (97). Although it was conducted at nine beaches, this secondary analysis focuses on the seven beaches studied between 2003 and 2007. In 2003 and 2004, NEEAR studies were conducted at four freshwater beaches: Huntington Beach on Lake Erie near Cleveland, Ohio; West Beach on Lake Michigan at Indiana Dunes National Seashore in Portage, Indiana; Silver Beach on Lake Michigan near St. Joseph, Michigan; and Washington Park Beach on Lake Michigan in Michigan City, Indiana. In 2005 and 2007, NEEAR studies were conducted at three temperate marine beaches: Edgewater Beach near Biloxi, Mississippi; Fairhope Municipal Beach in Fairhope, Alabama; and Goddard Beach near Warwick, Rhode Island.

Beaches that were impacted by sources of human fecal contamination were specifically selected. All of the beaches were located within 7 miles of WWTPs or sewage effluent discharges providing a point source that discharged into a receiving stream, or one of its tributaries, in the beach watershed. All beach sites were selected so that they had sufficient variability in water quality so that the relationship between water quality and illness could be investigated without a control beach. Each beach also had to be generally compliant with local or

state water quality guidelines. Beach site locations and descriptions are shown in Figure 4.1 and Table 4.1.

3. Data collection: Health surveys

Trained interviewers approached all beach visitors as they arrived between 11:00 AM and 5:00 PM, and were enrolled if they provided verbal informed consent. Each participant completed three surveys, with an adult (≥18 years old) answering questions for other household members. At baseline, each participant completed an enrollment questionnaire about illnesses in the three days prior to their beach visit. Upon departure, participants completed a beach questionnaire about beach activities, water exposure (extent, time, duration and location), presence of underlying acute and chronic health conditions (including allergies), food and drink consumption, animal contact in the past 48 h, contact with sick persons in the past 48 h, other swimming in the past week, and demographics. A low-cost incentive was offered after completion of the beach questionnaire. Follow-up telephone interviews were conducted 10–12 days after the beach interview to collect information about the enteric and non-enteric illnesses (gastrointestinal, diarrhea, upper respiratory, skin rash, ear, eye, urinary tract infection) each beachgoer experienced since the beach visit, burdens experienced as a result of illness (e.g. missed days of work), and other swimming or water related activities, contact with animals, and consumption of high-risk foods since the beach visit. Interviews were conducted on weekends and holidays between May and September. Because of the acute nature and short duration of the enteric and non-enteric symptoms and illnesses in this study, repeated enrollment of participants was allowed. However, participants were ineligible if they had already completed the study in

the previous 28 days, were unaccompanied minors (<18 years), or did not speak English or Spanish.

4. Data collection: Outcome assessments

In a telephone interview 10-12 days following beach exposure, interviewers asked beachgoers to self-report if they had experienced any gastrointestinal illness, diarrhea, upper respiratory illness, eye ailments, earache, skin rash or urinary tract infection since their beach interview. The time period accounts for pathogens with longer incubation times, such as *Cryptosporidium* spp., a common waterborne pathogen. These heath outcomes are consistent with previous reports investigating the association between fecal indicator organisms and illness, to facilitate comparison (56,57,62,65,98,99). Responses to questions about symptoms or illness could take the form of Yes, No, Refused, or Don't know.

"Gastrointestinal illness" (GI illness) refers to any of the following: diarrhea (≥3 loose stools in a 24-hour period); vomiting; nausea and stomachache; or nausea or stomachache and interference with regular activities (missed time from work/regular activities due to illness).

''Respiratory illness'' refers to any two of the following: sore throat, cough, runny nose, cold, or fever.

''Rash'' refers to a rash or itchy skin.

''Eye ailments'' refers to eye infection or watery eye.

''Earache'' refers to earache, ear infection, or runny ears.

"Urinary tract infection" (UTI) refers to urinary tract infection or burning sensation when urinating.

Diarrhea was also be considered as a stand-alone outcome because it is frequently used as a definition of gastroenteritis in population-based surveillance e.g.(100,101).
Participants ill within the three days prior to their beach visit were excluded from analysis of the health outcome related to their baseline symptoms, but were eligible to be included in analyses of other outcomes.

Aim 1: Human-associated *Bacteroides* indicators and risk of illness

1. Study population

Participants eligible to be included in this Aim were those who visited beaches in which human-associated *Bacteroides* indicators were collected: Fairhope, Goddard, Huntington, Silver, Washington Park, and West Beaches (n=25,288).

2. Definition of swimming

The primary exposure is detection of human-associated *Bacteroides* indicator from water exposure. Beach visitors self-reported water exposure in three different ways that were not mutually exclusive: "body immersion" (immersion to the waist or higher); "head immersion"; and "swallowed water." Although some studies include head immersion in their definition of swimming (e.g. Fleisher et al. (61)), a previous report on two of the NEEAR beaches did not find appreciable differences in risk between those who immersed their head vs. their body (55). Therefore, our main analysis considered those who reported "body immersion" as being exposed to water. Other categories of water exposure (i.e. head immersion, swallowed water) were considered in sensitivity analyses. Participants who reported no water contact (i.e. "non-

swimmers") and those who reported having water contact, but not "body immersion" were excluded from analysis because they comprise a group with heterogeneous water exposure.

3. Exposure assessment

Water sample collection and analysis

Water samples were collected three times a day (8:00 AM, 11:00 AM, and 3:00 PM) along three transects perpendicular to the shoreline (57,102). At each transect, one-liter of water was collected in waist-high water (1m deep) and one-liter was collected in shin-high water (0.3m deep). Transects were at least 60m apart to encompass the entire swimming area. After collection, samples were maintained on ice at 1-4°C in coolers for up to 6h before polycarbonate membrane filtration. Filters were kept at -20°C and shipped on dry ice to EPA, Cincinnati for qPCR analysis. Filters were stored at -40 °C for up to six years before analysis. DNA was extracted from the filters by a simple bead milling procedure and aliquots corresponding to two-thirds of the total crude extracts were concentrated 2-fold and purified using a commercially available 96-well silica column based system (DNeasy, Qiagen, Valencia, CA) with binding and elution buffers from another system (DNA-EZ, Gene-Rite, North Brunswick, NJ) essentially as previously described (103).

Purified DNA extracts were analyzed for total *Enterococcus* (102) using a previously described and validated qPCR calibrator cell equivalent (CCE) method (55) and *Bacteroidales* markers using five different qPCR assays—GenBac3, HF183, BsteriF1, BuniF2, and HumM2—as indicated below. Total *Enterococcus* and total *Bacteroidales* (GenBac3) genetic markers detect general, non-source-specific fecal pollution (Siefring et al. 2008). QPCR assays targeting 16S rRNA gene markers of human-associated *Bacteroides* species clusters included HF183

TaqMan (hereafter HF183), BsteriF1, and BuniF2 (104). Of those, the HF183 assay has shown promise because of its abundance in human feces and sewage (i.e. high sensitivity, detection in samples that are actually of human origin), low cross-reactivity in chicken and dog feces, and absence in many other animals including cattle, pig, gull, and cat feces (104-107). The BsteriF1 and BuniF2 assays have shown high sensitivity, but lower specificity due to cross-reactivity with animal feces (104,105). The HumM2 assay targets a hypothetical protein potentially involved in remodeling surface lipopolysaccharides and polysaccharides (71). It has been found to be highly sensitive and specific to human feces and wastewater samples, but cross-reacted with sheep and elk feces at levels approaching those in human feces (71,105).

All qPCR analyses were performed in an Applied Biosystems StepOnePlus® using the above-mentioned primer and TaqMan™ hybridization probe assays (71,104,108). QPCR amplification was performed by using 5 µL of purified DNA extracts in a total reaction volume of 25 µL. Reagent mixes were prepared by combining 12.5 µL of TaqMan® Universal Master Mix (Applied Biosystems, Foster City, CA), 2.5 µL of 2 mg/ml bovine serum albumin, 1 µM of each primer, and 80 nM of probe for each reaction. Amplification occurred with an initial incubation at 50°C for 2 min followed by 95°C for 10 min, then forty PCR cycles of 95°C for 15s and 60°C for 1 min. Serial dilutions of commericially prepared plasmid DNA templates (Integrated DNA Technologies, Coralville, IA) containing the amplicons for each assay were analyzed as positive controls in each reaction plate. Limits of detection for each assay were based on the estimated plasmid copy number per reaction of the highest dilution of these templates that was routinely analyzed and detected (6 copies per reaction). Extracts of blank filters that were prepared in the same manner as the sample extracts were also analyzed as negative controls in each reaction plate. Potential interferences by the sample extracts to the

qPCR analyses were assessed by analyses of each extract with a multiplex version of the HF183 assay using an internal amplification control (IAC) template and by analyses with the Sketa22 assay for salmon testes DNA which was added to each sample as a sample processing control (SPC) prior to extraction (104). Criteria for classifying sample measurements as being unacceptable were offset Ct values from corresponding control samples of >1.5 and >3.0 for the IAC and SPC assays, respectively, as previously described (57,104).

Exposure coding

Due to a large proportion of human-associated *Bacteroides* data that was below the detection limit (~50-90%), I considered categorical classifications. In order to be the most sensitive, I initially created a binary variable for each *Bacteroides* marker that took the value of '1' if it was detected in 1 or more samples, and '0' otherwise. This resulted in very few exposed swimmers with illness, and would have presented problems estimating associations. Therefore, I modified the categorization so that each marker took the value of '1' if it was detected in at least two samples per day, and '0' otherwise. Thus, the primary exposure of interest in Aim 1 was the presence/absence of human-associated *Bacteroides* fecal indicators measured in water samples as one of four assays (HF183, BsteriF1, BuniF2, and HumM2). Because non-swimmers are unexposed to fecal indicator organisms from water, this aim was restricted to body immersion swimmers only. Alternative classifications of water exposure were explored in sensitivity analyses.

4. Outcome assessment

As stated in the previous section, health outcomes were assessed in a telephone interview conducted 10-12 days following beach exposure: GI illness, diarrhea, respiratory illness, earache, eye ailment, rash, and UTI.

5. Covariate assessment

Potential confounding factors plausibly associated with poor water quality and illness were identified from published literature or those associated with outcome and available from the health/enrollment questionnaire included environmental as well as demographic and beach characteristics. Potentially relevant environmental and meteorological covariates were recorded at each sampling time (8:00 AM, 11:00 AM, and 3:00 PM). These measures were available for inclusion as covariates, and included time, date, air temperature, water temperature, ultraviolet radiation, rainfall, cloud cover, wind speed, wind direction, water current direction, wave height, turbidity, pH, bather density, number of boats, number of animals and birds, and presence of debris. Potentially relevant demographic and beach covariates for this analysis were collected during the beach questionnaire at the end of the day of the beach visit and at the follow up telephone interview. They include age; sex; race/ethnicity; swimming within 48h before the beach visit or between the beach visit and telephone interview; beach site; allergies; contact with animals; contact with other persons with gastrointestinal illness; number of other beach visits; any other chronic illnesses (GI, skin, asthma); presence of beach festivals; eating any food or drink while at the beach; bather density; and boat density. For respiratory illness, rash, and eye ailments, use of insect repellent and sunblock were also considered.

We used directed acyclic graphs (DAG) (109,110) (visualized using DAGity (111)) to analyze these potential environmental, demographic, and beach covariates for confounders that would need to be adjusted to achieve the least biased estimate of association (Figure 4.2 – 4.4). It is worth noting that because fecal indicators by nature are non-pathogenic and act as a proxy for disease-causing microbes, the primary path of interest on this DAG is non-causal: Indicator ← Human source → Pathogen → Outcome. Thus, the least biased estimates would be produced with an adjustment set that closed all other non-causal, back-door paths and included Pathogen as a variable in the set.

In the construction of the DAG, environmental risk factors were further evaluated for their plausible influence on the outcome independent of exposure, as well as amount of missing data. Sunlight, water/air temperature, and rainfall totals from 3 PM the previous day to 8 AM on the current day (hereafter, rainfall) were conditions with the fewest missing data and most plausible association with a subset of the health outcomes. The DAG analysis identified a minimally sufficient adjustment set for each exposure-outcome relationship: beach, bather density, rainfall, sand exposure, water temperature (for GI illness, diarrhea, earache, and UTI outcomes); and beach, bather density, rainfall, sand exposure (for respiratory, rash, and eye outcomes). A second adjustment set consisting of the covariates in the minimally sufficient set plus age was also evaluated because it can be argued that age encompasses certain characteristics associated with intensity of swimming exposure (which was not captured in the DAG), and thus exposure to *Bacteroides* (e.g. children swim longer, swallow more water (56)) as well as being strongly associated with most outcomes. Covariates were coded as follows: beach (indicator coding: Fairhope, Goddard, Huntington, Silver, West, Washington Park), age (0-4, 5-11, 12-19, 20-34, ≥35), mean bathers (continuous), sand exposure (digging in sand or burying body in the

sand) (binary), rainfall (continuous), and water temperature (continuous). Results from a study day that occurred during a festival at Silver Beach were dropped from analysis because they were not representative of typical beach days.

6. Effect measure modifiers

A potential effect measure modifier (EMM) of the association between human-associated *Bacteroides* and illness was identified a priori: type of water matrix (marine/saltwater vs. freshwater). Type of water matrix was investigated as an EMM due to the possibility that it influences the concentration of microbial fecal indicators in water, particularly for molecular markers used in this study. In addition, there is limited research on the persistence of genetic material of human-associated *Bacteroides* markers in various water matrices to inform a decision. Nevertheless, modification of these marker-illness effect estimates by water matrix was of secondary interest, so was assessed by stratification.

A priori, we were also interested in whether the human-associated *Bacteroidales* markers, which proportedly indicate human source, act as modifiers of the association between non-specific total *Enterococcus* assayed by qPCR Method 1611 (CCE/ml) and illness. For that modification analysis, *Enterococcus* was treated as the main exposure and the *Bacteroides* marker was the binary modifier. For the primary effect measure modification analyses with the general indicator *Enterococcus*, the quantitated values were dichotomized in two ways according to 2012 EPA recreational water quality guidelines: above and below a geometric mean of 470 CCE/100ml (for an estimated illness rate of 36/1000 primary contact recreators), and above and below a geometric mean of 300 CCE/100ml (for an estimated illness rate of 32/1000 primary contact recreators) (95). Secondary effect measure modification analyses were also performed

with *Enterococcus* coded as a continuous variable (average $\log_{10}$ count of *Enterococcus* per day (CCE/100ml)). Risk difference modification was estimated with product interactions of *Enterococcus* and *Bacteroides* markers and then assessed by an interaction contrast (i.e., difference of risk differences) (112). The interaction contrast takes on the value of zero when the joint effects of two factors are simply additive (112).

7. Data analysis

Univariate analyses were conducted to explore the distribution of demographic, covariate data, non-specific and human-associated *Bacteroides* indicators, and health outcomes to identify the completeness and consistency of the data. They were examined using frequencies and percents for categorical variables, and descriptive statistics for continuous variables. The frequency of missing data was also evaluated for each variable. To reconcile inconsistencies, the environmental microbiologist responsible for data collection was consulted as needed.

We sought to use a binomial model to directly estimate risk differences (RD) and 95% confidence intervals (95% CI) for the relationship between human-associated *Bacteroides* markers and risk of illness among swimmers. However, due to well-documented problems with non-convergence (113-116), we explored other recommended alternatives, including modified Poisson regression with an identity link (115,117), the COPY method (114,118) and inverse-probability of exposure weighting (119) but encountered non-convergence issues for some indicator-illness associations. We decided to use model-based standardization (116,120-122) to produce standardized marginal risks and RD with 95% CI estimated using the delta method (123) and the total group as the standard. Logistic regression was used to estimate predicted probabilities of the outcome for every value of observed confounders and then combined as a

weighted average separately for both levels of the binary exposure. Thus, the effect estimates are estimated using predicted probabilities standardized to the same confounder distribution. The predicted probabilities were subtracted to produce a marginal estimate of the risk difference comparing *Bacteroides* marker exposure to no exposure. Robust standard errors were used to account for dependence of observations within a household (124).

As previously mentioned, we excluded participants ill within the three days prior to their beach visit from analysis of the health outcome related to their baseline symptoms, but they were eligible to be included in analyses of other outcomes. All analyses were completed using SAS version 9.4 (SAS Institute, Inc., Cary, NC,) and Stata version 13 (StataCorp, College Station, TX).

8. Sensitivity analyses

We investigated the robustness of our estimates through sensitivity analyses that tested several alternate ways of classifying swimming and *Bacteroides* exposure. First, we repeated our analyses using two additional definitions of swimmer: as participants who reported immersing their head under water, and participants who reported swallowing water. Second, we explored alternate exposure classifications since our primary one did not take into account intensity (i.e. cannot distinguish between situations when human fecal contamination is detected in multiple samples per day vs. two samples). We therefore explored exposure defined as 1) quartiles of each *Bacteroides* marker, with the referent (1^{st}) quartile being non-detect; and 2) a count of the number of *Bacteroides* markers detected per day (ranging from 0 to 4), where "detected" meant 2 or more of the daily samples taken were positive for the marker. Ultimately, we were unable to

investigate quartiles because of limitations in the quantitation of the *Bacteroides* markers (See discussion of limitations in Chapter 5 "Discussion" section).

Aim 2: Human-associated chemical markers and risk of illness

1. Study population

Participants eligible to be included in this aim were those who visited beaches in which human-associated chemical markers were collected: Edgewater, Huntington, Silver, Washington Park, and West Beaches (n=17,753).

2. Definition of swimming

The primary exposure for aim 2 was detection of human-associated chemical markers from water exposure. Similar to aim 1, the main analysis for this aim considered those who reported "body immersion" as being exposed to water. Participants who reported no water contact (i.e. "non-swimmers") and those who reported having water contact, but not "body immersion" were excluded from analysis because they comprise a group with heterogeneous water exposure. Other categories of water exposure (i.e. head immersion, swallowed water) were considered in sensitivity analyses.

3. Exposure assessment

Water sample collection and chemical analysis

Water samples for chemical analysis were collected in baked amber glass bottles on the Sunday of the weekend collection at 11:00 AM (Glassmeyer, personal communication). At West

and Huntington beaches, three one-liter water samples were collected in waist-high water (1m), for a total of 3 samples per day. At Silver, Washington Park, and Edgewater beaches, water samples were collected along two transects perpendicular to the shoreline and closest to the effluent. Two samples were collected at waist depth and two samples at shin depth (0.3m deep), for a total of 4 samples per day. After collection, samples were packed in coolers with ice during transport and at ≤4 °C alongside a travel blank (de-ionized water) until the following day, when they were packed on dry ice and shipped to USGS National Water Quality Laboratory in Lakewood, Colorado and the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas for extraction and analysis.

Because of the different physiochemical properties of the chemical compounds, three different analytical methods were used (40). For wastewater compounds and some pharmaceutical compounds, a whole-water sample was extracted using continuous liquid-liquid extraction and then analyzed using gas chromatography/mass spectrometry (GC/ MS) (125). Most pharmaceutical compounds were extracted by first passing 500 – 1000 ml filtered water through solid-phase extraction cartridges, then eluent was concentrated, and the final extract was analyzed using liquid chromatography/mass spectrometry positive-ion electrospray (126). Antibiotic compounds were extracted and analyzed by solid-phase extraction using tandem cartridges, and analyzed by liquid chromatography/mass spectrometry positive-ion electrospray on a single quadrapole mass spectrometer (127). Concentration is reported in µg/L.

Chemical marker exposure coding

Although 56 chemicals were assayed across the five beaches, only nine chemicals were assayed at every beach: acetaminophen, beta-sitosterol, bisphenol A, caffeine, cholesterol,

diethoxyoctylphenol, DEET, phenol and tributyl phosphate. I evaluated continuous (log_{10} transformed), categorical, and binary coding schemes for chemical concentrations. Continuous chemical concentrations were log_{10}-transformed because they were right-skewed. To avoid implausible values once transformed, chemical concentrations that had a value of zero were imputed with ½ the minimum non-zero value for that chemical. A daily average chemical concentration was provided for each beach-day, computed as the average of the log_{10} concentrations of all samples collected that day.

Due to a high proportion of chemical concentrations that were below the detection limit (~50-90%), I explored only categorical classifications. Each chemical marker was dichotomized by giving it a value of '1' if it was detected in all samples per day, and 0 otherwise. Thus, the primary exposure of interest in Aim 2 is the presence/absence of these nine chemical compounds that are markers of human presence in water samples that were measured at all 5 beaches in participants with body immersion exposure (n=9,109). Alternative classifications of this primary exposure were explored in sensitivity analyses. For a secondary analysis, all 56 chemicals were grouped into five broad categories: pharmaceuticals, fecal sterols/stanols, household waste products, industrial waste products, and chemicals with a potential for runoff (hereafter, runoff). The value of each category was a count of the number of chemical compounds belonging to it that were detected in all samples per day. For example, for a given beach and day, a value of '2' for the pharmaceutical category meant that there were '2' pharmaceutical compounds that were detected in all samples collected that day. Non-swimmers were considered unexposed to chemical compounds from water, and therefore excluded from the analysis.

Fecal indicator bacteria

Intestinal enterococci are validated, nonspecific indicators of fecal pollution used to measure water quality throughout the world. Total *Enterococus* spp. by qPCR (calibrator cell equivalents (CCE)/100 ml) was enumerated following water sample collection and subsequent membrane filtration according to previously published protocols (57,102,108).

4. Outcome assessment

The outcomes assessed for aim 2 were identical to aim 1: GI illness, diarrhea, respiratory illness, earache, eye ailment, rash, and UTI.

5. Covariate assessment

The same DAG used in aim 1 was used for aim 2 for the reason that both the *Bacteroides* and chemical markers represent two types of indicators of human fecal contamination in water. Though the mechanisms may arguably differ, the research question was still to determine the association between potential human-associated fecal markers and health outcomes, so the same DAG and minimally sufficient sets were used.

6. Effect measure modifiers

The effect measure modifiers assessed for aim 2 were identical to aim 1. Type of water matrix was investigated as an EMM of the association between human-associated chemical markers and illness using stratification. And the human-associated chemical markers (primary analysis) or chemical categories (secondary analysis) were investigated as binary modifiers of

the association between non-specific total *Enterococcus* assayed by qPCR Method 1611 (CCE/ml) and illness using an interaction contrast. Thus, the chemical categories were dichotomized for the modification analyses as follows: a value of '1' any chemicals belonging to that category were detected in all samples per day, and '0' otherwise.

7. Data analysis

Univariate analyses were conducted to explore the distribution of demographic, covariate data, non-specific and human-associated chemical indicators, and health outcomes to identify the completeness and consistency of the data. They were examined using frequencies and percents for categorical variables, and descriptive statistics for continuous variables. The frequency of missing data was also evaluated for each variable. To reconcile inconsistencies, the environmental microbiologist responsible for data collection was consulted as needed.

Because 56 chemicals encompassing ten broad categories were analyzed, we intended to use empirical Bayes modeling, a form of hierarchical regression in which all of the parameters for the Bayesian prior are generated from the data. Empirical Bayes methods offers improvements over conventional statistical methods in analyses of multiple exposures (or outcomes), particularly if the exposures can be grouped into categories according to similarity of expected effects on a particular outcome (referred to as "exchangeability of effects"); and in analyses of correlated exposures when there is limited prior information on the exposure-disease relationships (128-131). However, due to the fact that only nine chemicals were assayed at all five beaches, we chose to focus on those nine because they were generally more frequently detected and would have a larger sample size than the remaining chemicals. So for this aim, we examined the effects of the nine human-associated chemical markers (acetaminophen, caffeine,

cholesterol, beta-sitosterol, bisphenol A, diethoxyoctylphenol, *n*-*n*-diethyl-meta-toluamide (DEET), phenol, and tributyl phosphate measured at all five beaches on self-reported illness among body immersion swimmers.

Similar to aim 1, we used model-based standardization (116,120-122) to produce standardized marginal risks and RD with 95% CI estimated using the delta method (123) and the total group as the standard. Logistic regression was used to estimate predicted probabilities of the outcome for every value of observed confounders and then combined as a weighted average separately for both levels of the binary exposure. Thus, the effect estimates are estimated using predicted probabilities standardized to the same confounder distribution. The predicted probabilities were subtracted to produce a marginal estimate of the risk difference comparing each chemical marker exposure to no exposure. Robust standard errors were used to account for dependence of observations within a household (124). As previously mentioned, we excluded participants ill within the three days prior to their beach visit from analysis of the health outcome related to their baseline symptoms, but they were eligible to be included in analyses of other outcomes. All analyses were completed using SAS version 9.4 (SAS Institute, Inc., Cary, NC) and Stata version 13 (StataCorp, College Station, TX).

8. Sensitivity analyses

To determine if estimates were robust to different exposure categorizations, we examined additional classifications of swimming and chemical exposure. First, we repeated our analyses using two additional definitions of swimmer: as participants who reported immersing their head under water, and participants who reported swallowing water. Second, we explored a more sensitive binary chemical classification where each chemical was given the value of '1' if it was

detected in 1 or more samples per day, and 0 otherwise. The data did not permit classifications that make use of quantitative values.

Tables and Figures

Figure 4.1. NEEAR beach sites included in this study

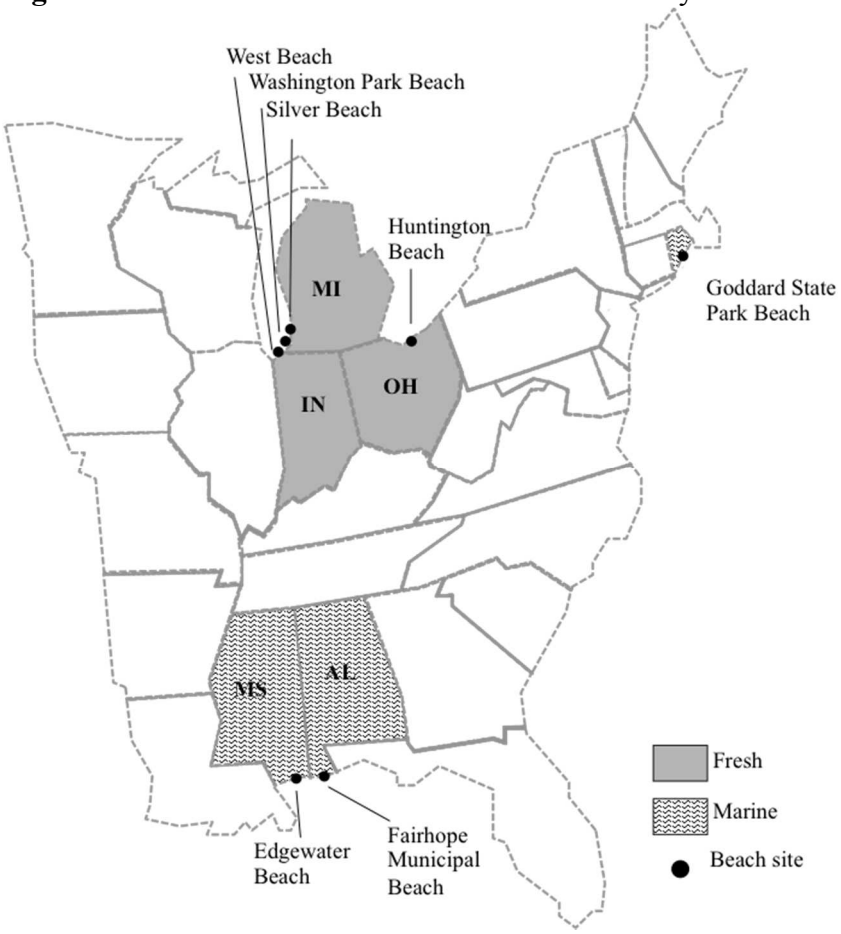


Table 4.1. Description of NEEAR beach sites included in this study

Beach	Year	Location	Water body type	Source of fecal pollution
Freshwater				
Huntington	2003	Lake Erie, (near Cleveland, OH)	Temperate	Treated WWTP
Silver	2004	Lake Michigan, (near St. Joseph, MI)	Temperate	Treated WWTP
Washington Park	2004	Lake Michigan, (in Michigan City, IN)	Temperate	Treated WWTP
West	2003	Lake Michigan, (Indiana Dunes National Seashore, IN)	Temperate	Treated WWTP
Marine				
Edgewater	2005	Biloxi, MS	Temperate	Treated WWTP
Fairhope	2007	Fairhope, AL	Temperate	Treated WWTP
Goddard	2007	West Warwick, RI	Temperate	Treated WWTP

Abbreviation: WWTP, wastewater treatment plant;

Figure 4.2. Directed acyclic graph - GI illness, diarrhea

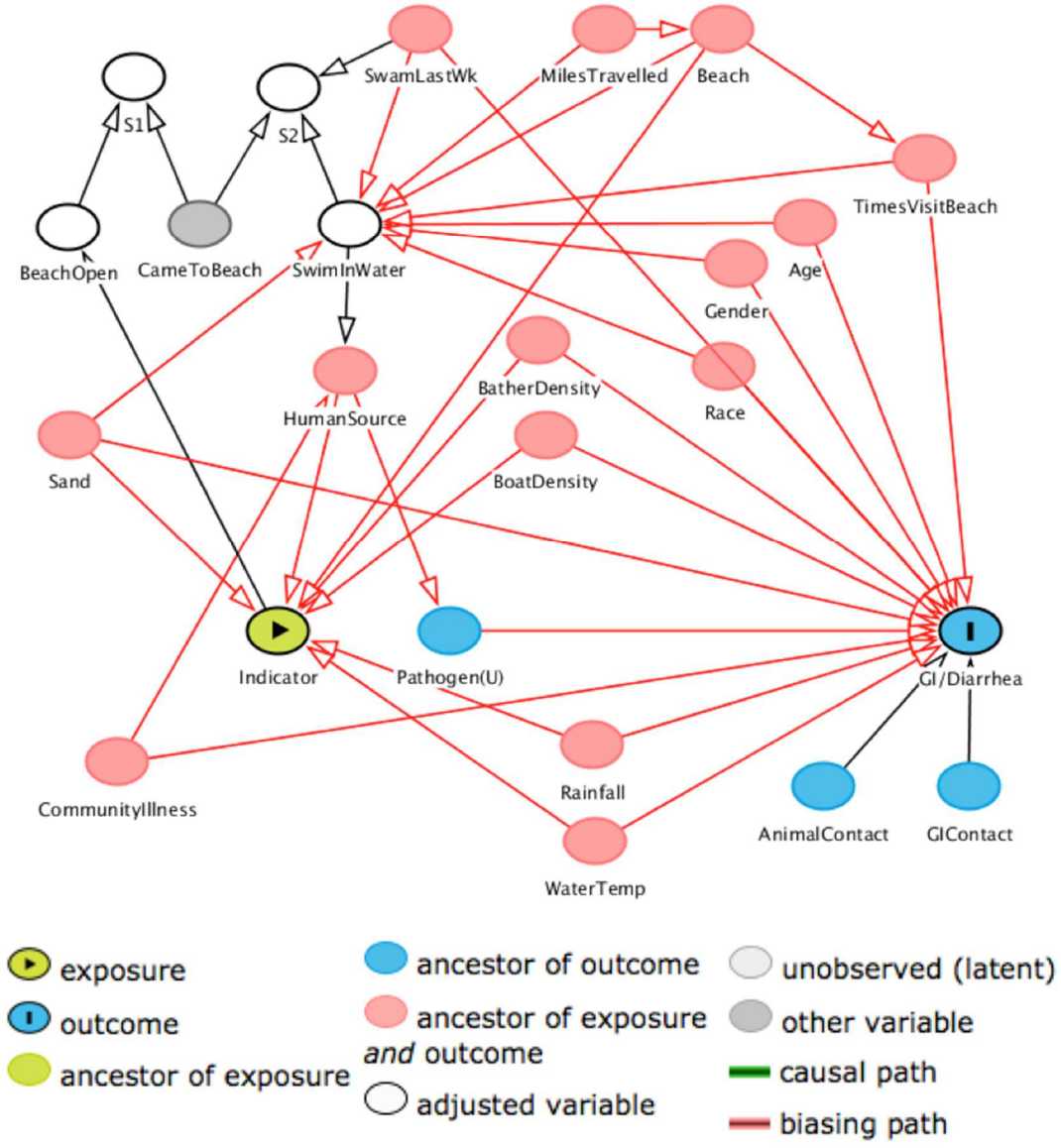


Figure 4.3. Directed acyclic graph - respiratory illness, eye ailment, rash

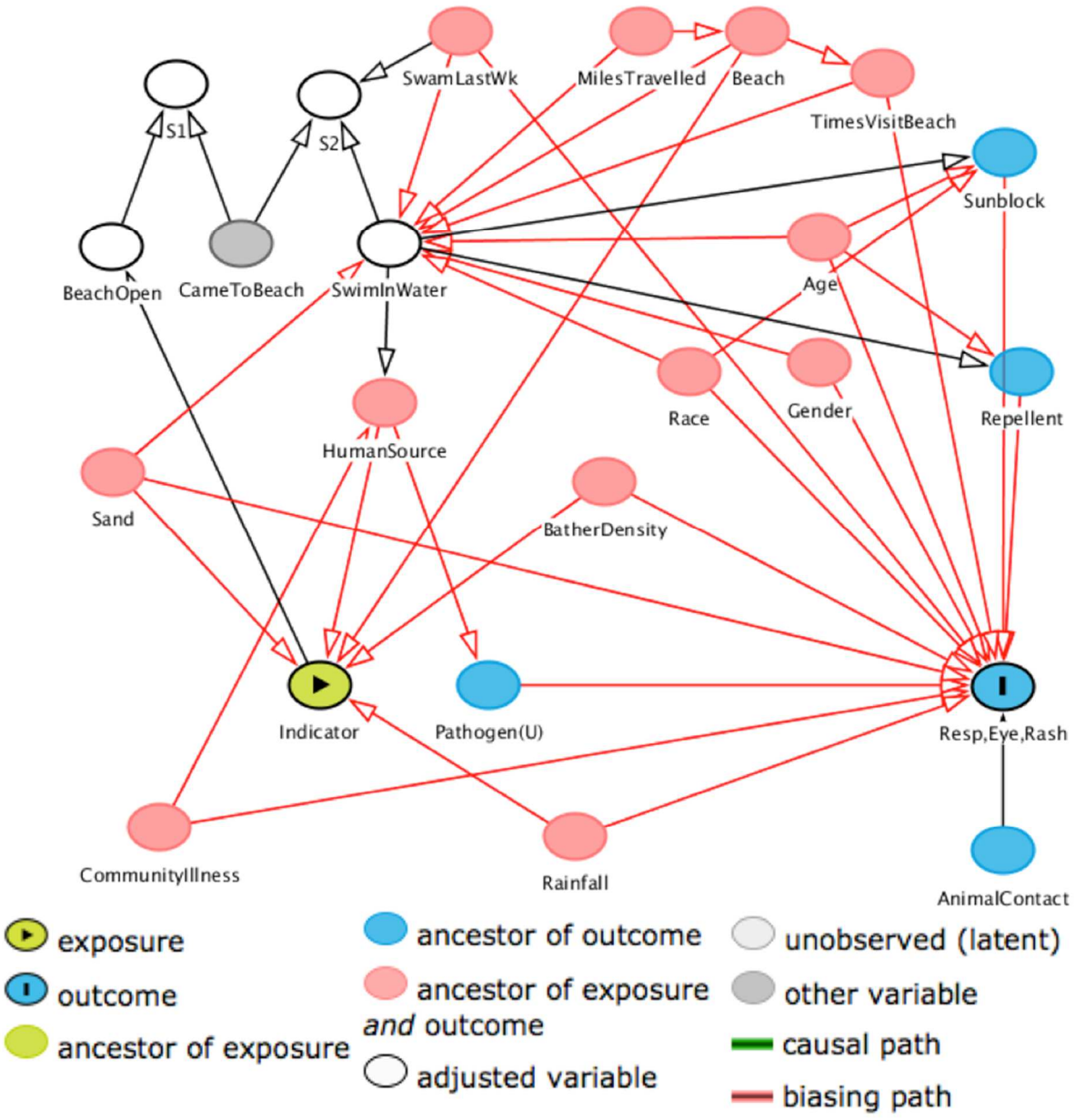
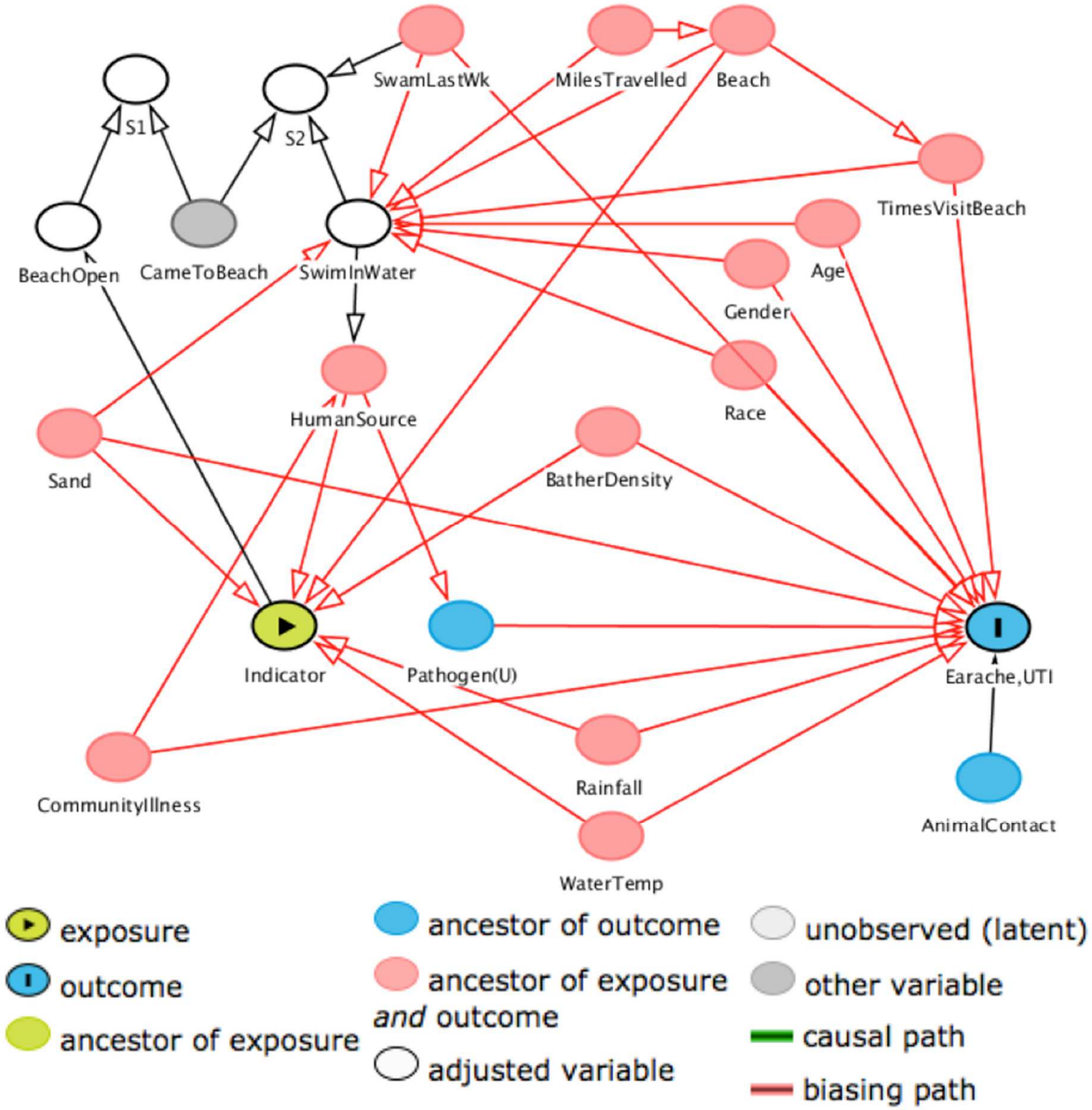


Figure 4.4. Directed acyclic graph - earache, UTI



CHAPTER 5. EXPOSURE TO HUMAN-ASSOCIATED FECAL INDICATORS AND SELF-REPORTED ILLNESS AMONG SWIMMERS AT RECREATIONAL BEACHES

Overview

Although fecal indicator bacteria are used to indicate the presence of fecal pollution and assess associated illness risks in recreational waters, few studies have examined illness risks associated with human-source-associated fecal bacteria. Our objective was to estimate associations between genetic markers of human-associated fecal bacteria and self-reported illness among swimmers at select U.S. beaches. We used data from 12,060 swimmers enrolled in the National Epidemiological and Environmental Assessment of Recreational Water study in 2003-2007. Participants were surveyed about beach activities, water exposure, and baseline symptoms on the day of their beach visit, and 10-12 days later, they were surveyed about illness symptoms experienced since the beach visit. Up to 18 water samples per day were tested for highly human-associated *Bacteroides* genetic markers using four assays (HF183, BsteriF1, BuniF2, HumM2). Adjusted standardized risk differences (RD) and 95% confidence intervals (CI) for the *Bacteroides*-illness associations among swimmers who immersed their bodies to the waist or higher were estimated using model-based standardization. *Bacteroides* markers were assessed as modifiers of the association between *Enterococcus* and illness using interaction contrast. A total of 2,422 water samples were analyzed for the four human-associated *Bacteroides* markers. The occurrence of the markers varied widely by beach and assay target. Among body immersion swimmers, we observed suggestive associations between risk of GI illness, diarrhea, and

respiratory illness and exposure to the human-associated *Bacteroides* marker BsteriF1. Small, positive associations were observed between the *Bacteroides* markers and earache and UTI, while small inverse associations were observed for HumM2 and HF183 markers and rash. Human-associated *Bacteroides* markers did not act as modifiers of general *Enterococcus* and illness. Patterns of risk were largely similar when stratified by water matrix (freshwater vs. saltwater). Sensitivity analyses indicated that risk estimates could be improved when combining multiple *Bacteroides* markers, although a clear dose-response pattern still did not emerge. It is not clear that these findings are generalizable to sites impacted predominantly by animal sources, runoff, or sporadic and diffuse sources of contamination.

Introduction

Fecal contamination of waters used for drinking, shellfish harvesting, and recreation is an important public health concern because of possible exposure to a wide range of disease-causing microorganisms. An estimated 170 million enteric and respiratory illnesses worldwide are attributed to swimming in and consuming shellfish from polluted water each year (1). Water pollution comes from a variety of point (e.g. sewage) and nonpoint (e.g. surface runoff, wildlife, leaky septic systems) sources. In recent decades, point source pollution and its effect on human health has received considerable attention due to legislation, such as the Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000 (132), a recent amendment to the Clean Water Act (133). However, the impacts of less-easily-identified-and-remedied nonpoint sources of pollution on water quality and health effects have not been addressed in current legislation; therefore, nonpoint sources are largely treated as if they were point sources. The growing demand for water resources has drawn attention to these issues and the need for more

information about this important aspect of water quality. Currently, the enumeration of fecal indicator bacteria (FIB) such as *Escherichia coli* (*E. coli*), fecal coliforms, and enterococci are used to monitor water bodies for the presence of and potential risk from exposure to waterborne pathogens that can cause human illness. These FIB have long been used because they are non-pathogenic, found in high levels in sewage and feces, and can be correlated with human health effects, but an important limitation is that they are found in both animal and human feces, and cannot be used to distinguish the source of pollution (15,134).

The identification of fecal pollution sources is vitally important for informing remediation of impaired water resources, in order to minimize the impact to public health. Human fecal contamination is generally considered of greater illness risk than contamination from non-human sources (9,97) since many waterborne pathogens transmitted via the fecal-oral route that cause human illnesses predominantly infect humans. In particular, much of the waterborne disease burden in developed countries is attributed to enteric viruses (e.g. Hepatitis A virus, Norwalk virus, and Norwalk-like virus) (7,15), which do not readily transmit infection to a host of a different species (2,3). Thus, elevated concentrations of FIB resulting from human sources are more likely to contain human-specific enteric pathogens (2,3) and be a major source of risk. Swimming in fecally-contaminated waters has been associated with self-limiting illness such as enteric and respiratory illness but can also result in more severe illness that warrants medical treatment, hospitalization, and lost days of school or work (135). Considering the approximately 301 million swimming visits made in the U.S. each year (136), the disease burden, even for self-limiting illness, is substantial.

Previous epidemiology studies that reported an increased risk of gastroenteritis (56,57,59,137), respiratory illness (138), ear ailments (139), and skin illness (59,61,62) among

swimmers exposed to increasing FIB levels relied on proximity to sewage effluent from wastewater treatment plants as a proxy for human fecal water contamination. In recent years, fecal source tracking (FST) tools capable of distinguishing human from animal fecal matter have been developed and validated (8,134). These tools include both new, host-associated microbial genetic markers, such as those from the genus *Bacteroides*, and new, rapid methods, such as quantitative polymerase chain reaction (qPCR) for detection of these markers. Critical questions that remain to be answered include whether these markers are associated with human illness and whether they represent an improvement over general, non-specific fecal indicator bacteria in terms of characterizing risk. To help determine the best applications for such human-associated markers, the relationship between these markers and human illness outcomes must be determined. The studies that have investigated this relationship are somewhat limited in size and scope (62,64,65,67). This paper seeks to address this gap. The primary objective of this study was to estimate the association between four human-associated *Bacteroidales* markers and self-reported illness among swimmers at six U.S. marine and fresh water beaches 10-12 days after exposure. A secondary objective was to determine whether these *Bacteroidales* markers, which purportedly indicate human source, act as modifiers of the association between a general *Enterococcus* indicator and illness.

Materials and Methods

Study design and beach descriptions

This study used data collected as part of the National Epidemiological and Environmental Assessment of Recreational Water (NEEAR) study from 2003-2007. The NEEAR study was a prospective cohort study that examined associations between microbial water quality and

swimming associated illnesses in visitors to freshwater and marine beaches. Beaches that were impacted by sources of human fecal contamination, including publicly owned treatment works, were specifically selected. The six beaches (four freshwater, two marine) used in this analysis were located within 7 miles of wastewater treatment plants or sewage effluent discharges believed to impact fecal contamination at the beach (Figure 5.1). In 2003 and 2004, NEEAR studies were conducted at four freshwater beaches: Huntington Beach on Lake Erie near Cleveland, Ohio; West Beach on Lake Michigan at Indiana Dunes National Seashore in Portage, Indiana; Silver Beach on Lake Michigan near St. Joseph, Michigan; and Washington Park Beach on Lake Michigan in Michigan City, Indiana. In 2007, NEEAR studies were conducted at two temperate marine beaches: Fairhope Beach in Fairhope, Alabama; and Goddard Beach near Warwick, Rhode Island. Criteria for beach selection are described previously (55-57,96).

Data collection

Data collection methods have been described previously (55-57). Briefly: all beachgoers were approached as they arrived, and were enrolled once they provided verbal informed consent. Each household group completed three surveys, with an adult (≥18 years old) answering questions for other household members. Upon arrival, each household completed an enrollment questionnaire about illnesses experience in the three days prior to their beach visit. Upon departure, participants completed an exit interview about beach activities, water exposure (extent, time, duration and location), presence of underlying acute and chronic health conditions (including allergies), food and drink consumption, animal contact in the past 48 h, contact with sick persons in the past 48 h, and demographic information for each household member. Follow-up telephone interviews were conducted 10–12 days after the beach interview to collect

information about the illness symptoms each household member experienced since the beach visit. Interviews were conducted on weekends and holidays between May and September. Respondents were ineligible if they had already completed the study in the previous 28 days, were unaccompanied minors (<18 years), or did not speak English or Spanish.

Study procedures, questionnaires, protocols and consent process were reviewed and approved by the Institutional Review Board (IRB) of the Centers for Disease Control and Prevention for the original study. For the analyses in this paper, IRB exemption was granted by University of North Carolina at Chapel Hill as the dataset was de-identified (Study# 13-2274).

Swim exposure definitions

Because we were interested in microbial markers present in fecally-contaminated water, this analysis was restricted to swimmers. For the purposes of this analysis, "swimmers" were those who reported "body immersion", defined as immersion to the waist or higher. Participants who reported no water contact (i.e. "non-swimmers") and those who reported having water contact, but not "body immersion" were excluded from analysis because they comprise a group with heterogeneous water exposure. Other categories of water exposure (i.e. head immersion, swallowed water) were considered in sensitivity analyses.

Health outcomes

In the telephone interview 10-12 days following beach exposure, several health outcomes were assessed, consistent with previous reports (56,57,65,98). "Gastrointestinal (GI) illness" referred to any of the following: diarrhea (≥3 loose stools in a 24-hour period); vomiting; nausea and stomachache; or nausea or stomachache and interference with regular activities (missed time

from work/regular activities due to illness). Diarrhea alone was also assessed as a separate outcome. ‘‘Respiratory illness’’ referred to any two of the following: sore throat, cough, runny nose, cold, or fever. ‘‘Rash’’ referred to a rash or itchy skin. ‘‘Eye ailments’’ referred to eye infection or watery eye. ‘‘Earache’’ referred to earache, ear infection, or runny ears. In addition to these previously reported outcomes, “urinary tract infection” (UTI) was also assessed and referred to urinary tract infection or burning sensation when urinating.

Participants ill within the three days prior to their beach visit were excluded from analysis of the health outcome related to their baseline symptoms, but were eligible to be included in analyses of other outcomes (e.g. those sick with respiratory illness were excluded from the respiratory analyses, but included in analyses of GI, diarrhea, rash, eye illness, earache, and UTI).

Water sample collection and analysis

Procedures for water sample collection and filtration have been described elsewhere (Haugland et al. 2005; Wade et al. 2010). Briefly: water samples were collected three times a day (8:00 AM, 11:00 AM, and 3:00 PM) along three transects perpendicular to the shoreline. At each transect, one-liter of water was collected in waist-high water (1m deep) and one-liter was collected in shin-high water (0.3m deep). Transects were at least 60m apart within the swimming area. After collection, samples were maintained on ice at 1-4°C in coolers for up to 6h before polycarbonate membrane filtration. The filters were kept at -20°C and shipped on dry ice to EPA, Cincinnati for qPCR analysis. Filters were stored at -40 °C for approximately two to six years before analysis. DNA was extracted from the filters by a simple bead milling procedure and aliquots corresponding to two-thirds of the total crude extracts were concentrated 2-fold and

purified using a commercially available 96-well silica column based system (DNeasy, Qiagen, Valencia, CA) with binding and elution buffers from another system (DNA-EZ, Gene-Rite, North Brunswick, NJ) essentially as previously described (103).

Purified DNA extracts were analyzed for *Bacteroidales* markers using four qPCR assays—HF183, BsteriF1, BuniF2, and HumM2— as indicated below. QPCR assays targeting 16S rRNA gene markers of highly human-associated *Bacteroides* species clusters included HF183 TaqMan (hereafter HF183), BsteriF1, and BuniF2 (104) while the HumM2 assay targets a hypothetical protein potentially involved in remodeling surface lipopolysaccharides and polysaccharides in other unidentified, highly human-associated *Bacteroides* species (71). Among these assays, the HF183 and HumM2 assays have shown the greatest promise for human source tracking due to their high sensitivity in detecting samples that are actually of human origin (e.g. human feces and sewage) as well as their low or nondetectable cross-reactivity with feces from many other animals (71,104-107). The BsteriF1 and BuniF2 assays have similarly shown high human source sensitivity, but lower specificity due to substantial cross-reactivity with feces from several animal groups including cats and dogs for BsteriF1 and pigs, sheep and chickens for BuniF2 (71,105). In addition, total *Bacteroidales* genetic markers were also analyzed using the GenBac3 qPCR assay as a marker of general, nonsource-specific fecal pollution (108).

All qPCR analyses were performed in an Applied Biosystems StepOnePlus® using the above-mentioned primer and TaqMan™ hybridization probe assays (71,104,108). QPCR amplification was performed by using 5 µL of purified DNA extracts in a total reaction volume of 25 µL. Reagent mixes were prepared by combining 12.5 µL of TaqMan® Universal Master Mix (Applied Biosystems, Foster City, CA), 2.5 µL of 2 mg/ml bovine serum albumin, 1 µM of each primer, and 80 nM of probe for each reaction. Amplification occurred with an initial

incubation at 50°C for 2 min followed by 95°C for 10 min, then forty PCR cycles of 95°C for 15s and 60°C for 1 min. Serial dilutions of commericially prepared plasmid DNA templates (Integrated DNA Technologies, Coralville, IA) containing the amplicons for each assay were analyzed as positive controls in each reaction plate. Limits of detection for each assay were based on the estimated plasmid copy number per reaction of the highest dilution of these templates that was routinely analyzed and detected (6 copies per reaction). Extracts of blank filters that were prepared in the same manner as the sample extracts were also analyzed as negative controls in each reaction plate. Potential interferences by the sample extracts to the qPCR analyses were assessed by analyses of each extract with a multiplex version of the HF183 assay using an internal amplification control (IAC) template and by analyses with the Sketa22 assay for salmon testes DNA which was added to each sample as a sample processing control (SPC) prior to extraction (104). Criteria for classifying sample measurements as being unacceptable were offset Ct values from corresponding control samples of >1.5 and >3.0 for the IAC and SPC assays, respectively, as previously described (57,104). Out of a total of 2,422 water samples, 2,336 samples passed the acceptance criteria for the HF183/IAC and Sketa22 control assays.

Fecal indicator bacteria

Intestinal enterococci are validated, nonspecific indicators of fecal pollution used to measure water quality throughout the world. Total *Enterococcus* spp. by qPCR (calibrator cell equivalents (CCE)/100 ml) was enumerated following water sample collection and subsequent membrane filtration according to previously published protocols (57,102,108).

Statistical analysis

The exposure of interest was the presence (detected in ≥2 samples/day)/absence (detected in 0-1 sample/day) of human-associated *Bacteroides* markers. We made this determination because although all beaches showed some indication of human contamination, there was a high proportion of samples where the human-source associated *Bacteroides* assays failed to detect genetic markers (Table 5.2). Alternative classifications of exposure were explored in sensitivity analyses. The outcome was a binary indicator of illness. Potential confounding factors plausibly associated with poor water quality and illness identified in published literature or those associated with outcome and available from the health/enrollment questionnaire included age; sex; race/ethnicity; swimming within 48h before the beach visit or between the beach visit and telephone interview; allergies; contact with animals; contact with other persons with gastrointestinal illness; number of other beach visits; any other chronic illnesses (gastrointestinal, skin, asthma); presence of beach festivals; eating any food or drink while at the beach; bather density; boat density; and environmental conditions such as sunlight, water/air temperature, and rainfall totals from 3 PM the previous day to 8 AM on the current day. For respiratory illness, rash, and eye ailments, use of insect repellent (binary) and sunblock (binary) were considered. Indicator variables representing beach were included in all models to control for differences in baseline illness among beaches. We used directed acyclic graphs (109,110) (visualized using DAGity (111)) to analyze the potential confounders and identified a minimally sufficient adjustment set for each exposure-outcome relationship: beach, bather density, rainfall, sand exposure, water temperature (for GI, diarrhea, earache, and UTI outcomes); and beach, bather density, rainfall, sand exposure (for respiratory, rash, and eye outcomes). A second adjustment set consisting of the covariates in the minimally sufficient set plus age was also evaluated

because it can be argued that age encompasses certain characteristics associated with intensity/duration of swimming exposure, and thus exposure to *Bacteroides* (e.g. children swim longer, swallow more water (56)) as well as being strongly associated with most outcomes. Estimates were similar using both adjustment sets, therefore we only present estimates using the adjustment set with age (results using the alternate set provided upon request). Covariates were coded as follows: beach (categorical: Fairhope, Goddard, Huntington, Silver, West, Washington Park), age (0-4, 5-11, 12-19, 20-34, ≥35), mean bathers (continuous), sand exposure (digging in sand or burying body in the sand) (binary), rainfall (continuous), and water temperature (continuous). Robust standard errors were used to account for dependence of observations within a household (124).

We used model-based standardization (116,120-122) to estimate standardized marginal risks, risk differences (RD), and 95% confidence intervals (95% CI) using the delta method (123) and the total group as the standard. Logistic regression was used to estimate predicted probabilities of the outcome for every value of observed confounders and then combined as a weighted average separately for both levels of the binary exposure. Thus, the effect estimates are estimated using predicted probabilities standardized to the same confounder distribution. The predicted probabilities were subtracted to produce a marginal estimate of the risk difference comparing *Bacteroides* marker exposure to no exposure. Modification of these marker-illness effect estimates by water matrix (freshwater vs. saltwater) was of secondary interest, so was assessed by stratification.

Effect measure modification of the association between *Enterococcus* assayed by qPCR Method 1611 (CCE/ml) and illness was examined to evaluate whether the occurrence of each of the *Bacteroides* markers improved the association of the general indicator with illness. In this

analysis, *Enterococcus* was treated as the main exposure and the *Bacteroides* marker was the binary modifier. For the primary effect measure modification analyses with the general indicator *Enterococcus*, the quantitated values were dichotomized in two ways according to 2012 EPA recreational water quality guidelines: above and below a geometric mean of 470 CCE/100ml (for an estimated illness rate of 36/1000 primary contact recreators), and above and below a geometric mean of 300 CCE/100ml (for an estimated illness rate of 32/1000 primary contact recreators) (95). Secondary effect measure modification analyses were also performed with *Enterococcus* coded as a continuous variable (average $\log_{10}$ count of *Enterococcus* per day (CCE/100ml)). Risk difference modification was estimated with product interactions of *Enterococcus* and *Bacteroides* markers and then assessed by an interaction contrast (i.e., difference of risk differences) (112). The interaction contrast takes on the value of zero when the joint effects of two factors are simply additive (112).

As previously mentioned, we excluded participants ill within the three days prior to their beach visit from analysis of the health outcome related to their baseline symptoms, but they were eligible to be included in analyses of other outcomes. All analyses were completed using SAS version 9.4 (SAS Institute, Inc., Cary, NC) and Stata version 13 (StataCorp, College Station, TX).

Sensitivity analyses

We investigated the robustness of our estimates through sensitivity analyses that test several alternate ways of classifying swimming and *Bacteroides* exposure. First, we repeated our analyses using two additional definitions of swimmer: as participants who reported immersing their head under water, and participants who reported swallowing water. Second, we explored

alternate exposure classifications since our primary one did not take into account intensity (i.e. cannot distinguish between situations when human fecal contamination is detected in multiple samples per day vs. two samples). We therefore explored exposure defined as a count of the number of *Bacteroides* markers detected per day (ranging from 0 to 4), where "detected" meant 2 or more of the daily samples taken were positive for the marker.

Results

Demographic characteristics

Data were available for 25,288 participants at six beaches between 2003 and 2007 (Table 5.1). More than one-third of participants (36%) did not have any contact with the water during their visit. A total of 12,060 of them (48%) immersed their body up to the waist or higher during their visit. Compared to non-swimmers, swimmers were younger (mean age 22.8 years vs. 35.5 years; $p<0.0001$), male (48% vs. 37%) and Hispanic (13% vs. 10%; $p<0.0001$); travelled farther to get to the beach (mean of 45 miles vs. 38 miles; $p<0.0001$); and had more sand contact (56% vs. 21%; $p<0.0001$). A quarter of both swimmers and non-swimmers reported having a chronic illness at the beach interview. Few participants (≤6%) reported acute illnesses ranging from GI to rash in the three days prior to their beach visits. Though descriptive statistics are provided for all participants here, the final analysis was restricted to body immersion swimmers only (n=12,060).

Distribution of human-associated Bacteroides markers

While the human-associated *Bacteroides* markers were detected at all of the beaches, the frequency of samples with detected markers varied widely by beach and by marker target (Table 5.2; Figure 5.2). Silver and Goddard Beaches had the highest frequencies of detects, regardless

of marker, while Fairhope Beach had the lowest. Within-beach, BuniF2 and BsteriF1 assay markers were generally detected more frequently than HF183 and HumM2. For BuniF2, the proportion of human-associated *Bacteroides* detected ranged from 15% (Fairhope) to 63% (Silver) of samples; for BsteriF1, the range was 11% (Fairhope) to 46% (Goddard). HF183 markers were detected in between 4% (Fairhope) and 49% (Silver) of samples. HumM2 assay markers were detected least often across all beaches, with 2% (Fairhope) to 17% (Silver) of samples testing positive. Non-specific general *Bacteroides* fecal contamination was widely present in >98% of samples tested using the GenBac3 assay.

Illness risk associated with human-associated Bacteroides markers

Frequencies and standardized marginal estimates of the RD (95% CI) comparing exposure to each *Bacteroides* marker vs. no exposure and illness are shown in Figure 5.3 and Supplemental Table A.1a-c. The strongest associations were those with the BsteriF1 marker, and occasionally the BuniF2 marker. Across all beaches, we observed an increase in risk of GI illness, diarrhea, and respiratory illness associated with detection of human fecal contamination by the BsteriF1 marker (RD=1.9% (0.1%, 3.7%); RD=1.3% (-0.2%, 2.7%); and RD=1.1% (-0.2%, 2.5%), respectively). Smaller increases of <1.0% were seen for BsteriF1 and eye ailments, earache, and UTI. Unexpectedly, detection of human fecal contamination by the HumM2 and HF183 markers was associated with a decreased risk of rash (RD=-1.0% (-1.9%, -0.2%) and RD=-1.1% (-2.4%, 0.3%), respectively). In general, many estimates were close to the null, and estimates closer to the null were more precise than those farther from the null. Estimates of GI illness risk were most precise (reflecting the high incidence) and UTI estimates were least precise. Similar patterns were seen when fresh and marine water were examined separately with

the exception of the BuniF2 marker and respiratory illness, and the BuniF2 marker and eye ailments. Marine beach estimates were less precise than fresh water estimates.

Assessing modification of Enterococcus-*illness association with* Bacteroides *markers*

We investigated whether the presence of *Bacteroides* markers of human fecal contamination strengthened the previously observed association between the general *Enterococcus* indicator and illness (56,57). Standardized marginal estimates of the RD (95% CI) for the association of *Enterococcus* and GI illness, diarrhea, and respiratory illness modified by each *Bacteroides* marker are shown in Table 5.3 and Supplemental Table A.3a-d (for *Enterococcus* < and ≥470 CCE/ml). Overall, interaction contrast estimates were imprecise and did not suggest the presence of modification between strata of *Bacteroides* marker. However, one pattern that did emerge for GI illness and diarrhea were that RD estimates were closer to the null when human-associated *Bacteroides* markers were present than in the absence of the markers. Interaction contrast values for BuniF2 were unable to be estimated for diarrhea and respiratory illness due to small sample size. Similarly, the associations between *Enterococcus* dichotomized at 300 CCE/ml and illness did not vary by presence of any *Bacteroides* marker; interaction contrast estimates were imprecise (Table 5.4 and Supplemental Table A.4a-d). Results for modification with *Enterococcus* assessed continuously are shown in Supplemental Table A.5. As shown previously in 2008 and 2010 by Wade et al. (56,57), we see an increased risk of GI illness and diarrhea with each 1-$\log_{10}$ increase in *Enterococcus* qPCR value (RD=1.4% (0.6%, 2.3%) and RD=1.1% (0.6%, 1.7%), respectively). However, consistent with results from the analysis with binary *Enterococcus,* interaction contrast estimates are imprecise and do not

suggest that human-associated *Bacteroides* markers are modifying the association between *Enterococcus* and swimming-associated illnesses.

Sensitivity analyses

An exploration of two alternate categorizations of exposure in sensitivity analyses (as one, two, three, or four *Bacteroides* markers vs. no exposure) showed little evidence of association between *Bacteroides* markers and illness (Supplemental Tables A.2a-c). While a clear dose-response pattern was not observed, the greatest risk of illness appeared to occur when 2 or 3 *Bacteroides* markers were detected. Because intensity of water contact might determine the extent of exposure to general fecal indicators and human-associated *Bacteroides*, we also repeated our analysis among those who had immersed their head in water (Supplemental Table A.6) and among those who swallowed water (Supplemental Table A.7). Estimates for head immersion swimmers were consistent with what was found for body immersion swimmers, but more imprecise. Estimates for swimmers who swallowed water were generally farther from the null, and imprecise.

Discussion

The primary goal of this study was to describe the association between the occurrence of four different human-associated *Bacteroides* markers and self-reported illness among swimmers. In this study, we found little clear evidence of an association between these markers and illness, though we observed a pattern of increased risks for GI illness, diarrhea, and respiratory illness with BsteriF1 exposure, and a pattern of decreased risks with rash and HumM2 and HF183 detection. In addition, none of the four markers modified the association between the currently-

used general indicator, *Enterococcus* by qPCR, and the outcomes assessed. That finding suggests that having an indicator of human source does not add any additional information to the prediction of illness risks above and beyond what the general indicator provides.

Our findings of no association between human-associated *Bacteroides* markers and swimming-associated illness were unexpected in light of findings from previously published reports of general, non-specific *Enterococcus* and *Bacteroides* at NEEAR beaches. General *Enterococcus* by qPCR was associated with an increased risk of GI illness in Great Lakes beaches and marine beaches, and general *Bacteroides* qPCR was associated with increased risk of GI illness in marine beaches (56,57). While our findings may seem counter-intuitive, there are potential reasons for the disparate findings between the general and human-specific markers. First and perhaps most importantly, human-associated *Bacteroides* markers are less persistent and less abundant than general *Enterococcus* markers, which may account for why health associations have previously been established with general enterococci measured by qPCR, but not among *Bacteroides* markers in this analysis. Several authors have reported that general, non-specific fecal indicator organisms such as total *Enterococcus* qPCR (Entero1a), total *Bacteroides* qPCR (GenBac3, AllBac), and *E. coli* (140) persist longer compared to human-associated FST genetic markers, including HF183 (140-142), HumM2 (142), BacHum (141), and BuniF2 (143). These studies were largely conducted in river, marine, and freshwater microcosms spiked with human sewage, but the findings suggest that human-associated markers are most useful as a conservative indicator of indicators of recent human fecal contamination.

While relatively little is known about factors influencing the decay of human-associated *Bacteroides* markers in aquatic environments, as an obligate anaerobes, their survival in the ambient aquatic environment is thought to be limited (Kreader 1998; Korajkic 2014}. Lower

temperature is believed to result in longer persistence for both fecal indicators and human-associated markers (141,144), while the effect of sunlight is mixed. In general, ambient sunlight has not been found to affect the survival or persistence of molecular FST markers (141,143,145), but other studies report shorter persistence of *Bacteroides* molecular markers HF183 and HumM2 (142). The source of environmental factors (e.g. artificial vs. natural sunlight) may also have a profound effect on relative rates of decay of genetic markers.

Second, the human-associated *Bacteroides* markers in this analysis were detected at relatively low densities, which may have limited our ability to estimate associations. Between 58% and 90% of the *Bacteroides* samples were below the limit of detection of the assay, prompting us to dichotomize them for analysis. In contrast, general, nonsource specific *Bacteroides* and *Enterococcus* were detected at relatively high densities >98%. This hypothesis seems supported by the fact that among the four assays in this study, the ones that showed patterns of association consistent with what we would expect also tended to be the more commonly occurring targets (e.g. BsteriF1). In addition, low target densities were also the main explanation for a finding of no association from one of the few previous studies of illness risks and human-associated markers. In a study of 8,797 beach visitors at a non-point source beach in California, Colford et al. (64) concluded that the association between illness and human-specific viruses adenovirus 40, 41 and norovirus could not adequately be evaluated because the viruses were rarely detected.

A third possibility is that the sensitivity of the detection of human marker may have been impacted by long-term freezer storage at -40 °C, but it is difficult to predict the magnitude of the impact. Reduced sensitivity would mean a decreased ability to detect the marker if it was indeed present, leading to false negatives, an underestimation of *Bacteroides* markers, and possibly

resulting in bias towards the null, which is what we observed. Thus, our findings of no association may be a consequence of extended storage times, but it is impossible to know for certain.

Finally, it is possible that human specific markers may be better associated with illness at sites without a known source of sewage contamination, impacted by a wider range of fecal contaminants, or with lower levels of overall fecal contamination. This analysis was performed among beaches with known human sewage inputs and high nonspecific fecal contamination, as evidenced by >98% of samples being positive for general *Bacteroides*. The level of fecal contamination may have been so high that the addition of a human marker did not add any additional information to the estimation of illness risk. Indeed, in our analysis, Table 4 and 5 RD estimates were closer to the null when human-associated *Bacteroides* markers were present compared to when they were absent for GI illness and diarrhea. In beaches with lower levels of overall fecal contamination, perhaps human markers would be more informative.

Our findings will help inform the limited evidence base of studies estimating the association between human source-associated bacterial fecal indicators and human illnesses. Our result of no association is consistent with the findings from three previous studies, though each used assays targeting different human-associated markers and all were conducted at non-point-source beaches. In a study of a marine beach impacted by urban runoff, Arnold et al. found *Enterococcus faecium* and *Enterococcus faecalis* densities were not consistently associated with swimmer illness (67) using the Scorpion-2 qPCR illness. And in the California study mentioned earlier, Colford et al. found no association between viruses adenovirus and norovirus and illness. Similarly, in a small study of 1,303 beach visitors at a marine beach, Sinigalliano et al. (62) found no association with the HF8 and UCD *Bacteroides* markers. An additional study by

Colford in 2012 at a marine beach impacted by urban runoff did find an increased risk of enteric illness with exposure to human fecal contamination measured by the Scorption-2 *Enterococcus* qPCR marker. To the best of our knowledge, our study represent the largest study to date investigating human-associated fecal markers and risk of illness, and the first conducted in settings where sewage is the primary source of pollution. Future studies investigating HF183, HumM2, BsteriF1, and BuniF2 may need to be even larger to be able to estimate associations given the low abundance in this study.

Though there are few studies that have investigated illness risks from human-associated fecal indicators, numerous previous studies have demonstrated an increased risk of gastrointestinal, diarrhea, respiratory, skin, eye, and ear illnesses among swimmers exposed to elevated general fecal indicator bacteria levels (54-60). Although these studies demonstrated the value of fecal indicators, many relied on proximity to sewage effluent as a proxy for human presence. Findings from studies where non-point sources of pollution are the predominant contaminant have been more inconsistent, with some studies reporting an association due to point and non-point sources (59,61-63,65), while others do not (64,65). But even with these non-point sources, a known human source of fecal contamination may have been nearby ((59)). One strength of our study is that it did not rely on a proxy; instead, the source of fecal contamination was directly assessed from the water via the *Bacteroides* markers. This approach may be of particular interest for investigating water bodies that are impacted by non-point sources.

This study has several limitations. As a proxy for an individual swimmer's exposure, we relied on measures of daily average water quality. Although these average daily measures may not be indicative of actual individual exposure, characterizing individual exposure would have been difficult and impractical. Body immersion swimmers entered the water at multiple time

periods and locations and were exposed for varying durations of time (mean duration=65 min±60 min). The study design allowed for the collection of water samples three times a day (8:00AM, 11:00AM, and 3:00PM) and at two water depths (shin height (0.3m) and waist height (1.0m)) and three beach locations to capture the variety of fecal indicator exposures a participant may experience in the water. The cohort design also allowed us to measure water quality over a wide range of study days, so we were able to capture varying water quality conditions in a large study population.

Additionally, a common limitation of this type of large-scale study of water quality is the reliance on self-reported, non-specific symptoms and signs (e.g. eye ailment). Such broadly-defined symptoms may have obscured more specific effects of fecal indicators. However, the prospective nature allowed us to determine temporality and the 10-12 day follow up period reflected the incubation time for likely pathogens that would cause the symptoms of interest. In addition, the use of self-reported outcomes allowed us to capture the diversity of symptoms potentially associated with recreational water exposure. While the health outcomes may have been affected by recall bias, it is unlikely that recall would be differential by varying levels of water quality.

Among the strengths of this study was its focus on members of the *Bacteroidales* order as targets for qPCR methods to detect human-specific fecal pollution. Because *Bacteroidales* are among the most dominant bacteria in the human gut (70), these organisms have been at the forefront of efforts to develop methods that target human sources. HF183 and HumM2 are two of the most promising markers for human fecal source tracking (106). While less studied and showing apparently greater cross-reactivity with other animal sources, BsteriF1 and BuniF2 have also shown promise as potentially more environmentally abundant human associated markers

(71,104,105). Nevertheless, high frequencies of samples giving either non-detects or otherwise generally weak qPCR signals (high Ct measurements) were encountered with all of the assays in this study. Because of this, the *Bacteroides* markers were not analyzed as quantitative variables, which may have limited the ability to make inferences. The decision to dichotomize was also influenced by our uncertainty about the effects of the long-term freezer storage on the filter samples as mentioned previously, which may have also limited the ability to make inferences. To mitigate these potential limitations, sensitivity analyses were performed with exposure defined as a count of whether 0, 1, 2, 3, or 4 of the *Bacteroides* markers were detected per day. Findings were robust to different exposure definitions although risk estimates did improve with use of multiple markers. Nevertheless, findings from this study make an important contribution to the literature determining the suitability of these assays as alternative fecal indicators. Also, even without a strong association with health, human-associated markers may help identify the source of pollution, which provides water quality managers with information to efficiently and effectively focus remediation efforts.

Conclusion

In this study, we found that human-associated *Bacteroides* markers did not strongly improve associations with swimming-associated illness compared to general, non-source specific indicators already in use at beach sites impacted by sewage effluent. However, patterns of increased disease risks were observed for the BsteriF1 marker and several outcomes that deserve further investigation. These findings may have been influenced by long storage times of membrane filters or other methodological challenges that could be overcome in the future. Human-associated markers may also better characterize risk at sites without a known impact

from sewage, or at sites impacted by runoff or a broader range of fecal contamination. This is one of the first and largest studies to evaluate associations between exposure to human-associated *Bacteroides* markers and self-reported illness among swimmers.

Disclaimer: The views expressed in this paper do not necessarily reflect EPA policy

Tables and Figures

Figure 5.1. Freshwater and marine beach sites

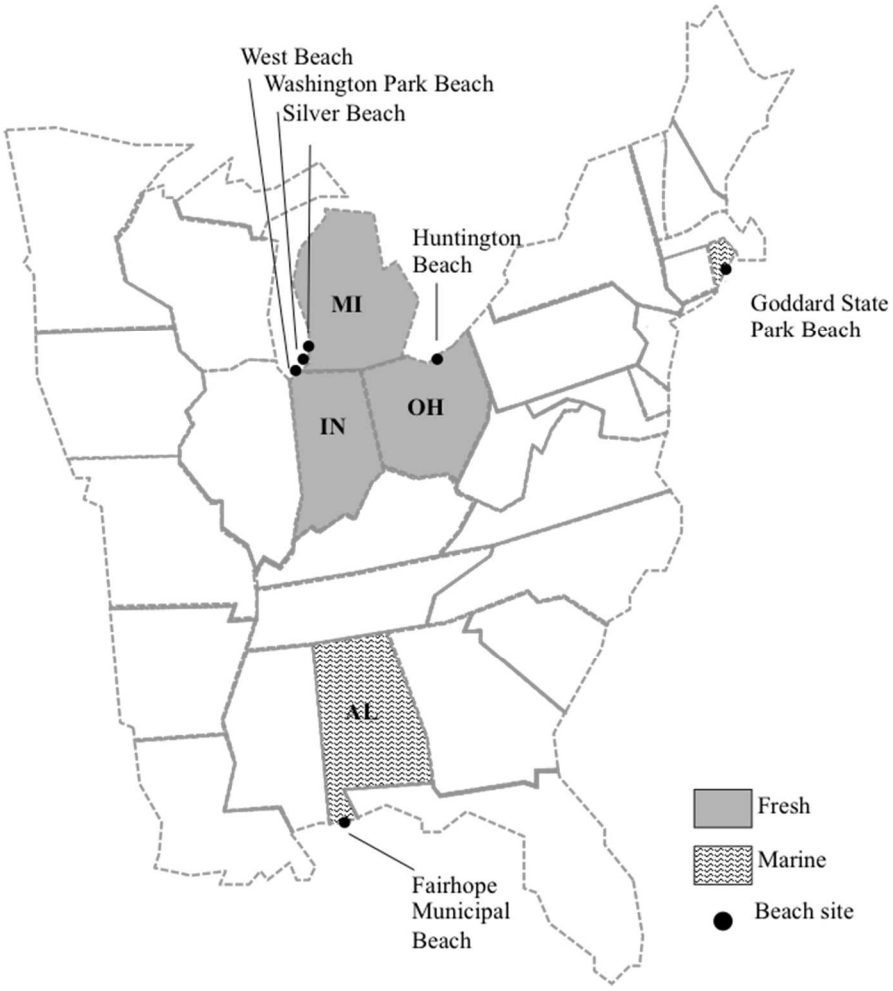


Table 5.1. Characteristics of NEEAR participants by body immersion status (n=25,288)

	No water contact	Water contact	
		No body immersion†	Body immersion†
	(n=9091)	(n=4137)	(n=12060)
	N* (%)	N* (%)	N* (%)
Sex			
Male	3729 (41)	1511 (37)	5814 (48)
Female	5356 (59)	2621 (63)	6225 (52)
Missing	6	5	21
Age in years (mean (SD), min/max)	35.5 (18), 0/101	31.9 (17.6), 0/85	22.8 (16.7), 0/103
0-4	503 (6)	364 (9)	1218 (10)
5-11	390 (4)	355 (9)	2953 (25)
12-19	911 (10)	367 (9)	1848 (16)
20-34	2399 (27)	991 (24)	2534 (22)
35 and over	4748 (53)	1981 (49)	3182 (27)
Missing	140	79	325
Race			
White	7266 (80)	3514 (85)	9501 (79)
Black	562 (6)	191 (5)	518 (4)
Asian	171 (2)	72 (2)	140 (1)
American Indian	22 (0)	17 (0)	29 (0)
Hispanic	905 (10)	248 (6)	1520 (13)
Multi-race / other	148 (2)	78 (2)	298 (2)
Missing	17	17	54
Illnesses in the 3 days prior to beach visit			
GI illness	247 (3)	93 (2)	221 (2)
Vomiting	94 (1)	50 (1)	123 (1)
Sore throat	510 (6)	227 (5)	676 (6)
Earache	114 (1)	39 (1)	167 (1)
Eye ailment	45 (0)	22 (1)	56 (0)
Rash	225 (2)	89 (2)	261 (2)
Urinary tract infection	44 (0)	22 (1)	49 (0)
Any history of chronic GI, skin, respiratory illness or allergies			
No	6521 (72)	2943 (71)	8970 (74)
Yes	2568 (28)	1192 (29)	3090 (26)
Missing	2	2	0
Swam in last week			

	No water contact	Water contact	
		No body immersion†	Body immersion†
	(n=9091)	(n=4137)	(n=12060)
	N* (%)	N* (%)	N* (%)
No	6689 (74)	2830 (69)	6844 (57)
Yes	2388 (26)	1295 (31)	5198 (43)
Missing	14	12	18
Miles travelled to beach			
0-20	5168 (58)	2114 (52)	5447 (46)
20-60	2531 (28)	1229 (30)	4141 (35)
60-100	561 (6)	349 (9)	1110 (9)
>100	720 (8)	408 (10)	1204 (10)
Missing	111	37	158
Frequency of travel to beach in summer			
0-1 times	2891 (32)	1648 (40)	4166 (35)
2-5 times	3071 (32)	1389 (34)	4483 (37)
>5 times	3110 (34)	1091 (26)	3396 (28)
Missing	0	0	0
Sand contact			
Dug in sand	1884 (21)	1797 (43)	6662 (55)
Buried body in sand	261 (3)	267 (6)	1871 (16)
Missing	0	0	0
Consumed food			
No	4613 (51)	1764 (43)	4226 (35)
Yes	4417 (49)	2348 (57)	7805 (65)
Missing	61	25	29
Animal contact 2 days prior to or after beach visit, or between beach visit and phone interview			
No	2586 (28)	942 (23)	2849 (24)
Yes	6505 (72)	3195 (77)	9211 (76)
Missing	0	0	0
All beaches			
Fairhope	853 (9)	340 (8)	823 (7)
Goddard	1584 (17)	305 (7)	1080 (9)
Huntington	1535 (17)	548 (13)	757 (6)
Silver	3140 (35)	1742 (42)	5372 (45)
West	722 (8)	475 (11)	1668 (14)
Washington Park	1257 (14)	727 (18)	2360 (20)

NEEAR, National Environmental and Epidemiologic Assessment of Recreational Water study; N, number; SD, standard deviation

* Sums may not add up to totals because of missing values

† Swimmers were those with body immersion (defined as immersion to the waist or higher). Those without water contact or with water contact but not body immersion were not included in the analysis but are shown in this descriptive table for completeness.

Table 5.2. Human *Bacteroides* markers detected by qPCR (n=2336 total samples)

Indicator	Detected in samples N (%)	Non-detected samples N (%)	Missing samples* N (%)	False positive rate† (%)
HumM2	233 (10)	2103 (90)	0	0.00
HF183	646 (28)	1690 (72)	0	0.15
BsteriF1	671 (29)	1665 (71)	0	0.20
BuniF2	972 (42)	1364 (58)	0	0.10

* Missing out of the 2,336 samples that passed quality control measures.
† Proportion of samples that test positive for the assay but are in fact negative.

Figure 5.2. Proportion of *Bacteroides* samples detected by beach

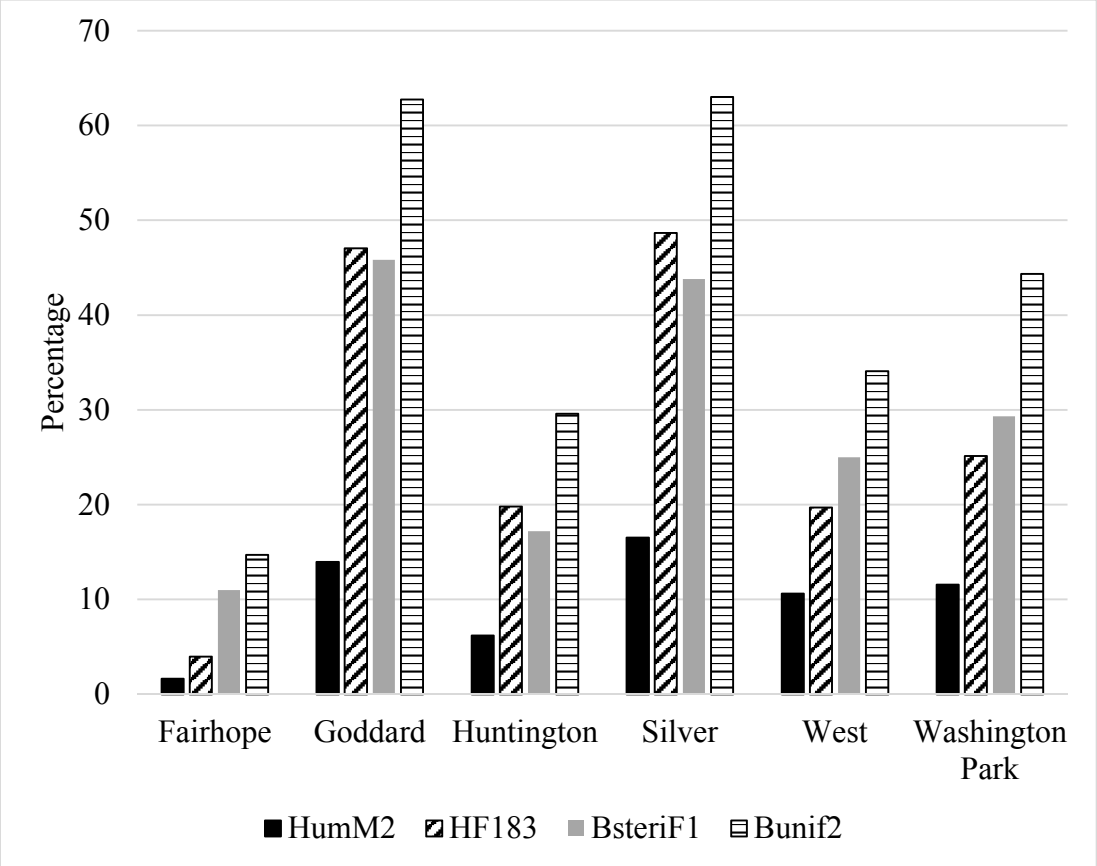
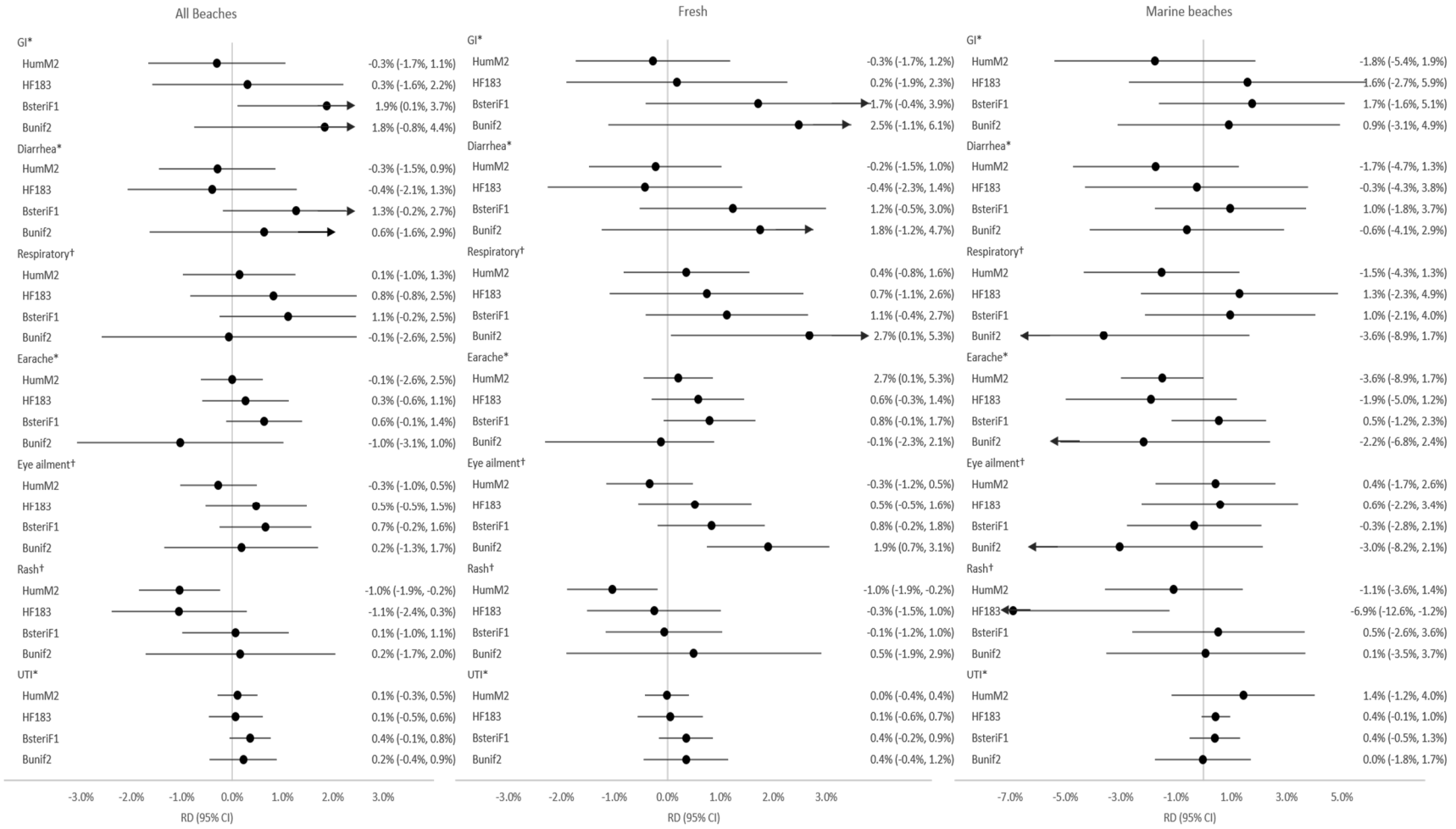


Figure 5.3. Standardized risk differences (95% CI) for the association between illness and human-associated *Bacteroides* markers among body immersion swimmers in all beaches, fresh water and marine beaches



Arrows show estimates that extend beyond field of vision of diagram.
*Adjusted for beach, age, mean bathers, sand, rain, water temperature
† Adjusted for beach, age, mean bathers, sand, rain

Table 5.3(a-c). Modification of the adjusted standardized RD (95% CI) for the association between illness and *Enterococcus* qPCR Method 1611 above and below EPA guidelines (geometric mean of 470 CCE/100ml for an illness rate of 36/1000) with detection/non-detection of *Bacteroides* markers among body immersion swimmers in all beaches

Table 5.3(a). **GI illness**

Gastrointestinal Illness							
Marker (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Crude Risk (%)**	**Adjusted Risk (%)***	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main association					0.6% (-2.5%, 3.7%)	
HumM2							
0-1	<470	466	5397	8.6	8.4	Ref	
	≥470	42	317	13.2	9.9	1.6% (-2.5%, 5.6%)	
≥ 2	<470	429	5781	7.4	8.2	Ref	
	≥470	20	195	10.3	7.5	-0.7% (-4.8%, 3.4%)	**-2.3% (-7.5%, 2.9%)**
HF183							
0-1	<470	216	2231	9.7	7.8	Ref	
	≥470	38	274	13.9	9.6	1.8% (-2.5%, 6.0%)	
≥ 2	<470	679	8947	7.6	8.5	Ref	
	≥470	24	238	10.1	7.7	-0.8% (-4.7%, 3.1%)	**-2.5% (-7.9%, 2.8%)**
BsteriF1							
0-1	<470	153	1796	8.5	7.0	Ref	
	≥470	4	72	5.6	3.7	-3.3% (-7.1%, 0.4%)	
≥ 2	<470	742	9382	7.9	8.6	Ref	
	≥470	58	440	13.2	9.3	0.7% (-2.8%, 4.2%)	**4.1% (-0.9%, 9.1%)**
BuniF2							
0-1	<470	50	559	8.9	6.8	Ref	
	≥470	1	17	5.9	3.9	-2.8% (-9.9%, 4.2%)	
≥ 2	<470	845	10619	8.0	8.4	Ref	
	≥470	61	495	12.3	8.8	0.3% (-2.9%, 3.6%)	**3.2% (-4.6%, 11%)**

NA, Not able to be estimated.

* Adjusted for beach, age, mean bathers, sand, rain, water temperature

Table 5.3(b). **Diarrhea**

Diarrhea							
Marker (samples)	Enterococcus (CCE/100ml)	Cases	N	Crude Risk (%)	Adjusted Risk (%)*	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--	Main association					0.5% (-2.0%, 3.1%)	
HumM2							
0-1	<470	322	5397	6.0	5.7	Ref	
	≥470	30	317	9.5	6.6	0.8% (-2.3%, 4.0%)	
≥ 2	<470	270	5781	4.7	5.4	Ref	
	≥470	17	195	8.7	5.7	0.3% (-3.4%, 3.9%)	**-0.6% (-4.9%, 3.7%)**
HF183							
0-1	<470	162	2231	7.3	5.8	Ref	
	≥470	28	274	10.2	6.7	0.9% (-2.7%, 4.4%)	
≥ 2	<470	430	8947	4.8	5.5	Ref	
	≥470	19	238	8.0	5.7	0.2% (-3.1%, 3.6%)	**-0.6% (-5.1%, 3.8%)**
BsteriF1							
0-1	<470	104	1796	5.8	4.8	Ref	
	≥470	2	72	2.8	1.9	-2.8% (-5.8%, 0.1%)	
≥ 2	<470	488	9382	5.2	5.8	Ref	
	≥470	45	440	10.2	6.5	0.7% (-2.1%, 3.5%)	**3.5% (-0.5%, 7.6%)**
BuniF2							
0-1	<470	38	559	6.8	5.3	Ref	
	≥470	0	17	0.0	0.0	NA	
≥ 2	<470	554	10619	5.2	5.6	Ref	
	≥470	47	495	9.5	6.3	0.7% (-2.1%, 3.4%)	**NA**

NA, not able to estimated.

* Adjusted for beach, age, mean bathers, sand, rain, water temperature

Table 5.3(c). **Respiratory illness**

Respiratory illness							
Marker (samples)	Enterococcus (CCE/100ml)	Cases	N	Crude Risk (%)	Adjusted Risk (%)*	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--	Main association					-1.3% (-4.0%, 1.4%)	
HumM2							
0-1	<470	321	5236	6.1	5.9	Ref	
	≥470	14	309	4.5	4.8	-1.1% (-4.2%, 2.0%)	
≥ 2	<470	328	5603	5.9	6.1	Ref	
	≥470	8	187	4.3	4.5	-1.7% (-6.4%, 3.1%)	**-0.5% (-6.1%, 5.1%)**
HF183							
0-1	<470	141	2182	6.5	5.6	Ref	
	≥470	8	268	3.0	3.0	-2.6% (-5.3%, 0.0%)	
≥ 2	<470	508	8657	5.9	6.2	Ref	
	≥470	14	228	6.1	6.3	0.1% (-4.5%, 4.8%)	**2.8% (-2.7%, 8.3%)**
BsteriF1							
0-1	<470	97	1726	5.6	5.1	Ref	
	≥470	2	68	2.9	2.5	-2.6% (-6.2%, 1.0%)	
≥ 2	<470	552	9113	6.1	6.3	Ref	
	≥470	20	428	4.7	4.8	-1.4% (-4.4%, 1.6%)	**1.2% (-3.4%, 5.8%)**
BuniF2							
0-1	<470	38	546	7.0	6.0	Ref	
	≥470	0	17	0.0	0.0	NA	
≥ 2	<470	611	10293	5.9	6.0	Ref	
	≥470	22	479	4.6	4.9	-1.1% (-4.0%, 1.8%)	**NA**

NA, not able to estimated

*Adjusted for beach, age, mean bathers, sand, rain

Table 5.4(a-c). Modification of the adjusted standardized RD (95% CI) for the association between illness and *Enterococcus* qPCR Method 1611 above and below EPA guidelines (geometric mean of 300 CCE/100ml for an illness rate of 32/1000) with detection/non-detection of *Bacteroides* markers among body immersion swimmers in all beaches

Gastrointestinal Illness							
Marker (samples)	Enterococcus (CCE/100ml)	Cases	N	Crude Risk (%)	Adjusted Risk (%)*	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--	Main association					1.6% (-1.0%, 4.3%)	
HumM2							
0-1	<300	429	5104	5.8	8.2	Ref	
	≥300	79	610	6.7	10.8	2.6% (-0.6%, 5.8%)	
≥ 2	<300	428	5752	5.7	8.1	Ref	
	≥300	21	224	4.5	7.5	-0.7% (-4.6%, 3.2%)	**-3.3% (-7.9%, 1.4%)**
HF183							
0-1	<300	195	2048	6.1	7.8	Ref	
	≥300	59	457	5.5	10.3	2.6% (-1.1%, 6.3%)	
≥ 2	<300	662	8808	5.6	8.3	Ref	
	≥300	41	377	6.9	8.8	0.4% (-3.1%, 3.9%)	**-2.1% (-7%, 2.7%)**
BsteriF1							
0-1	<300	131	1643	4.9	6.5	Ref	
	≥300	26	225	8.0	9.3	2.8% (-1.9%, 7.4%)	
≥ 2	<300	726	9213	5.9	8.6	Ref	
	≥300	74	609	5.4	9.1	0.5% (-2.4%, 3.5%)	**-2.2% (-7.6%, 3.1%)**
BuniF2							
0-1	<300	33	444	5.9	5.6	Ref	
	≥300	18	132	9.1	12.0	6.4% (-0.6%, 13.3%)	
≥ 2	<300	824	10412	5.7	8.4	Ref	
	≥300	82	702	5.6	8.8	0.3% (-2.4%, 3.1%)	**-6.0% (-13.4%, 1.3%)**

NA, Not able to be estimated.

* Adjusted for beach, age, mean bathers, sand, rain, water temperature

Table 5.4(b)**. Diarrhea**

Diarrhea							
Marker (samples)	Enterococcus (CCE/100ml)	Cases	N	Crude Risk (%)	Adjusted Risk (%)*	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--	Main association					2.0% (-0.4%, 4.3%)	
HumM2							
0-1	<300	291	5104	5.7	5.5	Ref	
	≥300	61	610	10.0	8.2	2.6% (-0.2%, 5.4%)	
≥ 2	<300	269	5752	4.7	5.3	Ref	
	≥300	18	224	8.0	6.0	0.6% (-3.0%, 4.2%)	**-2.0% (-6.2%, 2.1%)**
HF183							
0-1	<300	144	2048	7.0	5.7	Ref	
	≥300	46	457	10.1	8.4	2.7% (-0.8%, 6.1%)	
≥ 2	<300	416	8808	4.7	5.3	Ref	
	≥300	33	377	8.8	6.9	1.6% (-1.6%, 4.7%)	**-1.1% (-5.5%, 3.2%)**
BsteriF1							
0-1	<300	87	1643	5.3	4.4	Ref	
	≥300	19	225	8.4	7.4	3.0% (-1.3%, 7.3%)	
≥ 2	<300	473	9213	5.1	5.7	Ref	
	≥300	60	609	9.9	6.9	1.2% (-1.4%, 3.8%)	**-1.8% (-6.5%, 3.0%)**
BuniF2							
0-1	<300	25	444	5.6	4.5	Ref	
	≥300	13	132	9.8	10.2	5.7% (-1.6%, 13.0%)	
≥ 2	<300	535	10412	5.1	5.5	Ref	
	≥300	66	702	9.4	6.8	1.3% (-1.2%, 3.8%)	**-4.4% (-12.1%, 3.3%)**

NA, not able to estimated

* Adjusted for beach, age, mean bathers, sand, rain, water temperature

Table 5.4(c)**. Respiratory illness**

Respiratory Illness							
Marker (samples)	Enterococcus (CCE/100ml)	Cases	N	Crude Risk (%)	Adjusted Risk (%)*	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--	Main association					0.2% (-2.1%, 2.6%)	
HumM2							
0-1	<300	294	4947	5.9	5.8	Ref	
	≥300	41	598	6.9	6.5	0.6% (-1.9%, 3.2%)	
≥ 2	<300	326	5572	5.9	6.1	Ref	
	≥300	10	218	4.6	5.1	-0.9% (-5.5%, 3.7%)	**-1.6% (-6.6%, 3.4%)**
HF183							
0-1	<300	124	2001	6.2	5.5	Ref	
	≥300	25	449	5.6	5.0	-0.5% (-3.1%, 2.1%)	
≥ 2	<300	496	8518	5.8	6.1	Ref	
	≥300	26	367	7.1	6.9	0.8% (-3.0%, 4.6%)	**1.3% (-3.3%, 5.9%)**
BsteriF1							
0-1	<300	81	1577	5.1	4.8	Ref	
	≥300	18	217	8.3	6.7	1.9% (-2.2%, 5.9%)	
≥ 2	<300	539	8942	6.0	6.2	Ref	
	≥300	33	599	5.5	5.5	-0.8% (-3.3%, 1.8%)	**-2.6% (-7.2%, 1.9%)**
BuniF2							
0-1	<300	26	435	6.0	5.5	Ref	
	≥300	12	128	9.4	6.0	2.3% (-3.6%, 8.2%)	
≥ 2	<300	594	10084	5.9	6.0	Ref	
	≥300	39	688	5.7	5.8	-0.2% (-2.7%, 2.3%)	**-2.5% (-8.7%, 3.8%)**

NA, not able to estimated

*Adjusted for beach, age, mean bathers, sand, rain

CHAPTER 6. EXPOSURE TO HUMAN-ASSOCIATED CHEMICAL INDICATORS OF FECAL CONTAMINATION AND SELF-REPORTED ILLNESS AMONG SWIMMERS AT RECREATIONAL BEACHES

Overview

Fecal indicator bacteria, commonly used to regulate recreational water quality, cannot discriminate among sources of contamination. The use of anthropomorphic chemicals as host-specific indicators of fecal contamination requires an understanding of relationships with illness risks; however, this research has not been conducted to date. We estimated associations between chemical markers of human fecal pollution and self-reported illness among body immersion swimmers at five U.S. beaches enrolled in the National Epidemiological and Environmental Assessment of Recreational Water. NEEAR participants were surveyed about beach activities, water exposure, and baseline symptoms on the day of their beach visit, and illness symptoms experienced 10-12 days later. RDs were estimated using model-based standardization, adjusted for beach, bather density, sand contact, rain at 8A.M., and water temperature. Robust standard errors were calculated due to clustering within household. Chemical markers were assessed as modifiers of the association between *Enterococcus* and illness using interaction contrast. Human-associated chemical markers were detected at all beaches at low levels (parts per billion or smaller). We observed little evidence of association between chemical markers and illness but several patterns were visible. For the more plausible outcomes of GI illness and diarrhea, bisphenol A and cholesterol showed positive associations of approximately 1.7% and 1.0%,

respectively. Implausible inverse associations were also observed between several chemicals and respiratory illness. Risk differences for the association between general *Enterococcus* and GI illness, eye ailments, and respiratory illness were greater in magnitude by 3-5% in the presence of phenol than in the absence of phenol. Among the chemical categories, exposure to household wastewater chemicals was associated with an increased risk of respiratory illness. All other chemical markers and chemical categories were not consistently associated with elevated risks of illness, nor were they an improvement over general *Enterococcus* at beaches impacted by human sources of fecal contamination. Under the conditions observed in this study, human-associated chemicals were not consistently associated with swimming-associated illness. Additional research is needed to support the use of chemical biomarkers to quantify risk of illness and identify sources contributing to fecal pollution of recreational water.

Introduction

The quality of water used for drinking and recreation is currently monitored through the enumeration of fecal indicator bacteria (FIB), which indicate the probable presence of pathogenic contaminants associated with human and animal waste. Fecal waste is a major cause of poor water quality resulting in environmental degradation, economic losses (146,147), and illness risks such as gastrointestinal, respiratory, eye, ear, and skin infections (1,60,135,148). In the US, *E.coli* and *enterococcus* are the FIB recommended for detection of fecal contamination in fresh and marine recreational waters (95). Culture-based methods of measuring these traditional indicators require 24-48 hours to complete and the indicators cannot be used to differentiate between sources of fecal contamination (15,134), which are often necessary for effective remediation because contamination can arise from numerous human and non-human

sources. In recent years, pollution from non-point sources such as surface runoff, agricultural deposits and leaky septic systems, has surpassed that from point sources, which are remediated through federal regulation, as the leading cause of water quality problems (149). Accurate and reliable methods of identifying pollution sources will provide an indication of types of pathogens that may be expected and risk of infection from them.

To address the limitations of traditional FIB, rapid methods for identifying fecal contamination sources that target host-specific microbial or chemical markers have been developed (2,11,36,42,150-152). Much of the source-tracking research has focused on host-specific gene products of microbial markers such as members of the genus *Bacteriodales* or *Bifidobacterium* using rapid methods such as real-time or quantitative polymerase chain reaction (11,144). In addition, chemical compounds such as caffeine (40,153), pharmaceuticals (73,154), personal care products (40,73), and industrial chemicals (40) associated with septic, manure and wastewater treatment plant effluent as well as fecal sterols and their derivatives (42,155,156) have also been suggested as anthropogenic markers in sewage. These compounds provide evidence as to source because they are associated with human metabolism, activity or sanitary sewage. They fall into three broad categories: compounds produced and excreted by humans (e.g. coprostanol); compounds ingested almost exclusively by humans (e.g. caffeine, carbamazepine); and those that make it into the human waste stream (e.g. fluorescent whitening agents). As many as 35 compounds have been shown to be useful as indicators of anthropogenic pollution in wastewater effluent in the US (40) and river and coastal environments in Japan (73).

The differing patterns of fate, transport, survival, and persistence between human-source chemical markers and microbial markers means they may be able to be used in combination as part of a source tracking "toolbox" to yield greater confidence in source-water quality

assessment since no single indicator is ideal (11,157). Chemicals have the advantage of low detection limits, more rapid sample preparation and analysis times than culture methods, and some may be more temporally or geographically stable (11,75). Further, chemicals do not have problems of regrowth in the environment (144), though they may degrade (75,158) or persist downstream of the effluent (40). However, the relationship between chemical compounds (as an indicator of human-derived fecal pollution) and illnesses caused by waterborne human fecal pollution (e.g. gastroenteritis) is unknown. The lack of this information limits the utility of chemicals as a fecal source marker. This paper seeks to address that gap.

To determine if there is a link between chemical concentration and negative health impacts associated with exposure to waterborne pathogens, we used data from a large, multi-site cohort study. Our primary objectives were to (1) estimate the association between chemical markers of human-derived fecal pollution and self-reported illness among bathers, and (2) determine whether chemical markers were able to identify source when used in combination with conventional fecal indicator *Enterococcus* by qPCR and culture. The investigation of an association between chemical source tracking markers and incidence of illness is an important step in the evaluation of these chemicals to serve as indicators of human fecal material.

Materials and Methods

Study design and population

This study uses data gathered from beachgoers participating in the National Epidemiological and Environmental Assessment of Recreational Water (NEEAR) study from 2003-2005 (56,57). The NEEAR study was a prospective cohort study that examined associations between microbial water quality and swimming associated illnesses in visitors to

freshwater and marine beaches impacted by sewage. Participants included in this analysis include those enrolled four freshwater beaches: Huntington Beach on Lake Erie near Cleveland, Ohio; West Beach on Lake Michigan at Indiana Dunes National Seashore in Portage, Indiana; Silver Beach on Lake Michigan near St. Joseph, Michigan; and Washington Park Beach on Lake Michigan in Michigan City, Indiana; and one temperate marine beach, Edgewater Beach near Biloxi, Mississippi.

Data collection

Data collection methods have been described previously (55-57). Briefly: all beachgoers were approached as they arrived, and were enrolled once they provided verbal informed consent. Each household group completed three surveys, with an adult (≥18 years old) answering questions for other household members. Upon arrival, each household completed an enrollment questionnaire about illnesses experience in the three days prior to their beach visit. Upon departure, participants completed an exit interview about beach activities, water exposure (extent, time, duration and location), presence of underlying acute and chronic health conditions (including allergies), food and drink consumption, animal contact in the past 48 h, contact with sick persons in the past 48 h, and demographic information for each household member. Follow-up telephone interviews were conducted 10–12 days after the beach interview to collect information about the illness symptoms each household member experienced since the beach visit. Interviews were conducted on weekends and holidays between May and September. Respondents were ineligible if they had already completed the study in the previous 28 days, were unaccompanied minors (<18 years), or did not speak English or Spanish.

The study procedures, questionnaires, protocols and consent process were reviewed and approved by the Institutional Review Board (IRB) of the Centers for Disease Control and Prevention for the original study. For the analyses in this report, IRB exemption was granted by University of North Carolina at Chapel Hill (Study #13-2274).

Swim exposure definitions

We were interested in exposure to these potential chemical markers from swimming in fecally-contaminated water. Therefore, this analysis was restricted to swimmers who reported "body immersion", defined as immersion to the waist or higher. Non-swimmers (i.e. those who reported no water contact) and all participants who reported going in the water but not at least having body immersion were excluded because they represent a heterogeneous level of water exposure. Other categories of water exposure (i.e. head immersion, those who swallowed water) were considered in sensitivity analyses.

Health outcomes

In the telephone interview 10-12 days following beach exposure, several health outcomes were assessed, consistent with previous reports (56,57,65,98). "Gastrointestinal illness" (GI illness) referred to any of the following: diarrhea (≥3 loose stools in a 24-hour period); vomiting; nausea and stomachache; or nausea or stomachache and interference with regular activities (missed time from work/regular activities due to illness). Diarrhea was also assessed as a stand-alone outcome because it is frequently used as a definition of gastroenteritis in population-based surveillance, e.g.(100,101). ''Respiratory illness'' referred to any two of the following: sore throat, cough, runny nose, cold, or fever. ''Rash'' referred to a rash or itchy skin.

‘‘Eye ailments’’ referred to eye infection or watery eye. ‘‘Earache’’ referred to earache, ear infection, or runny ears. “Urinary tract infection” (UTI) was also assessed and referred to urinary tract infection or burning sensation when urinating.

Participants ill within the three days prior to their beach visit were excluded from analysis of the health outcome related to their baseline symptoms, but were eligible to be included in analyses of other outcomes.

Water sample collection and chemical analysis

Water samples for chemical analysis were collected in baked amber glass bottles at 11:00 AM on weekends and holidays between May and September at each beach. Specifically, at West and Huntington beaches, three one-liter water samples were collected in waist-high water (1 m), for a total of 3 samples per day. At Silver, Washington Park, and Edgewater beaches, one-liter water samples were collected along two transects perpendicular to the shoreline and closest to the effluent. Two samples were collected at waist depth and two samples at shin depth (0.3 m deep), for a total of 4 samples per day. Four additional quality control (QC) samples were collected on alternate weekends. After collection, samples were packed in coolers with ice during transport and at ≤4 °C alongside a travel blank (de-ionized water) until the following day, when they were packed on dry ice and shipped to USGS National Water Quality Laboratory in Lakewood, Colorado and the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas for extraction and analysis.

Chemical analysis has been previously described (40). Briefly: because of the different physiochemical properties of the chemical compounds, three different analytical methods were used. For wastewater compounds and some pharmaceutical compounds, a whole-water sample

was extracted using continuous liquid-liquid extraction and then analyzed using gas chromatography/mass spectrometry (GC/ MS) (125). Most pharmaceutical compounds were extracted by first passing 500 – 1000 ml filtered water through solid-phase extraction cartridges, then eluent was concentrated, and the final extract was analyzed using liquid chromatography/mass spectrometry (LC/MS) positive-ion electrospray (126). Antibiotic compounds were extracted and analyzed by solid-phase extraction using tandem cartridges, and analyzed by LC/MS positive-ion electrospray on a single quadrapole mass spectrometer (127). Concentrations were reported in μg/L.

Fecal indicator bacteria

Intestinal enterococci are validated, nonspecific indicators of fecal pollution used to measure water quality throughout the world. Total *Enterococcus* spp. by qPCR (calibrator cell equivalents (CCE)/100 ml) was enumerated following water sample collection and subsequent membrane filtration according to previously published protocols (57,102,108).

Statistical analysis

We examined the effects of the nine human-associated chemical markers measured at all five beaches on self-reported illness among body immersion swimmers; they can be grouped into the following broad chemical categories: (1) pharmaceuticals (acetaminophen, caffeine); (2) fecal sterols/stanols (cholesterol, beta-sitosterol); (3) compounds associated with household waste (bisphenol A, diethoxyoctylphenol, *n*-*n*-diethyl-meta-toluamide (DEET)); and (4) compounds associated with industrial waste (phenol, tributyl phosphate). Due to a high proportion of samples where chemical assays failed to detect a signal (concentrations were below

the detection limit) (Table 6.2), each chemical marker was dichotomized by giving it a value of '1' if it was detected in all samples per day, and 0 otherwise. Thus, the primary exposure of interest was the presence/absence of these nine chemical compounds that function as markers of human presence in water samples. Alternative classifications of this primary exposure were explored in sensitivity analyses. For a secondary analysis, we grouped all 56 chemicals into five broad categories: pharmaceuticals, fecal sterols/stanols, household waste products, industrial waste products, and chemicals with a potential for runoff (hereafter, runoff). Prior to grouping, the collinearity of each pair-wise combination of chemical markers was investigated using Spearman rank correlations. The value of each category was a count of the number of chemical compounds belonging to it that were detected in all samples per day. For example, for a given beach and day, a value of '2' for the pharmaceutical category meant that there were '2' pharmaceutical compounds that were detected in all samples collected that day. Non-swimmers represent a distinct group from swimmers and could not have been exposed to chemical compounds from water; thus non-swimmers were therefore excluded from the analysis.

Potential confounding factors plausibly associated with poor water quality and illness identified in published literature or those associated with outcome and available from the health/enrollment questionnaire included age; sex; race/ethnicity; swimming within 48 h before the beach visit or between the beach visit and telephone interview; allergies; contact with animals; contact with other persons with gastrointestinal illness; number of other beach visits; any other chronic illnesses (gastrointestinal, skin, asthma); presence of beach festivals; eating any food or drink while at the beach; bather density; boat density; and environmental conditions such as sunlight, water/air temperature, and rainfall totals from 3pm the previous day to 8 am on the current day. Indicator variables representing beach were included in all models to control for

differences in baseline illness among beaches. We used directed acyclic graphs (109,110) to analyze the potential confounders and identified minimally sufficient adjustment sets for each chemical-illness pair: beach, bather density, sand contact, rain at 8A.M., and water temperature (for GI, diarrhea, earache, and UTI outcomes); and beach, bather density, rainfall, and sand (for respiratory, rash, and eye outcomes). A second adjustment set consisting of the covariates in the minimally sufficient set plus age was also evaluated because it can be argued that age encompasses certain characteristics associated with swimming exposure, and thus exposure to chemicals in water (e.g. children swim longer, swallow more water (56)) as well as being strongly associated with most outcomes. Estimates were similar using both adjustment sets, therefore we only present estimates using the adjustment set without age for reasons of parsimony. Covariates were coded as follows: beach (categorical: Fairhope, Goddard, Huntington, Silver, West, Washington Park), age (0-4, 5-11, 12-19, 20-34, ≥35), mean bathers (continuous), sand (binary), rainfall (continuous), and water temperature (continuous). Robust standard errors were used to account for dependence of observations within a household (124).

To examine the association between human-associated chemical markers and swimming-associated illness, model-based standardization (116,120-122) was performed to estimate standardized marginal risks, risk differences (RD), and 95% confidence intervals (95% CI) using the delta method (123) and the total group as the standard. Logistic regression was used to estimate predicted probabilities of the outcome for every value of observed confounders and then combined as a weighted average separately for both levels of the binary exposure. Thus, the effect estimates are estimated using predicted probabilities standardized to the same confounder distribution. The predicted probabilities were subtracted to produce a marginal estimate of the risk difference comparing chemical marker exposure to no exposure. Modification of these

marker-illness effect estimates by water matrix (freshwater vs. saltwater) was of secondary interest, so was assessed by stratification. As previously mentioned, we excluded participants ill within the three days prior to their beach visit from analysis of the health outcome related to their baseline symptoms, but they were eligible to be included in analyses of other outcomes. All analyses were completed using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina) and Stata version 13 (StataCorp, College Station, TX).

Effect measure modification of the association between *Enterococcus* assayed by qPCR Method 1611 (CCE/ml) and illness was examined to evaluate whether the occurrence of each of the chemical markers improved the association of the general indicator with illness. In both the primary (chemical marker) and secondary (chemical categories) analyses, *Enterococcus* was treated as the main exposure and the chemical marker/category was the binary modifier. Thus, the chemical categories were dichotomized for the modification analyses as follows: a value of '1' any chemicals belonging to that category were detected in all samples per day, and '0' otherwise. For the primary effect measure modification analyses with the general indicator *Enterococcus*, the quantitated values were dichotomized in two ways according to 2012 EPA recreational water quality guidelines: above and below a geometric mean of 470 CCE/100ml (for an estimated illness rate of 36/1000 primary contact recreators), and above and below a geometric mean of 300 CCE/100ml (for an estimated illness rate of 32/1000 primary contact recreators) (95). Secondary effect measure modification analyses were also performed with *Enterococcus* coded as a continuous variable (average $\log_{10}$ count of *Enterococcus* per day (CCE/100ml)). Risk difference modification was estimated with product interactions of *Enterococcus* and chemical markers and then assessed by an interaction contrast (i.e., difference

of risk differences) (112). The interaction contrast takes on the value of zero when the joint effects of two factors are simply additive (112).

Sensitivity Analyses

To determine if estimates were robust to different exposure categorizations, we examined additional classifications of swimming and chemical exposure. First, we repeated our analyses using two additional definitions of swimmer: as participants who reported immersing their head under water, and participants who reported swallowing water. Second, we explored a more sensitive binary chemical classification where each chemical was given the value of '1' if it was detected in 1 or more samples per day, and 0 otherwise. The data did not permit classifications that make use of quantitative values.

Results

Demographic characteristics

Data were available for 17,753 participants at five beaches between 2003 and 2005, including 9,109 swimmers (body immersion), 5,591 non-swimmers and 3,053 beach visitors who had water contact but no body immersion (Table 6.1). Compared to non-swimmers, more swimmers were younger (mean age 23 years vs. 35 years), male (49% vs. 41%), Hispanic (11% vs. 8%); and travelled farther to get to the beach (mean of 56 miles vs. 46 miles). Similar proportions of swimmers and non-swimmers reported having a chronic illness, and few reported acute illnesses in the three days prior to the beach visit. Because non-swimmers may represent a group distinct from swimmers in several behavioral characteristics and they would not, by

definition, have been exposed to chemical indicators in water, the final analysis was restricted to body immersion swimmers only (n=9,109).

Distribution of human-associated chemical markers in recreational waters

Chemicals detected by beach. A total of 318 chemical samples were collected over 88 days: 18 days at Edgewater, Silver, and Washington Park Beaches, 15 days at Huntington Beach, and 19 days at West Beach. Human-associated chemical markers were found at all beaches. They were detected in at least 1 sample almost every day samples were collected (87/88 days), but rarely detected in *all* of a day's samples (27/88 days), and were quantified at low levels (Table 6.2).

The least amount of chemical contamination occurred at Silver and Washington Park beaches, where for 12 and 13 days, respectively, no chemical markers were detected. The greatest amount of chemical contamination occurred at Huntington Beach, where at least two chemicals were detected in all samples every day chemicals were measured.

Prevalence of chemical markers. According to chemical category, non-prescription pharmaceuticals were the chemical compounds detected most often, followed by industry wastewater products and household wastewater products. Detergents and prescription pharmaceuticals were detected least often. Of the 9 chemicals measured at all five beaches, DEET, caffeine, and phenol were detected most frequently, in 59%, 54%, and 53% of non-missing samples respectively (Table 6.2). The proportion of samples with non-detectable (below limit of detection) concentrations exceeded 40% for all chemicals, ranging from a low of 41% for DEET to a high of 93% for diethoxyoctylphenol. Average daily concentrations varied widely by type of chemical and beach, as evidenced by geometric means ranging from 0.019 µg/L for

acetaminophen to 1.438 μg/L for cholesterol. Of the group of 11 chemicals measured at 4 out of the 5 beaches, cotinine was the only chemical detected in more than 50% of non-missing samples; the remaining chemicals were detected in less than 40% of samples. Average daily concentrations among these 11 chemicals were smaller than those in the 9 chemicals at each beach; geometric means ranged from 0.0004 μg/L for pharmaceuticals cotinine and diphenhydramine to 0.481 μg/L for monoethoxyoctylphenol. The remaining chemicals were detected infrequently and/or at low concentrations. For our investigation of chemicals and illness, we focused on the subset of 9 chemicals measured at all 5 beaches.

Illness risk associated with presence/absence of human-associated chemical markers

Frequencies and standardized marginal estimates of the RD (95% CI) comparing exposure to each chemical marker vs. no exposure and illness are shown in Figure 3 and Supplemental Table B.1. In general, across all beaches, RD estimates crossed the null, and 95% CI were narrow. We observed little evidence of association between chemical markers and illness but several patterns were visible. For the more plausible outcomes of GI illness and diarrhea, bisphenol A and cholesterol showed positive associations of approximately 1.7% and 1.0% respectively. Additional positive associations were seen for less plausible non-enteric outcomes such as respiratory illness and rash with tributyl phosphate exposure; for rash with phenol exposure; and earache with acetaminophen and caffeine exposure. Inverse associations for respiratory illness were also observed with exposure to bisphenol A (RD=-1.9%; -3.0%, -0.5%), phenol (RD=-2.4%; -4.4%, -0.3%; respectively), and cholesterol (RD=-1.8%; -3.0%, -0.5%). Due to a high percentage of non-detects, associations with diethoxyoctylphenol were imprecise and unavailable for associations with rash and UTI, the least prevalent outcomes.

Modification of Enterococcus-*illness association with chemical markers as indicators of source*

We investigated whether the presence of chemical markers of human fecal contamination or human presence strengthened the association of the general *Enterococcus* indicator with illness. Interaction contrast estimates (95% CI) for the association of *Enterococcus* (dichotomized at < and ≥470 CCE/ml) and GI illness, diarrhea, and respiratory illness modified by each chemical marker are shown in Table 6.3; the remaining outcomes are in Supplemental Table B.2. Overall, interaction contrast estimates were imprecise and did not suggest the presence of modification between strata of most chemical markers, with the exception of phenol. Exposure to phenol modified the association for GI illness by 5.2% (ICR=5.2%; 0.3%, 10.2%) and for eye ailments by 3.0% (ICR=3.0%; 0.8%, 5.2%). Modification by phenol was also suggested for associations with *Enterococcus* dichotomized at < and ≥300 CCE/ml and respiratory illness (ICR=4.2%; 1.0%, 7.4%) and eye ailments (ICR=2.4%; 0.1%, 4.6%), but not GI illness (ICR=4.4%; -0.2%, 9.1%) (Supplemental Table B.3).

Results for modification with *Enterococcus* assessed as a daily average QPCR CCE concentration (CCE/100ml) are shown in Supplemental Table B.4. As shown previously in 2008 and 2010 by Wade et al. (56,57), we see an increased risk of GI illness and diarrhea with each 1-$\log_{10}$ increase in daily average QPCR CCE *Enterococcus* concentration (RD=1.3% (0.2%, 2.4%) and RD=1.1% (0.4%, 1.7%), respectively) (Supplemental Table B.4a-b). No modification by phenol was observed for GI illness, respiratory illness or eye ailment. Other chemicals did not show strong or consistent modification of the association between *Enterococcus* and the remaining outcomes. Interaction contrast estimates were imprecise overall, particularly for chemicals that were infrequently detected (e.g. acetaminophen, beta-sitosterol, and diethoxyoctylphenol).

Illness risk associated with categories of human-associated chemical markers

Frequencies and standardized marginal estimates of the RD (95% CI) comparing participants exposed to increasing counts of chemicals detected within each category vs. no chemicals detected in that category for each illness outcome are shown in Supplemental Table B.5. As the number of chemicals detected increased in each category, the adjusted risk of illness for most outcomes was relatively flat, indicating that a dose-response relationship was not present. Notable exceptions include chemicals in the household wastewater category, which showed a peak in adjusted risk of illness at 4 or 5 chemicals for GI illness, diarrhea, respiratory illness, and eye ailments. Across all beaches, we observed little evidence to suggest an association between chemical categories and illness.

Modification of Enterococcus-*illness association with categories of chemical markers as indicators of source*

Risk difference estimates for the association between *Enterococcus* and illness were similar among participants exposed and unexposed to chemical marker categories; no modification of RD estimates was observed. This was true for *Enterococcus* assessed dichotomously at < and ≥470 CCE/ml (Supplementary Table B.6) and continuously (Supplementary Table B.7). Interaction contrast estimates were imprecise overall.

Sensitivity analyses

Because intensity of water contact might determine the extent of exposure to human-associated chemical markers, we also repeated our analysis among those who had immersed their head in water (Supplemental Table B.8) and among those who swallowed water (Supplemental

Table B.9). Estimates for head immersion swimmers were consistent with what was found for body immersion swimmers, but more imprecise. Estimates for swimmers who swallowed water were generally farther from the null, and very imprecise.

Exploration of a more sensitive, less stringent categorization of exposure showed that RD estimates were moderately affected by choice of dichotomization category (Supplemental Tables B.10). Overall results were similar to the primary analysis but greater in magnitude (though not always in the same direction (e.g. bisphenol A and GI illness)) and precision. Similar to the primary analysis, most RD estimates crossed the null and had narrow 95% CIs; little evidence of association was observed except for respiratory illness. As before, cholesterol and phenol were associated with decreased risk of respiratory illness (RD=-3.0%; -4.3%, -1.7% and RD=-3.7%; -5.2%, -2.2%, respectively). However, bisphenol A was no longer associated with respiratory illness. Instead DEET was associated with a decreased risk of respiratory illness (RD=-1.4%; -2.7%, -0.2%). Additional chemicals that showed suggestive evidence of association included acetaminophen and diarrhea, DEET and eye ailments, and caffeine and rash/earache**.**

Discussion

We analyzed exposure to a select group of anthropomorphic chemical markers as indicators of human fecal contamination and incidence of swimming-associated illnesses in a well-characterized cohort of visitors to US beaches. Overall our findings demonstrate little clear evidence that the individual chemical markers or categories of chemical markers were associated with swimming-associated illness, though we observed a pattern of increased risks for several outcomes: GI illness and bisphenol A, GI illness and cholesterol, respiratory illness and household wastewater products, respiratory illness and tributyl phosphate, and rash and tributyl

phosphate. At the same time, several implausible, inverse associations were observed with respiratory illness. Phenol was the only chemical that may act as a modifier in associations between the currently-used FIB, general *Enterococcus,* and GI illness, eye ailments and respiratory illness.

To the best of our knowledge, this study is the first to investigate the illness risks associated with exposure to chemical markers of human fecal pollution. This research question is of public health importance because there is a recognized need for alternative fecal indicators (1) that can be used to distinguish the sources of fecal pollution to help direct remediation efforts efficiently; (2) whose survival and fate correlate better with viral pathogens that cause waterborne illness; and (3) that can be rapidly assessed so that beach advisory and closing decisions can be made in real-time (11,29,40,151,159). While a wide range of chemicals specific to human wastewater have been investigated for potential differentiation of fecal sources in aquatic environments (40,42,45,73,156,159), the relationship of these chemical compounds to the incidence of illness has not been determined. In this study, some of the most promising chemical markers in the literature – caffeine and fecal sterols/stanols – were measured at all 5 beaches. Though detected relatively frequently, the concentrations detected were low and did not show an association with risk of any measured health illness. No chemical marker investigated was associated with enteric illnesses, which are the illnesses most commonly associated with swimming in fecally-contaminated water (55-57,60,61,99), though patterns of increased illness were identified with bisphenol A, an industrial wastewater compound used in the manufacture of polycarbonate resins; cholesterol, a plant and animal sterol; and tributyl phosphate, an antifoaming agent and flame retardant.

Several chemical markers showed an inverse association with respiratory illness. They included bisphenol A; phenol, a disinfectant and industrial wastewater compound; and cholesterol. The magnitudes of the inverse associations were small (~2%) and the significance of this finding is unclear. Similarly, the significance of the finding that phenol modified the association between binary *Enterococcus* and several illnesses is also unclear. Given that modification by phenol was not present with continuous *Enterococcus*, this finding may be an artifact of dichotomization, though the cut-points used coincide with recreational water quality criteria levels set by the EPA for determining fecal contamination that result in illness (95). Although there are no epidemiology studies that have examined the relationship between chemical markers and incidence of illness, several studies have identified specific chemicals and groups of chemicals that have the greatest potential to assess human-origin pollution (see (11) for a review). Bisphenol A, phenol, cholesterol, and tributyl phosphate were among 35 chemicals suggested as potentially useful indicators of human fecal contamination in an extensive survey of 110 chemicals from wastewater effluent samples collected in 10 rivers in the US (40) due to being abundant and present in sufficient concentration. In fact, chemical markers investigated in this study included 27 of the 35 compounds suggested as potential indicators by Glassmeyer et al. The finding that most chemical markers we investigated were not associated with illness is not unexpected, given that chemicals specific to human waste streams are often at low concentrations and are further diluted below detection limits once wastewater enters environmental waters (11). This was true in our study, where, although human-associated chemical markers were detected in at least 1 sample almost every day samples were collected, chemical concentrations were in parts per billion or smaller (Table 6.2). For this reason, it is unlikely that human-associated chemical compounds will replace microbial source tracking

markers in determining source of fecal contamination. Chemical markers will most likely be used in combination with microbial source tracking fecal markers or to validate results obtained using microbial markers, as part of a source-tracking "toolbox" approach. In such an approach, a suite of source tracking tools that includes both microbial and chemical human-associated indicators is more likely to provide information about source-specificity than any one indicator (11,75,144,151,159). Each indicator has varying patterns of fate, transport, survival, and persistence that together may yield greater confidence in an assessment of water quality source.

This study has several strengths and limitations. For our exposure, we dichotomized exposure into presence/absence based on the number of daily samples in which the chemical concentration was above zero (where presence means detected in all samples). This measure then became a proxy for an individual swimmer's exposure to chemical markers. Although these dichotomized daily measures may not be indicative of actual individual exposure, characterizing individual exposure would have been difficult, costly and impractical given the size of the NEEAR cohort. The study design allowed for the collection of 3-4 water samples at two water depths (shin height (0.3m) and waist height (1.0m)) in an attempt to capture some of the variety of chemical exposures a participant may experience in the water at a time when participants would likely have arrived. The cohort design also allowed us to measure water quality over a wide range of study days, so we were able to capture varying water quality conditions over the summer months. Nevertheless, exposure classification based on a single time point is imperfect and the results may reflect residual or unmeasured confounding.

Related to that, though measured quantitatively, a high proportion of chemical samples were below the limit of detection and could not be analyzed quantitatively, thus our ability to make inferences was limited. While quantitative categorizations of exposure were explored,

ultimately the low frequencies of detection necessitated the decision to dichotomize. To mitigate these potential limitations, sensitivity analyses were performed with exposure dichotomized using a more sensitive definition, where a chemical was given the value of '1' if it was detected in ≥1 samples collected that day. The choice of a dichotomization cutpoint moderately affected the estimation of RD estimates by affecting the proportion of cases with chemical exposure. When using the more sensitive, but less stringent categorization where '1' means detected in 1 or more samples per day, the proportion of cases with chemical exposure increased substantially in some cases. A striking example is diethoxyoctylphenol, whose estimates were unstable, imprecise, and often unable to be estimated when using the more sensitive categorization, but well-behaved when using the less sensitive categorization. This issue was likely exacerbated because chemicals were not present in high levels. Ideally the amount of non-detection would have been low enough to permit us to use quantitative exposure levels. Future studies should make every effort to use quantitative measures of chemical exposure, particularly when concentrations are low.

An additional limitation was a reliance on non-specific, self-reported illness symptoms (e.g. eye ailment) as outcomes rather than confirmed diagnoses. This was done in an effort to reflect the diversity of symptoms potentially associated with recreational water exposure, especially since most are self-limiting and infrequently result in doctor's visits. Such broadly-defined symptoms may have obscured more specific effects of human-associated fecal indicators. However, the prospective nature of the study allowed us to determine temporality and the 10-12 day follow up period reflected the incubation time for likely pathogens that would cause the symptoms of interest. It is possible that our health outcomes may also have been

affected by recall bias, though it is unlikely that recall would be differential by varying levels of water quality/chemical exposure.

Conclusion

Despite these limitations, findings from this study of beach sites impacted by sewage effluent highlight the need for further epidemiology studies to investigate the relationship between human-associated chemical markers and swimming-related illnesses. Few human-associated chemicals were associated with swimming-associated illness; however, bisphenol A, cholesterol, tributyl phosphate, and phenol may deserve further study because we observed a pattern of increased risk for several outcomes. Similarly, phenol was the only chemical to improve associations with swimming-associated illness compared to general, non-source specific *Enterococcus* indicators already in use at beach sites. These findings may have been influenced by low/no abundance of chemical markers or indicate that human-associated markers are better suited to characterize risk at sites impacted by non-point sources of fecal contamination. To our knowledge, this is the first study to evaluate associations between exposure to human-associated chemical markers of fecal contamination and health outcomes among swimmers. This study suggests that additional research is needed to support the use of chemical biomarkers to identify human sources contributing to fecal pollution of recreational water.

Disclaimer: The views expressed in this paper do not necessarily reflect EPA policy.

Tables and Figures

Figure 6.1. Freshwater and marine beach sites

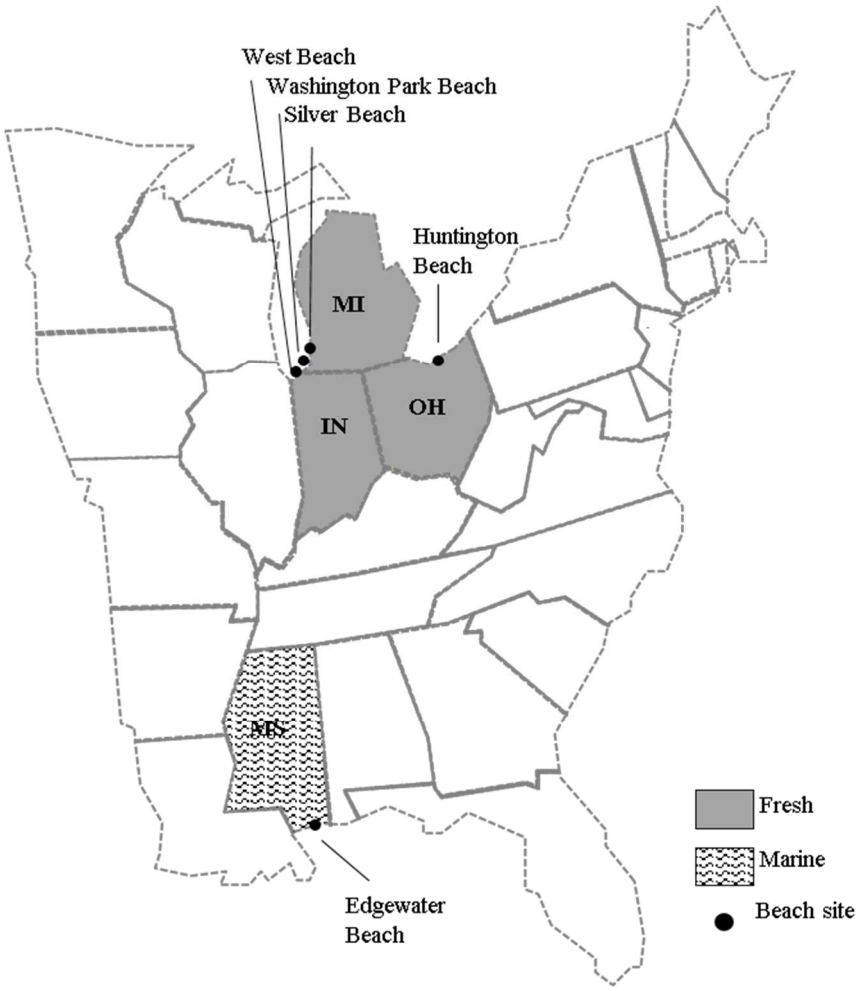


Table 6.1. Characteristics of NEEAR participants by body immersion status (n=17,753)

	No water contact	Water contact	
		No body immersion†	Body immersion†
	(n=5591) N* (%)	(n=3053) N* (%)	(n=9109) N* (%)
Sex			
Male	2315 (41)	1118 (37)	4437 (49)
Female	3276 (59)	1933 (63)	4652 (51)
Missing	0	2	20
Age in years (mean, SD, min/max)	34.8 (17), 0/93	32.3 (17), 0/85	23.4 (17), 0/103
0-4	283 (5)	248 (8)	851 (9)
5-11	241 (4)	235 (8)	2115 (23)
12-19	606 (11)	291 (10)	1424 (16)
20-34	1440 (26)	743 (24)	1978 (22)
35 and over	2945 (53)	1478 (48)	2496 (27)
Missing	76	58	245
Race			
White	4695 (84)	2626 (86)	7434 (82)
Black	276 (5)	137 (4)	357 (4)
Asian	96 (2)	52 (2)	112 (1)
American Indian	15 (0)	14 (0)	20 (0)
Hispanic	428 (8)	163 (5)	975 (11)
Multi-race	39 (1)	22 (1)	106 (1)
Other	34 (1)	28 (1)	68 (1)
Missing	8	11	37
Illnesses in the 3 days prior to beach visit			
GI illness	150 (3)	70 (2)	163 (2)
Vomiting	46 (1)	32 (1)	88 (1)
Sore throat	295 (5)	174 (6)	515 (6)
Earache	74 (1)	25 (1)	129 (1)
Eye ailment	33 (1)	13 (0)	41 (0)
Rash	124 (2)	60 (2)	203 (2)
Urinary tract infection	27 (0)	14 (0)	38 (0)
History of chronic GI, skin, respiratory illness or allergies	1583 (28)	865 (28)	2293 (25)
Miles travelled to beach			
0-20	2987 (53)	1450 (47)	3765 (41)
20-60	1503 (27)	843 (28)	2979 (33)
60-100	425 (8)	300 (10)	983 (11)
>100	614 (11)	442 (14)	1278 (14)
Missing	62	18	104
Swam in last week	1446 (26)	922 (30)	3848 (42)
Sand contact			

	No water contact	Water contact	
	(n=5591) N* (%)	No body immersion† (n=3053) N* (%)	Body immersion† (n=9109) N* (%)
Dug in sand	1288 (23)	1300 (43)	5179 (57)
Buried body in sand	170 (3)	201 (7)	1469 (16)
Consumed food	2629 (47)	1677 (55)	5729 (63)
Animal contact 2 days prior to or after beach visit, or between beach visit and phone interview	4120 (74)	2349 (77)	7022 (77)
All beaches			
Edgewater Beach	305 (5)	202 (7)	639 (7)
Huntington Beach	1535 (27)	548 (18)	757 (8)
Silver Beach	2224 (40)	1328 (44)	4281 (47)
West Beach	689 (12)	468 (15)	1665 (18)
Washington Park Beach	838 (15)	507 (17)	1767 (19)

NEEAR, National Environmental and Epidemiologic Assessment of Recreational Water study; N, number; SD, standard deviation

* Sums may not add up to totals because of missing values.

† Swimmers were those with body immersion (defined as immersion to the waist or higher). Those without water contact or with water contact but not body immersion were not included in the analysis but are shown in this descriptive table for completeness.

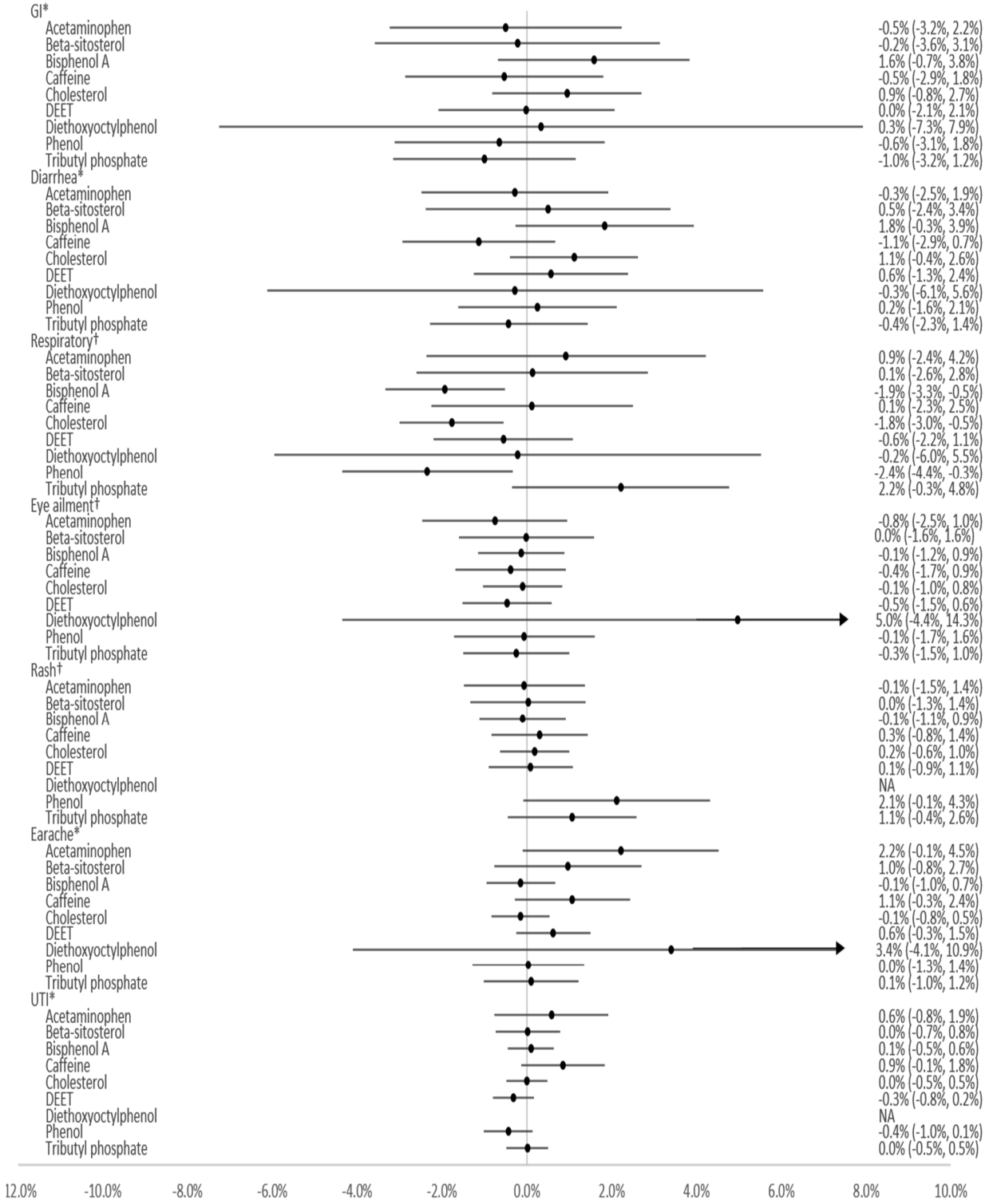
Table 6.2. Concentrations of chemicals in the NEEAR study (µg/L)

	Category	N samples collected at beach	N samples collected of chemical*	Chemical samples detected				Missing N	Non-detects N (%)†
				N	Min	Max	Geo Mean		
Chemicals measured at all 5 beaches									
Acetaminophen	Pharmaceutical	318	315	67 (21)	0.0005	0.5	0.019	3	248 (79)
Beta sitosterol	Fecal sterol/stanol	318	290	48 (17)	0.4	2	1.075	28	242 (83)
Bisphenol A	Industrial waste	318	278	92 (33)	0.06	1	0.185	40	186 (67)
Caffeine	Pharmaceutical	318	315	171 (54)	0.0004	0.3	0.021	3	144 (46)
Cholesterol	Fecal sterol/stanol	318	290	132 (46)	0.2	20	1.438	28	158 (54)
Diethoxyoctylphenol	Detergent/ Household waste	318	290	20 (7)	0.05	0.2	0.096	28	270 (93)
DEET	Insect repellent /Household waste	318	290	172 (59)	0.01	20	0.074	28	118 (41)
Phenol	Industrial waste	318	286	151 (53)	0.08	3	0.512	32	135 (47)
Tributyl phosphate	Flame retardant/ Household waste	318	290	116 (40)	0.02	0.2	0.045	28	174 (60)
Chemicals measured at 4 beaches									
2-methylnaphthalene	PAH/ Runoff	261	240	18 (8)	0.01	0.2	0.039	21	222 (93)
Cotinine	Pharmaceutical	246	246	128 (52)	0.0001	0.01	0.004	0	118 (48)
Diphenhydramine	Pharmaceutical	261	258	19 (7)	0.0001	0.04	0.004	3	239 (93)
Fluoranthene	PAH/ Runoff	246	225	60 (27)	0.003	4	0.027	21	165 (73)
Isophorone	Industrial waste	261	240	35 (15)	0.005	0.07	0.013	21	205 (85)
Metolachlor	Pesticide; runoff	246	226	86 (38)	0.02	0.5	0.062	20	140 (62)
Monoethoxyoctylphenol	Detergent/ Household waste	246	222	9 (4)	0.07	0.7	0.481	24	213 (96)
Naphthalene	PAH/ Runoff	261	240	25 (10)	0.01	0.2	0.042	21	215 (90)
P-cresol	Inudstrial waste product	246	221	22 (10)	0.01	0.6	0.038	25	199 (90)
Phenanthrene	PAH/ Runoff	261	240	23 (10)	0.006	2	0.028	21	217 (90)
Pyrene	PAH/ Runoff	246	225	57 (25)	0.003	3	0.026	21	168 (75)
Chemicals measured at 3 beaches									
1,7-dimethylxanthine	Pharmaceutical	174	174	31 (18)	0.02	0.1	0.038	144	143 (82)
1-methylnaphthalene	PAH/ Runoff	189	172	13 (8)	0.01	0.2	0.033	146	159 (92)
3-beta-coprostanol	Fecal sterol/stanol	174	161	4 (2)	0.6	0.8	0.670	157	157 (98)
Beta-stigmastanol	Fecal sterol/stanol	174	158	5 (3)	0.4	1	0.784	160	153 (97)
Diethoxynonylphenol (total)	Detergent/ Household waste	174	157	8 (5)	1	3	1.883	161	149 (95)
Fluoxetine	Pharmaceutical	189	189	10 (5)	0.01	0.2	0.033	129	179 (95)
Tri(2-chloroethyl) phosphate	Flame retardant/ Household waste	174	157	9 (6)	0.02	0.07	0.040	161	148 (94)
Triclosan	Household waste	189	175	9 (5)	0.02	0.2	0.073	143	166 (95)

	Category	N samples collected at beach	N samples collected of chemical*	Chemical samples detected				Missing N	Non-detects N (%)†
				N	Min	Max	Geo Mean		
Triphenyl phosphate	Industrial waste	174	157	15 (10)	0.005	0.09	0.017	161	142 (90)
Chemicals measured at 2 beaches									
2,6-dimethylnaphthalene	PAH Runoff	117	107	12 (11)	0.006	0.03	0.013	211	95 (89)
4-tert-octylphenol	Detergent/ Household waste	129	115	2 (2)	0.08	0.2	0.126	203	113 (98)
AHTN	Fragrance/ Household waste	144	132	25 (19)	0.01	0.08	0.020	186	107 (81)
Anthracene	PAH/ Runoff	117	111	4 (4)	0.04	0.8	0.204	207	107 (96)
Benz(a)pyrene	PAH/ Runoff	102	93	6 (6)	0.01	2	0.059	225	87 (94)
Benzophenone	Fragrance/ Household waste	117	107	45 (42)	0.008	0.2	0.028	211	62 (58)
Camphor	Fragrance/ Household waste	117	107	11 (10)	0.006	0.01	0.009	211	96 (90)
Carbamazapine	Pharmaceutical	102	102	16 (16)	0.0003	0.02	0.006	216	86 (84)
Codeine	Pharmaceutical	117	117	5 (4)	0.003	0.01	0.005	201	112 (96)
D-limonene	Household waste	117	107	5 (5)	0.01	0.03	0.014	211	102 (95)
Pentachlorophenol	Industrial waste	117	111	1 (1)	0.2	0.2	0.200	207	110 (99)
Tri(2-butoxyethyl) phosphate	Flame retardant/ Household waste	117	107	29 (27)	0.1	6	0.495	211	78 (73)
Tri(dichloroisopropyl) phosphate	Flame retardant/ Household waste	117	107	20 (19)	0.01	0.09	0.041	211	87 (81)
Chemicals measured at 1 beach									
1,4-dichlorobenzene	Household waste	45	43	10 (23)	0.04	0.4	0.111	275	33 (77)
5-methyl-1h-benzotriazole	Industrial waste	72	64	1 (2)	0.5	0.5	0.500	254	63 (98)
Anthraquinone	Pesticide; runoff product	72	64	10 (16)	0.02	0.05	0.032	254	54 (84)
Carbazole	Industrial waste	45	43	1 (2)	0.2	0.2	0.180	275	42 (98)
Dehydronifedipine	Pharmaceutical	57	57	4 (7)	0.001	0.002	0.002	261	53 (93)
Diltiazem	Pharmaceutical	72	72	1 (1)	0.004	0.004	0.004	246	71 (99)
Isopropylbenzene (cumene)	Industrial waste	72	64	6 (9)	0.006	0.02	0.012	254	58 (91)
Menthol	Fragrance/ Household waste	72	64	6 (9)	0.02	0.03	0.024	254	58 (91)
Methyl salicylate	Fragrance/ Household waste	72	64	13 (20)	0.007	0.02	0.010	254	51 (80)
Miconazole	Pharmaceutical	72	72	1 (1)	0.007	0.007	0.007	246	71 (99)
Para-nonylphenol (total)	Detergent/ Household waste	72	68	1 (1)	0.3	0.3	0.300	250	67 (99)
Sulfamethoxazole	Pharmaceutical	45	45	4 (9)	0.0003	0.001	0.001	273	41 (91)
Tetrachloroethylene	Industrial waste	72	64	3 (5)	0.01	0.02	0.016	254	61 (95)
Trimethoprim	Pharmaceutical	45	45	1 (2)	0.003	0.003	0.003	273	44 (98)

Min, minimum; Max, maximum; N, number; PAH, polycyclic aromatic hydrocarbon. * Number of samples collected of chemical = Number of chemical samples detected + Non-detects. † Percent of non-detects out of non-missing samples collected of chemical

Figure 6.2. Standardized risk differences (95% CI) for the association between illness and human-associated chemical markers (detected in all daily samples vs. <all) among body immersion swimmers in all beaches



NA, not able to estimated.* Adjusted for beach, age, mean bathers, sand, rain, water temperature
† Adjusted for beach, age, mean bathers, sand, rain

Table 6.3(a-c). Modification of the adjusted standardized RD (95% CI) for the association between illness and *Enterococcus* qPCR Method 1611 above and below EPA guidelines (geometric mean of 470 CCE/100ml for an illness rate of 36/1000) with chemical markers (detected in all daily samples vs. <all) among body immersion swimmers in all beaches

		GI Illness				
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adj Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main assoc				0.7% (-2.3%, 3.6%)	
Acetaminophen						
Not detected	<470	641	7884	8.6	Ref	
	≥470	57	457	10.3	1.7% (-1.9%, 5.3%)	
Detected in all	<470	43	383	9.2	Ref	
	≥470	13	150	6.6	-2.5% (-7.4%, 2.3%)	**-4.3% (-10.4%, 1.8%)**
Beta-sitosterol						
Not detected	<470	640	7750	8.6	Ref	
	≥470	63	528	9.5	1.0% (-2.3%, 4.2%)	
Detected in all	<470	39	428	8.9	Ref	
	≥470	7	79	7.6	-1.2% (-8.0%, 5.5%)	**-2.2% (-9.7%, 5.3%)**
Bisphenol A						
Not detected	<470	562	6800	8.4	Ref	
	≥470	66	565	9.2	0.8% (-2.2%, 3.9%)	
Detected in all	<470	110	1297	10.0	Ref	
	≥470	4	42	8.9	-1.1% (-10.7%, 8.4%)	**-1.9% (-11.6%, 7.8%)**
Caffeine						
Not detected	<470	502	6593	8.5	Ref	
	≥470	41	287	11.6	3.1% (-1.6%, 7.8%)	
Detected in all	<470	182	1674	8.8	Ref	
	≥470	29	320	7.5	-1.3% (-4.9%, 2.2%)	**-4.4% (-10.1%, 1.3%)**
Cholesterol						
Not detected	<470	461	5931	8.3	Ref	
	≥470	31	292	8.9	0.5% (-3.4%, 4.4%)	
Detected in all	<470	218	2247	9.2	Ref	
	≥470	39	315	10.0	0.8% (-3.3%, 4.9%)	**0.3% (-5.0%, 5.5%)**
DEET						
Not detected	<470	474	6081	8.7	Ref	
	≥470	10	108	7.7	-1.0% (-6.9%, 4.9%)	
Detected in all	<470	205	2097	8.5	Ref	
	≥470	60	499	9.6	1.1% (-2.2%, 4.3%)	**2.1% (-4.4%, 8.6%)**
Diethoxyoctylphenol						
Not detected	<470	672	8123	8.6	Ref	
	≥470	70	607	9.3	0.7% (-2.3%, 3.6%)	
Detected in all	<470	7	55	9.0	Ref	
	≥470	0	0	NA	NA	**NA**
Phenol						
Not detected	<470	487	6284	9.4	Ref	
	≥470	22	241	6.4	-3.0% (-7.1%, 1.1%)	
Detected in all	<470	192	1894	7.3	Ref	
	≥470	48	366	9.5	2.3% (-1.2%, 5.7%)	**5.2% (0.3%, 10.2%)**
Tributyl phosphate						
Not detected	<470	609	7384	8.7	Ref	
	≥470	52	413	9.5	0.7% (-2.7%, 4.1%)	
Detected in all	<470	70	794	7.8	Ref	
	≥470	18	194	8.1	0.4% (-4.3%, 5.0%)	**-0.4% (-5.9%, 5.2%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain, water temperature

Table 6.3(b). **Diarrhea**

		Diarrhea				
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adj Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main assoc				1.1% (-1.5%, 3.7%)	
Acetaminophen						
Not detected	<470	415	7884	5.7	Ref	
	≥470	46	457	7.9	2.2% (-1.0%, 5.5%)	
Detected in all	<470	32	383	6.5	Ref	
	≥470	10	150	4.5	-2.1% (-6.0%, 1.9%)	**-4.3% (-9.4%, 0.8%)**
Beta-sitosterol						
Not detected	<470	410	7750	5.6	Ref	
	≥470	51	528	7.3	1.7% (-1.3%, 4.6%)	
Detected in all	<470	33	428	6.9	Ref	
	≥470	5	79	5.5	-1.4% (-7.2%, 4.5%)	**-3.0% (-9.6%, 3.5%)**
Bisphenol A						
Not detected	<470	364	6800	5.5	Ref	
	≥470	52	565	6.6	1.1% (-1.6%, 3.7%)	
Detected in all	<470	75	1297	7.3	Ref	
	≥470	4	42	9.1	1.7% (-8.1%, 11.6%)	**0.7% (-9.3%, 10.6%)**
Caffeine						
Not detected	<470	319	6593	5.9	Ref	
	≥470	33	287	8.4	2.5% (-1.6%, 6.6%)	
Detected in all	<470	128	1674	5.3	Ref	
	≥470	23	320	5.3	0.0% (-2.9%, 2.9%)	**-2.5% (-7.2%, 2.2%)**
Cholesterol						
Not detected	<470	281	5931	5.3	Ref	
	≥470	26	292	6.8	1.5% (-2.0%, 5.0%)	
Detected in all	<470	162	2247	6.5	Ref	
	≥470	30	315	7.4	0.9% (-2.7%, 4.5%)	**-0.6% (-5.2%, 4.0%)**
DEET						
Not detected	<470	292	6081	5.5	Ref	
	≥470	8	108	6.2	0.7% (-5.0%, 6.4%)	
Detected in all	<470	151	2097	6.1	Ref	
	≥470	48	499	7.4	1.3% (-1.7%, 4.3%)	**0.6% (-5.6%, 6.8%)**
Diethoxyoctylphenol						
Not detected	<470	438	8123	5.7	Ref	
	≥470	56	607	6.8	1.1% (-1.5%, 3.7%)	
Detected in all	<470	5	55	5.5	Ref	
	≥470	0	0	NA	NA	**NA**
Phenol						
Not detected	<470	295	6284	5.7	Ref	
	≥470	18	241	5.3	-0.5% (-4.2%, 3.3%)	
Detected in all	<470	148	1894	5.7	Ref	
	≥470	38	366	8.0	2.3% (-1.2%, 5.8%)	**2.8% (-1.8%, 7.3%)**
Tributyl phosphate						
Not detected	<470	394	7384	5.8	Ref	
	≥470	41	413	6.7	0.9% (-2.1%, 3.8%)	
Detected in all	<470	49	794	5.3	Ref	
	≥470	15	194	7.0	1.7% (-2.8%, 6.2%)	**0.9% (-4.3%, 6.0%)**

NA, not able to estimated

* Adjusted for beach, mean bathers, sand, rain, water temperature

Table 6.3(c). **Respiratory illness**

Respiratory Illness						
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adj Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main assoc				-1.8% (-4.2%, 0.6%)	
Acetaminophen						
Not detected	<470	474	7628	6.1	Ref	
	≥470	17	441	4.2	-1.9% (-4.3%, 0.5%)	
Detected in all	<470	25	377	6.9	Ref	
	≥470	6	148	5.2	-1.7% (-9.2%, 5.7%)	**0.1% (-7.7%, 8.0%)**
Beta-sitosterol						
Not detected	<470	466	7496	6.1	Ref	
	≥470	19	509	4.1	-2.0% (-4.7%, 0.7%)	
Detected in all	<470	21	423	5.8	Ref	
	≥470	4	80	5.5	-0.4% (-6.1%, 5.4%)	**1.6% (-4.8%, 8.0%)**
Bisphenol A						
Not detected	<470	429	6577	6.5	Ref	
	≥470	20	545	4.1	-2.4% (-5.0%, 0.1%)	
Detected in all	<470	57	1260	4.4	Ref	
	≥470	3	44	6.1	1.7% (-5.4%, 8.7%)	**4.1% (-3.2%, 11.5%)**
Caffeine						
Not detected	<470	385	6388	6.1	Ref	
	≥470	11	282	4.3	-1.8% (-5.1%, 1.6%)	
Detected in all	<470	114	1617	6.3	Ref	
	≥470	12	307	4.4	-1.9% (-5.5%, 1.7%)	**-0.1% (-5.1%, 4.8%)**
Cholesterol						
Not detected	<470	378	5730	6.6	Ref	
	≥470	14	280	5.4	-1.2% (-5.3%, 3.0%)	
Detected in all	<470	109	2189	4.9	Ref	
	≥470	9	309	3.1	-1.9% (-4.1%, 0.4%)	**-0.7% (-5.3%, 4.0%)**
DEET						
Not detected	<470	369	5874	6.3	Ref	
	≥470	3	102	2.7	-3.6% (-6.7%, -0.6%)	
Detected in all	<470	118	2045	5.6	Ref	
	≥470	20	487	4.5	-1.1% (-4.0%, 1.8%)	**2.6% (-1.6%, 6.7%)**
Diethoxyoctylphenol						
Not detected	<470	483	7866	6.1	Ref	
	≥470	23	589	4.4	-1.8% (-4.2%, 0.7%)	
Detected in all	<470	4	53	5.8	Ref	
	≥470	0	0	NA	NA	**NA**
Phenol						
Not detected	<470	394	6079	7.3	Ref	
	≥470	9	233	3.2	-4.1% (-7.6%, -0.6%)	
Detected in all	<470	93	1840	3.8	Ref	
	≥470	14	356	3.3	-0.6% (-2.7%, 1.5%)	**3.5% (-0.4%, 7.5%)**
Tributyl phosphate						
Not detected	<470	428	7147	5.9	Ref	
	≥470	12	401	3.3	-2.6% (-5.3%, 0.1%)	
Detected in all	<470	59	772	7.8	Ref	
	≥470	11	188	8.0	0.2% (-5.9%, 6.2%)	**2.8% (-3.8%, 9.3%)**

NA, not able to estimated

* Adjusted for beach, mean bathers, sand, rain

CHAPTER 7. CONCLUSIONS

Summary of overall study aims and findings

This study examined the associations between human-associated *Bacteroides* and chemical markers of fecal contamination, and the risk of swimming-associated illness among body immersion swimmers using a large prospective cohort. We estimated risk differences for exposure to four *Bacteroides* assays: HumM2, HF183, BsteriF1, and BuniF2 in the first aim. In the second aim, our primary analysis estimated risk differences for exposure to nine chemicals (acetaminophen, caffeine, cholesterol, beta-sitosterol, bisphenol A, diethoxyoctylphenol, DEET, phenol, and tributyl phosphate) and five chemical categories (pharmaceuticals, fecal sterols/stanols, household waste products, industrial waste products, and chemicals with a potential for runoff). For both aims, we also investigated whether the human marker modified the association between general *Enterococcus* and each illness.

To accomplish the first aim, we estimated risk differences for body immersion swimmers who swam on days when human-associated *Bacteroides* markers were detected in 2 or more samples in the water vs. on days when markers were detected in 0-1 samples. Among body immersion swimmers, we observed suggestive associations between risk of GI illness, diarrhea, and respiratory illness and exposure to the human-associated *Bacteroides* marker BsteriF1. We did not observe consistent associations between disease risk from fecally-contaminated water and occurrence of other human-associated *Bacteroides* markers, nor did we see an improvement

over general *Enterococcus* at beaches impacted by human sources of fecal contamination. Patterns in disease risk were largely similar when stratified by water matrix (freshwater vs. saltwater). Sensitivity analyses indicated that risk estimates could be improved when combining multiple *Bacteroides* markers, although a clear dose-response pattern still did not emerge.

Results from our first aim were unexpected in light of findings from previous NEEAR findings that *Enterococcus* by qPCR was associated with an increased risk of GI illness in sewage-impacted freshwater and marine beaches, and general *Bacteroides* qPCR was associated with increased risk of GI illness in sewage-impacted marine beaches (56,57). However, there are a number of plausible explanations. Human-associated *Bacteroides* markers used in this study (HF183, HumM2, BuniF2) are reported to be less persistent in than culture-based or qPCR-based general *Enterococcus* (141-143). Although this evidence comes from microcosm studies using water from a variety of settings spiked with sewage, they include *in situ* microcosms. This finding is believed to extend to waters in the ambient environment. If human-associated *Bacteroides* markers are indeed less persistent and abundant than general *Enterococcus*, this may have limited our ability to estimate associations with swimming-associated illness. Extended freezer storage times may also have affected the sensitivity or abundance of *Bacteroides* samples, but the impact is impossible to know for certain. A final possibility is that human-specific markers may be more strongly associated with illness at sites without a known source of sewage contamination, impacted by a wider range of fecal contaminants, or with lower levels of overall fecal contamination. Thus it is not clear that our findings are generalizable to those other settings.

Our findings were consistent with three previous studies of human-associated fecal markers and swimming-associated illness, despite a number of differences between our study

and those previous. In particular, previous studies were smaller, used different assays targeting either *Enterococcus* or *Bacteroides*, and the source of fecal contamination was from non-point sources (62,64,67). Our study was not consistent with an additional study (65) which did find an increased risk of enteric illness with exposure to a human-associated *Enterococcus* marker. Given our findings and the limited number of existing studies, future research investigating HF183, HumM2, BsteriF1, and BuniF2 in point and non-point source-impacted beaches may need to be even larger to estimate consistent associations.

To accomplish the second aim, we examined exposure to human-associated chemicals and chemical categories, and estimated risk differences for associations with swimming-related illness. Overall we observed little evidence of association between chemical markers and illness, but there were several chemicals that did show a pattern of increased risks, including bisphenol A and GI illness, cholesterol and GI illness, household wastewater products and respiratory illness, and tributyl phosphate and respiratory illness. At the same time, several implausible, inverse associations were observed with respiratory illness. Phenol exposure increased the magnitude of association between general *Enterococcus* dichotomized at policy-relevant cut-points and GI illness, eye ailments, and respiratory illness by 3-5%. All other chemical markers and chemical categories were not consistently associated with elevated risks of illness, nor were they an improvement over general *Enterococcus* at beaches impacted by human sources of fecal contamination. To the best of our knowledge, our study is the first investigation of the relationship between human-associated chemical markers and swimming-related illnesses.

The finding that most chemical markers we investigated were not associated with illness is not unexpected, given that chemicals specific to human waste streams are often at low concentrations and are further diluted below detection limits once wastewater enters

environmental waters (11). This was true in our study, where, although human-associated chemical markers were detected in at least 1 sample almost every day samples were collected, chemical concentrations were low (Table 6.2). Thus, chemical markers may be most appropriate when used in combination with microbial source tracking fecal markers or to validate results obtained using microbial markers, as part of a source-tracking "toolbox" approach to yield greater confidence in an assessment of water quality source.

Strengths

This research makes use of an existing prospective cohort with objective exposure measurements for a wide range of potential human-associated *Bacteroides* and chemical indicators and multiple health outcomes to investigate the research aims. Thus, we did not rely on proxy measures of human fecal contamination (i.e. proximity to effluent from sewage treatment plants) to assign exposure; instead, exposure was assessed directly from the water using fecal indicators. The *Bacteroides* fecal indicator measures included were those that are considered highly human-associated (HF183, HumM2, BsteriF1 and BuniF2) and make use of rapid, qPCR-based molecular methods for detection. The analysis included several sensitivity analyses testing alternate exposure categorizations and results were robust to intensity of swimming exposure as defined by head immersion and swallowing water.

Strengths of the study design included its prospective nature, which allowed us to establish a temporal relationship between the presence/concentration of human-associated markers and subsequent risk of illness, and thus we were able to investigate their association with the risk of illness. The 10-12 day follow-up period of the study reflected the incubation time for likely waterborne pathogens that cause gastrointestinal, respiratory, rash, ear, eye, and

urinary tract symptoms that are our outcomes of interest. The study design allowed for the collection of water samples multiple times per day at two water depths (shin height (0.3 m) and waist height (1.0 m)) and three beach locations to capture the variety of fecal indicator exposures a beachgoer may experience in the water. This study is also the largest and most comprehensive investigation of associations between human-associated microbial and chemical markers (12,060 body immersion swimmers in 4 freshwater and 2 marine beaches for Aim 1-*Bacteroides*; 9,109 swimmers in 4 freshwater and 1 marine beaches for Aim 2-chemicals).

Limitations

Despite the large cohort, our results may not be generalizable to sites affected by fecal contamination from other, non-point sources (e.g. bird-impacted), settings (e.g. tropical climates, estuaries), or geographical locations. Additionally, analyses of less frequent, non-enteric illnesses (i.e. rash, eye ailment, earache, urinary tract infection), and sensitivity analyses among swimmers with head immersion exposure or who swallowed water was limited by smaller sample sizes, and thus produced less precise estimates. The smaller sample size of certain illnesses also led to instability of binomial regression models and limited our ability to estimate the risk difference directly as a measure of association. We therefore used model-based standardization to estimate risk differences adjusted for covariates identified by directed acyclic graph. Our ability to make inferences was also limited by the high proportion of human-associated markers that were below the limit of detection, particularly for chemical markers. We therefore dichotomized exposure to human-associated markers and examined other categorizations in sensitivity analyses. These exposures do not necessarily reflect each swimmer's individual exposure; however, characterizing individual exposures would have been costly and logistically difficult. There is

likely some exposure misclassification, but the magnitude is difficult to predict. In an effort to capture the diversity of health outcomes potentially associated with recreational water exposure, particularly because they can be self-limiting and of short duration, the health outcomes examined in this research focus on self-reported symptomology rather than physician- or laboratory-confirmed cases. While health outcomes may have been affected by recall bias, it is likely nondifferential with respect to water quality fecal indicator exposure because swimmers were unaware of the water quality values recorded in the samples on the day of their beach visit. Lastly, it is unknown what effect freezing and long-term storage had on the concentration of human-associated *Bacteroides* indicators measured by qPCR, but the concentration of general *Enterococcus* samples similarly stored was lower than the concentration of samples assayed soon after collection. Thus, the concentration of human-associated *Bacteroides* may also have lowered after storage.

Public health impact

This investigation of the relationship between human-associated markers and human illness outcomes was conducted to estimate effects related to human fecal exposure, and use that information to help determine the best applications for such markers. Alternative fecal indicators that can distinguish sources of fecal pollution and are measured using rapid methods are an active area of water quality research. This research provides some insight into two critical questions that remain to be answered: (1) are human-associated fecal markers associated with human illness?; and (2) do human-associated markers represent an improvement over general, non-specific fecal indicator bacteria, such as *E. coli* and enterococci, in terms of characterizing risk? For the human-associated *Bacteroides* indicators HumM2, HF183, BsteriF1, and BuniF2,

several patterns of disease risk were suggested for GI illness, diarrhea, respiratory illness, and rash. A combination of *Bacteroides* markers seemed to improve the strength of risk estimates. For the human-associated chemical indicators, bisphenol A, phenol, and cholesterol were inversely associated with respiratory illness. Thus, our results suggest that human-associated markers may be associated with human illness, but the importance of the associations we observed remains unclear and in some cases unexpected. As indicators of human-source, no single *Bacteroides* indicator or chemical marker strongly improved associations between general, non-specific *Enterococcus* and risk of swimming-related illnesses. These findings highlight the need for further epidemiology studies to investigate the illness risks associated with human-associated markers in different geographical locations, at rivers and estuarine settings, at sites dominated by non-point sources, and at sites impacted by a broader range of fecal contamination. The true public health impact may not be readily apparent until that time. Until then, our findings offer an initial step toward the goal of using microbial and chemical markers to identify fecal sources.

Future directions

This research represents an initial investigation of the illness risks associated with several promising human-associated fecal indicators. The elucidation of illness risks associated with human-associated indicators is an important research question that can benefit from further research in several directions. First, because we observed patterns of increased risk for GI illness, diarrhea, and respiratory illness associated with BsteriF1 detection, and patterns of decreased risk for rash and HumM2 and HF183 detection, future studies should collect quantitative fecal indicator measures by qPCR to further clarify the potential illness risks we

observed. If the markers are more abundant in the beaches chosen by the replication study than in this study and if quantitative measures can be collected, then the ability to make inferences will improve. This could lead to more refined exposure classification than was possible at the NEEAR beaches. Similarly, replicating our investigation using a different suite of human-associated fecal markers, such as F+ specific coliphage, enteric viruses, *Bifidobacteria*, and *Methanobrevibacter smithii*, and different chemical markers might reveal novel associations with illness risk that could inform the use of a fecal source tracking toolbox.

Another research question that deserves further investigation is whether the relationships observed at the beaches in this study can be extended to other settings where sewage is not believed to be the primary source of pollution. We hypothesized that one reason for our findings of no association could be that human-associated markers may be better associated with illness at sites which do not have a known source of sewage contamination, are impacted by a wider range of fecal contaminants, or have lower levels of overall fecal contamination. To answer these questions, future studies should be conducted at beaches impacted predominantly by animal sources, runoff-impacted beaches, or beaches impacted by sporadic and diffuse sources of contamination. Studies conducted in other settings such as rivers, estuaries, temperate beaches outside the US, and tropical beaches are also needed. Evaluating relationships at beaches in a variety of settings may also help to clarify the extent to which human-associated indicators are associated with swimming-associated illness.

Improving outcome classification can also strengthen future studies by removing subjectivity. While a study focused on physician- or laboratory-confirmed cases would underestimate health outcomes, the incorporation of saliva samples may be a practical, non-invasive way to obtain objective information on the production of antibodies to common enteric

illnesses associated with swimming, including *Giardia*, norovirus, legionella, rotavirus, and *Cryptosporidium*) (160,161). Because of its non-invasive nature, it has the added advantage that it can be collected from non-swimmers as well, providing the investigator with a measure of the amount of disease circulating in the community.

Lastly, although our findings suggest little consistent association between human-associated *Bacteroides/*chemical markers and illness risks, the markers we investigated may be useful as part of a predictive model for human fecal contamination. Since no single microbial or chemical marker has been shown to determine the source of fecal pollution on its own (151,162,163), a predictive model that combines both chemical and microbial host-specific markers as well as environmental parameters can enable a more confident discrimination of human source.

Final conclusions

This is one of the first and largest studies to evaluate associations between exposure to human-associated *Bacteroides* markers and self-reported illness among swimmers, and the first study, to our knowledge, to evaluate associations between exposure to human-associated chemical markers and illness. Overall, neither human-associated *Bacteroides* markers nor chemical markers were consistently associated with swimming-associated illnesses, although a pattern of increased illness risk was observed for BsteriF1, bisphenol A, tributyl phosphate, and cholesterol. In addition, when phenol exposure was detected, the associations between general, non-source specific *Enterococcus* and several illnesses were greater in magnitude than when phenol was not detected, indicating that it might be a useful addition to estimating risk at beach sites impacted by sewage effluent. Collecting quantitative fecal indicator measures, replicating

our investigation using different indicators and in beaches influenced by non-point sources, and improving outcome classification are a few ways to clarify the associations between human fecal contamination and illness among swimmers. These improvements could help inform the use of human-associated fecal indicators in determining illness risks and remediating fecal pollution.

APPENDIX A. CHAPTER 5 SUPPLEMENTAL TABLES AND FIGURES

This appendix contains supplemental tables and figures associated with analyses involving human-associated *Bacteroides* markers shown in Chapter 5.

Table A.1a. Frequencies and standardized RD (95% CI) for the association between illness and human-associated Bacteroides markers among body immersion swimmers in all beaches

		All beaches				
		Cases	**N**	**Crude Risk**	**Adjusted Risk**	**Adjusted RD (95% CI)**
GI illness*						
HumM2	0-1 samples	508	5712	8.9%	8.5%	Ref
	≥2 samples	449	5976	7.5%	8.2%	-0.3% (-1.7%, 1.1%)
HF183	0-1 samples	254	2503	10.1%	8.1%	Ref
	≥2 samples	703	9185	7.7%	8.4%	0.3% (-1.6%, 2.2%)
BsteriF1	0-1 samples	51	575	8.9%	6.8%	Ref
	≥2 samples	906	11113	8.2%	8.7%	1.9% (0.1%, 3.7%)
BuniF2	0-1 samples	696	8453	8.2%	6.6%	Ref
	≥2 samples	261	3235	8.1%	8.5%	1.8% (-0.8%, 4.4%)
Diarrhea*						
HumM2	0-1 samples	352	5707	6.2%	5.8%	Ref
	≥2 samples	287	5971	4.8%	5.5%	-0.3% (-1.5%, 0.9%)
HF183	0-1 samples	190	2501	7.6%	5.9%	Ref
	≥2 samples	449	9177	4.9%	5.5%	-0.4% (-2.1%, 1.3%)
BsteriF1	0-1 samples	38	575	6.6%	4.6%	Ref
	≥2 samples	601	11103	5.4%	5.9%	1.3% (-0.2%, 2.7%)
BuniF2	0-1 samples	462	8449	5.5%	5.1%	Ref
	≥2 samples	177	3229	5.5%	5.7%	0.6% (-1.6%, 2.9%)
Respiratory†						
HumM2	0-1 samples	335	5543	6.0%	5.9%	Ref
	≥2 samples	336	5790	5.8%	6.0%	0.1% (-1.0%, 1.3%)
HF183	0-1 samples	149	2448	6.1%	5.3%	Ref
	≥2 samples	522	8885	5.9%	6.2%	0.8% (-0.8%, 2.5%)
BsteriF1	0-1 samples	38	562	6.8%	5.1%	Ref
	≥2 samples	633	10771	5.9%	6.2%	1.1% (-0.2%, 2.5%)
BuniF2	0-1 samples	472	8202	5.8%	6.0%	Ref
	≥2 samples	199	3131	6.4%	6.0%	-0.1% (-2.6%, 2.5%)
Earache*						

		All beaches				
		Cases	N	Crude Risk	Adjusted Risk	Adjusted RD (95% CI)
HumM2	0-1 samples	107	5775	1.9%	1.9%	Ref
	≥2 samples	114	6058	1.9%	1.9%	0.0% (-0.6%, 0.6%)
HF183	0-1 samples	46	2541	1.8%	1.7%	Ref
	≥2 samples	175	9292	1.9%	2.0%	0.3% (-0.6%, 1.1%)
BsteriF1	0-1 samples	15	574	2.6%	1.4%	Ref
	≥2 samples	206	11259	1.8%	2.0%	0.6% (-0.1%, 1.4%)
BuniF2	0-1 samples	157	8554	1.8%	2.9%	Ref
	≥2 samples	64	3279	2.0%	1.9%	-1.0% (-3.1%, 1.0%)
Eye ailment†						
HumM2	0-1 samples	171	5834	2.9%	2.9%	Ref
	≥2 samples	157	6112	2.6%	2.7%	-0.3% (-1.0%, 0.5%)
HF183	0-1 samples	69	2558	2.7%	2.4%	Ref
	≥2 samples	259	9388	2.8%	2.9%	0.5% (-0.5%, 1.5%)
BsteriF1	0-1 samples	17	582	2.9%	2.3%	Ref
	≥2 samples	311	11364	2.7%	2.9%	0.7% (-0.2%, 1.6%)
BuniF2	0-1 samples	247	8640	2.9%	2.6%	Ref
	≥2 samples	81	3306	2.5%	2.8%	0.2% (-1.3%, 1.7%)
Rash†						
HumM2	0-1 samples	201	5740	3.5%	3.7%	Ref
	≥2 samples	166	5995	2.8%	2.6%	-1.0% (-1.9%, -0.2%)
HF183	0-1 samples	87	2523	3.4%	4.0%	Ref
	≥2 samples	280	9212	3.0%	2.9%	-1.1% (-2.4%, 0.3%)
BsteriF1	0-1 samples	17	576	3.0%	3.1%	Ref
	≥2 samples	350	11159	3.1%	3.1%	0.1% (-1.0%, 1.1%)
BuniF2	0-1 samples	272	8491	3.2%	3.0%	Ref
	≥2 samples	95	3244	2.9%	3.1%	0.2% (-1.7%, 2.0%)
UTI*						
HumM2	0-1 samples	34	5845	0.6%	0.6%	Ref
	≥2 samples	42	6099	0.7%	0.7%	0.1% (-0.3%, 0.5%)
HF183	0-1 samples	16	2557	0.6%	0.6%	Ref
	≥2 samples	60	9387	0.6%	0.7%	0.1% (-0.5%, 0.6%)
BsteriF1	0-1 samples	3	585	0.5%	0.4%	Ref
	≥2 samples	73	11359	0.6%	0.7%	0.4% (-0.1%, 0.8%)
BuniF2	0-1 samples	50	8640	0.6%	0.4%	Ref
	≥2 samples	26	3304	0.8%	0.7%	0.2% (-0.4%, 0.9%)

GI, gastrointestinal; NA, not able to be estimated; UTI, urinary tract infection.

* Adjusted for beach, age, mean bathers, sand, rain, water temperature

† Adjusted for beach, age, mean bathers, sand, rain

Table A.1b. Frequencies and standardized risk differences (95% CI) for the association between illness and human-associated Bacteroides markers among body immersion swimmers in fresh water beaches

		Freshwater beaches				
		Cases	**N**	**Crude Risk**	**Adjusted Risk**	**Adjusted RD (95% CI)**
GI illness*						
HumM2	0-1 samples	403	4474	9.0%	8.7%	Ref
	≥2 samples	416	5373	7.7%	8.4%	-0.3% (-1.7%, 1.2%)
HF183	0-1 samples	186	1734	10.7%	8.4%	Ref
	≥2 samples	633	8113	7.8%	8.6%	0.2% (-1.9%, 2.3%)
BsteriF1	0-1 samples	19	208	9.1%	7.0%	Ref
	≥2 samples	800	9639	8.3%	8.7%	1.7% (-0.4%, 3.9%)
BuniF2	0-1 samples	585	6925	8.4%	6.1%	Ref
	≥2 samples	234	2922	8.0%	8.6%	2.5% (-1.1%, 6.1%)
Diarrhea*						
HumM2	0-1 samples	282	4469	6.3%	5.9%	Ref
	≥2 samples	265	5368	4.9%	5.7%	-0.2% (-1.5%, 1.0%)
HF183	0-1 samples	142	1732	8.2%	6.1%	Ref
	≥2 samples	405	8105	5.0%	5.7%	-0.4% (-2.3%, 1.4%)
BsteriF1	0-1 samples	13	208	6.3%	4.7%	Ref
	≥2 samples	534	9629	5.5%	6.0%	1.2% (-0.5%, 3.0%)
BuniF2	0-1 samples	388	6921	5.6%	4.1%	Ref
	≥2 samples	159	2916	5.5%	5.8%	1.8% (-1.2%, 4.7%)
Respiratory†						
HumM2	0-1 samples	253	4319	5.9%	5.8%	Ref
	≥2 samples	312	5197	6.0%	6.2%	0.4% (-0.8%, 1.6%)
HF183	0-1 samples	99	1686	5.9%	5.4%	Ref
	≥2 samples	466	7830	6.0%	6.1%	0.7% (-1.1%, 2.6%)
BsteriF1	0-1 samples	7	201	3.5%	5.0%	Ref
	≥2 samples	558	9315	6.0%	6.1%	1.1% (-0.4%, 2.7%)
BuniF2	0-1 samples	388	6700	5.8%	3.4%	Ref
	≥2 samples	177	2816	6.3%	6.1%	2.7% (0.1%, 5.3%)
Earache*						
HumM2	0-1 samples	78	4512	1.7%	1.8%	Ref
	≥2 samples	107	5440	2.0%	2.0%	0.2% (-0.5%, 0.9%)
HF183	0-1 samples	28	1750	1.6%	1.4%	Ref
	≥2 samples	157	8202	1.9%	2.0%	0.6% (-0.3%, 1.4%)
BsteriF1	0-1 samples	5	204	2.5%	1.2%	Ref
	≥2 samples	180	9748	1.8%	2.0%	0.8% (-0.1%, 1.7%)
BuniF2	0-1 samples	126	6996	1.8%	2.0%	Ref

		Freshwater beaches				
		Cases	N	Crude Risk	Adjusted Risk	Adjusted RD (95% CI)
	≥2 samples	59	2956	2.0%	1.9%	-0.1% (-2.3%, 2.1%)
Eye ailment†						
HumM2	0-1 samples	135	4561	3.0%	3.0%	Ref
	≥2 samples	139	5493	2.5%	2.6%	-0.3% (-1.2%, 0.5%)
HF183	0-1 samples	48	1762	2.7%	2.4%	Ref
	≥2 samples	226	8292	2.7%	2.9%	0.5% (-0.5%, 1.6%)
BsteriF1	0-1 samples	3	209	1.4%	2.1%	Ref
	≥2 samples	271	9845	2.8%	2.9%	0.8% (-0.2%, 1.8%)
BuniF2	0-1 samples	202	7072	2.9%	0.9%	Ref
	≥2 samples	72	2982	2.4%	2.8%	1.9% (0.7%, 3.1%)
Rash†						
HumM2	0-1 samples	156	4484	3.5%	3.6%	Ref
	≥2 samples	143	5390	2.7%	2.5%	-1% (-1.9%, -0.2%)
HF183	0-1 samples	56	1733	3.2%	3.2%	Ref
	≥2 samples	243	8141	3.0%	2.9%	-0.3% (-1.5%, 1.0%)
BsteriF1	0-1 samples	6	204	2.9%	3.0%	Ref
	≥2 samples	293	9670	3.0%	3.0%	-0.1% (-1.2%, 1.0%)
BuniF2	0-1 samples	219	6946	3.2%	2.5%	Ref
	≥2 samples	80	2928	2.7%	3.0%	0.5% (-1.9%, 2.9%)
UTI*						
HumM2	0-1 samples	28	4570	0.6%	0.7%	Ref
	≥2 samples	35	5481	0.6%	0.7%	0.0% (-0.4%, 0.4%)
HF183	0-1 samples	12	1759	0.7%	0.6%	Ref
	≥2 samples	51	8292	0.6%	0.7%	0.1% (-0.6%, 0.7%)
BsteriF1	0-1 samples	1	209	0.5%	0.4%	Ref
	≥2 samples	62	9842	0.6%	0.7%	0.4% (-0.2%, 0.9%)
BuniF2	0-1 samples	44	7070	0.6%	0.3%	Ref
	≥2 samples	19	2981	0.6%	0.7%	0.4% (-0.4%, 1.2%)

GI, gastrointestinal; NA, not able to be estimated; UTI, urinary tract infection.
* Adjusted for beach, age, mean bathers, sand, rain, water temperature
† Adjusted for beach, age, mean bathers, sand, rain

Table A.1c. Frequencies and standardized risk differences (95% CI) for the association between illness and human-associated Bacteroides markers among body immersion swimmers in marine beaches

		Marine beaches				
		Cases	N	Crude Risk	Adjusted Risk	Adjusted RD (95% CI)
GI illness*						
HumM2	0-1 samples	105	1238	8.5%	7.9%	Ref
	≥2 samples	33	603	5.5%	6.1%	-1.8% (-5.4%, 1.9%)
HF183	0-1 samples	68	769	8.8%	6.7%	Ref
	≥2 samples	70	1072	6.5%	8.3%	1.6% (-2.7%, 5.9%)
BsteriF1	0-1 samples	32	367	8.7%	6.3%	Ref
	≥2 samples	106	1474	7.2%	8.0%	1.7% (-1.6%, 5.1%)
BuniF2	0-1 samples	111	1528	7.3%	6.7%	Ref
	≥2 samples	27	313	8.6%	7.6%	0.9% (-3.1%, 4.9%)
Diarrhea*						
HumM2	0-1 samples	70	1238	5.7%	5.3%	Ref
	≥2 samples	22	603	3.6%	3.6%	-1.7% (-4.7%, 1.3%)
HF183	0-1 samples	48	769	6.2%	5.0%	Ref
	≥2 samples	44	1072	4.1%	4.7%	-0.3% (-4.3%, 3.8%)
BsteriF1	0-1 samples	25	367	6.8%	4.3%	Ref
	≥2 samples	67	1474	4.5%	5.2%	1.0% (-1.8%, 3.7%)
BuniF2	0-1 samples	74	1528	4.8%	5.3%	Ref
	≥2 samples	18	313	5.8%	4.7%	-0.6% (-4.1%, 2.9%)
Respiratory†						
HumM2	0-1 samples	82	1224	6.7%	6.3%	Ref
	≥2 samples	24	593	4.0%	4.7%	-1.5% (-4.3%, 1.3%)
HF183	0-1 samples	50	762	6.6%	5.3%	Ref
	≥2 samples	56	1055	5.3%	6.5%	1.3% (-2.3%, 4.9%)
BsteriF1	0-1 samples	31	361	8.6%	5.2%	Ref
	≥2 samples	75	1456	5.2%	6.2%	1.0% (-2.1%, 4%)
BuniF2	0-1 samples	84	1502	5.6%	8.7%	Ref
	≥2 samples	22	315	7.0%	5.1%	-3.6% (-8.9%, 1.7%)
Earache*						
HumM2	0-1 samples	29	1263	2.3%	2.5%	Ref
	≥2 samples	7	618	1.1%	1.0%	-1.5% (-3.0%, 0.0%)
HF183	0-1 samples	18	791	2.3%	3.3%	Ref
	≥2 samples	18	1090	1.7%	1.4%	-1.9% (-5.0%, 1.2%)
BsteriF1	0-1 samples	10	370	2.7%	1.6%	Ref
	≥2 samples	26	1511	1.7%	2.1%	0.5% (-1.2%, 2.3%)
BuniF2	0-1 samples	31	1558	2.0%	3.8%	Ref

		Marine beaches				
		Cases	N	Crude Risk	Adjusted Risk	Adjusted RD (95% CI)
	≥2 samples	5	323	1.5%	1.6%	-2.2% (-6.8%, 2.4%)
Eye ailment†						
HumM2	0-1 samples	36	1273	2.8%	2.8%	Ref
	≥2 samples	18	619	2.9%	3.2%	0.4% (-1.7%, 2.6%)
HF183	0-1 samples	21	796	2.6%	2.6%	Ref
	≥2 samples	33	1096	3.0%	3.2%	0.6% (-2.2%, 3.4%)
BsteriF1	0-1 samples	14	373	3.8%	3.1%	Ref
	≥2 samples	40	1519	2.6%	2.8%	-0.3% (-2.8%, 2.1%)
BuniF2	0-1 samples	45	1568	2.9%	5.5%	Ref
	≥2 samples	9	324	2.8%	2.5%	-3.0% (-8.2%, 2.1%)
Rash†						
HumM2	0-1 samples	45	1256	3.6%	4.2%	Ref
	≥2 samples	23	605	3.8%	3.1%	-1.1% (-3.6%, 1.4%)
HF183	0-1 samples	31	790	3.9%	9.4%	Ref
	≥2 samples	37	1071	3.5%	2.5%	-6.9% (-12.6%, -1.2%)
BsteriF1	0-1 samples	11	372	3.0%	3.4%	Ref
	≥2 samples	57	1489	3.8%	3.9%	0.5% (-2.6%, 3.6%)
BuniF2	0-1 samples	53	1545	3.4%	3.7%	Ref
	≥2 samples	15	316	4.7%	3.8%	0.1% (-3.5%, 3.7%)
UTI*						
HumM2	0-1 samples	6	1275	0.5%	0.5%	Ref
	≥2 samples	7	618	1.1%	1.9%	1.4% (-1.2%, 4.0%)
HF183	0-1 samples	4	798	0.5%	0.5%	Ref
	≥2 samples	9	1095	0.8%	1.0%	0.4% (-0.1%, 1.0%)
BsteriF1	0-1 samples	2	376	0.5%	0.5%	Ref
	≥2 samples	11	1517	0.7%	0.8%	0.4% (-0.5%, 1.3%)
BuniF2	0-1 samples	6	1570	0.4%	0.8%	Ref
	≥2 samples	7	323	2.2%	0.8%	0.0% (-1.8%, 1.7%)

GI, gastrointestinal; NA, not able to be estimated; UTI, urinary tract infection.
* Adjusted for beach, age, mean bathers, sand, rain, water temperature
† Adjusted for beach, age, mean bathers, sand, rain

Table A.2a. Frequencies and standardized risk differences (95% CI) for the association between illness and number of human-associated *Bacteroides* markers among body immersion swimmers in all beaches

All beaches				
Number of Markers Detected	**Cases**	**N**	**Adjusted Risk**	**Adjusted RD (95% CI)**
GI illness*				
0	39	440	6.4%	Ref
1	82	899	7.0%	0.7% (-2.4%, 3.7%)
2	121	1044	9.3%	2.9% (-0.2%, 6.1%)
3	326	4112	8.8%	2.5% (-0.7%, 5.6%)
4	389	5193	8.3%	1.9% (-1.2%, 5.1%)
Diarrhea*				
0	29	440	5.1%	Ref
1	61	897	4.9%	-0.2% (-2.9%, 2.6%)
2	88	1044	6.7%	1.7% (-1.1%, 4.4%)
3	211	4109	5.8%	0.8% (-2.1%, 3.7%)
4	250	5188	5.5%	0.4% (-2.5%, 3.3%)
Respiratory†				
0	26	430	4.2%	Ref
1	52	868	4.7%	0.5% (-2.0%, 3.0%)
2	58	1029	4.4%	0.1% (-2.2%, 2.5%)
3	245	3964	6.9%	2.7% (0.1%, 5.2%)
4	290	5042	6.2%	2.0% (-0.6%, 4.5%)
Earache*				
0	12	437	2.3%	Ref
1	10	907	1.1%	-1.3% (-3.0%, 0.5%)
2	21	1068	1.8%	-0.5% (-2.4%, 1.3%)
3	78	4158	2.0%	-0.3% (-2.4%, 1.8%)
4	100	5263	1.9%	-0.4% (-2.5%, 1.6%)
Eye ailment†				
0	11	443	1.9%	Ref
1	20	910	2.0%	0.1% (-1.4%, 1.6%)
2	35	1078	2.9%	1.0% (-0.5%, 2.5%)
3	131	4208	3.2%	1.4% (-0.1%, 2.9%)
4	131	5307	2.7%	0.8% (-0.7%, 2.4%)
Rash†				
0	11	437	3.2%	Ref
1	27	903	3.5%	0.3% (-2.3%, 2.9%)

All beaches				
Number of Markers Detected	**Cases**	**N**	**Adjusted Risk**	**Adjusted RD (95% CI)**
2	44	1060	5.2%	2% (-0.7%, 4.7%)
3	147	4130	3.4%	0.2% (-2.3%, 2.7%)
4	138	5205	2.5%	-0.7% (-3.3%, 1.8%)
UTI*				
0	3	445	0.4%	Ref
1	4	914	0.4%	0.0% (-0.7%, 0.6%)
2	6	1075	0.4%	0.0% (-0.7%, 0.7%)
3	26	4205	0.8%	0.4% (-0.4%, 1.1%)
4	37	5305	0.8%	0.3% (-0.5%, 1.1%)

GI, gastrointestinal; NA, not able to be estimated; UTI, urinary tract infection.
* Adjusted for beach, age, mean bathers, sand, rain, water temperature
† Adjusted for beach, age, mean bathers, sand, rain

Table A.2b. Frequencies and standardized risk differences (95% CI) for the association between illness and number of human-associated *Bacteroides* markers among body immersion swimmers in fresh water beaches

Fresh water beaches				
Number of Markers Detected	**Cases**	**N**	**Adjusted Risk**	**Adjusted RD (95% CI)**
GI illness*				
0	19	208	6.3%	Ref
1	57	576	7.1%	0.8% (-3.4%, 5.0%)
2	91	765	9.7%	3.4% (-0.6%, 7.4%)
3	295	3683	8.9%	2.7% (-1.3%, 6.7%)
4	357	4615	8.4%	2.1% (-1.8%, 6.1%)
Diarrhea*				
0	13	208	4.4%	Ref
1	44	574	5.1%	0.7% (-3.0%, 4.3%)
2	69	765	7.0%	2.6% (-0.9%, 6.1%)
3	192	3680	6.0%	1.6% (-1.9%, 5.0%)
4	229	4610	5.6%	1.3% (-2.2%, 4.7%)
Respiratory†				
0	7	201	2.8%	Ref
1	35	556	4.9%	2.2% (-0.6%, 4.9%)
2	37	744	3.9%	1.1% (-1.3%, 3.5%)
3	219	3545	6.8%	4.0% (1.5%, 6.5%)
4	267	4470	6.2%	3.4% (1.0%, 5.9%)
Earache*				
0	5	204	1.6%	Ref
1	6	578	1.0%	0.0% (-0.03, 0.0%)
2	14	776	1.6%	-0.1% (-2.0%, 1.8%)
3	66	3724	2.0%	0.3% (-1.7%, 2.3%)
4	94	4670	2.1%	0.4% (-1.6%, 2.4%)
Eye ailment†				
0	3	209	0.9%	Ref
1	11	578	1.6%	0.8% (-0.7%, 2.2%)
2	28	783	3.0%	2.1% (0.5%, 3.7%)
3	118	3771	3.3%	2.4% (1.1%, 3.6%)
4	114	4713	2.6%	1.8% (0.5%, 3.1%)
Rash†				
0	6	204	3.0%	Ref
1	13	575	2.5%	-0.5% (-3.7%, 2.6%)

Fresh water beaches				
Number of Markers Detected	**Cases**	**N**	**Adjusted Risk**	**Adjusted RD (95% CI)**
2	32	766	4.6%	1.6% (-1.5%, 4.7%)
3	132	3704	3.5%	0.5% (-2.5%, 3.5%)
4	116	4625	2.4%	-0.6% (-3.6%, 2.5%)
UTI*				
0	1	209	0.2%	Ref
1	4	582	0.5%	0.3% (-0.6%, 1.1%)
2	4	780	0.4%	0.1% (-0.6%, 0.8%)
3	24	3768	0.9%	0.6% (-0.2%, 1.4%)
4	30	4712	0.7%	0.4% (-0.3%, 1.2%)

GI, gastrointestinal; NA, not able to be estimated; UTI, urinary tract infection.
* Adjusted for beach, age, mean bathers, sand, rain, water temperature
† Adjusted for beach, age, mean bathers, sand, rain

Table A.2c. Frequencies and standardized risk differences (95% CI) for the association between illness and number of human-associated *Bacteroides* markers among body immersion swimmers in marine beaches

Marine beaches				
Number of Markers Detected	**Cases**	**N**	**Adjusted Risk**	**Adjusted RD (95% CI)**
GI illness*				
0	20	232	6.5%	Ref
1	25	323	6.9%	0.4% (-4.3%, 5.2%)
2	30	279	9.1%	2.7% (-3.1%, 8.5%)
3	31	429	7.9%	1.4% (-4.1%, 6.9%)
4	32	578	6.8%	0.3% (-6.3%, 6.9%)
Diarrhea*				
0	16	232	6.1%	Ref
1	17	323	4.8%	-1.3% (-6.1%, 3.5%)
2	19	279	7.2%	1.2% (-4.5%, 6.8%)
3	19	429	4.6%	-1.5% (-7.1%, 4.2%)
4	21	578	3.3%	-2.8% (-9.2%, 3.6%)
Respiratory†				
0	19	229	6.9%	Ref
1	17	312	5.4%	-1.5% (-6.8%, 3.7%)
2	21	285	5.9%	-1.0% (-6.5%, 4.4%)
3	26	419	6.6%	-0.3% (-6.5%, 5.8%)
4	23	572	4.8%	-2.1% (-8.5%, 4.2%)
Earache*				
0	7	233	8.1%	Ref
1	4	329	1.9%	-6.3% (-16.2%, 3.7%)
2	7	292	7.6%	-0.5% (-11.4%, 10.3%)
3	12	434	2.4%	-5.7% (-17.9%, 6.6%)
4	6	593	0.6%	-7.5% (-19.4%, 4.4%)
Eye ailment†				
0	8	234	3.7%	Ref
1	9	332	3.3%	-0.4% (-4.3%, 3.4%)
2	7	295	2.5%	-1.2% (-4.9%, 2.5%)
3	13	437	2.6%	-1.1% (-5.8%, 3.6%)
4	17	594	2.9%	-0.8% (-5.9%, 4.3%)
Rash†				
0	5	233	6.2%	Ref
1	14	328	8.5%	2.3% (-4.8%, 9.5%)

Marine beaches				
Number of Markers Detected	**Cases**	**N**	**Adjusted Risk**	**Adjusted RD (95% CI)**
2	12	294	12.3%	6.0% (-4.5%, 16.5%)
3	15	426	2.7%	-3.5% (-11.1%, 4.1%)
4	22	580	2.4%	-3.9% (-11.6%, 3.9%)
UTI*				
0	2	236	0.5%	Ref
1	0	332	NA	NA
2	2	295	0.5%	-0.1% (-1.2%, 1.1%)
3	2	437	0.7%	0.2% (-0.7%, 1.1%)
4	7	593	3.0%	2.4% (-2.8%, 7.7%)

GI, gastrointestinal; NA, not able to be estimated; UTI, urinary tract infection.
* Adjusted for beach, age, mean bathers, sand, rain, water temperature
† Adjusted for beach, age, mean bathers, sand, rain

Table A.3(a-d). Modification of the adjusted standardized RD (95% CI) for the association between illness and *Enterococcus* qPCR Method 1611 above and below EPA guidelines (geometric mean of 470 CCE/100ml for an illness rate of 36/1000) with detection/non-detection of *Bacteroides* markers among body immersion swimmers in all beaches

Table A.3(a). **Eye ailments**

Eye Ailments							
Marker (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Crude Risk (%)**	**Adjusted Risk (%)***	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main association					-1.3% (-2.6%, 0.0%)	
HumM2							
0-1	<470	164	5515	3.0	3.0	Ref	
	≥470	7	321	2.2	2.7	-1.2% (-3.0%, 0.5%)	
≥ 2	<470	154	5917	2.6	2.7	Ref	
	≥470	3	195	1.5	1.3	-1.4% (-3.1%, 0.2%)	**-0.2% (-2.5%, 2.1%)**
HF183							
0-1	<470	64	2283	2.8	2.5	Ref	
	≥470	5	277	1.8	1.3	-1.2% (-2.9%, 0.5%)	
≥ 2	<470	254	9149	2.8	3.0	Ref	
	≥470	5	239	2.1	1.7	-1.3% (-2.9%, 0.4%)	**-0.1% (-2.4%, 2.2%)**
BsteriF1							
0-1	<470	47	1820	2.6	2.2	Ref	
	≥470	1	73	1.4	0.9	-1.3% (-3.2%, 0.6%)	
≥ 2	<470	271	9612	2.8	3.0	Ref	
	≥470	9	443	2.0	1.6	-1.5% (-2.9%, -0.1%)	**-0.1% (-2.5%, 2.2%)**
BuniF2							
0-1	<470	17	565	3.0	2.5	Ref	
	≥470	0	18	0.0	0.0	NA	
≥ 2	<470	301	10867	2.8	2.9	Ref	
	≥470	10	498	2.0	1.6	-1.3% (-2.6%, 0.1%)	**NA**

NA, not able to be estimated. * Adjusted for beach, age, mean bathers, sand, rain

Table A.3(b). **Rash**

Rash							
Marker (samples)	Enterococcus (CCE/100ml)	Cases	N	Crude Risk (%)	Adjusted Risk (%)*	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--	Main association					-0.3% (-1.9%, 1.3%)	
HumM2							
0-1	<470	188	5424	3.5	3.7	Ref	
	≥470	13	318	4.1	3.7	0.0% (-2.4%, 2.4%)	
≥ 2	<470	162	5810	2.8	2.7	Ref	
	≥470	4	192	2.1	1.9	-0.7% (-2.7%, 1.3%)	**-0.7% (-3.7%, 2.2%)**
HF183							
0-1	<470	78	2251	3.5	4.1	Ref	
	≥470	9	274	3.3	3.2	-0.9% (-3.4%, 1.6%)	
≥ 2	<470	272	8983	3.0	2.9	Ref	
	≥470	8	236	3.4	3.1	0.2% (-2.3%, 2.7%)	**1.1% (-2.3%, 4.6%)**
BsteriF1							
0-1	<470	51	1796	2.8	2.9	Ref	
	≥470	4	73	5.5	5.1	2.2% (-2.9%, 7.3%)	
≥ 2	<470	299	9438	3.2	3.2	Ref	
	≥470	13	437	3.0	2.5	-0.7% (-2.4%, 1.0%)	**-2.9% (-8.1%, 2.3%)**
BuniF2							
0-1	<470	15	559	2.7	2.5	Ref	
	≥470	2	18	11.1	9.1	6.6% (-5.8%, 18.9%)	
≥ 2	<470	335	10675	3.1	3.2	Ref	
	≥470	15	492	3.0	2.6	-0.6% (-2.3%, 1.0%)	**-7.2% (-19.5%, 5.1%)**

NA, not able to estimated. * Adjusted for beach, age, mean bathers, sand, rain

Table A.3(c). **Earache**

		Earache					
Marker (samples)	Enterococcus (CCE/100ml)	Cases	N	Crude Risk (%)	Adjusted Risk (%)	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--	Main association					0.0% (-1.5%, 1.6%)	
HumM2							
0-1	<470	102	5463	1.9	1.9	Ref	
	≥470	5	316	1.6	1.5	-0.5% (-2.0%, 1.1%)	
≥ 2	<470	109	5866	1.9	1.9	Ref	
	≥470	5	192	2.6	2.8	0.9% (-1.8%, 3.7%)	**1.4% (-1.5%, 4.2%)**
HF183							
0-1	<470	44	2272	1.9	1.9	Ref	
	≥470	2	273	0.7	0.6	-1.3% (-2.5%, -0.1%)	
≥ 2	<470	167	9057	1.8	1.9	Ref	
	≥470	8	235	3.4	3.5	1.6% (-1.2%, 4.4%)	**2.9% (-0.1%, 5.9%)**
BsteriF1							
0-1	<470	29	1802	1.6	1.4	Ref	
	≥470	1	72	1.4	1.0	-0.4% (-2.5%, 1.7%)	
≥ 2	<470	182	9527	1.9	2.0	Ref	
	≥470	9	436	2.1	1.9	-0.1% (-1.7%, 1.5%)	**0.3% (-2.2%, 2.8%)**
BuniF2							
0-1	<470	15	558	2.7	3.1	Ref	
	≥470	0	17	0.0	0.0	NA	
≥ 2	<470	196	10771	1.8	1.8	Ref	
	≥470	10	491	2.0	2.2	0.4% (-1.4%, 2.2%)	**NA**

NA, not able to estimated. * Adjusted for beach, age, mean bathers, sand, rain, water temperature

Table A.3(d). **Urinary tract infection**

Urinary tract infection							
Marker (samples)	Enterococcus (CCE/100ml)	Cases	N	Crude Risk (%)	Adjusted Risk (%)	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--	Main association					-0.2% (-0.9%, 0.5%)	
HumM2							
0-1	<470	33	5529	0.6	0.6	Ref	
	≥470	1	320	0.3	0.3	-0.3% (-1.0%, 0.4%)	
≥ 2	<470	41	5910	0.7	0.7	Ref	
	≥470	1	196	0.5	0.7	-0.1% (-1.4%, 1.3%)	**0.2% (-1.3%, 1.8%)**
HF183							
0-1	<470	16	2289	0.7	0.7	Ref	
	≥470	0	277	0.0	0.0	NA	
≥ 2	<470	58	9150	0.6	0.7	Ref	
	≥470	2	239	0.8	1.0	0.3% (-1.2%, 1.8%)	**NA**
BsteriF1							
0-1	<470	9	1820	0.5	0.4	Ref	
	≥470	0	73	0.0	0.0	NA	
≥ 2	<470	65	9619	0.7	0.7	Ref	
	≥470	2	443	0.5	0.5	-0.2% (-1.0%, 0.5%)	**NA**
BuniF2							
0-1	<470	3	570	0.5	0.5	Ref	
	≥470	0	18	0.0	0.0	NA	
≥ 2	<470	71	10869	0.7	0.7	Ref	
	≥470	2	498	0.4	0.5	-0.2% (-1.0%, 0.6%)	**NA**

NA, not able to estimated. Note estimates are based on small cell sizes. * Adjusted for beach, age, mean bathers, sand, rain, water temperature

Table A.4(a-d). Modification of the adjusted standardized RD (95% CI) for the association between illness and *Enterococcus* qPCR Method 1611 above and below EPA guidelines (geometric mean of 300 CCE/100ml for an illness rate of 32/1000) with detection/non-detection of *Bacteroides* markers among body immersion swimmers in all beaches

Table A.4(a). **Eye Ailment**

Eye Ailment							
Marker (samples)	Enterococcus (CCE/100ml)	Cases	N	Crude Risk (%)	Adjusted Risk (%)	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--	Main association					-0.8% (-2.0%, 0.3%)	
HumM2							
0-1	<300	155	5215	3.0	3.0	Ref	
	≥300	16	621	2.6	2.3	-0.7% (-2.1%, 0.7%)	
≥ 2	<300	153	5886	2.6	2.7	Ref	
	≥300	4	226	1.8	1.6	-1.1% (-2.9%, 0.6%)	**-0.5% (-2.6%, 1.7%)**
HF183							
0-1	<300	59	2096	2.8	2.5	Ref	
	≥300	10	464	2.2	1.7	-0.8% (-2.3%, 0.6%)	
≥ 2	<300	249	9005	2.8	3.0	Ref	
	≥300	10	383	2.6	2.2	-0.8% (-2.4%, 0.8%)	**0.0% (-2.1%, 2.2%)**
BsteriF1							
0-1	<300	40	1667	2.4	2.0	Ref	
	≥300	8	226	3.5	2.8	0.8% (-1.4%, 2.9%)	
≥ 2	<300	268	9434	2.8	3.1	Ref	
	≥300	12	621	1.9	1.6	-1.5% (-2.8%, -0.3%)	**-2.3% (-4.5%, 0.0%)**
BuniF2							
0-1	<300	12	450	2.7	2.1	Ref	
	≥300	5	133	3.8	3.1	1.0% (-2.0%, 4.0%)	
≥ 2	<300	296	10651	2.8	2.9	Ref	
	≥300	15	714	2.1	1.7	-1.2% (-2.4%, 0.0%)	**-2.2% (-5.3%, 1.0%)**

NA, not able to estimated.
* Adjusted for beach, age, mean bathers, sand, rain

Table A.4(b). **Rash**

				Rash			
Marker (samples)	Enterococcus (CCE/100ml)	Cases	N	Crude Risk (%)	Adjusted Risk (%)	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--	Main association					-0.8% (-2.1%, 0.5%)	
HumM2							
0-1	<300	187	5124	3.6	3.8	Ref	
	≥300	14	618	2.3	2.3	-1.5% (-3.0%, 0.1%)	
≥ 2	<300	158	5778	2.7	2.6	Ref	
	≥300	8	224	3.6	3.1	0.5% (-2.1%, 3.0%)	**1.9% (-1.0%, 4.8%)**
HF183							
0-1	<300	77	2066	3.7	4.3	Ref	
	≥300	10	459	2.2	2.3	-2.0% (-3.9%, -0.1%)	
≥ 2	<300	268	8836	3.0	2.9	Ref	
	≥300	12	383	3.1	3.1	0.2% (-2.0%, 2.4%)	**2.2% (-0.7%, 5.1%)**
BsteriF1							
0-1	<300	50	1645	3.0	3.1	Ref	
	≥300	5	224	2.2	2.1	-0.9% (-3.2%, 1.3%)	
≥ 2	<300	295	9257	3.2	3.2	Ref	
	≥300	17	618	2.8	2.4	-0.8% (-2.4%, 0.7%)	**0.1% (-2.5%, 2.7%)**
BuniF2							
0-1	<300	15	445	3.4	3.1	Ref	
	≥300	2	132	1.5	1.5	-1.6% (-4.2%, 1.0%)	
≥ 2	<300	330	10457	3.2	3.2	Ref	
	≥300	20	710	2.8	2.5	-0.7% (-2.2%, 0.8%)	**0.9% (-2.2%, 4.0%)**

NA, not able to estimated.
* Adjusted for beach, age, mean bathers, sand, rain

Table A.4(c). **Earache**

Earache							
Marker (samples)	Enterococcus (CCE/100ml)	Cases	N	Crude Risk (%)	Adjusted Risk (%)	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--	Main association					0.5% (-0.9%, 1.8%)	
HumM2							
0-1	<300	94	5165	1.8	1.9	Ref	
	≥300	13	614	2.1	2.2	3.0% (-1.2%, 1.7%)	
≥ 2	<300	108	5834	1.9	1.8	Ref	
	≥300	6	224	2.7	3.0	1.2% (-1.5%, 3.8%)	**0.9% (0.9%, 3.6%)**
HF183							
0-1	<300	38	2086	1.8	1.8	Ref	
	≥300	8	459	1.7	1.6	3.2% (-1.6%, 1.1%)	
≥ 2	<300	164	8913	1.8	1.9	Ref	
	≥300	11	379	2.9	3.2	1.3% (-0.8%, 3.4%)	**1.5% (-0.9%, 3.9%)**
BsteriF1							
0-1	<300	25	1650	1.5	1.3	Ref	
	≥300	5	224	2.2	1.8	0.5% (-1.3%, 2.3%)	
≥ 2	<300	177	9349	1.9	2.0	Ref	
	≥300	14	614	2.3	2.2	0.2% (-1.2%, 1.7%)	**-0.3% (-2.4%, 1.8%)**
BuniF2							
0-1	<300	11	443	2.5	3.0	Ref	
	≥300	4	132	3.0	3.8	0.7% (-3.3%, 4.8%)	
≥ 2	<300	191	10556	1.8	1.8	Ref	
	≥300	15	706	2.1	2.5	0.6% (-0.9%, 2.2%)	**-0.1% (-4.4%, 4.3%)**

NA, not able to estimated

* Adjusted for beach, age, mean bathers, sand, rain, water temperature

Table A.4(d). **Urinary tract infection**

Urinary tract infection							
Marker (samples)	Enterococcus (CCE/100ml)	Cases	N	Crude Risk (%)	Adjusted Risk (%)	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--	Main association					0.2% (-0.6%, 1.0%)	
HumM2							
0-1	<300	30	5227	0.6	0.6	Ref	
	≥300	4	622	0.6	0.7	0.1% (-0.7%, 0.8%)	
≥ 2	<300	40	5879	0.7	0.7	Ref	
	≥300	2	227	0.9	1.2	0.5% (-1.3%, 2.2%)	**0.4% (-1.4%, 2.2%)**
HF183							
0-1	<300	14	2102	0.7	2.2	Ref	
	≥300	2	464	0.4	0.5	-0.2% (-0.9%, 0.6%)	
≥ 2	<300	56	9004	0.6	0.6	Ref	
	≥300	4	385	1.0	1.1	0.5% (-0.9%, 1.8%)	**0.7% (-0.9%, 2.2%)**
BsteriF1							
0-1	<300	8	1666	0.5	0.4	Ref	
	≥300	1	227	0.4	0.4	0.0% (-0.8%, 0.9%)	
≥ 2	<300	62	9440	0.7	0.7	Ref	
	≥300	5	622	0.8	0.8	0.1% (-0.8%, 1.0%)	**0.1% (-1.1%, 1.2%)**
BuniF2							
0-1	<300	2	454	0.4	0.4	Ref	
	≥300	1	134	0.7	0.7	0.3% (0.1%, 1.8%)	
≥ 2	<300	68	10652	0.6	0.7	Ref	
	≥300	5	715	0.7	0.8	0.1% (-0.7%, 1.0%)	**-0.1% (-1.9%, 1.6%)**

NA, not able to estimated. Note estimates are based on small cell sizes.
* Adjusted for beach, age, mean bathers, sand, rain, water temperature

Table A.5. Risk difference modification of the association between *Enterococcus* general indicator measured continuously by qPCR (CCE/100ml) and illness with human-associated *Bacteroides* markers in all beaches

GI illness			
Marker (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		1.4% (0.6%, 2.3%)	
HumM2			
0-1	4.4	Ref	
	6.3	1.8% (1.0%, 2.7%)	
≥ 2	5.9	Ref	
	6.9	1.0% (-0.5%, 2.4%)	**-0.9% (-2.3%, 0.6%)**
HF183			
0-1	4.4	Ref	
	6.1	1.7% (0.6%, 2.7%)	
≥ 2	5.7	Ref	
	6.9	1.3% (0.2%, 2.4%)	**-0.4% (-1.7%, 0.9%)**
BsteriF1			
0-1	7.2	Ref	
	7.2	0.0% (-3.7%, 3.7%)	
≥ 2	5.6	Ref	
	7.0	1.4% (0.4%, 2.4%)	**1.4% (-2.4%, 5.1%)**
BuniF2			
0-1	1.1	Ref	
	2.5	1.5% (0.4%, 2.5%)	
≥ 2	5.7	Ref	
	7.0	1.3% (0.3%, 2.3%)	**-0.2% (-1.5%, 1.1%)**
Diarrhea			
Marker (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		1.1% (0.6%, 1.7%)	
HumM2			
0-1	2.6	Ref	
	4.0	1.4% (0.9%, 1.9%)	
≥ 2	3.3	Ref	
	4.2	0.9% (0.0%, 1.8%)	**-0.5% (-1.4%, 0.4%)**
HF183			
0-1	3.6	Ref	
	4.8	1.2% (0.1%, 2.3%)	
≥ 2	2.7	Ref	
	3.9	1.2% (0.7%, 1.7%)	**0% (-1.1%, 1.1%)**

BsteriF1			
0-1	5.5	Ref	
	5.2	-0.3% (-4.0%, 3.5%)	
≥ 2	3.0	Ref	
	4.2	1.2% (0.7%, 1.7%)	**1.5% (-2.3%, 5.2%)**
BuniF2			
0-1	0.9	Ref	
	2.1	1.2% (0.3%, 2.1%)	
≥ 2	3.2	Ref	
	4.3	1.1% (0.5%, 1.7%)	**-0.1% (-1.1%, 0.9%)**

Respiratory

Marker (samples)	Adjusted Risk (%)	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--		0.6% (-0.7%, 1.8%)	
HumM2			
0-1	6.4	Ref	
	6.4	0.0% (-2.0%, 2.0%)	
≥ 2	4.6	Ref	
	5.6	1.0% (-0.4%, 2.3%)	**0.9% (-1.2%, 3.1%)**
HF183			
0-1	13.9	Ref	
	9.5	-4.4% (-10.8%, 2.0%)	
≥ 2	3.9	Ref	
	5.1	1.3% (0.4%, 2.1%)	**5.7% (-0.7%, 12%)**
BsteriF1			
0-1	8.7	Ref	
	6.7	-1.9% (-7.8%, 4.0)	
≥ 2	5.7	Ref	
	6.2	0.5% (-0.9%, 1.9%)	**2.5% (-3.3%, 8.3%)**
BuniF2			
0-1	1.5	Ref	
	2.7	1.2% (0.7%, 1.8%)	
≥ 2	5.6	Ref	
	6.1	0.5% (-0.9%, 1.8%)	**-0.8% (-2.1%, 0.5%)**

Eye

Marker (samples)	Adjusted Risk (%)	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
--		-1.8% (-4.2%, 0.7%)	
HumM2			
0-1	6.8	Ref	

	4.3	-2.4% (-6.5%, 1.6%)	
≥ 2	4.5	Ref	
	3.4	-1.0% (-3.5%, 1.4%)	**1.4% (-3.1%, 5.9%)**
HF183			
0-1	6.0	Ref	
	3.7	-2.3% (-7.8%, 3.3%)	
≥ 2	5.9	Ref	
	4.1	-1.8% (-4.5%, 1.0%)	**0.5% (-5.6%, 6.6%)**
BsteriF1			
0-1	5.2	Ref	
	3.2	-2.0% (-7.5%, 3.6%)	
≥ 2	6.7	Ref	
	4.4	-2.3% (-5.4%, 0.8%)	**-0.4% (-6.5%, 5.7%)**
BuniF2			
0-1	1.9	Ref	
	2.0	0.1% (-2.8%, 3.1%)	
≥ 2	5.9	Ref	
	4.0	-1.9% (-4.5%, 0.7%)	**-2.0% (-5.8%, 1.8%)**

Rash

Marker (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		-0.2% (-1.3%, 1.0%)	
HumM2			
0-1	4.4	Ref	
	4.0	-0.4% (-2.4%, 1.6%)	
≥ 2	2.1	Ref	
	2.4	0.3% (-0.5%, 1.0%)	**0.7% (-1.3%, 2.7%)**
HF183			
0-1	5.9	Ref	
	4.9	-1% (-4.6%, 2.6%)	
≥ 2	2.7	Ref	
	2.8	0.1% (-0.8%, 1.1%)	**1.1% (-2.5%, 4.8%)**
BsteriF1			
0-1	8.0	Ref	
	4.8	-3.2% (-12.6%, 6.3%)	
≥ 2	3.2	Ref	
	3.2	0.0% (-1.1%, 1.0%)	**3.1% (-6.2%, 12.5%)**
BuniF2			
0-1	9.1	Ref	
	5.6	-3.6% (-29.2%, 22%)	
≥ 2	3.4	Ref	

	3.2	-0.1% (-1.3%, 1.0%)	**3.5% (-22.1%, 29%)**
Earache			
Marker (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		0.1% (-0.6%, 0.8%)	
HumM2			
0-1	1.7	Ref	
	1.8	0.1% (-0.7%, 1.0%)	
≥ 2	1.7	Ref	
	1.8	0.1% (-0.9%, 1.1%)	**0.0% (-1.2%, 1.1%)**
HF183			
0-1	1.5	Ref	
	1.6	0.1% (-0.7%, 0.9%)	
≥ 2	1.8	Ref	
	1.9	0.1% (-0.8%, 1.0%)	**0.0% (-1.1%, 1.0%)**
BsteriF1			
0-1	0.5	Ref	
	0.8	0.3% (0.1%, 0.6%)	
≥ 2	2.2	Ref	
	2.1	-0.1% (-1.2%, 1.0%)	**-0.4% (-1.4%, 0.6%)**
BuniF2			
0-1	0.1	Ref	
	0.4	0.3% (-0.2%, 0.8%)	
≥ 2	1.8	Ref	
	1.8	0.0% (-0.8%, 0.8%)	**-0.3% (-1.2%, 0.6%)**
Urinary tract infection			
Marker (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		-0.2% (-1.2%, 0.7%)	
HumM2			
0-1	1.4	Ref	
	0.9	-0.5% (-2.4%, 1.3%)	
≥ 2	0.9	Ref	
	0.8	-0.1% (-1.0%, 0.9%)	**0.4% (-1.5%, 2.4%)**
HF183			
0-1	2.0	Ref	
	1.1	-0.9% (-4.1%, 2.3%)	
≥ 2	0.9	Ref	
	0.8	-0.1% (-0.9%, 0.7%)	**0.8% (-2.4%, 4.0%)**
BsteriF1			

0-1	0.8	Ref	
	0.5	-0.3% (-2.4%, 1.8%)	
≥ 2	1.5	Ref	
	1.0	-0.5% (-1.9%, 1.0%)	**-0.2% (-2.7%, 2.3%)**
BuniF2			
0-1	0.5	Ref	
	0.4	0.0% (-2.1%, 2.0%)	
≥ 2	1.1	Ref	
	0.9	-0.3% (-1.3%, 0.7%)	**-0.2% (-2.6%, 2.1%)**

GI, gastrointestinal; NA, not able to estimated.
* Adjusted for beach, age, mean bathers, sand, rain, water temperature
† Adjusted for beach, age, mean bathers, sand, rain

Table A.6. Frequencies and standardized risk differences (95% CI) for the association between illness and human-associated *Bacteroides* markers among <u>*head immersion*</u> swimmers in all beaches

		All beaches				
		Cases	**N**	**Crude Risk**	**Adjusted Risk**	**Adjusted RD (95% CI)**
GI illness*						
HumM2	0-1 samples	365	4115	8.9%	8.4%	Ref
	≥2 samples	301	4230	7.1%	7.9%	-0.6% (-2.1%, 1.0%)
HF183	0-1 samples	173	1776	9.7%	7.1%	Ref
	≥2 samples	493	6569	7.5%	8.6%	1.6% (-0.4%, 3.5%)
BsteriF1	0-1 samples	111	1328	8.4%	6.6%	Ref
	≥2 samples	555	7017	7.9%	8.5%	1.9% (-0.1%, 3.9%)
BuniF2	0-1 samples	39	424	9.2%	6.8%	Ref
	≥2 samples	627	7921	7.9%	8.3%	1.4% (-1.7%, 4.6%)
Diarrhea*						
HumM2	0-1 samples	250	4112	6.1%	5.7%	Ref
	≥2 samples	180	4227	4.3%	4.9%	-0.8% (-2.1%, 0.5%)
HF183	0-1 samples	128	1774	7.2%	5.2%	Ref
	≥2 samples	302	6565	4.6%	5.4%	0.3% (-1.4%, 2.0%)
BsteriF1	0-1 samples	73	1326	5.5%	4.6%	Ref
	≥2 samples	357	7013	5.1%	5.5%	1.0% (-0.6%, 2.6%)
BuniF2	0-1 samples	30	424	7.1%	5.6%	Ref
	≥2 samples	400	7915	5.1%	5.4%	-0.3% (-3.2%, 2.6%)
Respiratory†						
HumM2	0-1 samples	241	4015	6.0%	5.9%	Ref
	≥2 samples	246	4092	6.0%	6.3%	0.4% (-1.0%, 1.7%)
HF183	0-1 samples	107	1745	6.1%	5.4%	Ref
	≥2 samples	380	6362	6.0%	6.3%	0.9% (-1.2%, 2.9%)
BsteriF1	0-1 samples	71	1273	5.6%	5.0%	Ref
	≥2 samples	416	6834	6.1%	6.3%	1.3% (-0.3%, 2.9%)
BuniF2	0-1 samples	28	413	6.8%	6.1%	Ref
	≥2 samples	459	7694	6.0%	6.1%	0.0% (-3.0%, 3.0%)
Earache*						
HumM2	0-1 samples	83	4165	2.0%	2.1%	Ref
	≥2 samples	78	4280	1.8%	1.8%	-0.3% (-1.0%, 0.4%)
HF183	0-1 samples	35	1808	1.9%	2.0%	Ref
	≥2 samples	126	6637	1.9%	1.9%	0.0% (-1.1%, 1.0%)
BsteriF1	0-1 samples	22	1330	1.7%	1.7%	Ref
	≥2 samples	139	7115	2.0%	2.0%	0.3% (-0.5%, 1.2%)
BuniF2	0-1 samples	12	423	2.8%	3.8%	Ref

		All beaches				
		Cases	N	Crude Risk	Adjusted Risk	Adjusted RD (95% CI)
	≥2 samples	149	8022	1.9%	1.9%	-1.9% (-4.8%, 1.0%)
Eye ailment†						
HumM2	0-1 samples	120	4205	2.9%	3.0%	Ref
	≥2 samples	103	4318	2.4%	2.4%	-0.6% (-1.5%, 0.3%)
HF183	0-1 samples	43	1819	2.4%	2.4%	Ref
	≥2 samples	180	6704	2.7%	2.7%	0.4% (-0.8%, 1.6%)
BsteriF1	0-1 samples	28	1341	2.1%	2.0%	Ref
	≥2 samples	195	7182	2.7%	2.8%	0.7% (-0.3%, 1.7%)
BuniF2	0-1 samples	13	429	3.0%	3.8%	Ref
	≥2 samples	210	8094	2.6%	2.6%	-1.2% (-3.6%, 1.3%)
Rash†						
HumM2	0-1 samples	141	4130	3.4%	3.5%	Ref
	≥2 samples	117	4238	2.8%	2.6%	-1.0% (-1.9%, -0.1%)
HF183	0-1 samples	59	1791	3.3%	4.1%	Ref
	≥2 samples	199	6577	3.0%	2.8%	-1.3% (-2.9%, 0.4%)
BsteriF1	0-1 samples	38	1327	2.9%	3.0%	Ref
	≥2 samples	220	7041	3.1%	3.0%	0.0% (-1.2%, 1.2%)
BuniF2	0-1 samples	8	424	1.9%	1.6%	Ref
	≥2 samples	250	7944	3.1%	3.1%	1.5% (0.1%, 2.9%)
UTI*						
HumM2	0-1 samples	25	4216	0.6%	0.6%	Ref
	≥2 samples	26	4314	0.6%	0.7%	0.1% (-0.3%, 0.5%)
HF183	0-1 samples	11	1819	0.6%	0.5%	Ref
	≥2 samples	40	6711	0.6%	0.6%	0.1% (-0.4%, 0.6%)
BsteriF1	0-1 samples	8	1343	0.6%	0.6%	Ref
	≥2 samples	43	7187	0.6%	0.6%	0.1% (-0.4%, 0.6%)
BuniF2	0-1 samples	4	431	0.9%	1.0%	Ref
	≥2 samples	47	8099	0.6%	0.6%	-0.4% (-1.7%, 0.8%)

GI, gastrointestinal; NA, not able to estimated; UTI, urinary tract infection.
* Adjusted for beach, age, mean bathers, sand, rain, water temperature
† Adjusted for beach, age, mean bathers, sand, rain

Table A.7. Frequencies and standardized risk differences (95% CI) for the association between illness and human-associated *Bacteroides* markers among swimmers who <u>*swallowed water*</u> in all beaches

		All beaches				
		Cases	**N**	**Crude Risk**	**Adjusted Risk**	**Adjusted RD (95% CI)**
GI illness*						
HumM2	0-1 samples	101	1009	10.0%	9.4%	Ref
	≥2 samples	99	1076	9.2%	10.2%	0.8% (-2.7%, 4.2%)
HF183	0-1 samples	52	493	10.5%	9.2%	Ref
	≥2 samples	148	1592	9.3%	10.0%	0.8% (-3.5%, 5.2%)
BsteriF1	0-1 samples	34	341	10.0%	8.1%	Ref
	≥2 samples	166	1744	9.5%	10.1%	2.0% (-2.4%, 6.5%)
BuniF2	0-1 samples	17	140	12.1%	12.7%	Ref
	≥2 samples	183	1945	9.4%	9.6%	-3.2% (-12%, 5.7%)
Diarrhea*						
HumM2	0-1 samples	75	1008	7.4%	7.1%	Ref
	≥2 samples	60	1076	5.6%	6.1%	-1.0% (-3.8%, 1.8%)
HF183	0-1 samples	39	493	7.9%	7.1%	Ref
	≥2 samples	96	1591	6.0%	6.5%	-0.6% (-4.4%, 3.3%)
BsteriF1	0-1 samples	24	341	7.0%	5.4%	Ref
	≥2 samples	111	1743	6.4%	6.9%	1.5% (-2.0%, 5.1%)
BuniF2	0-1 samples	13	140	9.3%	9.3%	Ref
	≥2 samples	122	1944	6.3%	6.4%	-2.8% (-10.4%, 4.7%)
Respiratory†						
HumM2	0-1 samples	79	981	8.1%	7.4%	Ref
	≥2 samples	88	1029	8.6%	9.3%	1.9% (-1.4%, 5.2%)
HF183	0-1 samples	40	481	8.3%	6.4%	Ref
	≥2 samples	127	1529	8.3%	9.1%	2.7% (-1.8%, 7.2%)
BsteriF1	0-1 samples	28	319	8.8%	7.9%	Ref
	≥2 samples	139	1691	8.2%	8.4%	0.5% (-3.2%, 4.3%)
BuniF2	0-1 samples	17	133	12.8%	14.4%	Ref
	≥2 samples	150	1877	8.0%	7.9%	-6.5% (-17.3%, 4.3%)
Earache*						
HumM2	0-1 samples	26	1028	2.5%	2.7%	Ref
	≥2 samples	26	1093	2.4%	2.3%	-0.4% (-2.2%, 1.3%)
HF183	0-1 samples	10	506	2.0%	1.7%	Ref
	≥2 samples	42	1615	2.6%	2.9%	1.2% (-0.7%, 3.1%)
BsteriF1	0-1 samples	9	347	2.6%	2.8%	Ref
	≥2 samples	43	1774	2.4%	2.5%	-0.4% (-2.7%, 2.0%)
BuniF2	0-1 samples	6	141	4.3%	8.6%	Ref

		All beaches				
		Cases	N	Crude Risk	Adjusted Risk	Adjusted RD (95% CI)
	≥2 samples	46	1980	2.3%	2.3%	-6.3% (-18.1%, 5.5%)
Eye ailment†						
HumM2	0-1 samples	36	1037	3.5%	3.6%	Ref
	≥2 samples	34	1102	3.1%	3.0%	-0.6% (-2.5%, 1.3%)
HF183	0-1 samples	15	510	2.9%	2.9%	Ref
	≥2 samples	55	1629	3.4%	3.4%	0.5% (-2.1%, 3.1%)
BsteriF1	0-1 samples	13	347	3.7%	4.1%	Ref
	≥2 samples	57	1792	3.2%	3.1%	-1.0% (-3.7%, 1.7%)
BuniF2	0-1 samples	6	143	4.2%	6.3%	Ref
	≥2 samples	64	1996	3.2%	3.1%	-3.2% (-9.0%, 2.6%)
Rash†						
HumM2	0-1 samples	35	1017	3.4%	4.0%	Ref
	≥2 samples	44	1081	4.1%	3.6%	-0.4% (-2.6%, 1.8%)
HF183	0-1 samples	12	500	2.4%	3.1%	Ref
	≥2 samples	67	1598	4.2%	3.9%	0.7% (-2.1%, 3.6%)
BsteriF1	0-1 samples	11	341	3.2%	4.3%	Ref
	≥2 samples	68	1757	3.9%	3.7%	-0.7% (-3.5%, 2.1%)
BuniF2	0-1 samples	2	140	1.4%	3.7%	Ref
	≥2 samples	77	1958	3.9%	3.7%	0.0% (-5.0%, 5.0%)
UTI*						
HumM2	0-1 samples	13	1042	1.2%	1.2%	Ref
	≥2 samples	11	1095	1.0%	1.1%	-0.1% (-1.2%, 0.9%)
HF183	0-1 samples	8	504	1.6%	1.8%	Ref
	≥2 samples	16	1633	1.0%	1.0%	-0.7% (-2.3%, 0.8%)
BsteriF1	0-1 samples	6	345	1.7%	1.8%	Ref
	≥2 samples	18	1792	1.0%	1.0%	-0.8% (-2.5%, 0.9%)
BuniF2	0-1 samples	3	144	2.1%	2.7%	Ref
	≥2 samples	21	1993	1.1%	1.1%	-1.6% (-6.1%, 2.9%)

GI, gastrointestinal; NA, not able to estimated; UTI, urinary tract infection.
* Adjusted for beach, age, mean bathers, sand, rain, water temperature
† Adjusted for beach, age, mean bathers, sand, rain

APPENDIX B. CHAPTER 6 SUPPLEMENTAL TABLES AND FIGURES

This appendix contains supplemental tables and figures associated with analyses involving human-associated chemical markers shown in Chapter 6.

Table B.1(a-g). Frequencies and standardized risk differences (95% CI) for the association between illness and human-associated chemical markers (detected in all daily samples vs. <all) among body immersion swimmers in all beaches

Table B.1(a). **GI illness**

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
GI illness*					
Acetaminophen					
Not detected	685	8176	8.4%	8.7%	Ref
Detected§	55	519	10.6%	8.2%	-0.5% (-3.2%, 2.2%)
Beta-sitosterol					
Not detected	694	8189	8.5%	8.7%	Ref
Detected§	46	506	9.1%	8.5%	-0.2% (-3.6%, 3.1%)
Bisphenol A					
Not detected	627	7360	8.5%	8.4%	Ref
Detected§	113	1335	8.5%	10.0%	1.6% (-0.7%, 3.8%)
Caffeine					
Not detected	536	6814	7.9%	8.8%	Ref
Detected§	204	1881	10.8%	8.3%	-0.5% (-2.9%, 1.8%)
Cholesterol					
Not detected	483	6137	7.9%	8.4%	Ref
Detected§	257	2558	10.0%	9.3%	0.9% (-0.8%, 2.7%)
DEET					
Not detected	484	6187	7.8%	8.7%	Ref
Detected§	256	2508	10.2%	8.7%	0.0% (-2.1%, 2.1%)
Diethoxyoctylphenol					
Not detected	733	8640	8.5%	8.7%	Ref
Detected§	7	55	12.7%	9.0%	0.3% (-7.3%, 7.9%)
Phenol					
Not detected	505	6491	7.8%	8.9%	Ref
Detected§	235	2204	10.7%	8.2%	-0.6% (-3.1%, 1.8%)
Tributyl phosphate					
Not detected	653	7710	8.5%	8.8%	Ref
Detected§	87	985	8.8%	7.8%	-1.0% (-3.2%, 1.2%)

* Adjusted for beach, mean bathers, sand, rain, water temperature
§ Detected in all daily samples

Table B.1(b). **Diarrhea**

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
Diarrhea*					
Acetaminophen					
Not detected	453	8176	5.5%	5.9%	Ref
Detected§	42	519	8.1%	5.6%	-0.3% (-2.5%, 1.9%)
Beta-sitosterol					
Not detected	457	8189	5.6%	5.8%	Ref
Detected§	38	506	7.5%	6.3%	0.5% (-2.4%, 3.4%)
Bisphenol A					
Not detected	416	7360	5.7%	5.6%	Ref
Detected§	79	1335	5.9%	7.4%	1.8% (-0.3%, 3.9%)
Caffeine					
Not detected	347	6814	5.1%	6.2%	Ref
Detected§	148	1881	7.9%	5.1%	-1.1% (-2.9%, 0.7%)
Cholesterol					
Not detected	303	6137	4.9%	5.4%	Ref
Detected§	192	2558	7.5%	6.6%	1.1% (-0.4%, 2.6%)
DEET					
Not detected	300	6187	4.8%	5.6%	Ref
Detected§	195	2508	7.8%	6.2%	0.6% (-1.3%, 2.4%)
Diethoxyoctylphenol					
Not detected	490	8640	5.7%	5.8%	Ref
Detected§	5	55	9.1%	5.6%	-0.3% (-6.1%, 5.6%)
Phenol					
Not detected	312	6491	4.8%	5.7%	Ref
Detected§	183	2204	8.3%	6.0%	0.2% (-1.6%, 2.1%)
Tributyl phosphate					
Not detected	431	7710	5.6%	5.9%	Ref
Detected§	64	985	6.5%	5.5%	-0.4% (-2.3%, 1.4%)

* Adjusted for beach, mean bathers, sand, rain, water temperature

§ Detected in all daily samples

Table B.1(c). **Respiratory illness**

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
Respiratory illness*					
Acetaminophen					
Not detected	479	7914	6.1%	6.0%	Ref
Detected§	30	511	5.9%	6.9%	0.9% (-2.4%, 4.2%)
Beta-sitosterol					
Not detected	484	7923	6.1%	6.0%	Ref
Detected§	25	502	5.0%	6.1%	0.1% (-2.6%, 2.8%)
Bisphenol A					
Not detected	449	7121	6.3%	6.4%	Ref
Detected§	60	1304	4.6%	4.4%	-1.9% (-3.3%, -0.5%)
Caffeine					
Not detected	393	6607	5.9%	6.0%	Ref
Detected§	116	1818	6.4%	6.1%	0.1% (-2.3%, 2.5%)
Cholesterol					
Not detected	391	5928	6.6%	6.5%	Ref
Detected§	118	2497	4.7%	4.7%	1.5% (-1.4%, 4.3%)
DEET					
Not detected	372	5976	6.2%	6.2%	Ref
Detected§	137	2449	5.6%	5.6%	-0.6% (-2.2%, 1.1%)
Diethoxyoctylphenol					
Not detected	505	8372	6.0%	6.0%	Ref
Detected§	4	53	7.5%	5.8%	-0.2% (-6.0%, 5.5%)
Phenol					
Not detected	402	6281	6.4%	6.7%	Ref
Detected§	107	2144	5.0%	4.3%	-2.4% (-4.4%, -0.3%)
Tributyl phosphate					
Not detected	439	7465	5.9%	5.8%	Ref
Detected§	70	960	7.3%	8.0%	2.2% (-0.3%, 4.8%)

* Adjusted for beach, mean bathers, sand, rain

§ Detected in all daily samples

Table B.1(d). **Eye illness**

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
Eye ailment					
Acetaminophen					
Not detected	234	8364	2.8%	2.8%	Ref
Detected§	12	524	2.3%	2.1%	-0.8% (-2.5%, 1.0%)
Beta-sitosterol					
Not detected	230	8364	2.7%	2.7%	Ref
Detected§	16	524	3.1%	3.0%	0.0% (-1.6%, 1.6%)
Bisphenol A					
Not detected	211	7513	2.8%	2.8%	Ref
Detected§	35	1375	2.5%	2.6%	-0.1% (-1.2%, 0.9%)
Caffeine					
Not detected	195	6976	2.8%	2.9%	Ref
Detected§	51	1912	2.7%	2.3%	-0.4% (-1.7%, 0.9%)
Cholesterol					
Not detected	171	6275	2.7%	2.7%	Ref
Detected§	75	2613	2.9%	2.8%	-0.1% (-1.0%, 0.8%)
DEET					
Not detected	177	6333	2.8%	2.8%	Ref
Detected§	69	2555	2.7%	2.5%	-0.5% (-1.5%, 0.6%)
Diethoxyoctylphenol					
Not detected	242	8831	2.7%	2.7%	Ref
Detected§	4	57	7.0%	7.2%	5.0% (-4.4%, 14.3%)
Phenol					
Not detected	179	6657	2.7%	2.6%	Ref
Detected§	67	2231	3.0%	3.0%	-0.1% (-1.7%, 1.6%)
Tributyl phosphate					
Not detected	221	7875	2.8%	2.7%	Ref
Detected§	25	1013	2.5%	2.6%	-0.3% (-1.5%, 1.0%)

* Adjusted for beach, mean bathers, sand, rain

§ Detected in all daily samples

Table B.1(e). **Rash**

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
Rash					
Acetaminophen					
Not detected	216	8216	2.6%	2.7%	Ref
Detected§	18	517	3.5%	2.6%	-0.1% (-1.5%, 1.4%)
Beta-sitosterol					
Not detected	215	8215	2.6%	2.7%	Ref
Detected§	19	518	3.7%	2.7%	0.0% (-1.3%, 1.4%)
Bisphenol A					
Not detected	202	7387	2.7%	2.7%	Ref
Detected§	32	1346	2.4%	2.6%	-0.1% (-1.1%, 0.9%)
Caffeine					
Not detected	177	6851	2.6%	2.6%	Ref
Detected§	57	1882	3.0%	3.0%	0.3% (-0.8%, 1.4%)
Cholesterol					
Not detected	156	6149	2.5%	2.6%	Ref
Detected§	78	2584	3.0%	2.8%	0.2% (-0.6%, 1.0%)
DEET					
Not detected	151	6208	2.4%	2.7%	Ref
Detected§	83	2525	3.3%	2.7%	0.1% (-0.9%, 1.1%)
Diethoxyoctylphenol					
Not detected	234	8677	2.7%	NA	Ref
Detected§	0	56	0.0%	NA	NA
Phenol					
Not detected	150	6521	2.3%	2.2%	Ref
Detected§	84	2212	3.8%	4.3%	2.1% (-0.1%, 4.3%)
Tributyl phosphate					
Not detected	195	7742	2.5%	2.6%	Ref
Detected§	39	991	3.9%	3.7%	1.1% (-0.4%, 2.6%)

* Adjusted for beach, mean bathers, sand, rain

§ Detected in all daily samples

Table B.1(f). **Earache**

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
Earache*					
Acetaminophen					
Not detected	145	8278	1.8%	1.7%	Ref
Detected§	15	525	2.9%	3.9%	2.2% (-0.1%, 4.5%)
Beta-sitosterol					
Not detected	147	8287	1.8%	1.8%	Ref
Detected§	13	516	2.5%	2.7%	1.0% (-0.8%, 2.7%)
Bisphenol A					
Not detected	138	7448	1.9%	1.8%	Ref
Detected§	22	1355	1.6%	1.7%	-0.1% (-1.0%, 0.7%)
Caffeine					
Not detected	122	6912	1.8%	1.6%	Ref
Detected§	38	1891	2.0%	2.7%	1.1% (-0.3%, 2.4%)
Cholesterol					
Not detected	116	6217	1.9%	1.9%	Ref
Detected§	44	2586	1.7%	1.7%	-0.1% (-0.8%, 0.5%)
DEET					
Not detected	110	6272	1.8%	1.7%	Ref
Detected§	50	2531	2.0%	2.3%	0.6% (-0.3%, 1.5%)
Diethoxyoctylphenol					
Not detected	158	8746	1.8%	1.8%	Ref
Detected§	2	57	3.5%	5.2%	3.4% (-4.1%, 10.9%)
Phenol					
Not detected	122	6590	1.9%	1.8%	Ref
Detected§	38	2213	1.7%	1.8%	0.0% (-1.3%, 1.4%)
Tributyl phosphate					
Not detected	143	7801	1.8%	1.8%	Ref
Detected§	17	1002	1.7%	1.9%	0.1% (-1.0%, 1.2%)

* Adjusted for beach, mean bathers, sand, rain, water temperature

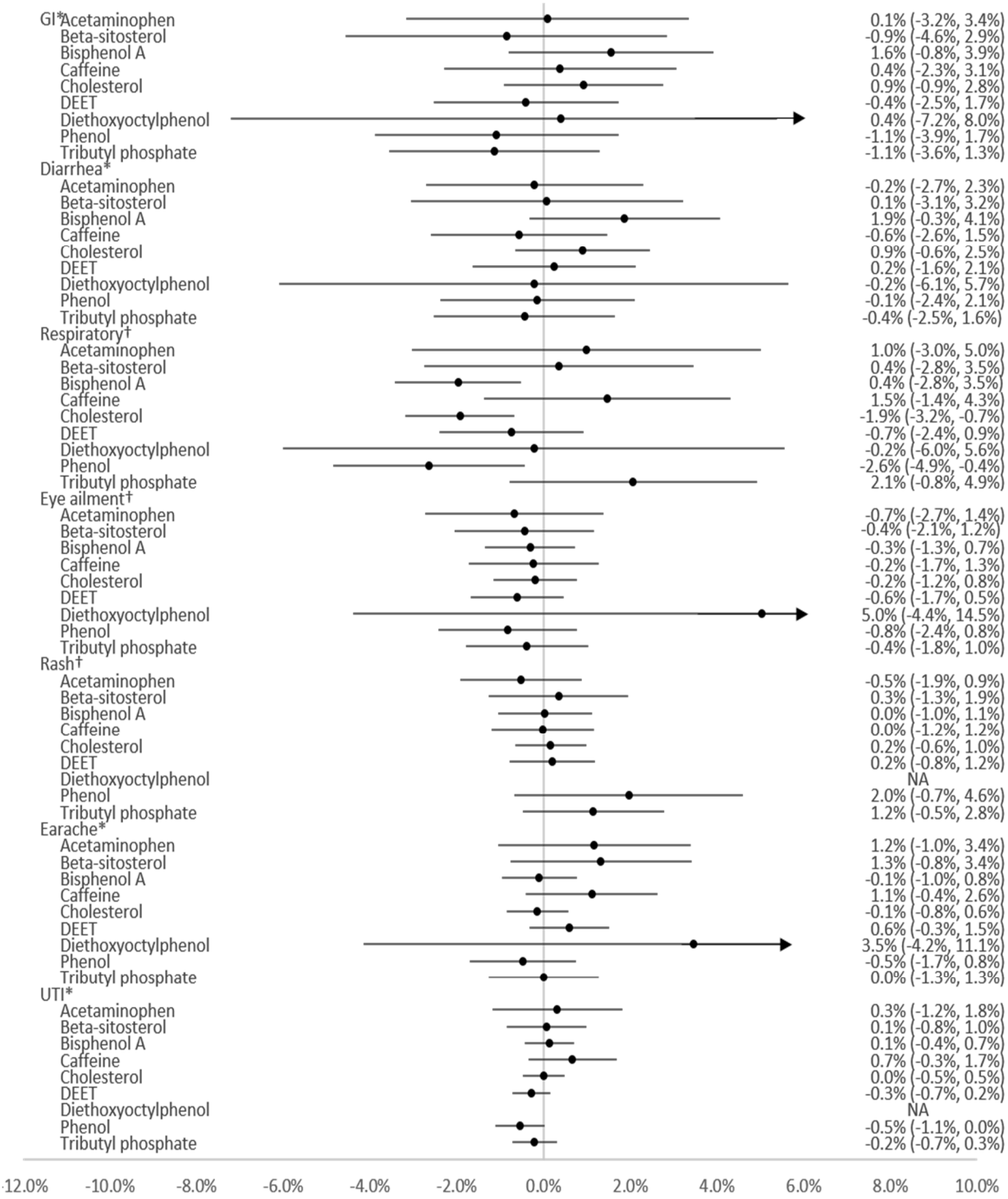
§ Detected in all daily samples

Table B.1(g). **Urinary tract infection**

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
Urinary tract infection					
Acetaminophen					
Not detected	51	8355	0.6%	0.6%	Ref
Detected§	6	530	1.1%	1.2%	0.6% (-0.8%, 1.9%)
Beta-sitosterol					
Not detected	53	8360	0.6%	0.6%	Ref
Detected§	4	525	0.8%	0.7%	0.0% (-0.7%, 0.8%)
Bisphenol A					
Not detected	48	7512	0.6%	0.6%	Ref
Detected§	9	1373	0.7%	0.7%	0.1% (-0.5%, 0.6%)
Caffeine					
Not detected	38	6965	0.5%	0.5%	Ref
Detected§	19	1920	1.0%	1.4%	0.9% (-0.1%, 1.8%)
Cholesterol					
Not detected	41	6269	0.7%	0.6%	Ref
Detected§	16	2616	0.6%	0.6%	0.0% (-0.5%, 0.5%)
DEET					
Not detected	41	6322	0.6%	0.8%	Ref
Detected§	16	2563	0.6%	0.4%	-0.3% (-0.8%, 0.2%)
Diethoxyoctylphenol					
Not detected	57	8828	0.6%	NA	Ref
Detected§	0	57	0.0%	NA	NA
Phenol					
Not detected	45	6645	0.7%	0.8%	Ref
Detected§	12	2240	0.5%	0.4%	-0.4% (-1.0%, 0.1%)
Tributyl phosphate					
Not detected	48	7870	0.6%	0.6%	Ref
Detected§	9	1015	0.9%	0.6%	0.0% (-0.5%, 0.5%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature
§ Detected in all daily samples

Figure B.1. Standardized risk differences (95% CI) for the association between illness and human-associated chemical markers (detected in all daily samples vs. <all) among body immersion swimmers in freshwater beaches



NA, not able to estimated.

* Adjusted for beach, mean bathers, sand, rain, water temperature

† Adjusted for beach, mean bathers, sand, rain

Table B.2(a-d). Modification of the adjusted standardized RD (95% CI) for the association between illness and *Enterococcus* qPCR Method 1611 above and below EPA guidelines (geometric mean of 470 CCE/100ml for an illness rate of 36/1000) with chemical markers (detected in all daily samples vs. <all) among body immersion swimmers in all beaches – Table B.2(a) **Eye ailment**

Eye Ailment						
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adj Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main assoc				-0.7% (-2.1%, 0.8%)	
Acetaminophen						
Not detected	<470	223	8067	2.8	Ref	
	≥470	13	463	2.7	-0.1% (-2.0%, 1.8%)	
Detected in all	<470	11	388	2.7	Ref	
	≥470	1	150	0.5	-2.1% (-4.7%, 0.4%)	**-2.0% (-5.3%, 1.2%)**
Beta-sitosterol						
Not detected	<470	220	7918	2.8	Ref	
	≥470	11	532	1.8	-1.0% (-2.5%, 0.5%)	
Detected in all	<470	13	444	2.5	Ref	
	≥470	3	81	3.4	0.9% (-3.2%, 5.1%)	**1.9% (-2.4%, 6.3%)**
Bisphenol A						
Not detected	<470	199	6945	2.9	Ref	
	≥470	12	569	1.9	-1.0% (-2.4%, 0.4%)	
Detected in all	<470	33	1331	2.6	Ref	
	≥470	2	44	5.5	2.9% (-4.9%, 10.8%)	**3.9% (-4.0%, 11.8%)**
Caffeine						
Not detected	<470	185	6749	2.8	Ref	
	≥470	10	291	3.2	0.4% (-2.2%, 3.0%)	
Detected in all	<470	49	1706	2.8	Ref	
	≥470	4	322	1.2	-1.7% (-3.4%, 0.0%)	**-2.1% (-5.2%, 1.0%)**
Cholesterol						
Not detected	<470	167	6067	2.9	Ref	
	≥470	5	294	1.6	-1.3% (-2.8%, 0.3%)	
Detected in all	<470	66	2295	2.7	Ref	
	≥470	9	319	2.4	-0.2% (-2.4%, 1.9%)	**1.0% (-1.5%, 3.5%)**
DEET						
Not detected	<470	176	6221	3.0	Ref	
	≥470	1	112	0.8	-2.2% (-3.9%, -0.5%)	
Detected in all	<470	57	2141	2.4	Ref	
	≥470	13	501	2.1	-0.3% (-1.8%, 1.3%)	**1.9% (-0.4%, 4.2%)**
Diethoxyoctylphenol						
Not detected	<470	229	8305	2.8	Ref	
	≥470	14	613	2.1	-0.7% (-2.1%, 0.7%)	
Detected in all	<470	4	57	7.7	Ref	
	≥470	0	0	0.0	NA	**NA**
Phenol						
Not detected	<470	177	6444	3.2	Ref	
	≥470	2	244	0.6	-2.7% (-4.2%, -1.1%)	
Detected in all	<470	56	1918	2.1	Ref	
	≥470	12	369	2.4	0.3% (-1.4%, 2.1%)	**3.0% (0.8%, 5.2%)**
Tributyl phosphate						
Not detected	<470	213	7547	2.9	Ref	
	≥470	9	415	1.8	-1.1% (-2.6%, 0.5%)	
Detected in all	<470	20	815	2.4	Ref	
	≥470	5	198	2.7	0.3% (-2.3%, 2.9%)	**1.3% (-1.6%, 4.3%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.2(b). **Rash**

		Rash				
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adj Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main assoc				0.0% (-1.4%, 1.3%)	
Acetaminophen						
Not detected	<470	202	7919	2.7	Ref	
	≥470	17	456	2.6	-0.1% (-1.7%, 1.5%)	
Detected in all	<470	13	383	2.6	Ref	
	≥470	6	148	2.8	0.2% (-2.8%, 3.2%)	**0.3% (-3.2%, 3.7%)**
Beta-sitosterol						
Not detected	<470	200	7772	2.7	Ref	
	≥470	18	524	2.3	-0.4% (-1.8%, 1.0%)	
Detected in all	<470	14	439	2.3	Ref	
	≥470	5	80	4.4	2.1% (-2.1%, 6.2%)	**2.5% (-1.8%, 6.8%)**
Bisphenol A						
Not detected	<470	182	6827	2.7	Ref	
	≥470	20	561	2.5	-0.2% (-1.6%, 1.1%)	
Detected in all	<470	29	1303	2.5	Ref	
	≥470	3	43	4.9	2.4% (-3.6%, 8.4%)	**2.6% (-3.4%, 8.7%)**
Caffeine						
Not detected	<470	168	6625	2.6	Ref	
	≥470	11	288	2.6	0.0% (-1.9%, 1.8%)	
Detected in all	<470	47	1677	2.9	Ref	
	≥470	12	316	2.9	-0.1% (-2.1%, 2.0%)	**0.0% (-2.8%, 2.7%)**
Cholesterol						
Not detected	<470	149	5943	2.6	Ref	
	≥470	10	287	2.5	-0.1% (-2.0%, 1.9%)	
Detected in all	<470	65	2268	2.8	Ref	
	≥470	13	317	2.8	-0.1% (-1.9%, 1.8%)	**0.0% (-2.6%, 2.6%)**
DEET						
Not detected	<470	146	6101	2.6	Ref	
	≥470	5	107	4.0	1.4% (-3.0%, 5.8%)	
Detected in all	<470	68	2110	2.9	Ref	
	≥470	18	497	2.5	-0.4% (-1.9%, 1.2%)	**-1.8% (-6.5%, 2.9%)**
Diethoxyoctylphenol						
Not detected	<470	214	8155	NA	Ref	
	≥470	23	604	NA	NA	
Detected in all	<470	0	56	NA	Ref	
	≥470	0	0	NA	NA	**NA**
Phenol						
Not detected	<470	141	6315	2.0	Ref	
	≥470	9	237	5.2	3.2% (-1.7%, 8.1%)	
Detected in all	<470	73	1896	5.8	Ref	
	≥470	14	367	4.6	-1.2% (-4.4%, 1.9%)	**-4.4% (-10.6%, 1.7%)**
Tributyl phosphate						
Not detected	<470	186	7413	2.6	Ref	
	≥470	12	411	2.0	-0.6% (-2.0%, 0.8%)	
Detected in all	<470	28	798	3.4	Ref	
	≥470	11	193	4.8	1.4% (-4.5%, 5.0%)	**1.9% (-1.8%, 5.7%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.2(c). **Earache**

Earache						
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adj Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main assoc				-0.9% (-1.8%, 0.1%)	
Acetaminophen						
Not detected	<470	142	7986	1.8	Ref	
	≥470	4	455	0.9	-0.9% (-2.0%, 0.2%)	
Detected in all	<470	13	388	4.0	Ref	
	≥470	3	151	2.0	-1.9% (-5.2%, 1.3%)	**-1.1% (-4.5%, 2.3%)**
Beta-sitosterol						
Not detected	<470	143	7843	1.8	Ref	
	≥470	5	527	0.9	-0.9% (-1.9%, 0.2%)	
Detected in all	<470	11	438	3.0	Ref	
	≥470	2	79	2.6	-0.4% (-4.5%, 3.6%)	**0.4% (-3.7%, 4.6%)**
Bisphenol A						
Not detected	<470	132	6886	2.0	Ref	
	≥470	6	563	0.9	-1.0% (-2.0%, -0.1%)	
Detected in all	<470	21	1312	1.7	Ref	
	≥470	1	43	3.0	1.4% (-5.1%, 7.8%)	**2.4% (-3.9%, 8.8%)**
Caffeine						
Not detected	<470	121	6686	1.7	Ref	
	≥470	2	289	0.6	-1.1% (-2.2%, -0.1%)	
Detected in all	<470	34	1688	2.6	Ref	
	≥470	5	317	1.7	-0.9% (-2.8%, 1.0%)	**0.2% (-1.9%, 2.3%)**
Cholesterol						
Not detected	<470	113	6008	1.9	Ref	
	≥470	4	292	1.4	-0.6% (-2.1%, 1.0%)	
Detected in all	<470	41	2273	1.8	Ref	
	≥470	3	314	0.7	-1.1% (-2.1%, 0.0%)	**-0.5% (-2.3%, 1.2%)**
DEET						
Not detected	<470	109	6165	1.7	Ref	
	≥470	1	107	1.2	-0.6% (-3.0%, 1.9%)	
Detected in all	<470	45	2116	2.3	Ref	
	≥470	6	499	1.2	-1.1% (-2.3%, 0.2%)	**-0.5% (-3.2%, 2.2%)**
Diethoxyoctylphenol						
Not detected	<470	152	8224	1.9	Ref	
	≥470	7	606	1.0	-0.9% (-1.8%, 0.1%)	
Detected in all	<470	2	57	5.3	Ref	
	≥470	0	0	NA	NA	**NA**
Phenol						
Not detected	<470	118	6380	1.7	Ref	
	≥470	4	241	1.2	-0.6% (-3.0%, 1.9%)	
Detected in all	<470	36	1901	2.3	Ref	
	≥470	3	365	1.2	-1.1% (-2.3%, 0.2%)	**-0.5% (-3.2%, 2.2%)**
Tributyl phosphate						
Not detected	<470	138	7477	1.8	Ref	
	≥470	6	408	1.7	-0.2% (-2.2%, 1.9%)	
Detected in all	<470	16	804	2.0	Ref	
	≥470	1	198	0.7	-1.3% (-2.5%, -0.1%)	**-1.1% (-3.7%, 1.5%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.2(d). **Urinary tract infection**

Urinary tract infection						
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adj Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main assoc				0.1% (-0.7%, 1.0%)	
Acetaminophen						
Not detected	<470	48	8064	0.6	Ref	
	≥470	3	463	0.6	0.0% (-0.8%, 0.8%)	
Detected in all	<470	4	392	0.9	Ref	
	≥470	2	152	2.4	1.5% (-2.3%, 5.3%)	**1.5% (-2.3%, 5.3%)**
Beta-sitosterol						
Not detected	<470	49	7919	0.6	Ref	
	≥470	4	534	0.7	0.1% (-0.7%, 1.0%)	
Detected in all	<470	3	445	0.9	Ref	
	≥470	1	81	1.1	0.2% (-2.3%, 2.6%)	**0.0% (-2.4%, 2.5%)**
Bisphenol A						
Not detected	<470	43	6950	0.6	Ref	
	≥470	5	571	0.8	0.1% (-0.7%, 1.0%)	
Detected in all	<470	9	1329	0.9	Ref	
	≥470	0	44	0.0	NA	**NA**
Caffeine						
Not detected	<470	38	6743	0.5	0.5	
	≥470	0	291	0.0	NA	
Detected in all	<470	14	1713	1.0	1.0	
	≥470	5	324	1.8	0.8% (-1.1%, 2.8%)	**NA**
Cholesterol						
Not detected	<470	38	6067	0.6	Ref	
	≥470	3	295	1.0	0.4% (-0.9%, 1.7%)	
Detected in all	<470	14	2297	0.8	Ref	
	≥470	2	320	0.6	-0.2% (-1.2%, 0.8%)	**-0.6% (-2.1%, 1.0%)**
DEET						
Not detected	<470	40	6219	0.8	Ref	
	≥470	1	111	0.4	-0.3% (-1.3%, 0.6%)	
Detected in all	<470	12	2145	0.4	Ref	
	≥470	4	504	0.6	0.2% (-0.6%, 1.0%)	**0.6% (-0.6%, 1.7%)**
Diethoxyoctylphenol						
Not detected	<470	52	8307	NA	Ref	
	≥470	5	615	NA	NA	
Detected in all	<470	0	57	NA	Ref	
	≥470	0	0	NA	NA	**NA**
Phenol						
Not detected	<470	42	6436	0.8	Ref	
	≥470	3	245	1.0	0.4% (-1.2%, 1.7%)	
Detected in all	<470	10	1928	0.4	Ref	
	≥470	2	370	0.4	0.0% (-0.7%, 0.6%)	**-0.3% (-1.9%, 1.3%)**
Tributyl phosphate						
Not detected	<470	45	7547	0.0	Ref	
	≥470	3	417	0.8	0.2% (-0.9%, 1.2%)	
Detected in all	<470	7	817	0.7	Ref	
	≥470	2	198	0.7	0.1% (-1.3%, 1.4%)	**-0.1% (-1.7%, 1.5%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.3(a-g). Modification of the adjusted standardized RD (95% CI) for the association between illness and *Enterococcus* qPCR Method 1611 above and below EPA guidelines (geometric mean of 300 CCE/100ml for an illness rate of 32/1000) with chemical markers (detected in all daily samples vs. <all) among body immersion swimmers in all beaches – Table B.3(a) **GI illness**

GI Illness						
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adj Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main assoc				0.4% (-2.3%, 3.1%)	
Acetaminophen						
Not detected	<300	617	7639	8.6	Ref	
	≥300	81	702	9.8	1.2% (-2.1%, 4.6%)	
Detected in all	<300	43	383	9.2	Ref	
	≥300	13	150	6.6	-2.6% (-7.5%, 2.2%)	**-3.9% (-10.0%, 2.2%)**
Beta-sitosterol						
Not detected	<300	631	7639	8.6	Ref	
	≥300	72	639	9.2	0.6% (-2.6%, 3.7%)	
Detected in all	<300	24	294	8.8	Ref	
	≥300	22	213	8.5	-0.3% (-6.8%, 6.2%)	**-0.9% (-8.2%, 6.5%)**
Bisphenol A						
Not detected	<300	552	6699	8.4	Ref	
	≥300	76	666	8.8	0.4% (-2.4%, 3.2%)	
Detected in all	<300	103	1234	10.0	Ref	
	≥300	11	105	10.0	0.0% (-8.3%, 8.2%)	**-0.4% (-8.6%, 7.8%)**
Caffeine						
Not detected	<300	492	6508	8.4	Ref	
	≥300	51	372	11.8	3.5% (-1.2%, 8.2%)	
Detected in all	<300	168	1514	9.1	Ref	
	≥300	43	480	7.6	-1.6% (-4.8%, 1.7%)	**-5.1% (-10.4%, 0.3%)**
Cholesterol						
Not detected	<300	445	5757	8.3	Ref	
	≥300	47	466	8.8	0.5% (-3.1%, 4.1%)	
Detected in all	<300	210	2176	9.3	Ref	
	≥300	47	386	9.6	0.3% (-3.3%, 3.9%)	**-0.1% (-4.8%, 4.5%)**
DEET						
Not detected	<300	474	6081	8.7	Ref	
	≥300	10	108	7.7	-1.0% (-6.9%, 5.0%)	
Detected in all	<300	181	1852	8.5	Ref	
	≥300	84	744	9.1	0.6% (-2.3%, 3.5%)	**1.6% (-4.7%, 7.8%)**
Diethoxyoctylphenol						
Not detected	<300	648	7878	8.6	Ref	
	≥300	94	852	9.0	0.4% (-2.4%, 3.1%)	
Detected in all	<300	7	55	9.0	Ref	
	≥300	0	0	NA	NA	**NA**
Phenol						
Not detected	<300	476	6163	9.6	Ref	
	≥300	33	362	6.7	-2.9% (-7.0%, 1.1%)	
Detected in all	<300	179	1770	7.2	Ref	
	≥300	61	490	8.7	1.5% (-1.5%, 4.5%)	**4.4% (-0.2%, 9.1%)**
Tributyl phosphate						
Not detected	<300	594	7232	8.8	Ref	
	≥300	67	565	8.9	0.1% (-2.9%, 3.1%)	
Detected in all	<300	61	701	7.7	Ref	
	≥300	27	287	8.4	0.8% (-3.7%, 5.3%)	**0.7% (-4.3%, 5.6%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.3(b). **Diarrhea**

Diarrhea						
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adj Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main assoc				0.8% (-1.6%, 3.2%)	
Acetaminophen						
Not detected	<300	398	7639	5.6	Ref	
	≥300	63	702	7.3	1.7% (-1.2%, 4.6%)	
Detected in all	<300	32	383	6.5	Ref	
	≥300	10	150	4.4	-2.2% (-6.1%, 1.8%)	**-3.9% (-8.9%, 1.2%)**
Beta-sitosterol						
Not detected	<300	405	7639	5.6	Ref	
	≥300	56	639	6.9	1.3% (-1.6%, 4.1%)	
Detected in all	<300	21	294	7.1	Ref	
	≥300	17	213	6.2	-0.9% (-6.7%, 4.8%)	**-2.2% (-8.9%, 4.5%)**
Bisphenol A						
Not detected	<300	355	6699	5.5	Ref	
	≥300	61	666	6.4	0.9% (-1.5%, 3.2%)	
Detected in all	<300	71	1234	7.4	Ref	
	≥300	8	105	7.3	-0.1% (-7.7%, 7.4%)	**-1.0% (-8.6%, 6.5%)**
Caffeine						
Not detected	<300	312	6508	5.8	Ref	
	≥300	40	372	8.6	2.7% (-1.3%, 6.7%)	
Detected in all	<300	118	1514	5.5	Ref	
	≥300	33	480	5.1	-0.3% (-2.9%, 2.3%)	**-3.1% (-7.4%, 1.3%)**
Cholesterol						
Not detected	<300	272	5757	5.3	Ref	
	≥300	35	466	6.3	0.9% (-2.2%, 4.1%)	
Detected in all	<300	154	2176	6.5	Ref	
	≥300	38	386	7.3	0.8% (-2.4%, 4.0%)	**-0.2% (-4.2%, 3.9%)**
DEET						
Not detected	<300	292	6081	5.5	Ref	
	≥300	8	108	6.3	0.8% (-5.0%, 6.6%)	
Detected in all	<300	134	1852	6.1	Ref	
	≥300	65	744	6.9	0.8% (-1.8%, 3.4%)	**0.0% (-6.0%, 6.0%)**
Diethoxyoctylphenol						
Not detected	<300	421	7878	5.7	Ref	
	≥300	73	852	6.5	0.8% (-1.6%, 3.2%)	
Detected in all	<300	5	55	5.5	Ref	
	≥300	0	0	NA	NA	**NA**
Phenol						
Not detected	<300	289	6163	5.9	Ref	
	≥300	24	362	5.0	-0.9% (-4.5%, 2.7%)	
Detected in all	<300	137	1770	5.5	Ref	
	≥300	49	490	7.2	1.7% (-1.2%, 4.6%)	**2.6% (-1.6%, 6.7%)**
Tributyl phosphate						
Not detected	<300	382	7232	5.8	Ref	
	≥300	53	565	6.4	0.6% (-2.0%, 3.1%)	
Detected in all	<300	44	701	5.3	Ref	
	≥300	20	287	6.7	1.4% (-2.8%, 5.6%)	**0.8% (-3.7%, 5.4%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.3(c). **Respiratory illness**

Respiratory illness						
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adj Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main assoc				-2.6% (-4.5%, -0.7%)	
Acetaminophen						
Not detected	<300	465	7383	6.3	Ref	
	≥300	26	686	3.4	-2.8% (-4.7%, -0.9%)	
Detected in all	<300	25	377	6.4	Ref	
	≥300	6	148	5.0	-1.4% (-8.5%, 5.7%)	**1.4% (-6.0%, 8.8%)**
Beta-sitosterol						
Not detected	<300	464	7385	6.2	Ref	
	≥300	21	620	3.4	-2.9% (-5.1%, -0.6%)	
Detected in all	<300	14	289	6.6	Ref	
	≥300	11	214	4.5	-2.1% (-7.0%, 2.8%)	**0.8% (-4.6%, 6.2%)**
Bisphenol A						
Not detected	<300	423	6475	6.6	Ref	
	≥300	26	647	4.1	-2.4% (-4.6%, -0.2%)	
Detected in all	<300	55	1199	4.5	Ref	
	≥300	5	105	4.1	-0.5% (-5.3%, 4.3%)	**2.0% (-3.1%, 7.0%)**
Caffeine						
Not detected	<300	383	6305	6.2	Ref	
	≥300	13	365	3.5	-2.7% (-5.4%, 0.0%)	
Detected in all	<300	107	1455	6.3	Ref	
	≥300	19	469	3.8	-2.6% (-5.3%, 0.1%)	**0.2% (-3.7%, 4.0%)**
Cholesterol						
Not detected	<300	374	5558	6.8	Ref	
	≥300	18	452	3.7	-3.0% (-6.0%, -0.1%)	
Detected in all	<300	104	2116	4.9	Ref	
	≥300	14	382	3.4	-1.6% (-3.6%, 0.5%)	**1.5% (-1.9%, 4.9%)**
DEET						
Not detected	<300	369	5874	6.4	Ref	
	≥300	3	102	2.5	-3.9% (-6.8%, -1.0%)	
Detected in all	<300	109	1800	5.9	Ref	
	≥300	29	732	3.7	-2.2% (-4.4%, 0.0%)	**1.8% (-1.7%, 5.2%)**
Diethoxyoctylphenol						
Not detected	<300	474	7621	6.3	Ref	
	≥300	32	834	3.7	-2.6% (-4.6%, -0.6%)	
Detected in all	<300	4	53	5.6	Ref	
	≥300	0	0	NA	NA	**NA**
Phenol						
Not detected	<300	390	5958	7.8	Ref	
	≥300	13	354	2.6	-5.2% (-7.9%, -2.4%)	
Detected in all	<300	88	1716	3.7	Ref	
	≥300	19	480	2.8	-1.0% (-2.6%, 0.7%)	**4.2% (1.0%, 7.4%)**
Tributyl phosphate						
Not detected	<300	422	6992	6.0	Ref	
	≥300	18	556	3.4	-2.6% (-4.9%, -0.4%)	
Detected in all	<300	56	682	7.9	Ref	
	≥300	14	278	5.6	-2.3% (-6.9%, 2.3%)	**0.3% (-4.5%, 5.2%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain

Table B.3(d). **Eye ailment**

Eye Ailment						
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adj Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main assoc				-1.0% (-2.2%, 0.1%)	
Acetaminophen						
Not detected	<300	220	7810	2.9	Ref	
	≥300	16	720	2.1	-0.8% (-2.2%, 0.7%)	
Detected in all	<300	11	388	2.5	Ref	
	≥300	1	150	0.5	-2.0% (-4.4%, 0.4%)	**-1.2% (-4.3%, 1.8%)**
Beta-sitosterol						
Not detected	<300	219	7802	2.9	Ref	
	≥300	12	648	1.6	-1.3% (-2.6%, 0.0%)	
Detected in all	<300	11	303	2.8	Ref	
	≥300	5	222	2.1	-0.7% (-3.3%, 1.9%)	**0.6% (-2.4%, 3.6%)**
Bisphenol A						
Not detected	<300	199	6840	2.9	Ref	
	≥300	12	674	1.6	-1.3% (-2.5%, -0.1%)	
Detected in all	<300	31	1265	2.6	Ref	
	≥300	4	110	4.2	1.6% (-3.4%, 6.5%)	**2.9% (-2.1%, 7.9%)**
Caffeine						
Not detected	<300	183	6660	2.8	Ref	
	≥300	12	380	3.0	0.2% (-2.1%, 2.5%)	
Detected in all	<300	48	1538	3.0	Ref	
	≥300	5	490	1.0	-2.0% (-3.5%, -0.6%)	**-2.2% (-5.0%, 0.6%)**
Cholesterol						
Not detected	<300	164	5885	2.9	Ref	
	≥300	8	476	1.5	-1.4% (-2.7%, -0.1%)	
Detected in all	<300	66	2220	2.8	Ref	
	≥300	9	394	1.9	-0.9% (-2.5%, 0.8%)	**0.6% (-1.5%, 2.6%)**
DEET						
Not detected	<300	176	6221	3.0	Ref	
	≥300	1	112	0.8	-2.3% (-3.9%, -0.6%)	
Detected in all	<300	54	1884	2.5	Ref	
	≥300	16	758	1.7	-0.8% (-2.0%, 0.4%)	**1.5% (-0.5%, 3.5%)**
Diethoxyoctylphenol						
Not detected	<300	226	8048	2.8	Ref	
	≥300	17	870	1.7	-1.1% (-2.2%, 0.0%)	
Detected in all	<300	4	57	7.5	Ref	
	≥300	0	0	NA	NA	**NA**
Phenol						
Not detected	<300	175	6317	3.4	Ref	
	≥300	4	371	0.8	-2.6% (-4.3%, -1.0%)	
Detected in all	<300	55	1788	2.1	Ref	
	≥300	13	499	1.8	-0.3% (-1.6%, 1.0%)	**2.4% (0.1%, 4.6%)**
Tributyl phosphate						
Not detected	<300	212	7386	2.9	Ref	
	≥300	10	576	1.5	-1.5% (-2.7%, -0.2%)	
Detected in all	<300	18	719	2.3	Ref	
	≥300	7	294	2.3	0.0% (-2.2%, 2.1%)	**1.5% (-0.9%, 3.8%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain

Table B.3(e). **Rash**

Rash						
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adj Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main assoc				-0.7% (-1.8%, 0.3%)	
Acetaminophen						
Not detected	<300	199	7672	2.9	Ref	
	≥300	20	703	1.8	-1.0% (-2.3%, 0.2%)	
Detected in all	<300	13	383	2.3	Ref	
	≥300	6	148	2.6	0.3% (-2.6%, 3.1%)	**1.3% (-2.0%, 4.6%)**
Beta-sitosterol						
Not detected	<300	197	7663	2.8	Ref	
	≥300	21	633	2.1	-0.7% (-1.9%, 0.6%)	
Detected in all	<300	14	301	3.2	Ref	
	≥300	5	218	1.7	-1.4% (-3.9%, 1.0%)	**-0.7% (-3.5%, 2.0%)**
Bisphenol A						
Not detected	<300	182	6723	2.8	Ref	
	≥300	20	665	2.0	-0.8% (-1.9%, 0.4%)	
Detected in all	<300	29	1241	2.8	Ref	
	≥300	3	105	1.6	-1.2% (-3.5%, 1.1%)	**-0.5% (-2.9%, 2.0%)**
Caffeine						
Not detected	<300	167	6541	2.7	Ref	
	≥300	12	372	2.1	-0.7% (-2.2%, 0.9%)	
Detected in all	<300	45	1514	3.1	Ref	
	≥300	14	479	2.2	-0.9% (-2.5%, 0.7%)	**-0.2% (-2.4%, 2.0%)**
Cholesterol						
Not detected	<300	146	5772	2.8	Ref	
	≥300	13	458	1.7	-1.0% (-2.4%, 0.3%)	
Detected in all	<300	65	2192	2.9	Ref	
	≥300	13	393	2.3	-0.6% (-2.1%, 1.0%)	**0.5% (-1.5%, 2.4%)**
DEET						
Not detected	<300	146	6101	2.7	Ref	
	≥300	5	107	3.7	1.0% (-3.0%, 5.1%)	
Detected in all	<300	65	1863	3.1	Ref	
	≥300	21	744	1.9	-1.1% (-2.4%, 0.1%)	**-2.2% (-6.4%, 2.0%)**
Diethoxyoctylphenol						
Not detected	<300	211	7908	NA	Ref	
	≥300	26	851	NA	NA	
Detected in all	<300	0	56	NA	Ref	
	≥300	0	0	NA	NA	**NA**
Phenol						
Not detected	<300	141	6194	2.2	Ref	
	≥300	9	358	2.4	0.3% (-2.3%, 2.8%)	
Detected in all	<300	70	1770	4.9	Ref	
	≥300	17	493	3.5	-1.4% (-3.8%, 1.0%)	**-1.7% (-5.4%, 2.1%)**
Tributyl phosphate						
Not detected	<300	183	7256	2.7	Ref	
	≥300	15	568	1.9	-0.7% (-2.0%, 0.5%)	
Detected in all	<300	28	708	3.7	Ref	
	≥300	11	283	2.7	-1.1% (-3.5%, 1.4%)	**-0.3% (-3.0%, 2.3%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain

Table B.3(f). **Earache**

Chemical (samples)	Enterococcus (CCE/100ml)	Cases	N	Adj Risk (%)	Adjusted RD (95% CI)*	Interaction Contrast (95% CI)
			Earache			
--	Main assoc				-0.9% (-1.8%, 0.1%)	
Acetaminophen						
Not detected	<300	139	7736	1.8	Ref	
	≥300	7	705	1.1	-0.7% (-1.8%, 0.4%)	
Detected in all	<300	13	388	3.9	Ref	
	≥300	3	151	2.0	-1.8% (-5.0%, 1.4%)	**-1.1% (-4.5%, 2.3%)**
Beta-sitosterol						
Not detected	<300	142	7730	1.8	Ref	
	≥300	6	640	0.9	-0.9% (-2.0%, 0.1%)	
Detected in all	<300	9	301	3.7	Ref	
	≥300	4	216	2.0	-1.7% (-5.1%, 1.7%)	**-0.8% (-4.4%, 2.8%)**
Bisphenol A						
Not detected	<300	130	6783	2.0	Ref	
	≥300	8	666	1.1	-0.9% (-1.9%, 0.1%)	
Detected in all	<300	21	1248	1.8	Ref	
	≥300	1	107	1.0	-0.8% (-3.1%, 1.5%)	**0.1% (-2.1%, 2.4%)**
Caffeine						
Not detected	<300	121	6600	1.8	Ref	
	≥300	2	375	0.5	-1.3% (-2.2%, -0.4%)	
Detected in all	<300	31	1524	2.5	Ref	
	≥300	8	481	1.8	-0.7% (-2.5%, 1.0%)	**0.6% (-1.3%, 2.5%)**
Cholesterol						
Not detected	<300	112	5831	2.0	Ref	
	≥300	5	469	1.0	-1.0% (-2.3%, 0.3%)	
Detected in all	<300	39	2200	1.8	Ref	
	≥300	5	387	1.0	-0.7% (-1.9%, 0.4%)	**0.3% (-1.3%, 1.8%)**
DEET						
Not detected	<300	109	6165	1.8	Ref	
	≥300	1	107	1.1	-0.7% (-3.0%, 1.6%)	
Detected in all	<300	42	1866	2.3	Ref	
	≥300	9	749	1.2	-1.1% (-2.3%, 0.1%)	**-0.4% (-2.8%, 2.0%)**
Diethoxyoctylphenol						
Not detected	<300	149	7974	1.9	Ref	
	≥300	10	856	1.0	-0.9% (-1.9%, 0.1%)	
Detected in all	<300	2	57	5.1	Ref	
	≥300	0	0	NA	NA	**NA**
Phenol						
Not detected	<300	118	6255	2.0	Ref	
	≥300	4	366	1.0	-1.1% (-2.5%, 0.4%)	
Detected in all	<300	33	1776	1.7	Ref	
	≥300	6	490	0.9	-0.8% (-1.9%, 0.3%)	**0.3% (-1.5%, 2.1%)**
Tributyl phosphate						
Not detected	<300	135	7321	1.9	Ref	
	≥300	9	564	1.4	-0.5% (-1.8%, 0.7%)	
Detected in all	<300	16	710	2.2	Ref	
	≥300	1	292	0.3	-1.9% (-3.2%, -0.6%)	**-1.4% (-3.2%, 0.5%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.3(g). **Urinary tract infection**

Urinary tract infection						
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adj Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main assoc				0.2% (-0.6%, 1.1%)	
Acetaminophen						
Not detected	<300	45	7807	0.6	Ref	
	≥300	6	720	0.8	0.2% (-0.6%, 1.0%)	
Detected in all	<300	4	392	1.0	Ref	
	≥300	2	152	2.6	1.6% (-2.6%, 5.8%)	**1.4% (-2.7%, 5.5%)**
Beta-sitosterol						
Not detected	<300	48	7804	0.6	Ref	
	≥300	5	649	0.7	0.1% (-0.8%, 0.9%)	
Detected in all	<300	1	303	0.7	Ref	
	≥300	3	223	1.1	0.4% (-1.5%, 2.4%)	**0.4% (-1.7%, 2.4%)**
Bisphenol A						
Not detected	<300	41	6844	0.6	Ref	
	≥300	7	677	0.9	0.3% (-0.6%, 1.1%)	
Detected in all	<300	8	1263	0.9	Ref	
	≥300	1	110	0.6	-0.3% (-1.8%, 1.2%)	**-0.6% (-2.1%, 0.9%)**
Caffeine						
Not detected	<300	37	6654	0.5	Ref	
	≥300	1	380	0.4	-0.2% (-0.9%, 0.6%)	
Detected in all	<300	12	1545	1.1	Ref	
	≥300	7	492	1.8	0.7% (-1.2%, 2.5%)	**0.9% (-1.0%, 2.7%)**
Cholesterol						
Not detected	<300	36	5886	0.6	Ref	
	≥300	5	476	1.0	0.4% (-1.0%, 1.7%)	
Detected in all	<300	13	2221	0.7	Ref	
	≥300	3	396	0.7	0.0% (-1.0%, 1.0%)	**-0.4% (-1.8%, 1.0%)**
DEET						
Not detected	<300	40	6219	0.8	Ref	
	≥300	1	111	0.5	-0.3% (-1.3%, 0.7%)	
Detected in all	<300	9	1888	0.4	Ref	
	≥300	7	761	0.6	0.2% (-0.5%, 1.0%)	**0.6% (-0.6%, 1.7%)**
Diethoxyoctylphenol						
Not detected	<300	49	8050	NA	Ref	
	≥300	8	872	NA	NA	
Detected in all	<300	0	57	NA	Ref	
	≥300	0	0	NA	NA	**NA**
Phenol						
Not detected	<300	40	6310	0.8	Ref	
	≥300	5	371	1.0	0.2% (-1.0%, 1.4%)	
Detected in all	<300	9	1797	0.4	Ref	
	≥300	3	501	0.4	0.0% (-0.6%, 0.7%)	**-0.2% (-1.5%, 1.1%)**
Tributyl phosphate						
Not detected	<300	44	7386	0.6	Ref	
	≥300	4	578	0.7	0.1% (-0.8%, 0.9%)	
Detected in all	<300	5	721	0.6	Ref	
	≥300	4	294	1.1	0.5% (-1.3%, 2.2%)	**0.4% (-1.3%, 2.1%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.4(a-g). Risk difference modification of the association between *Enterococcus* general indicator measured continuously (CCE/100ml) and illness with human-associated chemical markers (detected in all daily samples vs. <all) in all beaches – Table B.4(a) **GI illness**

		GI Illness	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		1.3% (0.2%, 2.4%)	
Acetaminophen			
Not detected	5.3	Ref	
	6.8	1.6% (0.7%, 2.5%)	
Detected in all	25.1	Ref	
	17.1	-8.1% (-30.5%, 14.4%)	**-9.6% (-32.1%, 12.9%)**
Beta-sitosterol			
Not detected	5.8	Ref	
	7.2	1.3% (0.3%, 2.4%)	
Detected in all	9.7	Ref	
	9.5	-0.2% (-13.9%, 13.5%)	**-1.6% (-15.3%, 12.2%)**
Bisphenol A			
Not detected	5.8	Ref	
	7.0	1.2% (0.2%, 2.3%)	
Detected in all	5.1	Ref	
	7.3	2.2% (-0.2%, 4.6%)	**1.0% (-1.5%, 3.4%)**
Caffeine			
Not detected	4.9	Ref	
	6.7	1.7% (0.8%, 2.7%)	
Detected in all	8.0	Ref	
	8.3	0.3% (-2.5%, 3.1%)	**-1.4% (-4.2%, 1.3%)**
Cholesterol			
Not detected	5.0	Ref	
	6.5	1.5% (0.4%, 2.5%)	
Detected in all	6.4	Ref	
	7.9	1.5% (0.0%, 3.0%)	**0.0% (-1.6%, 1.6%)**
DEET			
Not detected	6.0	Ref	
	7.3	1.2% (-0.3%, 2.8%)	
Detected in all	5.8	Ref	
	7.1	1.3% (0.0%, 2.6%)	**0.1% (-1.9%, 2.0%)**
Diethoxyoctylphenol			
Not detected	5.9	Ref	
	7.2	1.3% (0.2%, 2.4%)	
Detected in all	NA	Ref	
	NA	NA	**NA**
Phenol			
Not detected	7.2	Ref	
	8.0	0.8% (-1.1%, 2.7%)	
Detected in all	4.8	Ref	
	6.3	1.5% (0.4%, 2.5%)	**0.7% (-1.2%, 2.6%)**
Tributyl phosphate			
Not detected	6.3	Ref	
	7.5	1.2% (0.0%, 2.4%)	
Detected in all	3.1	Ref	
	4.9	1.8% (0.9%, 2.6%)	**0.6% (-0.7%, 1.9%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.4(b). **Diarrhea**

		Diarrhea	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		1.1% (0.4%, 1.7%)	
Acetaminophen			
Not detected	3.0	Ref	
	4.2	1.3% (0.7%, 1.8%)	
Detected in all	15.5	Ref	
	10.9	-4.6% (-21.3%, 12.2%)	**-5.8% (-22.5%, 10.9%)**
Beta-sitosterol			
Not detected	3.3	Ref	
	4.4	1.1% (0.5%, 1.8%)	
Detected in all	9.3	Ref	
	8.3	-0.9% (-17.7%, 15.8%)	**-2.0% (-18.9%, 14.8%)**
Bisphenol A			
Not detected	3.3	Ref	
	4.3	1.0% (0.4%, 1.7%)	
Detected in all	2.5	Ref	
	4.4	1.9% (1.0%, 2.8%)	**0.9% (-0.1%, 2.0%)**
Caffeine			
Not detected	2.8	Ref	
	NA	NA	
Detected in all	3.8	Ref	
	4.4	0.7% (-0.7%, 2.0%)	**NA**
Cholesterol			
Not detected	2.5	Ref	
	3.7	1.2% (0.7%, 1.7%)	
Detected in all	3.8	Ref	
	5.1	1.4% (0.4%, 2.3%)	**0.2% (-0.8%, 1.1%)**
DEET			
Not detected	3.2	Ref	
	4.3	1.1% (0.1%, 2.0%)	
Detected in all	3.6	Ref	
	4.8	1.2% (0.3%, 2.0%)	**0.1% (-1.1%, 1.3%)**
Diethoxyoctylphenol			
Not detected	3.4	Ref	
	4.5	1.1% (0.4%, 1.7%)	
Detected in all	NA	Ref	
	NA	NA	**NA**
Phenol			
Not detected	3.8	Ref	
	4.6	0.8% (-0.3%, 1.9%)	
Detected in all	2.9	Ref	
	4.2	1.3% (0.7%, 2.0%)	**0.5% (-0.5%, 1.5%)**
Tributyl phosphate			
Not detected	3.6	Ref	
	4.6	1.0% (0.3%, 1.7%)	
Detected in all	1.5	Ref	
	2.8	1.3% (0.9%, 1.8%)	**0.3% (-0.5%, 1.1%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.4(c). **Respiratory illness**

		Respiratory illness	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		-0.1% (-1.9%, 1.7%)	
Acetaminophen			
Not detected	6.2	Ref	
	6.1	-0.1% (-1.9%, 1.7%)	
Detected in all	18.0	Ref	
	12.5	-5.5% (-40.4%, 29.3%)	**-5.4% (-40.3%, 29.5%)**
Beta-sitosterol			
Not detected	6.1	Ref	
	6.1	0.0% (-1.8%, 1.8%)	
Detected in all	24.0	Ref	
	14.8	-9.2% (-67.3%, 48.9%)	**-9.2% (-67.4%, 49.0%)**
Bisphenol A			
Not detected	7.0	Ref	
	6.8	-0.3% (-2.2%, 1.7%)	
Detected in all	4.2	Ref	
	4.2	0.1% (-4.1%, 4.2%)	**0.3% (-4.1%, 4.7%)**
Caffeine			
Not detected	4.6	Ref	
	5.3	NA	
Detected in all	16.6	Ref	
	11.0	-5.5% (-16.9%, 5.8%)	**NA**
Cholesterol			
Not detected	6.4	Ref	
	6.6	0.2% (-1.9%, 2.3%)	
Detected in all	8.2	Ref	
	6.1	-2.1% (-6.1%, 1.9%)	**-2.3% (-6.6%, 2.0%)**
DEET			
Not detected	4.7	Ref	
	5.6	0.9% (-0.5%, 2.2%)	
Detected in all	9.1	Ref	
	7.2	-1.9% (-6.4%, 2.6%)	**-2.8% (-7.4%, 1.9%)**
Diethoxyoctylphenol			
Not detected	6.2	Ref	
	6.2	-0.1% (-1.9%, 1.7%)	
Detected in all	0.0	Ref	
	0.0	NA	**NA**
Phenol			
Not detected	5.8	Ref	
	6.2	0.5% (-1.4%, 2.3%)	
Detected in all	8.0	Ref	
	6.1	-1.8% (-6.1%, 2.4%)	**-2.3% (-6.9%, 2.3%)**
Tributyl phosphate			
Not detected	5.8	Ref	
	5.8	0.1% (-1.6%, 1.7%)	
Detected in all	10.6	Ref	
	9.3	-1.4% (-10.8%, 8.1%)	**-1.5% (-10.7%, 7.8%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain

Table B.4(d). **Eye ailment**

		Eye Ailment	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		-1.4% (-3.8%, 0.9%)	
Acetaminophen			
Not detected	4.5	Ref	
	3.5	-1.0% (-3.1%, 1.1%)	
Detected in all	45.2	Ref	
	15.5	-29.7% (-104.1%, 44.7%)	**-28.7% (-103.2%, 45.8%)**
Beta-sitosterol			
Not detected	5.3	Ref	
	3.8	-1.6% (-4.2%, 1.0%)	
Detected in all	15.8	Ref	
	8.1	-7.7% (-58.9%, 43.5%)	**-6.1% (-57.6%, 45.4%)**
Bisphenol A			
Not detected	5.7	Ref	
	3.9	-1.8% (-4.5%, 0.9%)	
Detected in all	1.6	Ref	
	2.1	0.5% (-0.9%, 2.0%)	**2.3% (-0.5%, 5.2%)**
Caffeine			
Not detected	4.1	Ref	
	3.3	-0.7% (-2.9%, 1.4%)	
Detected in all	10.8	Ref	
	5.6	-5.2% (-16.2%, 5.8%)	**-4.5% (-15.4%, 6.4%)**
Cholesterol			
Not detected	7.4	Ref	
	4.5	-2.9% (-8.0%, 2.2%)	
Detected in all	3.9	Ref	
	3.2	-0.7% (-2.9%, 1.5%)	**2.2% (-2.9%, 7.3%)**
DEET			
Not detected	7.9	Ref	
	4.6	-3.2% (-8.8%, 2.3%)	
Detected in all	3.2	Ref	
	2.7	-0.5% (-2.2%, 1.2%)	**2.8% (-2.9%, 8.4%)**
Diethoxyoctylphenol			
Not detected	5.2	Ref	
	3.7	-1.5% (-3.9%, 1.0%)	
Detected in all	0.0	Ref	
	0.0	NA	**NA**
Phenol			
Not detected	10.7	Ref	
	5.5	-5.2% (-13.6%, 3.3%)	
Detected in all	2.3	Ref	
	2.2	-0.1% (-1.3%, 1.2%)	**5.1% (-3.4%, 13.5%)**
Tributyl phosphate			
Not detected	5.7	Ref	
	3.9	-1.8% (-4.6%, 0.9%)	
Detected in all	1.6	Ref	
	1.9	0.3% (-1.0%, 1.6%)	**2.1% (-0.6%, 4.9%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain

Table B.4(e). **Rash**

		Rash	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		0.0% (-1.0%, 1.0%)	
Acetaminophen			
Not detected	2.6	Ref	
	2.6	0.0% (-1.0%, 1.1%)	
Detected in all	3.8	Ref	
	3.3	-0.5% (-6.6%, 5.6%)	**-0.5% (-6.8%, 5.7%)**
Beta-sitosterol			
Not detected	2.8	Ref	
	2.7	-0.1% (-1.2%, 1.0%)	
Detected in all	0.4	Ref	
	0.8	0.4% (-0.2%, 1.1%)	**0.5% (-0.7%, 1.7%)**
Bisphenol A			
Not detected	2.5	Ref	
	2.6	0.0% (-0.9%, 1.0%)	
Detected in all	4.2	Ref	
	3.4	-0.8% (-6.9%, 5.2%)	**-0.9% (-6.8%, 5.0%)**
Caffeine			
Not detected	2.6	Ref	
	2.6	0.0% (-1.1%, 1.1%)	
Detected in all	3.6	Ref	
	3.3	-0.3% (-2.8%, 2.1%)	**-0.3% (-2.8%, 2.2%)**
Cholesterol			
Not detected	1.7	Ref	
	2.1	0.4% (-0.2%, 1.0%)	
Detected in all	3.8	Ref	
	3.3	-0.5% (-2.6%, 1.6%)	**-0.8% (-2.9%, 1.2%)**
DEET			
Not detected	1.0	Ref	
	1.7	0.7% (0.4%, 0.9%)	
Detected in all	6.4	Ref	
	4.4	-2.0% (-5.7%, 1.8%)	**-2.6% (-6.4%, 1.1%)**
Diethoxyoctylphenol			
Not detected	NA	Ref	
	NA	NA	
Detected in all	NA	Ref	
	NA	NA	**NA**
Phenol			
Not detected	0.9	Ref	
	1.4	0.5% (0.2%, 0.8%)	
Detected in all	8.0	Ref	
	6.5	-1.5% (-6.0%, 3.0%)	**-2.0% (-6.5%, 2.5%)**
Tributyl phosphate			
Not detected	2.6	Ref	
	2.6	-0.1% (-1.2%, 1.0%)	
Detected in all	2.7	Ref	
	3.1	0.4% (-1.7%, 2.5%)	**0.5% (-1.6%, 2.6%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain

Table B.4(f). **Earache**

		Earache	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		-0.2% (-1.4%, 0.9%)	
Acetaminophen			
Not detected	2.4	Ref	
	2.0	-0.4% (-1.9%, 1.1%)	
Detected in all	22.4	Ref	
	11.3	-11.1% (-57.0%, 34.7%)	**-10.7% (-56.4%, 34.9%)**
Beta-sitosterol			
Not detected	2.0	Ref	
	1.9	-0.2% (-1.2%, 0.9%)	
Detected in all	78.4	Ref	
	36.0	-42.4% (-68.0%, -16.7%)	**-42.2% (-67.8%, -16.6%)**
Bisphenol A			
Not detected	2.2	Ref	
	2.0	-0.2% (-1.3%, 1.0%)	
Detected in all	2.8	Ref	
	2.1	-0.7% (-7.0%, 5.7%)	**-0.5% (-6.7%, 5.7%)**
Caffeine			
Not detected	3.0	Ref	
	2.2	-0.8% (-2.9%, 1.2%)	
Detected in all	2.3	Ref	
	2.3	0.1% (-1.7%, 1.8%)	**0.9% (-1.5%, 3.3%)**
Cholesterol			
Not detected	2.4	Ref	
	2.1	-0.3% (-2.0%, 1.4%)	
Detected in all	2.0	Ref	
	1.8	-0.2% (-1.5%, 1.1%)	**0.1% (-1.7%, 1.9%)**
DEET			
Not detected	2.3	Ref	
	1.9	-0.4% (-2.3%, 1.5%)	
Detected in all	2.3	Ref	
	2.2	-0.1% (-1.5%, 1.4%)	**0.3% (-2.0%, 2.6%)**
Diethoxyoctylphenol			
Not detected	2.1	Ref	
	1.9	-0.2% (-1.3%, 0.9%)	
Detected in all	0.0	Ref	
	0.0	NA	**NA**
Phenol			
Not detected	2.3	Ref	
	2.0	-0.3% (-2.0%, 1.4%)	
Detected in all	2.0	Ref	
	1.9	-0.1% (-1.3%, 1.1%)	**0.1% (-1.8%, 2.1%)**
Tributyl phosphate			
Not detected	2.1	Ref	
	1.9	-0.1% (-1.2%, 0.9%)	
Detected in all	3.9	Ref	
	2.8	-1.2% (-6.8%, 4.4%)	**-1.0% (-6.4%, 4.3%)**

NA, not able to estimated;* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.4(g). **Urinary tract infection**

		Urinary tract infection	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		-0.4% (-1.6%, 0.9%)	
Acetaminophen			
Not detected	1.6	Ref	
	1.0	-0.6% (-2.4%, 1.2%)	
Detected in all	0.4	Ref	
	0.6	0.2% (-0.2%, 0.6%)	**0.8% (-1.0%, 2.6%)**
Beta-sitosterol			
Not detected	1.6	Ref	
	1.0	-0.6% (-2.3%, 1.1%)	
Detected in all	0.4	Ref	
	0.5	0.1% (-0.3%, 0.6%)	**0.7% (-0.9%, 2.4%)**
Bisphenol A			
Not detected	1.3	Ref	
	0.9	-0.4% (-1.8%, 1.0%)	
Detected in all	6.8	Ref	
	2.4	-4.4% (-25.1%, 16.3%)	**-4.0% (-24.4%, 16.4%)**
Caffeine			
Not detected	4.6	Ref	
	1.4	-3.3% (-9.7%, 3.1%)	
Detected in all	0.5	Ref	
	0.7	0.2% (-0.2%, 0.6%)	**3.5% (-2.9%, 9.8%)**
Cholesterol			
Not detected	2.4	Ref	
	1.2	-1.2% (-5.1%, 2.8%)	
Detected in all	0.9	Ref	
	0.8	-0.1% (-1.0%, 0.8%)	**1.1% (-2.7%, 4.9%)**
DEET			
Not detected	5.6	Ref	
	1.9	-3.7% (-12.5%, 5.1%)	
Detected in all	0.2	Ref	
	0.3	0.1% (-0.1%, 0.2%)	**3.8% (-5.0%, 12.6%)**
Diethoxyoctylphenol			
Not detected	NA	Ref	
	NA	NA	
Detected in all	NA	Ref	
	NA	NA	**NA**
Phenol			
Not detected	4.4	Ref	
	2.0	-2.4% (-9.6%, 4.7%)	
Detected in all	0.4	Ref	
	0.3	0.0% (-0.5%, 0.4%)	**2.4% (-4.6%, 9.4%)**
Tributyl phosphate			
Not detected	1.7	Ref	
	1.0	-0.7% (-2.7%, 1.3%)	
Detected in all	0.2	Ref	
	0.3	0.1% (0.0%, 0.2%)	**0.8% (-1.2%, 2.9%)**

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.5(a-g). Frequencies and risk differences (95% CI) for the association between illness and categories of human-associated chemical markers among body immersion swimmers in all beaches

Table B.5(a). **GI illness**

	All beaches				
Count of chemicals in each category	**Cases**	**N**	**(%)**	**Adjusted Risk(%)**	**Adjusted RD (95% CI)**
GI illness					
Pharmaceuticals					
0	309	4155	7.4%	9.1%	Ref
1	19	309	6.1%	8.8%	-0.3% (-2.6%, 2.0%)
2	73	701	10.4%	7.1%	-1.9% (-4.7%, 0.9%)
3	26	211	12.3%	9.1%	0.0% (-5.0%, 5.1%)
4	14	105	13.3%	9.2%	0.1% (-5.9%, 6.2%)
Fecal Sterols/Stanols					
0	314	4141	7.6%	8.3%	Ref
1	114	1220	9.3%	9.5%	1.1% (-0.7%, 2.9%)
2	13	120	10.8%	8.4%	0.0% (-3.7%, 3.7%)
Household wastewater					
0	293	3874	7.6%	8.8%	Ref
1	64	654	9.8%	8.8%	-0.1% (-2.6%, 2.5%)
2	24	285	8.4%	6.6%	-2.3% (-5.2%, 0.7%)
3	28	406	6.9%	8.1%	-0.7% (-3.9%, 2.4%)
4	16	106	15.1%	12.2%	3.4% (-3.6%, 10.4%)
5	9	93	9.7%	9.1%	0.3% (-6.5%, 7.0%)
6	7	63	11.1%	10.5%	1.7% (-9.1%, 12.5%)
Industrial wastewater					
0	265	3583	7.4%	8.3%	Ref
1	167	1792	9.3%	9.2%	0.8% (-1.0%, 2.7%)
2	9	106	8.5%	7.5%	-0.9% (-5.0%, 3.2%)
Runoff					
0	286	3825	7.5%	8.6%	Ref
1	95	1150	8.3%	8.3%	-0.3% (-2.2%, 1.7%)
2	32	272	11.8%	9.9%	1.3% (-3.0%, 5.7%)
3	28	234	12.0%	9.5%	0.9% (-2.8%, 4.5%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.5(b). **Diarrhea**

	All beaches				
Count of chemicals in each category	**Cases**	**N**	**(%)**	**Adjusted Risk(%)**	**Adjusted RD (95% CI)**
Diarrhea					
Pharmaceuticals					
0	188	4155	4.5%	6.2%	Ref
1	15	309	4.9%	6.0%	-0.3% (-2.2%, 1.7%)
2	55	701	7.8%	4.6%	-1.7% (-3.9%, 0.6%)
3	22	211	10.4%	6.7%	0.5% (-3.9%, 4.9%)
4	8	105	7.6%	4.7%	-1.6% (-5.6%, 2.5%)
Fecal Sterols/Stanols					
0	195	4141	4.7%	5.4%	Ref
1	81	1220	6.6%	6.5%	1.1% (-0.4%, 2.6%)
2	12	120	10.0%	6.6%	1.2% (-2.1%, 4.4%)
Household wastewater					
0	179	3874	4.6%	5.8%	Ref
1	52	654	8.0%	6.3%	0.5% (-1.7%, 2.7%)
2	15	285	5.3%	4.4%	-1.4% (-3.9%, 1.1%)
3	20	406	4.9%	6.1%	0.3% (-2.7%, 3.2%)
4	13	106	12.3%	9.2%	3.4% (-2.7%, 9.4%)
5	5	93	5.4%	5.3%	-0.5% (-5.7%, 4.8%)
6	4	63	6.3%	6.1%	0.3% (-9.3%, 9.8%)
Industrial wastewater					
0	157	3583	4.4%	5.3%	Ref
1	123	1792	6.9%	6.5%	1.2% (-0.3%, 2.8%)
2	8	106	7.5%	6.0%	0.7% (-2.9%, 4.4%)
Runoff					
0	175	3825	4.6%	5.7%	Ref
1	69	1150	6.0%	6.0%	0.3% (-1.4%, 2.1%)
2	23	272	8.5%	6.4%	0.7% (-2.9%, 4.3%)
3	21	234	9.0%	6.2%	0.5% (-2.4%, 3.4%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.5(c). **Respiratory illness**

	All beaches				
Count of chemicals in each category	**Cases**	**N**	**(%)**	**Adjusted Risk(%)**	**Adjusted RD (95% CI)**
Respiratory illness					
Pharmaceuticals					
0	256	3986	6.4%	5.8%	Ref
1	21	305	6.9%	7.2%	1.4% (-0.8%, 3.5%)
2	43	686	6.3%	5.5%	-0.4% (-3.4%, 2.7%)
3	17	209	8.1%	7.2%	1.4% (-3.8%, 6.5%)
4	9	101	8.9%	7.0%	1.1% (-5.4%, 7.7%)
Fecal Sterols/Stanols					
0	272	3984	6.8%	6.4%	Ref
1	66	1180	5.6%	4.6%	-1.8% (-3.1%, -0.6%)
2	8	123	6.5%	6.2%	-0.3% (-3.1%, 2.6%)
Household wastewater					
0	247	3717	6.6%	6.1%	Ref
1	32	641	5.0%	4.4%	-1.7% (-3.4%, 0.0%)
2	17	276	6.2%	6.4%	0.4% (-3.1%, 3.8%)
3	29	398	7.3%	7.7%	1.6% (-1.9%, 5.1%)
4	9	100	9.0%	9.4%	3.3% (-4.1%, 10.7%)
5	10	94	10.6%	15.2%	9.1% (-2.1%, 20.4%)
6	2	61	3.3%	5.4%	-0.7% (-11.4%, 10.1%)
Industrial wastewater					
0	241	3440	7.0%	6.7%	Ref
1	91	1739	5.2%	4.7%	-2.0% (-3.5%, -0.5%)
2	14	108	13.0%	6.8%	0.0% (-4.8%, 4.9%)
Runoff					
0	242	3660	6.6%	6.3%	Ref
1	75	1127	6.7%	5.7%	-0.6% (-2.3%, 1.0%)
2	17	270	6.3%	5.4%	-0.9% (-4.1%, 2.3%)
3	12	230	5.2%	2.9%	-3.4% (-5.2%, -1.5%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.5(d). **Eye ailment**

	All beaches				
Count of chemicals in each category	**Cases**	**N**	**(%)**	**Adjusted Risk(%)**	**Adjusted RD (95% CI)**
Eye ailment					
Pharmaceuticals					
0	115	4256	2.7%	2.9%	Ref
1	5	318	1.6%	2.6%	-0.3% (-1.4%, 0.9%)
2	17	713	2.4%	2.1%	-0.8% (-2.5%, 0.9%)
3	6	216	2.8%	2.4%	-0.5% (-3.3%, 2.3%)
4	3	106	2.8%	2.4%	-0.5% (-3.9%, 2.8%)
Fecal Sterols/Stanols					
0	108	4236	2.5%	2.7%	Ref
1	35	1247	2.8%	2.8%	0.1% (-0.9%, 1.0%)
2	3	126	2.4%	2.9%	0.2% (-1.7%, 2.0%)
Household wastewater					
0	108	3961	2.7%	2.9%	Ref
1	13	676	1.9%	2.1%	-0.7% (-1.9%, 0.5%)
2	5	284	1.8%	2.4%	-0.4% (-2.2%, 1.3%)
3	9	417	2.2%	2.4%	-0.4% (-2.1%, 1.3%)
4	7	107	6.5%	7.7%	4.8% (-2.2%, 11.8%)
5	2	98	2.0%	2.9%	0.0% (-4.5%, 4.5%)
6	2	66	3.0%	4.0%	1.2% (-5.0%, 7.4%)
Industrial wastewater					
0	94	3672	2.6%	2.7%	Ref
1	50	1831	2.7%	2.8%	0.1% (-0.8%, 1.0%)
2	2	106	1.9%	1.9%	-0.8% (-2.7%, 1.2%)
Runoff					
0	104	3913	2.7%	2.9%	Ref
1	29	1178	2.5%	2.1%	-0.7% (-1.7%, 0.2%)
2	7	279	2.5%	2.8%	-0.1% (-2.4%, 2.3%)
3	6	239	2.5%	2.3%	-0.6% (-2.6%, 1.4%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.5(e). **Rash**

	All beaches				
Count of chemicals in each category	**Cases**	**N**	**(%)**	**Adjusted Risk(%)**	**Adjusted RD (95% CI)**
Rash					
Pharmaceuticals					
0	97	4176	2.3%	2.4%	Ref
1	11	313	3.5%	3.4%	1.0% (-0.3%, 2.2%)
2	25	695	3.6%	3.6%	1.2% (-0.7%, 3.1%)
3	1	212	0.5%	0.5%	-1.9% (-3.0%, -0.8%)
4	3	104	2.9%	3.6%	1.1% (-3.2%, 5.4%)
Fecal Sterols/Stanols					
0	114	4147	2.7%	2.7%	Ref
1	19	1227	1.5%	2.6%	-0.1% (-0.9%, 0.7%)
2	4	126	3.2%	3.2%	0.6% (-1.1%, 2.2%)
Household wastewater					
0	93	3884	2.4%	2.6%	Ref
1	11	666	1.7%	2.5%	-0.1% (-1.1%, 0.9%)
2	12	278	4.3%	3.8%	1.2% (-0.8%, 3.2%)
3	15	407	3.7%	3.3%	0.7% (-1.5%, 3.0%)
4	1	106	0.9%	0.9%	-1.7% (-3.6%, 0.1%)
5	5	97	5.2%	3.3%	0.7% (-3.0%, 4.3%)
6	0	62	0.0%	NA	NA
Industrial wastewater					
0	87	3597	2.4%	2.5%	Ref
1	46	1796	2.6%	2.9%	0.4% (-0.5%, 1.2%)
2	4	107	3.7%	4.8%	2.3% (-0.9%, 5.6%)
Runoff					
0	99	3823	2.6%	2.8%	Ref
1	25	1166	2.1%	2.4%	-0.5% (-1.4%, 0.5%)
2	11	273	4.0%	3.4%	0.6% (-2.0%, 3.2%)
3	2	238	0.8%	1.5%	-1.4% (-2.7%, 0.0%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.5(f). **Earache**

	All beaches				
Count of chemicals in each category	**Cases**	**N**	**(%)**	**Adjusted Risk(%)**	**Adjusted RD (95% CI)**
Earache					
Pharmaceuticals					
0	71	4215	1.7%	1.6%	Ref
1	5	311	1.6%	2.2%	0.6% (-0.5%, 1.8%)
2	7	703	1.0%	2.7%	1.1% (-1.0%, 3.2%)
3	5	211	2.4%	6.0%	4.5% (-2.4%, 11.3%)
4	5	105	4.8%	11.4%	9.8% (-3.6%, 23.2%)
Fecal Sterols/Stanols					
0	72	4191	1.7%	1.8%	Ref
1	17	1232	1.4%	1.5%	-0.4% (-1.0%, 0.3%)
2	4	122	3.3%	2.9%	1.1% (-0.9%, 3.0%)
Household wastewater					
0	67	3922	1.7%	1.7%	Ref
1	9	666	1.4%	1.9%	0.2% (-0.8%, 1.2%)
2	7	279	2.5%	3.8%	2.1% (-0.2%, 4.4%)
3	6	408	1.5%	1.7%	0.0% (-1.6%, 1.7%)
4	2	108	1.9%	3.2%	1.5% (-3.3%, 6.3%)
5	2	98	2.0%	3.6%	1.9% (-3.8%, 7.6%)
6	0	64	0.0%	NA	NA
Industrial wastewater					
0	61	3634	1.7%	1.8%	Ref
1	31	1804	1.7%	1.8%	0.0% (-0.8%, 0.7%)
2	1	107	0.9%	1.1%	-0.7% (-2.3%, 0.8%)
Runoff					
0	67	3871	1.7%	1.9%	Ref
1	17	1163	1.5%	1.5%	-0.4% (-1.2%, 0.4%)
2	3	279	1.1%	1.3%	-0.6% (-2.2%, 1.1%)
3	6	232	2.6%	1.7%	-0.2% (-1.6%, 1.1%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.5(g). **Urinary tract infection**

	All beaches				
Count of chemicals in each category	**Cases**	**N**	**(%)**	**Adjusted Risk (%)**	**Adjusted RD (95% CI)**
Urinary tract infection					
Pharmaceuticals					
0	24	4255	0.6%	0.5%	Ref
1	2	317	0.6%	0.8%	0.3% (-0.2%, 0.9%)
2	7	718	1.0%	1.3%	0.8% (-0.5%, 2.1%)
3	2	217	0.9%	1.2%	0.7% (-1.9%, 3.4%)
4	2	106	1.9%	2.6%	2.1% (-2.9%, 7.2%)
Fecal Sterols/Stanols					
0	26	4236	0.6%	0.6%	Ref
1	9	1250	0.7%	0.7%	0.0% (-0.5%, 0.5%)
2	2	127	1.6%	0.6%	0.0% (-0.9%, 0.8%)
Household wastewater					
0	23	3963	0.6%	0.8%	Ref
1	5	673	0.7%	0.4%	-0.4% (-1.0%, 0.2%)
2	5	290	1.7%	0.6%	-0.2% (-1.0%, 0.6%)
3	3	416	0.7%	0.4%	-0.4% (-1.1%, 0.3%)
4	0	107	0.0%	NA	NA
5	0	98	0.0%	NA	NA
6	1	66	1.5%	0.5%	-0.3% (-1.6%, 1.0%)
Industrial wastewater					
0	22	3670	0.6%	0.7%	Ref
1	15	1834	0.8%	0.6%	0.0% (-0.5%, 0.5%)
2	0	109	0.0%	NA	NA
Runoff					
0	27	3912	0.7%	0.7%	Ref
1	5	1177	0.4%	0.4%	-0.3% (-0.7%, 0.1%)
2	2	281	0.7%	0.4%	-0.3% (-1.0%, 0.4%)
3	3	243	1.2%	0.5%	-0.2% (-0.9%, 0.6%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.6(a-g). Modification of the adjusted standardized RD (95% CI) for the association between illness and *Enterococcus* qPCR Method 1611 above and below EPA guidelines (geometric mean of 470 CCE/100ml for an illness rate of 36/1000) with categories of chemical markers among body immersion swimmers in all beaches

Table B.6(a). **GI illness**

					GI Illness	
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main association				0.7% (-2.3%, 3.6%)	
Pharmaceuticals						
Not detected	<470	448	5923	8.5	Ref	
	≥470	40	270	12.1	3.5% (-1.7%, 8.7%)	
Detected in all	<470	236	2344	8.7	Ref	
	≥470	30	337	7.4	-1.3% (-4.5%, 1.9%)	**-4.8% (-10.6%, 0.9%)**
Fecal sterols						
Not detected	<470	459	5957	8.3	Ref	
	≥470	31	292	8.9	0.7% (-3.3%, 4.6%)	
Detected in all	<470	225	2310	9.3	Ref	
	≥470	39	315	10.1	0.8% (-3.2%, 4.9%)	**0.2% (-5.1%, 5.4%)**
Household waste						
Not detected	<470	477	6071	8.7	Ref	
	≥470	8	53	11.3	2.6% (-6.7%, 11.9%)	
Detected in all	<470	207	2196	8.5	Ref	
	≥470	62	554	8.9	0.4% (-2.6%, 3.4%)	**-2.2% (-12.0%, 7.6%)**
Industrial waste						
Not detected	<470	391	5183	8.4	Ref	
	≥470	21	225	7.5	-0.9% (-5.1%, 3.3%)	
Detected in all	<470	293	3084	8.8	Ref	
	≥470	49	382	10.8	2.0% (-1.9%, 5.8%)	**2.9% (-2.3%, 8.0%)**
Runoff						
Not detected	<470	536	6630	8.7	Ref	
	≥470	14	150	8.1	-0.6% (-5.6%, 4.4%)	
Detected in all	<470	148	1637	8.4	Ref	
	≥470	56	457	9.8	1.4% (-2.6%, 5.4%)	**2.0% (-4.3%, 8.3%)**

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.6(b). **Diarrhea**

		Diarrhea				
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main association				1.1% (-1.5%, 3.7%)	
Pharmaceuticals						
Not detected	<470	277	5923	5.6	Ref	
	≥470	33	270	9.1	3.5% (-1.1%, 8.1%)	
Detected in all	<470	170	2344	5.8	Ref	
	≥470	23	337	5.3	-0.5% (-3.3%, 2.3%)	**-4.0% (-9.0%, 1.0%)**
Fecal sterols						
Not detected	<470	281	5957	5.3	Ref	
	≥470	26	292	6.9	1.6% (-1.9%, 5.1%)	
Detected in all	<470	166	2310	6.5	Ref	
	≥470	30	315	7.5	0.9% (-2.7%, 4.6%)	**-0.7% (-5.3%, 4.0%)**
Household waste						
Not detected	<470	295	6071	5.6	Ref	
	≥470	7	53	10.0	4.4% (-4.8%, 13.7%)	
Detected in all	<470	152	2196	6.0	Ref	
	≥470	49	554	6.7	0.7% (-2.0%, 3.3%)	**-3.8% (-13.4%, 5.9%)**
Industrial waste						
Not detected	<470	231	5183	5.2	Ref	
	≥470	17	225	5.7	0.6% (-3.1%, 4.3%)	
Detected in all	<470	216	3084	6.3	Ref	
	≥470	39	382	8.7	2.4% (-1.3%, 6.1%)	**1.8% (-2.8%, 6.4%)**
Runoff						
Not detected	<470	341	6630	5.7	Ref	
	≥470	12	150	7.2	1.5% (-3.5%, 6.5%)	
Detected in all	<470	106	1637	5.9	Ref	
	≥470	44	457	6.7	0.8% (-2.7%, 4.3%)	**-0.7% (-6.8%, 5.4%)**

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.6(c). **Respiratory illness**

		Respiratory illness				
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main association				-1.8% (-4.2%, 0.6%)	
Pharmaceuticals						
Not detected	<470	341	5732	5.8	Ref	
	≥470	11	265	5.0	-0.8% (-4.7%, 3.1%)	
Detected in all	<470	158	2273	6.9	Ref	
	≥470	12	324	4.4	-2.4% (-6.1%, 1.2%)	**-1.6% (-7.0%, 3.8%)**
Fecal sterols						
Not detected	<470	388	5755	6.6	Ref	
	≥470	14	280	5.3	-1.4% (-5.4%, 2.7%)	
Detected in all	<470	111	2250	4.9	Ref	
	≥470	9	309	3.0	-1.9% (-4.1%, 0.4%)	**-0.5% (-5.1%, 4.1%)**
Household waste						
Not detected	<470	377	5862	6.4	Ref	
	≥470	2	51	3.1	-3.3% (-7.2%, 0.7%)	
Detected in all	<470	122	2143	5.5	Ref	
	≥470	21	538	4.2	-1.3% (-3.9%, 1.3%)	**1.9% (-2.7%, 6.6%)**
Industrial waste						
Not detected	<470	350	5009	7.2	Ref	
	≥470	8	217	3.4	-3.8% (-7.6%, 0.0%)	
Detected in all	<470	149	2996	4.7	Ref	
	≥470	15	372	3.7	-0.9% (-3.3%, 1.4%)	**2.9% (-1.5%, 7.2%)**
Runoff						
Not detected	<470	399	6400	6.4	Ref	
	≥470	5	140	3.3	-3.1% (-5.9%, -0.3%)	
Detected in all	<470	100	1605	5.2	Ref	
	≥470	18	449	4.8	-0.4% (-3.9%, 3.1%)	**2.7% (-1.8%, 7.2%)**

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain

Table B.6(d). **Eye ailment**

		Eye Ailment				
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main association				-0.7% (-2.1%, 0.8%)	
Pharmaceuticals						
Not detected	<470	164	6070	2.8	Ref	
	≥470	10	273	3.5	0.7% (-2.1%, 3.6%)	
Detected in all	<470	70	2385	2.8	Ref	
	≥470	4	340	1.1	-1.7% (-3.1%, -0.3%)	**-2.4% (-5.6%, 0.7%)**
Fecal sterols						
Not detected	<470	166	6094	2.8	Ref	
	≥470	5	294	1.6	-1.2% (-2.8%, 0.4%)	
Detected in all	<470	68	2361	2.7	Ref	
	≥470	9	319	2.5	-0.2% (-2.4%, 1.9%)	**1.0% (-1.6%, 3.5%)**
Household waste						
Not detected	<470	175	6215	3.0	Ref	
	≥470	1	54	1.4	-1.6% (-4.4%, 1.3%)	
Detected in all	<470	59	2240	2.4	Ref	
	≥470	13	559	2.0	-0.4% (-1.8%, 1.0%)	**1.2% (-2.0%, 4.3%)**
Industrial waste						
Not detected	<470	147	5315	3.0	Ref	
	≥470	2	228	0.7	-2.3% (-3.6%, -0.9%)	
Detected in all	<470	87	3140	2.5	Ref	
	≥470	12	385	2.7	0.2% (-1.8%, 2.2%)	**2.5% (0.3%, 4.7%)**
Runoff						
Not detected	<470	197	6783	3.0	Ref	
	≥470	2	153	1.3	-1.6% (-3.5%, 0.2%)	
Detected in all	<470	37	1672	2.1	Ref	
	≥470	12	460	2.4	0.3% (-1.7%, 2.3%)	**1.9% (-0.9%, 4.7%)**

NA, not able to estimated. * Adjusted for beach, mean bathers, sand, rain

Table B.6(e). **Rash**

		Rash				
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main association				0.0% (-1.4%, 1.3%)	
Pharmaceuticals						
Not detected	<470	140	5953	2.4	Ref	
	≥470	9	270	2.5	0.1% (-2.0%, 2.2%)	
Detected in all	<470	75	2349	3.3	Ref	
	≥470	14	334	3.5	0.2% (-2.0%, 2.3%)	**0.1% (-2.8%, 3.0%)**
Fecal sterols						
Not detected	<470	150	5972	2.7	Ref	
	≥470	10	287	2.5	-0.1% (-2.1%, 1.8%)	
Detected in all	<470	65	2330	2.7	Ref	
	≥470	13	317	2.8	0.1% (-1.8%, 1.9%)	**0.2% (-2.4%, 2.8%)**
Household waste						
Not detected	<470	143	6093	2.6	Ref	
	≥470	2	54	4.0	1.4% (-4.2%, 7.0%)	
Detected in all	<470	72	2209	2.9	Ref	
	≥470	21	550	2.7	-0.2% (-1.7%, 1.3%)	**-1.6% (-7.5%, 4.3%)**
Industrial waste						
Not detected	<470	118	5210	2.5	Ref	
	≥470	6	220	2.2	-0.3% (-2.5%, 1.9%)	
Detected in all	<470	97	3092	3.0	Ref	
	≥470	17	384	3.2	0.2% (-1.6%, 2.1%)	**0.5% (-2.3%, 3.3%)**
Runoff						
Not detected	<470	182	6650	2.8	Ref	
	≥470	6	149	3.1	0.3% (-2.8%, 3.4%)	
Detected in all	<470	33	1652	2.2	Ref	
	≥470	17	455	2.6	0.3% (-1.5%, 2.1%)	**0.0% (-3.7%, 3.7%)**

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain

Table B.6(f). **Earache**

		Earache				
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main association				-0.9% (-1.8%, 0.1%)	
Pharmaceuticals						
Not detected	<470	108	6013	1.7	Ref	
	≥470	2	272	0.7	-1.0% (-2.2%, 0.2%)	
Detected in all	<470	47	2361	2.5	Ref	
	≥470	5	334	1.6	-0.9% (-2.6%, 0.8%)	**0.1% (-1.9%, 2.1%)**
Fecal sterols						
Not detected	<470	114	6037	2.0	Ref	
	≥470	4	292	1.3	-0.6% (-2.1%, 0.9%)	
Detected in all	<470	41	2337	1.8	Ref	
	≥470	3	314	0.7	-1.0% (-2.1%, 0.0%)	**-0.4% (-2.1%, 1.3%)**
Household waste						
Not detected	<470	110	6159	1.8	Ref	
	≥470	1	50	2.2	0.4% (-4.1%, 4.8%)	
Detected in all	<470	45	2215	2.2	Ref	
	≥470	6	556	1.0	-1.2% (-2.3%, 0.0%)	**-1.6% (-6.2%, 3.1%)**
Industrial waste						
Not detected	<470	99	5268	2.0	Ref	
	≥470	4	224	1.6	-0.3% (-2.2%, 1.6%)	
Detected in all	<470	56	3106	1.8	Ref	
	≥470	3	382	0.6	-1.2% (-2.1%, -0.3%)	**-0.9% (-2.9%, 1.1%)**
Runoff						
Not detected	<470	130	6724	2.0	Ref	
	≥470	1	147	0.7	-1.2% (-2.8%, 0.4%)	
Detected in all	<470	25	1650	1.6	Ref	
	≥470	6	459	1.1	-0.5% (-1.9%, 0.9%)	**0.7% (-1.3%, 2.8%)**

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.6(g). **Urinary tract infection**

		Urinary tract infection				
Chemical (samples)	**Enterococcus (CCE/100ml)**	**Cases**	**N**	**Modeled Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--	Main association				0.1% (-0.7%, 1.0%)	
Pharmaceuticals						
Not detected	<470	35	6062	0.6	Ref	
	≥470	0	273	NA	NA	
Detected in all	<470	17	2394	0.8	Ref	
	≥470	5	342	1.5	0.6% (-0.9%, 2.1%)	**NA**
Fecal sterols						
Not detected	<470	37	6093	0.6	Ref	
	≥470	3	295	1.1	0.5% (-0.9%, 1.9%)	
Detected in all	<470	15	2363	0.8	Ref	
	≥470	2	320	0.6	-0.2% (-1.2%, 0.9%)	**-0.7% (-2.3%, 1.0%)**
Household waste						
Not detected	<470	40	6212	0.0	Ref	
	≥470	0	54	NA	NA	
Detected in all	<470	12	2244	0.0	Ref	
	≥470	5	561	0.0	0.3% (-0.6%, 1.3%)	**NA**
Industrial waste						
Not detected	<470	33	5307	0.6	Ref	
	≥470	2	228	1.0	0.5% (-1.1%, 2.0%)	
Detected in all	<470	19	3149	0.7	Ref	
	≥470	3	387	0.7	0.0% (-1.0%, 1.0%)	**-0.4% (-2.2%, 1.4%)**
Runoff						
Not detected	<470	44	6781	0.7	Ref	
	≥470	2	153	0.7	0.0% (-1.1%, 1.1%)	
Detected in all	<470	8	1675	0.4	Ref	
	≥470	3	462	0.8	0.4% (-0.8%, 1.6%)	**0.4% (-1.2%, 2.0%)**

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.7(a-g). Risk difference modification of the association between *Enterococcus* general indicator measured continuously (CCE/100ml) and illness with categories of human-associated chemicals in all beaches

Table B.7(a). **GI illness**

		GI illness	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		1.3% (0.2%, 2.4%)	
Pharmaceuticals			
Not detected	4.7%	Ref	
	6.6%	1.9% (1.0%, 2.7%)	
Detected in all	8.1%	Ref	
	8.3%	0.2% (-2.2%, 2.7%)	**-1.6% (-4.1%, 0.8%)**
Fecal sterols			
Not detected	5.0%	Ref	
	6.5%	1.5% (0.4%, 2.5%)	
Detected in all	6.3%	Ref	
	7.9%	1.5% (0.1%, 3.0%)	**0.1% (-1.5%, 1.6%)**
Household waste			
Not detected	5.5%	Ref	
	7.0%	1.5% (0.2%, 2.9%)	
Detected in all	6.2%	Ref	
	7.3%	1.1% (-0.4%, 2.6%)	**-0.4% (-2.5%, 1.6%)**
Industrial waste			
Not detected	5.9%	Ref	
	7.0%	1.1% (-0.3%, 2.5%)	
Detected in all	5.5%	Ref	
	7.1%	1.6% (0.4%, 2.8%)	**0.5% (-1.0%, 2.1%)**
Runoff			
Not detected	6.2%	Ref	
	7.4%	1.2% (-0.3%, 2.7%)	
Detected in all	5.5%	Ref	
	6.9%	1.4% (0.2%, 2.6%)	**0.2% (-1.5%, 1.9%)**

* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.7(b). **Diarrhea**

		Diarrhea	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		1.1% (0.4%, 1.7%)	
Pharmaceuticals			
Not detected	2.5%	Ref	
	3.9%	1.5% (1.1%, 1.9%)	
Detected in all	5.1%	Ref	
	5.4%	0.3% (-1.5%, 2.2%)	**-1.2% (-3.0%, 0.7%)**
Fecal sterols			
Not detected	2.5%	Ref	
	3.7%	1.2% (0.7%, 1.7%)	
Detected in all	3.8%	Ref	
	5.1%	1.3% (0.4%, 2.3%)	**0.2% (-0.8%, 1.1%)**
Household waste			
Not detected	2.9%	Ref	
	4.1%	1.2% (0.4%, 2.0%)	
Detected in all	3.9%	Ref	
	4.9%	1.0% (0.0%, 2.0%)	**-0.2% (-1.5%, 1.0%)**
Industrial waste			
Not detected	3.3%	Ref	
	NA	0.9% (0.0%, 1.8%)	
Detected in all	3.0%	Ref	
	4.5%	1.5% (0.9%, 2.1%)	**0.6% (-0.3%, 1.5%)**
Runoff			
Not detected	3.1%	Ref	
	4.2%	1.2% (0.4%, 1.9%)	
Detected in all	3.8%	Ref	
	4.8%	1.0% (0.0%, 2.0%)	**-0.2% (-1.3%, 0.9%)**

* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.7(c). **Respiratory illness**

		Respiratory illness	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		-0.1% (-1.9%, 1.7%)	
Pharmaceuticals			
Not detected	4.2%	Ref	
	5.0%	0.8% (-0.3%, 1.9%)	
Detected in all	16.4%	Ref	
	11.1%	-5.3% (-14.8%, 4.1%)	**-6.1% (-15.5%, 3.2%)**
Fecal sterols			
Not detected	6.5%	Ref	
	6.6%	0.1% (-2.0%, 2.3%)	
Detected in all	8.4%	Ref	
	6.2%	-2.2% (-6.3%, 1.9%)	**-2.4% (-6.7%, 2.0%)**
Household waste			
Not detected	4.3%	Ref	
	5.4%	1.1% (0.0%, 2.3%)	
Detected in all	10.2%	Ref	
	7.5%	-2.6% (-7.8%, 2.6%)	**-3.8% (-9.1%, 1.6%)**
Industrial waste			
Not detected	6.3%	Ref	
	6.7%	0.4% (-1.4%, 2.3%)	
Detected in all	8.4%	Ref	
	6.3%	-2.1% (-6.2%, 1.9%)	**-2.5% (-6.7%, 1.6%)**
Runoff			
Not detected	5.8%	Ref	
	6.2%	0.3% (-1.5%, 2.2%)	
Detected in all	6.2%	Ref	
	5.7%	-0.5% (-3.4%, 2.3%)	**-0.9% (-4.1%, 2.3%)**

* Adjusted for beach, mean bathers, sand, rain

Table B.7(d). **Eye ailment**

		Eye Ailment	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		-1.4% (-3.8%, 0.9%)	
Pharmaceuticals			
Not detected	3.9%	Ref	
	3.3%	-0.6% (-2.7%, 1.5%)	
Detected in all	9.0%	Ref	
	5.0%	-4.0% (-11.0%, 3.0%)	**-3.4% (-10.4%, 3.6%)**
Fecal sterols			
Not detected	7.2%	Ref	
	4.4%	-2.8% (-7.8%, 2.1%)	
Detected in all	3.6%	Ref	
	3.1%	-0.6% (-2.5%, 1.4%)	**2.3% (-2.7%, 7.2%)**
Household waste			
Not detected	7.4%	Ref	
	4.5%	-2.9% (-8.2%, 2.4%)	
Detected in all	3.2%	Ref	
	2.7%	-0.5% (-2.2%, 1.2%)	**2.4% (-3.1%, 7.8%)**
Industrial waste			
Not detected	8.9%	Ref	
	4.8%	-4.1% (-9.9%, 1.8%)	
Detected in all	2.9%	Ref	
	2.7%	-0.2% (-1.7%, 1.3%)	**3.9% (-1.8%, 9.5%)**
Runoff			
Not detected	6.9%	Ref	
	4.4%	-2.5% (-6.9%, 1.9%)	
Detected in all	2.6%	Ref	
	2.4%	-0.2% (-1.8%, 1.3%)	**2.3% (-2.4%, 6.9%)**

* Adjusted for beach, mean bathers, sand, rain

Table B.7(e). **Rash**

		Rash	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		0.0% (-1.0%, 1.0%)	
Pharmaceuticals			
Not detected	2.5%	Ref	
	2.4%	0.0% (-1.3%, 1.2%)	
Detected in all	4.0%	Ref	
	3.6%	-0.4% (-2.5%, 1.8%)	**-0.3% (-2.6%, 2.0%)**
Fecal sterols			
Not detected	1.6%	Ref	
	2.0%	0.4% (-0.1%, 0.9%)	
Detected in all	4.1%	Ref	
	3.4%	-0.7% (-3.2%, 1.7%)	**-1.1% (-3.5%, 1.2%)**
Household waste			
Not detected	1.0%	Ref	
	1.7%	0.7% (0.4%, 0.9%)	
Detected in all	6.0%	Ref	
	4.3%	-1.7% (-5.1%, 1.7%)	**-2.4% (-5.8%, 1.1%)**
Industrial waste			
Not detected	1.8%	Ref	
	2.1%	0.2% (-0.6%, 1.0%)	
Detected in all	3.2%	Ref	
	3.2%	-0.1% (-1.6%, 1.5%)	**-0.3% (-1.8%, 1.2%)**
Runoff			
Not detected	2.1%	Ref	
	2.4%	0.3% (-0.6%, 1.2%)	
Detected in all	3.1%	Ref	
	2.8%	-0.4% (-2.1%, 1.4%)	**-0.7% (-2.5%, 1.2%)**

* Adjusted for beach, mean bathers, sand, rain

Table B.7(f). **Earache**

		Earache	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		-0.2% (-1.4%, 0.9%)	
Pharmaceuticals			
Not detected	3.5%	Ref	
	2.3%	-1.2% (-4.0%, 1.5%)	
Detected in all	1.7%	Ref	
	2.0%	0.3% (-0.6%, 1.2%)	**1.5% (-1.1%, 4.2%)**
Fecal sterols			
Not detected	2.4%	Ref	
	2.1%	-0.3% (-1.9%, 1.4%)	
Detected in all	2.2%	Ref	
	1.9%	-0.3% (-1.8%, 1.2%)	**0.0% (-1.9%, 1.8%)**
Household waste			
Not detected	2.2%	Ref	
	1.9%	-0.2% (-2.0%, 1.5%)	
Detected in all	2.6%	Ref	
	2.3%	-0.3% (-2.0%, 1.5%)	**0.0% (-2.4%, 2.4%)**
Industrial waste			
Not detected	2.2%	Ref	
	2.1%	-0.2% (-1.7%, 1.3%)	
Detected in all	2.3%	Ref	
	2.0%	-0.3% (-1.8%, 1.1%)	**-0.2% (-1.9%, 1.6%)**
Runoff			
Not detected	2.0%	Ref	
	2.0%	0.0% (-1.2%, 1.1%)	
Detected in all	2.0%	Ref	
	1.7%	-0.3% (-1.8%, 1.3%)	**-0.2% (-2.0%, 1.5%)**

* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.7(g). **Urinary tract infection**

		Urinary Tract Infection	
Chemical (samples)	**Adjusted Risk (%)**	**Adjusted RD (95% CI)***	**Interaction Contrast (95% CI)**
--		-0.4% (-1.6%, 0.9%)	
Pharmaceuticals			
Not detected	3.9%	Ref	
	1.3%	-2.7% (-8.2%, 2.8%)	
Detected in all	0.6%	Ref	
	0.7%	0.1% (-0.5%, 0.7%)	**2.8% (-2.7%, 8.2%)**
Fecal sterols			
Not detected	2.3%	Ref	
	1.2%	-1.1% (-4.9%, 2.7%)	
Detected in all	0.8%	Ref	
	0.7%	0.0% (-0.8%, 0.7%)	**1.1% (-2.6%, 4.7%)**
Household waste			
Not detected	8.8%	Ref	
	2.2%	-6.6% (-19.9%, 6.8%)	
Detected in all	0.1%	Ref	
	0.2%	0.1% (0.0%, 0.2%)	**6.7% (-6.7%, 20.0%)**
Industrial waste			
Not detected	1.8%	Ref	
	1.0%	-0.8% (-3.8%, 2.2%)	
Detected in all	0.9%	Ref	
	0.8%	-0.2% (-1.1%, 0.8%)	**0.6% (-2.3%, 3.6%)**
Runoff			
Not detected	3.4%	Ref	
	1.5%	-1.9% (-6.3%, 2.6%)	
Detected in all	0.2%	Ref	
	0.3%	0.1% (-0.1%, 0.3%)	**2.0% (-2.5%, 6.4%)**

* Adjusted for beach, mean bathers, sand, rain, water temperature

Table B.8(a-g). Frequencies and standardized risk differences (95% CI) for the association between illness and human-associated chemical markers *head immersion* swimmers in all beaches

Table B.8(a). **Gastrointestinal illness**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
GI illness*					
Acetaminophen					
Not detected	475	5786	8.2	8.6	Ref
Detected§	41	375	10.9	8.0	-0.5% (-3.7%, 2.6%)
Beta-sitosterol					
Not detected	485	5817	8.3	8.5	Ref
Detected§	31	344	9.0	9.1	0.6% (-3.7%, 4.9%)
Bisphenol A					
Not detected	431	5185	8.3	8.2	Ref
Detected§	85	976	8.7	10.6	2.4% (-0.5%, 5.2%)
Caffeine					
Not detected	371	4872	7.6	8.4	Ref
Detected§	145	1289	11.2	8.8	0.3% (-2.6%, 3.2%)
Cholesterol					
Not detected	332	4299	7.7	8.2	Ref
Detected§	184	1862	9.9	9.3	1.1% (-1.0%, 3.1%)
DEET					
Not detected	334	4335	7.7	8.7	Ref
Detected§	182	1826	10.0	8.4	-0.3% (-2.7%, 2.1%)
Diethoxyoctylphenol					
Not detected	513	6127	8.4	8.6	Ref
Detected§	3	34	8.8	5.8	-2.8% (-9.5%, 4.0%)
Phenol					
Not detected	345	4554	7.6	8.8	Ref
Detected§	171	1607	10.6	8.1	-0.7% (-3.7%, 2.3%)
Tributyl phosphate					
Not detected	455	5504	8.3	8.6	Ref
Detected§	61	657	9.3	8.3	-0.2% (-2.9%, 2.4%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature
§ Detected in all samples per day

Table B.8(b). **Diarrhea**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
Diarrhea*					
Acetaminophen					
Not detected	300	5786	5.2	5.6	Ref
Detected§	30	375	8.0	5.2	-0.4% (-2.8%, 2.0%)
Beta-sitosterol					
Not detected	306	5817	5.3	5.5	Ref
Detected§	24	344	7.0	6.6	1.1% (-2.6%, 4.8%)
Bisphenol A					
Not detected	268	5185	5.2	5.1	Ref
Detected§	62	976	6.4	8.5	3.4% (0.6%, 6.1%)
Caffeine					
Not detected	231	4872	4.7	5.7	Ref
Detected§	99	1289	7.7	5.1	3.4% (0.6%, 6.1%)
Cholesterol					
Not detected	194	4299	4.5	5.0	Ref
Detected§	136	1862	7.3	6.5	-0.6% (-2.7%, 1.5%)
DEET					
Not detected	196	4335	4.5	5.3	Ref
Detected§	134	1826	7.3	5.9	1.5% (-0.2%, 3.3%)
Diethoxyoctylphenol					
Not detected	329	6127	5.4	5.6	Ref
Detected§	1	34	2.9	1.7	0.6% (-1.5%, 2.7%)
Phenol					
Not detected	197	4554	4.3	5.0	Ref
Detected§	133	1607	8.3	6.6	-3.9% (-7.3%, -0.5%)
Tributyl phosphate					
Not detected	288	5504	5.2	5.5	Ref
Detected§	42	657	6.4	5.8	1.6% (-1.2%, 4.4%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature
§ Detected in all samples per day

Table B.8(c). **Respiratory illness**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
Respiratory illness*					
Acetaminophen					
Not detected	357	5589	6.4	6.3	Ref
Detected§	23	373	6.2	8.0	1.8% (-2.9%, 6.4%)
Beta-sitosterol					
Not detected	360	5620	6.4	6.3	Ref
Detected§	20	342	5.8	7.4	1.1% (-2.5%, 4.7%)
Bisphenol A					
Not detected	336	5007	6.7	6.8	Ref
Detected§	44	955	4.6	4.3	-2.5% (-4.1%, -0.9%)
Caffeine					
Not detected	300	4717	6.4	6.5	Ref
Detected§	80	1245	6.4	5.9	-0.5% (-3.5%, 2.4%)
Cholesterol					
Not detected	291	4139	7.0	7.0	Ref
Detected§	89	1823	4.9	4.9	-2.1% (-3.6%, -0.6%)
DEET					
Not detected	276	4176	6.6	6.5	Ref
Detected§	104	1786	5.8	5.9	-0.7% (-2.6%, 1.3%)
Diethoxyoctylphenol					
Not detected	377	5929	6.4	6.3	Ref
Detected§	3	33	9.1	7.4	1.1% (-7.9%, 10.0%)
Phenol					
Not detected	300	4394	6.8	7.1	Ref
Detected§	80	1568	5.1	4.5	-2.6% (-5.2%, 0.1%)
Tributyl phosphate					
Not detected	328	5320	6.2	6.1	Ref
Detected§	52	642	8.1	8.6	2.5% (-0.5%, 5.5%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain
§ Detected in all samples per day

Table B.8(d). **Eye illness**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
Eye ailment					
Acetaminophen					
Not detected	160	5898	2.7	2.7	Ref
Detected§	9	381	2.4	2.1	-0.6% (-2.8%, 1.6%)
Beta-sitosterol					
Not detected	160	5926	2.7	2.7	Ref
Detected§	9	353	2.5	2.2	-0.5% (-2.3%, 1.3%)
Bisphenol A					
Not detected	142	5278	2.7	2.7	Ref
Detected§	27	1001	2.7	2.7	0.0% (-1.2%, 1.3%)
Caffeine					
Not detected	138	4973	2.8	2.8	Ref
Detected§	31	1306	2.4	2.2	-0.6% (-2.1%, 0.8%)
Cholesterol					
Not detected	116	4381	2.6	2.7	Ref
Detected§	53	1898	2.8	2.7	0.0% (-1.1%, 1.1%)
DEET					
Not detected	121	4423	2.7	2.8	Ref
Detected§	48	1856	2.6	2.3	-0.5% (-1.8%, 0.7%)
Diethoxyoctylphenol					
Not detected	167	6244	2.7	2.7	Ref
Detected§	2	35	5.7	7.0	4.3% (-5.9%, 14.5%)
Phenol					
Not detected	123	4651	2.6	2.7	Ref
Detected§	46	1628	2.8	2.7	0.0% (-1.8%, 1.7%)
Tributyl phosphate					
Not detected	153	5608	2.7	2.7	Ref
Detected§	16	671	2.4	2.4	-0.3% (-1.8%, 1.2%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain
§ Detected in all samples per day

Table B.8(e). **Rash**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
Rash					
Acetaminophen					
Not detected	153	5794	2.6	2.7	Ref
Detected§	15	376	4.0	3.2	0.5% (-1.3%, 2.3%)
Beta-sitosterol					
Not detected	156	5819	2.7	2.7	Ref
Detected§	12	351	3.4	2.6	-0.1% (-1.8%, 1.6%)
Bisphenol A					
Not detected	143	5191	2.8	2.7	Ref
Detected§	25	979	2.6	2.8	0.1% (-1.2%, 1.3%)
Caffeine					
Not detected	129	4887	2.6	2.7	Ref
Detected§	39	1283	3.0	2.9	0.2% (-1.2%, 1.6%)
Cholesterol					
Not detected	114	4295	2.7	2.7	Ref
Detected§	54	1875	2.9	2.7	-0.1% (-1.0%, 0.9%)
DEET					
Not detected	104	4333	2.4	2.5	Ref
Detected§	64	1837	3.5	3.1	0.5% (-0.7%, 1.8%)
Diethoxyoctylphenol					
Not detected	168	6135	2.7	NA	Ref
Detected§	0	35	0.0	NA	NA
Phenol					
Not detected	111	4557	2.4	2.5	Ref
Detected§	57	1613	3.5	3.4	0.9% (-1.3%, 3.0%)
Tributyl phosphate					
Not detected	139	5512	2.5	2.5	Ref
Detected§	29	658	4.4	4.3	1.8% (-0.1%, 3.7%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain
§ Detected in all samples per day

Table B.8(f). **Earache**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
Earache					
Acetaminophen					
Not detected	104	5836	1.8	1.8	Ref
Detected§	11	380	2.9	2.6	5.2% (-1.1%, 2.6%)
Beta-sitosterol					
Not detected	107	5868	1.8	1.8	Ref
Detected§	8	348	2.3	2.6	0.7% (-1.3%, 2.7%)
Bisphenol A					
Not detected	98	5226	1.9	1.9	Ref
Detected§	17	990	1.7	1.8	-0.1% (-1.1%, 0.8%)
Caffeine					
Not detected	89	4930	1.8	1.8	Ref
Detected§	26	1286	2.0	2.3	0.5% (-0.8%, 1.8%)
Cholesterol					
Not detected	81	4337	1.9	1.9	Ref
Detected§	34	1879	1.8	1.9	-0.2% (-1.0%, 0.7%)
DEET					
Not detected	75	4379	1.7	1.8	Ref
Detected§	40	1837	2.2	2.1	0.3% (-0.7%, 1.4%)
Diethoxyoctylphenol					
Not detected	114	6181	1.8	1.9	Ref
Detected§	1	35	2.9	4.6	2.8% (-6.6%, 12.1%)
Phenol					
Not detected	83	4603	1.8	1.9	Ref
Detected§	32	1613	2.0	1.9	0.1% (-1.4%, 1.6%)
Tributyl phosphate					
Not detected	101	5551	1.8	1.9	Ref
Detected§	14	665	2.1	2.1	0.2% (-1.0%, 1.5%)

* NA, not able to estimated. Adjusted for beach, mean bathers, sand, rain, water temperature
§ Detected in all samples per day

Table B.8(g). **Urinary tract infection**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
Urinary tract infection					
Acetaminophen					
Not detected	30	5897	0.5	0.5	Ref
Detected§	4	384	1.0	1.5	1.0% (-1.0%, 3.0%)
Beta-sitosterol					
Not detected	32	5927	0.5	0.5	Ref
Detected§	2	354	0.6	0.7	0.1% (-1.0%, 1.2%)
Bisphenol A					
Not detected	28	5282	0.5	0.5	Ref
Detected§	6	999	0.6	0.7	0.1% (-0.5%, 0.7%)
Caffeine					
Not detected	23	4969	0.5	0.4	Ref
Detected§	11	1312	0.8	1.5	1.0% (-0.3%, 2.4%)
Cholesterol					
Not detected	23	4380	0.5	0.5	Ref
Detected§	11	1901	0.6	0.6	0.1% (-0.4%, 0.6%)
DEET					
Not detected	24	4421	0.5	0.6	Ref
Detected§	10	1860	0.5	0.5	-0.1% (-0.6%, 0.4%)
Diethoxyoctylphenol					
Not detected	34	6246	0.5	NA	Ref
Detected§	0	35	0.0	NA	NA
Phenol					
Not detected	25	4647	0.5	0.5	Ref
Detected§	9	1634	0.6	0.6	0.0% (-0.9%, 0.9%)
Tributyl phosphate					
Not detected	29	5608	0.5	0.5	Ref
Detected§	5	673	0.7	0.7	0.2% (-0.5%, 0.8%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature
§ Detected in all samples per day

Table B.9(a-g). Frequencies and standardized risk differences (95% CI) for the association between illness and human-associated chemical markers among swimmers who *swallowed water* in all beaches

Table B.9(a). **Gastrointestinal illness**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
GI*					
Acetaminophen					
Not detected	140	1350	10.4	10.3	Ref
Detected§	11	90	12.2	13.4	3.1% (-6.7%, 12.9%)
Beta-sitosterol					
Not detected	145	1372	10.6	10.5	Ref
Detected§	6	68	8.8	10.2	-0.3% (-9.2%, 8.5%)
Bisphenol A					
Not detected	122	1214	10.0	10.0	Ref
Detected§	29	226	12.8	13.5	3.5% (-2.2%, 9.2%)
Caffeine					
Not detected	112	1138	9.8	10.5	Ref
Detected§	39	302	12.9	10.4	0.0% (-7.2%, 7.1%)
Cholesterol					
Not detected	108	1055	10.2	10.4	Ref
Detected§	43	385	11.2	10.9	0.5% (-4.2%, 5.2%)
DEET					
Not detected	105	1020	10.3	10.2	Ref
Detected§	46	420	11.0	11.4	1.2% (-4.7%, 7.0%)
Diethoxyoctylphenol					
Not detected	150	1432	10.5	10.5	Ref
Detected§	1	8	12.5	7.5	-3.0% (-18.8%, 12.8%)
Phenol					
Not detected	118	1112	10.6	11.4	Ref
Detected§	33	328	10.1	8.2	-3.3% (-8.5%, 2.0%)
Tributyl phosphate					
Not detected	134	1273	10.5	10.6	Ref
Detected§	17	167	10.2	9.7	-1.0% (-6.4%, 4.4%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature
§ Detected in all samples per day

Table B.9(b). **Diarrhea**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
Diarrhea*					
Acetaminophen					
Not detected	90	1350	6.7	6.5	Ref
Detected§	7	90	7.8	8.8	2.3% (-5.4%, 10.0%)
Beta-sitosterol					
Not detected	95	1372	6.9	6.8	Ref
Detected§	2	68	2.9	3.3	-3.5% (-8.5%, 1.6%)
Bisphenol A					
Not detected	77	1214	6.3	6.2	Ref
Detected§	20	226	8.8	10.0	3.8% (-1.4%, 8.9%)
Caffeine					
Not detected	75	1138	6.6	7.3	Ref
Detected§	22	302	7.3	5.0	-2.3% (-7.0%, 2.5%)
Cholesterol					
Not detected	65	1055	6.2	6.1	Ref
Detected§	32	385	8.3	8.1	2.0% (-2.3%, 6.3%)
DEET					
Not detected	65	1020	6.4	5.9	Ref
Detected§	32	420	7.6	8.8	2.9% (-2.7%, 8.6%)
Diethoxyoctylphenol					
Not detected	97	1432	6.8	NA	Ref
Detected§	0	8	0.0	NA	NA
Phenol					
Not detected	70	1112	6.3	6.1	Ref
Detected§	27	328	8.2	8.4	2.3% (-3.7%, 8.2%)
Tributyl phosphate					
Not detected	88	1273	6.9	6.7	Ref
Detected§	9	167	5.4	6.1	-0.7% (-5.3%, 4.0%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature
§ Detected in all samples per day

Table B.9(c). **Respiratory illness**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
Respiratory illness					
Acetaminophen					
Not detected	117	1291	9.1	8.9	Ref
Detected§	5	89	5.6	6.9	-2.0% (-9.2%, 5.3%)
Beta-sitosterol					
Not detected	117	1311	8.9	8.7	Ref
Detected§	5	69	7.2	9.1	0.4% (-8.8%, 9.6%)
Bisphenol A					
Not detected	107	1158	9.2	9.3	Ref
Detected§	15	222	6.8	6.6	-2.7% (-7.1%, 1.7%)
Caffeine					
Not detected	98	1094	9.0	9.8	Ref
Detected§	24	286	8.4	6.1	-3.7% (-9.6%, 2.3%)
Cholesterol					
Not detected	101	1002	10.1	9.9	Ref
Detected§	21	378	5.6	5.6	-4.4% (-7.7%, -1.1%)
DEET					
Not detected	89	966	9.2	9.3	Ref
Detected§	33	414	8.0	7.6	-1.6% (-6.7%, 3.5%)
Diethoxyoctylphenol					
Not detected	122	1373	8.9	NA	Ref
Detected§	0	7	0.0	NA	NA
Phenol					
Not detected	99	1062	9.3	9.3	Ref
Detected§	23	318	7.2	6.9	-2.4% (-7.7%, 2.9%)
Tributyl phosphate					
Not detected	100	1213	8.2	8.0	Ref
Detected§	22	167	13.2	15.3	7.3% (-1.3%, 15.8%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain
§ Detected in all samples per day

Table B.9(d). **Eye ailment**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
Eye ailment					
Acetaminophen					
Not detected	53	1389	3.8	3.8	Ref
Detected§	3	89	3.4	2.4	-1.4% (-4.8%, 2.0%)
Beta-sitosterol					
Not detected	52	1408	3.7	3.7	Ref
Detected§	4	70	5.7	5.7	2.0% (-4.3%, 8.4%)
Bisphenol A					
Not detected	48	1243	3.9	3.8	Ref
Detected§	8	235	3.4	3.5	-0.3% (-3.1%, 2.4%)
Caffeine					
Not detected	46	1172	3.9	4.1	Ref
Detected§	10	306	3.3	2.7	-1.4% (-4.6%, 1.7%)
Cholesterol					
Not detected	38	1083	3.5	3.5	Ref
Detected§	18	395	4.6	4.5	1.0% (-1.6%, 3.5%)
DEET					
Not detected	40	1047	3.8	4.2	Ref
Detected§	16	431	3.7	2.9	-1.3% (-3.8%, 1.3%)
Diethoxyoctylphenol					
Not detected	55	1470	3.7	3.7	Ref
Detected§	1	8	12.5	16.0	12.3% (-19.3%, 43.9%)
Phenol					
Not detected	39	1148	3.4	3.1	Ref
Detected§	17	330	5.2	7.2	4.1% (-1.9%, 10.1%)
Tributyl phosphate					
Not detected	49	1304	3.8	3.7	Ref
Detected§	7	174	4.0	4.5	0.8% (-2.9%, 4.5%)

NA, not able to estimated. Note estimates influenced by sample sizes<5. * Adjusted for beach, mean bathers, sand, rain

§ Detected in all samples per day

Table B.9(e). **Rash**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
Rash					
Acetaminophen					
Not detected	54	1362	4.0	4.0	Ref
Detected§	5	88	5.7	4.9	0.9% (-4.3%, 6.1%)
Beta-sitosterol					
Not detected	56	1380	4.1	4.1	Ref
Detected§	3	70	4.3	3.4	-0.7% (-4.9%, 3.5%)
Bisphenol A					
Not detected	52	1218	4.3	4.2	Ref
Detected§	7	232	3.0	3.2	-1.1% (-3.8%, 1.6%)
Caffeine					
Not detected	47	1150	4.1	4.2	Ref
Detected§	12	300	4.0	3.3	-0.9% (-4.1%, 2.4%)
Cholesterol					
Not detected	43	1057	4.1	4.1	Ref
Detected§	16	393	4.1	3.9	-0.2% (-2.5%, 2.0%)
DEET					
Not detected	40	1024	3.9	4.3	Ref
Detected§	19	426	4.5	3.6	-0.7% (-3.4%, 2.0%)
Diethoxyoctylphenol					
Not detected	59	1442	4.1	NA	Ref
Detected§	0	8	0.0	NA	NA
Phenol					
Not detected	43	1123	3.8	3.9	Ref
Detected§	16	327	4.9	4.3	0.4% (-3.8%, 4.5%)
Tributyl phosphate					
Not detected	48	1282	3.7	3.7	Ref
Detected§	11	168	6.5	6.6	2.9% (-1.3%, 7.2%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain
§ Detected in all samples per day

Table B.9(f). **Earache**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
Earache					
Acetaminophen					
Not detected	32	1377	2.3	2.6	Ref
Detected§	2	89	2.2	2.6	0.0% (-5.1%, 5.2%)
Beta-sitosterol					
Not detected	30	1397	2.1	2.3	Ref
Detected§	4	69	5.8	33.9	31.6% (-14.0%, 77.2%)
Bisphenol A					
Not detected	30	1232	2.4	2.6	Ref
Detected§	4	234	1.7	2.1	-0.6% (-2.8%, 1.7%)
Caffeine					
Not detected	26	1165	2.2	2.4	Ref
Detected§	8	301	2.7	3.4	1.0% (-2.5%, 4.5%)
Cholesterol					
Not detected	25	1072	2.3	2.6	Ref
Detected§	9	394	2.3	2.4	1.0% (-2.5%, 4.5%)
DEET					
Not detected	24	1036	2.3	2.3	Ref
Detected§	10	430	2.3	3.4	1.1% (-1.8%, 4.0%)
Diethoxyoctylphenol					
Not detected	34	1458	2.3	NA	Ref
Detected§	0	8	0.0	NA	NA
Phenol					
Not detected	25	1137	2.2	2.4	Ref
Detected§	9	329	2.7	3.0	0.6% (-3.3%, 4.5%)
Tributyl phosphate					
Not detected	32	1292	2.5	2.6	Ref
Detected§	2	174	1.1	2.4	-0.2% (-3.8%, 3.4%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature
§ Detected in all samples per day

Table B.9(g). **Urinary tract infection**

	Cases	N	(%)	Adjusted Risk(%)	Adjusted RD (95% CI)
Urinary tract infection					
Acetaminophen					
Not detected	13	1382	0.9	1.0	Ref
Detected§	2	91	2.2	2.8	1.9% (-2.7%, 6.4%)
Beta-sitosterol					
Not detected	15	1403	1.1	NA	Ref
Detected§	0	70	0.0	NA	NA
Bisphenol A					
Not detected	11	1239	0.9	0.9	Ref
Detected§	4	234	1.7	2.5	1.6% (-0.9%, 4.1%)
Caffeine					
Not detected	9	1164	0.8	0.7	Ref
Detected§	6	309	1.9	3.7	3.0% (-1.1%, 7.0%)
Cholesterol					
Not detected	9	1076	0.8	0.8	Ref
Detected§	6	397	1.5	1.8	1.0% (-0.8%, 2.8%)
DEET					
Not detected	11	1040	1.1	1.2	Ref
Detected§	4	433	0.9	0.8	-0.3% (-1.3%, 0.7%)
Diethoxyoctylphenol					
Not detected	15	1465	1.0	NA	Ref
Detected§	0	8	0.0	NA	NA
Phenol					
Not detected	11	1139	1.0	1.0	Ref
Detected§	4	334	1.2	1.1	0.1% (-2.4%, 2.7%)
Tributyl phosphate					
Not detected	13	1299	1.0	1.0	Ref
Detected§	2	174	1.1	1.2	0.2% (-1.3%, 1.7%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature
§ Detected in all samples per day

Table B.10(a-g). Frequencies and standardized risk differences (95% CI) for the association between illness and human-associated chemical markers (detected in ≥1 daily sample vs. none) among body immersion swimmers in all beaches – **Table B.10(a).** GI Illness

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
Gastrointestinal illness					
Acetaminophen					
Not detected	467	5291	8.8	9.0	Ref
Detected§	273	3404	8.0	8.1	-0.9% (-2.4%, 0.6%)
Beta-sitosterol					
Not detected	548	6818	8.0	8.5	Ref
Detected§	192	1877	10.2	9.1	0.6% (-1.6%, 2.8%)
Bisphenol A					
Not detected	341	3408	10.0	8.9	Ref
Detected§	399	5287	7.5	8.5	-0.4% (-2.4%, 1.7%)
Caffeine					
Not detected	261	3319	7.9	8.7	Ref
Detected§	479	5376	8.9	8.7	0.0% (-1.6%, 1.6%)
Cholesterol					
Not detected	331	4282	7.7	8.4	Ref
Detected§	409	4413	9.3	8.9	0.5% (-1.1%, 2.1%)
DEET					
Not detected	297	3713	8.0	8.7	Ref
Detected§	443	4982	8.9	8.6	-0.1% (-1.8%, 1.5%)
Diethoxyoctylphenol					
Not detected	663	7920	8.4	8.7	Ref
Detected§	77	775	9.9	8.7	0.1% (-2.5%, 2.6%)
Phenol					
Not detected	310	4178	7.4	8.4	Ref
Detected§	430	4517	9.5	8.9	0.5% (-1.3%, 2.4%)
Tributyl phosphate					
Not detected	459	5696	8.1	8.6	Ref
Detected§	281	2999	9.4	8.8	0.2% (-1.4%, 1.9%)

* Adjusted for beach, mean bathers, sand, rain, water temperature

§ Detected in 1 or more samples per day

Table B.10(b). Diarrhea

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
Diarrhea					
Acetaminophen					
Not detected	320	5291	6.0	6.3	Ref
Detected§	175	3404	5.1	5.2	-1.1% (-2.3%, 0.1%)
Beta-sitosterol					
Not detected	348	6818	5.1	5.6	Ref
Detected§	147	1877	7.8	6.6	1.0% (-0.8%, 2.9%)
Bisphenol A					
Not detected	248	3408	7.3	6.1	Ref
Detected§	247	5287	4.7	5.6	-0.5% (-2.3%, 1.2%)
Caffeine					
Not detected	183	3319	5.5	6.5	Ref
Detected§	312	5376	5.8	5.5	-1.0% (-2.4%, 0.4%)
Cholesterol					
Not detected	200	4282	4.7	5.4	Ref
Detected§	295	4413	6.7	6.2	0.8% (-0.5%, 2.2%)
DEET					
Not detected	188	3713	5.1	6.0	Ref
Detected§	307	4982	6.2	5.7	-0.3% (-1.7%, 1.1%)
Diethoxyoctylphenol					
Not detected	439	7920	5.5	5.8	Ref
Detected§	56	775	7.2	5.9	0.0% (-2.1%, 2.1%)
Phenol					
Not detected	186	4178	4.5	5.3	Ref
Detected§	309	4517	6.8	6.2	0.9% (-0.5%, 2.4%)
Tributyl phosphate					
Not detected	302	5696	5.3	5.8	Ref
Detected§	193	2999	6.4	5.8	0.0% (-1.3%, 1.3%)

* Adjusted for beach, mean bathers, sand, rain, water temperature

§ Detected in 1 or more samples per day

Table B.10(c). Respiratory illness

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
Respiratory illness					
Acetaminophen					
Not detected	322	5135	6.3	6.2	Ref
Detected§	187	3290	5.7	5.6	-0.6% (-1.8%, 0.6%)
Beta-sitosterol					
Not detected	427	6590	6.5	6.1	Ref
Detected§	82	1835	4.5	5.3	-0.8% (-2.7%, 1.1%)
Bisphenol A					
Not detected	195	3313	5.9	6.1	Ref
Detected§	314	5112	6.1	6.0	-0.1% (-1.8%, 1.5%)
Caffeine					
Not detected	197	3221	6.1	6.4	Ref
Detected§	312	5204	6.0	5.8	-0.7% (-2.0%, 0.6%)
Cholesterol					
Not detected	313	4130	7.6	7.5	Ref
Detected§	196	4295	4.6	4.5	-0.6% (-1.9%, 0.7%)
DEET					
Not detected	254	3597	7.1	6.8	Ref
Detected§	255	4828	5.3	5.4	-1.4% (-2.7%, -0.2%)
Diethoxyoctylphenol					
Not detected	458	7662	6.0	5.9	Ref
Detected§	51	763	6.7	7.2	1.3% (-1.1%, 3.7%)
Phenol					
Not detected	308	4036	7.6	8.0	Ref
Detected§	201	4389	4.6	4.3	-3.7% (-5.2%, -2.2%)
Tributyl phosphate					
Not detected	348	5504	6.3	6.3	Ref
Detected§	161	2921	5.5	5.4	-0.9% (-2.2%, 0.4%)

* Adjusted for beach, mean bathers, sand, rain

§ Detected in all daily samples

Table B.10(d). **Eye ailment**

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
Eye ailment					
Acetaminophen					
Not detected	148	5416	2.7	2.7	Ref
Detected§	98	3472	2.8	2.8	0.0% (-0.8%, 0.9%)
Beta-sitosterol					
Not detected	184	6975	2.6	2.7	Ref
Detected§	62	1913	3.2	3.0	0.3% (-1.0%, 1.6%)
Bisphenol A					
Not detected	107	3469	3.1	3.0	Ref
Detected§	139	5419	2.6	2.6	-0.4% (-1.6%, 0.8%)
Caffeine					
Not detected	105	3387	3.1	3.1	Ref
Detected§	141	5501	2.6	2.5	-0.5% (-1.4%, 0.3%)
Cholesterol					
Not detected	122	4374	2.8	2.9	Ref
Detected§	124	4514	2.7	2.6	-0.3% (-1.2%, 0.6%)
DEET					
Not detected	119	3803	3.1	3.3	Ref
Detected§	127	5085	2.5	2.4	-0.9% (-1.8%, 0.0%)
Diethoxyoctylphenol					
Not detected	221	8092	2.7	2.7	Ref
Detected§	25	796	3.1	2.9	0.2% (-1.3%, 1.7%)
Phenol					
Not detected	116	4280	2.7	2.9	Ref
Detected§	130	4608	2.8	2.7	-0.2% (-1.2%, 0.8%)
Tributyl phosphate					
Not detected	172	5819	3.0	3.0	Ref
Detected§	74	3069	2.4	2.3	-0.7% (-1.6%, 0.1%)

* Adjusted for beach, mean bathers, sand, rain

§ Detected in 1 or more samples per day

Table B.10(e). **Rash**

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
Rash					
Acetaminophen					
Not detected	141	5326	2.6	2.8	Ref
Detected§	93	3407	2.7	2.6	-0.2% (-1.0%, 0.6%)
Beta-sitosterol					
Not detected	166	6845	2.4	2.6	Ref
Detected§	68	1888	3.6	2.9	0.3% (-0.7%, 1.4%)
Bisphenol A					
Not detected	107	3418	3.1	2.7	Ref
Detected§	127	5315	2.4	2.7	-0.1% (-1.0%, 0.9%)
Caffeine					
Not detected	104	3338	3.1	3.2	Ref
Detected§	130	5395	2.4	2.4	-0.8% (-1.5%, 0.0%)
Cholesterol					
Not detected	110	4302	2.6	2.7	Ref
Detected§	124	4431	2.8	2.7	0.0% (-0.8%, 0.7%)
DEET					
Not detected	90	3740	2.4	2.6	Ref
Detected§	144	4993	2.9	2.8	0.2% (-0.6%, 1.0%)
Diethoxyoctylphenol					
Not detected	209	7949	2.6	NA	Ref
Detected§	0	784	0.0	NA	NA
Phenol					
Not detected	104	4206	2.5	2.7	Ref
Detected§	130	4527	2.9	2.6	-0.1% (-1.0%, 0.8%)
Tributyl phosphate					
Not detected	141	5715	2.5	2.6	Ref
Detected§	93	3018	3.1	2.8	0.2% (-0.6%, 1.0%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain
§ Detected in 1 or more samples per day

Table B.10(f). **Earache**

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
Earache					
Acetaminophen					
Not detected	92	5358	1.7	1.7	Ref
Detected§	68	3445	2.0	2.1	0.4% (-0.2%, 1.0%)
Beta-sitosterol					
Not detected	121	6905	1.8	1.7	Ref
Detected§	39	1898	2.1	2.1	0.4% (-0.7%, 1.4%)
Bisphenol A					
Not detected	61	3453	1.8	1.7	Ref
Detected§	99	5350	1.9	1.9	0.1% (-0.8%, 1.1%)
Caffeine					
Not detected	69	3366	2.0	2.1	Ref
Detected§	91	5437	1.7	1.7	-0.4% (-1.1%, 0.3%)
Cholesterol					
Not detected	83	4342	1.9	1.9	Ref
Detected§	77	4461	1.7	1.7	-0.2% (-0.9%, 0.5%)
DEET					
Not detected	67	3773	1.8	1.7	Ref
Detected§	93	5030	1.8	1.9	0.2% (-0.5%, 0.8%)
Diethoxyoctylphenol					
Not detected	143	8014	1.8	1.8	Ref
Detected§	17	789	2.2	2.5	0.7% (-0.6%, 2.1%)
Phenol					
Not detected	83	4247	2.0	2.1	Ref
Detected§	77	4556	1.7	1.6	-0.6% (-1.3%, 0.2%)
Tributyl phosphate					
Not detected	112	5772	1.9	2.0	Ref
Detected§	48	3031	1.6	1.6	-0.4% (-1.0%, 0.2%)

* Adjusted for beach, mean bathers, sand, rain, water temperature

§ Detected in 1 or more samples per day

Table B.10(g). **Urinary tract infection**

	Cases	N	(%)	Adjusted Risk (%)	Adjusted RD (95% CI)
Urinary Tract infection					
Acetaminophen					
Not detected	36	5411	0.7	0.6	Ref
Detected§	21	3474	0.6	0.7	0.0% (-0.4%, 0.4%)
Beta-sitosterol					
Not detected	41	6968	0.6	0.6	Ref
Detected§	16	1917	0.8	0.9	0.3% (-0.5%, 1.2%)
Bisphenol A					
Not detected	25	3472	0.7	0.6	Ref
Detected§	32	5413	0.6	0.7	0.1% (-0.3%, 0.6%)
Caffeine					
Not detected	22	3384	0.7	0.7	Ref
Detected§	35	5501	0.6	0.6	0.0% (-0.4%, 0.4%)
Cholesterol					
Not detected	26	4369	0.6	0.6	Ref
Detected§	31	4516	0.7	0.7	0.2% (-0.3%, 0.6%)
DEET					
Not detected	23	3800	0.6	0.6	Ref
Detected§	34	5085	0.7	0.7	0.1% (-0.4%, 0.5%)
Diethoxyoctylphenol					
Not detected	52	8088	0.6	NA	Ref
Detected§	0	797	0.0	NA	NA
Phenol					
Not detected	25	4271	0.6	0.6	Ref
Detected§	32	4614	0.7	0.7	0.0% (-0.5%, 0.6%)
Tributyl phosphate					
Not detected	36	5816	0.6	0.7	Ref
Detected§	21	3069	0.7	0.6	-0.1% (-0.6%, 0.3%)

NA, not able to estimated.* Adjusted for beach, mean bathers, sand, rain, water temperature
§ Detected in 1 or more samples per day

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