Exposure to human-associated chemical markers of fecal contamination and self-reported illness among swimmers at recreational beaches

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Abstract

Anthropogenic chemicals have been proposed as potential markers of human fecal contamination in recreational water. However, to date, there are no published studies describing their relationships with illness risks. Using a cohort of swimmers at seven U.S. beaches, we examined potential associations between the presence of chemical markers of human fecal pollution and self-reported gastrointestinal (GI) illness, diarrhea, and respiratory illness. Swimmers were surveyed about their beach activities, water exposure, and baseline symptoms on the day of their beach visit, and about any illness experienced 10–12 days later. Risk differences were estimated using model-based standardization and adjusted for the swimmer's age, beach site, sand contact,

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rainfall, and water temperature. Sixty-two chemical markers were analyzed from daily water samples at freshwater and marine beaches. Of those, 20 were found consistently. With the possible exception of bisphenol A and cholesterol, no chemicals were consistently associated with increased risks of illness. These two chemicals were suggestively associated with 2% and 1% increased risks of GI illness and diarrhea in both freshwater and marine beaches. Additional research using the more sensitive analytic methods currently available for a wider suite of analytes is needed to support the use of chemical biomarkers to quantify illness risk and identify fecal pollution sources.

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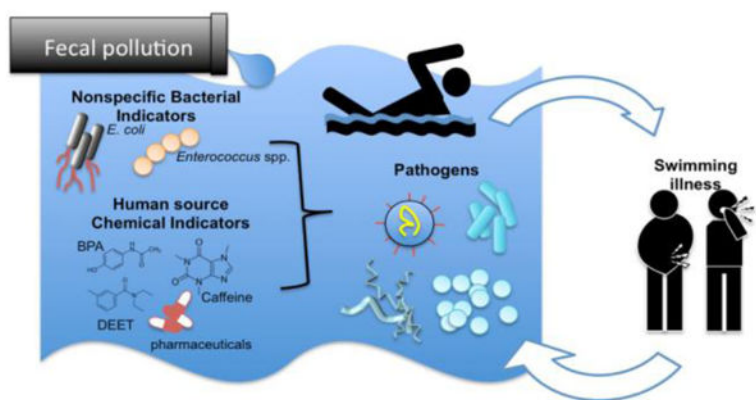
Keywords

chemical markers; fecal indicator bacteria; recreational water quality

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/or animal waste. Fecal waste is a major cause of poor water quality resulting in environmental degradation, economic losses (1,2), and illness risks such as gastrointestinal (GI), respiratory, eye, ear, and skin infections (3–6). In the U.S., *E.coli* and enterococci enumerated by culture are the FIB recommended for detection of fecal contamination in fresh and marine recreational waters (7). Culture-based methods of measuring these traditional indicators have two significant limitations: (1) the methods require 24–48 hours to complete and (2) the indicators cannot be used to differentiate between sources of fecal contamination (8,9). Importantly, the latter is 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 from agricultural land use have surpassed that from point sources, which are subject to discharge permit targets, as the leading cause of water quality problems (10). Accurate and reliable methods of identifying pollution sources would not only provide an indication

of types of pathogens that may be expected and their associated risks, but such information would also help determine potential remediation strategies.

To address the limitations of traditional culturable FIB, rapid methods for identifying fecal contamination sources that target host-specific microbial or chemical markers have been developed (11–17). Much of the source-tracking research has focused on host-specific gene products of microbial markers such as members of the genus *Bacteroidales* or *Bifidobacterium* using rapid methods such as real-time or quantitative polymerase chain reaction (qPCR) (8). Select studies have focused on viruses, such as coliphages, which may be attractive source tracking tools because they are important etiological agents of waterborne disease and are highly host-specific (8,18). Chemical compounds such as caffeine (19–23), pharmaceuticals (23–26), personal care products (19,24), and industrial chemicals (19,23) associated with septic, manure, and wastewater treatment plant effluent as well as fecal sterols and their derivatives, (23,27–30) have also been suggested as anthropogenic markers in sewage. These compounds provide evidence of a potential source because they are associated with human metabolism, activity, or sanitary sewage. Such chemical markers 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 U.S. (19) and river and coastal environments in Japan (24).

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 (i.e. multiple lines of supporting evidence), since no single indicator has been shown to be a perfect predictor of fecal contamination (16,31). Chemicals have the advantage of low detection limits, more rapid sample preparation and analysis times than bacterial culture methods, and can be more temporally or geographically stable (16,32). Furthermore, chemical markers do not have the problem of regrowth in the environment, as do bacteria (33). However, they may degrade (32,34) or persist for some distance downstream of their source (16), and require expensive analytical equipment operated by trained personnel (19). The relationship between chemicals (as an indicator of human-derived fecal pollution) and illnesses caused by waterborne human fecal pollution (e.g. gastroenteritis) is unknown. This lack of understanding limits the utility of chemicals as a potential fecal source marker.

Thus, to determine if there is a link between chemical markers of fecal contamination 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 recreational swimmers, and (2) determine whether chemical markers were able to identify human source when used in combination with conventional fecal indicator *Enterococcus* spp. that were detected by qPCR. 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 contamination.

MATERIALS AND METHODS

Study design and site information

This study used data gathered from the National Epidemiological and Environmental Assessment of Recreational Water (NEEAR) study (35,36), a prospective cohort study that examined associations between recreational water quality and swimming associated illnesses in visitors to freshwater and marine beaches impacted by sewage. Participants were enrolled at four freshwater beaches: Huntington Beach on Lake Erie near Cleveland, Ohio (2003); West Beach on Lake Michigan at Indiana Dunes National Seashore in Portage, Indiana (2003); Silver Beach on Lake Michigan near St. Joseph, Michigan (2004); and Washington Park Beach on Lake Michigan in Michigan City, Indiana (2004); and three temperate marine beaches: Edgewater Beach near Biloxi, Mississippi (2005); Fairhope Beach in Fairhope, Alabama (2007); and Goddard State Park Beach near Warwick, Rhode Island (2007) (Figure 1). Criteria for beach selection included being within 7 miles or less of treated sewage discharge outfalls believed to be a source of fecal pollution at the beach, variability in water quality based on historical records, a swimming season at least 90 days long, and several other factors (35–37). Due to resource and personnel constraints, all sites could not be sampled during the same year; however, data from all sites were designed to be combined since they were all located near treated sewage discharges.

Data collection

Data collection methods have been described previously (35–37). Briefly, all beachgoers were evaluated on weekends and holidays between May and September (2003–2007). An adult from each household provided information about demographics, beach activities, water exposure (extent, time, duration, and location), presence of underlying acute and chronic health conditions, food and drink consumption, pre-existing illnesses within three days of the beach visit, and contact with animals or sick persons in the past 48 h for each household member. Ten-to-twelve days later, participants were interviewed by telephone about illnesses experienced since the beach visit. Consistent with previous reports (35,36,38,39), "GI illness" was defined as any of the following: diarrhea (3 loose stools in a 24-hour period); vomiting; nausea and stomachache; or nausea or stomachache and 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. (40,41). "Respiratory illness" was defined as any two of the following: fever, cough, sore throat, runny nose, or cold symptoms. Respondents who already completed the study in the previous 28 days, were <18 years old, or did not speak English or Spanish were ineligible.

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).

Swimming exposure

Because we were interested in exposure to potential chemical markers from swimming in fecally-contaminated water, the main analysis was restricted to swimmers who reported "body immersion", defined as immersion to the waist or higher (n=10,723). Non-swimmers (i.e. those who reported no water contact, n=7,469) and all participants who reported going in the water but not "body immersion" (n=3,576) were excluded because they represent a more heterogeneous level of water exposure. Fewer participants reported other categories of water exposure (i.e. head immersion, swallowed water); they were considered in sensitivity analyses because of sample size concerns. Definitions were consistent with previous NEEAR studies (35,36). Participants ill within the three days prior to their beach visit were excluded from analysis of the illness outcome related to their baseline symptoms, but were eligible to be included in analyses of other outcomes.

Water sample collection and analysis

The exposures of interest were human-associated chemical markers. A total of 62 chemical markers were detected at least once at one of the beaches (Table S1); however, not all 62 were detected at each beach. Therefore, analyses in this paper were focused on the 20 most consistently detected chemicals (Table 1): (1) ten chemicals detected at all 4 freshwater beaches were included in freshwater analyses (acetaminophen, beta-sitosterol, bisphenol A, caffeine, cholesterol, *n,n*-diethyl-meta-toluamide (DEET), diethoxyoctylphenol, metolachlor, phenol and tributyl phosphate); (2) ten chemicals most frequently detected at all 3 marine beaches were included in marine analyses (acetaminophen, anthraquinone, benzophenone, caffeine, camphor, cotinine, DEET, methyl salicylate, phenol and tributyl phosphate); and (3) eight of these 20 most consistently detected chemicals were included in analyses that combined freshwater and marine beaches (acetaminophen, caffeine, DEET, phenol, tributyl phosphate, beta-sitosterol, bisphenol A, and cholesterol).

Water samples for chemical analysis were collected in washed, safety coated amber glass bottles at 11:00 AM on weekends and holidays between May and September at each beach in the year participants were enrolled. The NEEAR study had two transects parallel to the beach, one at waist-depth (1 m) and one at shin depth (0.3 m). Each transect had three sampling points that were sampled three times per day (8:00 AM, 11:00 AM, and 3:00 PM). In 2003, samples were collected at the three waist-depth sampling points only. In the other years, the two waist-depth and two shin-depth samples closest to the wastewater discharge were sampled. In all years, only the 11:00 AM sample was collected for chemical analysis. At each sampling point, two one-liter bottles of water were collected, one for each analytical method. Samples were collected by opening and filling bottles 6–12" below the surface of the water. Collection personnel were restricted from wearing sunscreen, insect repellant, and other personal care products to minimize contamination of the samples being collected (i.e. prevent false positives in the corresponding analytical results). Additional quality control samples were collected on alternate weekends. For each method, two extra bottles were collected. One was analyzed as a sample duplicate, the other as a laboratory fortified matrix sample (i.e. matrix spike(42,43) (for details see Supplementary Methods). Immediately following collection, all samples were packed in coolers with wet ice during transport to maintain an internal temperature of 4 °C until the following day, when they were repacked

on fresh wet ice and shipped to the U.S. Geological Survey (USGS) National Water Quality Laboratory in Lakewood, Colorado for sample extraction and chemical analysis.

Chemical analysis has been previously described (19). Briefly, different analytical methods were used because of the different physiochemical properties of the chemical compounds. For wastewater compounds, including some pharmaceutical compounds, a whole or filtered-water sample was extracted using continuous liquid-liquid extraction or solid phase extraction and then analyzed using gas chromatography/mass spectrometry (Method 1433) (43). Most other human health 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 (Method 2080) positive-ion electrospray (42,44). Concentrations were reported in µg/L (Table S2).

Enterococcus spp. assessment and analysis

Enterococcus spp. measured by qPCR (reported as calibrator cell equivalents (CCE)/100 ml) was enumerated following water sample collection at 8:00 AM, 11:00 AM, and 3:00 PM and subsequent membrane filtration according to previously published protocols (U. S. EPA Method 1611(45–47)). Analysis was performed at EMSL Analytical, Inc. Laboratory (Westmont, NJ) (data available upon request) (35,36).

Statistical analyses

The primary exposure of interest was the presence/absence of each of 20 chemicals that function as markers of human presence in water samples. The markers were dichotomized due to a high proportion of sample concentrations below the detection limit (Table 1) by giving it a value of '1' if detected in all samples per day, and 0 otherwise. Alternative classifications of this primary exposure were explored in sensitivity analyses.

As a secondary exposure of interest, we grouped all 62 chemicals into five broad categories: pharmaceuticals, fecal sterols/stanols, household waste products, industrial waste products, and chemical contaminants in surface runoff such as persistent organic pollutants and pesticides (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 two pharmaceutical compounds that were detected in all samples collected that day.

Factors plausibly associated with poor water quality and illness were identified and analyzed using directed acyclic graphs (48,49). Final models included: age (0–4, 5–11, 12–19, 20–34, 35), beach site (categorical: Edgewater, Fairhope, Goddard, Huntington, Silver, West, Washington Park), sand contact (binary where 1=digging in or burying body in sand), 17-hour rainfall totals (continuous; (3pm the previous day to 8am the present day),), and water temperature (continuous; (only for analyses with GI illness and diarrhea as outcomes)). Robust standard errors were used to account for dependence of observations within a household (50).

To examine the association between human-associated chemical markers and swimming-associated illness, model-based standardization (51–54) was performed to estimate standardized marginal risks, risk differences (RD), and 95% confidence intervals (95% CI) using the delta method (55) and the total group as the standard. We used logistic regression to estimate predicted probabilities of the outcome for every value of observed confounders, and they were 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. A marginal estimate of the risk difference comparing chemical marker exposure to no exposure was calculated by subtracting the predicted probabilities. We also used stratification to assess modification of these marker-illness effect estimates by water matrix (freshwater vs. marine).

Effect measure modification of the association between *Enterococcus* spp. measured by qPCR Method 1611(45) (CCE/100 ml) and illness was examined to evaluate whether the presence of each of the chemical markers strengthened the association of the general, nonsource-specific *Enterococcus* spp. with illness. In both the primary (chemical marker) and secondary (chemical category) analyses, *Enterococcus* spp. measured by qPCR was treated as the main exposure and the chemical marker/category was the binary modifier. For modification analyses, chemical categories were dichotomized with a value of '1' if any chemicals belonging to that category were detected in all samples per day, and '0' otherwise. The quantitated values of *Enterococcus* spp. measured by qPCR were dichotomized in two ways according to the Environmental Protection Agency's 2012 Recreational Water Quality Criteria recommendations (7): 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). RD modification was estimated with product interactions of *Enterococcus* spp. measured by qPCR and chemical markers and then assessed by an interaction contrast (i.e., difference of risk differences) (IC) (56), which is zero when the joint effects of two factors are simply additive (56).

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

All analyses were completed using Stata version 13 (StataCorp, College Station, TX).

RESULTS

Descriptive characteristics by body immersion status are provided in Table S3.

Distribution of human-associated chemical markers in recreational waters

Chemicals detected by beach.—Up to 478 chemical samples were collected over 128 weekend days throughout the course of a summer: 18 days at Edgewater (2005), Silver (2004), and Washington Park (2004) Beaches; 15 days at Huntington Beach (2003); 19 days at West Beach (2003); and 20 days at Fairhope and Goddard Beaches (2007). At least one of the 62 human-associated chemical markers was found at all beaches. Such chemicals were essentially ubiquitous on a daily basis (127/128 days), but rarely detected in *all* of a day's samples (26/128 days), and were generally present in low concentrations.

The lowest frequency of detection of chemical markers occurred at Silver and Washington Park Beaches. No chemical markers were detected on 12 out of 18 days (67%) at Silver Beach and 13 out of 18 days (72%) at Washington Park Beach. Huntington and Edgewater Beaches had the highest frequency of marker detection, where at least two chemicals were detected in all samples every day chemicals were measured.

Prevalence of chemical markers.—Of the five chemicals detected at all seven beaches, DEET, tributyl phosphate, and caffeine were detected most frequently, in 73%, 53%, and 47% of non-missing samples respectively (Table 1). The proportion of samples with left-censored (below limit of detection) concentrations exceeded 25% for all chemicals, ranging from a low of 27% for DEET to a high of 82% for acetaminophen. Average daily concentrations varied widely by type of chemical and beach (Table 1).

Of the chemicals detected at freshwater beaches, cholesterol was the only chemical detected in more than 50% of samples; the remaining chemicals were detected in less than 40% of samples. The chemicals detected at marine beaches were detected in less than 35% of samples.

The remaining chemicals were detected infrequently and/or at low concentrations. We present findings from analyses of the chemicals most frequently detected across fresh water and marine beaches.

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

In combined analyses of fresh and marine waters, there were 2 excess cases of GI illness and diarrhea per 100 swimmers (that is, 2% increased risk) on days when bisphenol A was present compared to when it was absent (Figure 2; Table S4). Cholesterol was associated with a 1% increased risk of GI illness and diarrhea, while caffeine was inversely associated with these same outcomes. For respiratory illness, none of the chemicals investigated were associated with any excess cases, but bisphenol A, cholesterol, and phenol were inversely associated.

In analyses of fresh water beaches alone, bisphenol A was associated with a 2% increased risk of GI illness and diarrhea (Figure 2; Table S5), while caffeine and tributyl phosphate were associated with 2% increased risks of respiratory illness. In marine beaches, benzophenone was associated with a 5% increase in risk of GI and respiratory illnesses and a 3% increased risk of diarrhea (Figure 2; Table S6). Methyl salicylate was associated with 5% and 4% increased risks of GI illness and diarrhea, respectively, and phenol was

associated with 2% and 4% increased risks of GI illness and diarrhea, respectively (Table S6). Caffeine, cotinine, and anthraquinone were the inverse associations of greatest magnitude for all three outcomes.

Modification of Illness-Enterococcus spp. as measured by qPCR association with presence/absence of chemical markers

Using the illness rate of 36/1000 swimmers, DEET was a potential modifier. On days when DEET was present, the difference in risk of respiratory illness between swimmers exposed to high vs. low *Enterococcus* spp. measured by qPCR was negligible (RD=0.2%; −2.5%, 2.9%); when DEET was absent, the risk difference was inverse (RD=−4.1; −6.9%, −1.2%). Thus, exposure to DEET modified the risk difference for respiratory illness by 4.3% (IC=4.3%; 0.4%, 8.2%) (Figure 3; Table S7). None of the other chemicals were modifiers for GI illness or diarrhea. Using an illness rate of 32/1000 swimmers, tributyl phosphate was a potential modifier of the risk difference with *Enterococcus* spp. measured by qPCR and GI illness (IC=4.3%; −0.2%, 8.7%) and diarrhea (IC=3.7%; −0.2%, 7.7) (Table S8).

Results for modification with *Enterococcus* spp. measured by qPCR assessed continuously as a daily average concentration (CCE/100ml) are shown in Table S9. As shown previously by Wade et al. (2008, 2010) (35,36), we see an increased risk of GI illness and diarrhea with each 1-log_{10} increase in daily average *Enterococcus* spp. measured by qPCR (CCE per 100 ml) (RD=1.3% (0.4%, 2.2%) and RD=1.1% (0.5%, 1.6%), respectively). However, no individual chemical showed strong or consistent modification of the association between *Enterococcus* spp. measured by qPCR and the outcomes. Interaction contrast estimates were imprecise, particularly for chemicals that were infrequently detected (e.g., acetaminophen).

Illness risk associated with categories of human-associated chemical markers

Across all beaches, we observed little evidence to suggest an association between chemical categories and illness. Exposure to a greater number of chemicals in a given category did not result in increased risk of the outcomes studied, suggesting the lack of a dose-response relationship. Trends were similar when stratified by freshwater and marine beaches. In addition, RD estimates for the association between *Enterococcus* spp. measured by qPCR and illness were similar among participants exposed and unexposed to chemical categories. This was true for *Enterococcus* spp. measured by qPCR assessed dichotomously at less than and greater than 470 CCE/100 ml and continuously.

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 and among those who swallowed water. Estimates for both were consistent with what was found for body immersion swimmers, but less precise (Tables S10–11).

Exploration of a more sensitive categorization of exposure showed that RD estimates were moderately affected by choice of dichotomization category (Table S12). Similar to the primary analysis, most RD estimates crossed the null and had narrow 95% CIs. Unlike the

primary analysis, we observed no suggestion of positive association between chemical markers and illness. We did observe several inverse associations. When acetaminophen was present, it was associated with an overall 1% lower risk of GI illness, diarrhea, and respiratory illness compared to when it was absent. Similarly, cholesterol, DEET, phenol, and tributyl phosphate were associated with 1–3% lower risks of respiratory illness in fresh water and marine beaches.

DISCUSSION

We analyzed possible associations between swimmer exposure to a select group of anthropogenic chemical markers as indicators of human fecal contamination and incident swimming-associated, microbial-caused illnesses, in a well-characterized cohort of visitors to U.S. beaches. Our findings suggest that the presence of human-associated chemical markers *may* be associated with illness, but, with a few exceptions, we did not observe consistent increased risks across fresh and marine beaches. Bisphenol A and cholesterol were associated with increased GI illness and diarrhea in both fresh water and marine beaches; caffeine and tributyl phosphate were associated with increased respiratory illness in fresh water beaches; and benzophenone-2, methyl salicylate, and phenol were associated with increased risk of all three outcomes in marine beaches. However, several implausible, inverse associations were observed as well, which indicate the positive associations we observed may also be due to chance alone.

To the best of our knowledge, this paper is the first to investigate the health risks from pathogen exposure associated with chemical markers of human fecal pollution. This research question remains an important public health issue, however, because correlating fecal indicator concentration to health risks is a key qualification for the adoption of any microbe or chemical as a fecal marker. Despite the gap between the collection and analysis of data for the NEEAR studies and this reporting of findings, there is still a recognized need for alternative fecal indicators that (1) 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) can be rapidly assessed so that beach advisory and closing decisions can be made in real-time (15,16,19,57,58). While a wide range of chemicals specific to human wastewater have been investigated for potential differentiation of fecal sources in aquatic environments (12,19,24,26,29,58–60), the relationship of these chemical compounds to the incidence of illness has not been determined in the intervening years. In this study, one of the most promising chemical markers in the literature – caffeine – was detected at all 7 beaches. Though detected in 47% of samples, the concentrations detected were low and did not show a positive association with risk of any measured health illness. Bisphenol A, an industrial wastewater compound used in the manufacture of polycarbonate resins and a component of paper receipts, and cholesterol, a plant and animal sterol, showed suggestive positive associations with enteric illnesses, which are the illnesses most commonly associated with swimming in fecally-contaminated water (3,35–37,61,62).

In our study, several chemical markers showed small inverse associations with illness, including caffeine and cholesterol. While the magnitudes of the inverse associations were

small (~2%), the implications of these findings, if any, are unclear. Any potential hypotheses are complicated by the fact that chemical compounds in this study serve as a proxy for human fecal contamination – either for human metabolism, human activity, or sanitary sewage at the beach sites. Because of this, and the potential for our findings to be due to random chance, we were not able to offer hypotheses for the inverse relationships.

Similarly, the significance of the finding that DEET was a modifier of the association between binary *Enterococcus* and respiratory illness is also unclear; the baseline risk of illness is null in the presence of DEET, and inverse in the absence of DEET so while DEET may act as modifier, the risks it modifies may not be significant enough to act on. In addition, given that modification by DEET 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 U.S. Environmental Protection Agency for determining fecal contamination that result in illness (7). It may also be that the recreational swimmers themselves could have contributed caffeine and DEET contamination to the water even though we took precaution with the sampling team.

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 (16,26) for a review). Bisphenol A, cholesterol, caffeine, DEET, benzophenone, 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 U.S., (19) due to being abundant and present in sufficient concentration at the time the NEEAR study was conducted. In fact, chemical markers investigated in this study included 33 of the 35 compounds suggested as potential indicators by Glassmeyer et al. 2005 (19). The finding that most chemical markers we investigated were not associated with illness is not unexpected, given that chemicals specific to human waste streams can occur at low concentrations and are further reduced once wastewater enters environmental waters through the combined actions of dilution, hydrolysis, sedimentation, and other factors (16). This was true in our study, where, although human-associated chemical markers were detected in at least one sample almost every day samples were collected, chemical concentrations were low. For this reason and others, it is unlikely that human-associated chemical compounds will replace microbial source tracking markers in determining the source of fecal contamination using the methods in this paper. In practice, chemical markers may 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 or an additional line of evidence about source-specificity than any one indicator (15,16,32,33,58). Each indicator has varying patterns of fate, transport, survival, and persistence that together may yield greater confidence in an assessment of water quality source.

Our ability to make inferences was limited by the fact that a high proportion of chemical samples was left-censored (i.e. below the limit of detection) and could not be analyzed

quantitatively. While we did explore quantitative categorizations of exposure, ultimately the low frequencies of detection necessitated the decision to dichotomize. 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. 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/day. The choice of a dichotomization cut-point moderately affected the estimation of RD estimates by affecting the proportion of cases with chemical exposure. When using the more sensitive categorization, the proportion of cases with chemical exposure increased substantially in some cases, as for acetaminophen and beta-sitosterol. This issue was likely also exacerbated because chemicals were not present in high levels; thus, the results may reflect residual or unmeasured confounding.

Ideally the amount of non-detections 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. One way to do this is to explore other beach sites in the U.S. where human fecal pollution is believed to be the dominant source of water pollution with a broader range of water qualities. We expect that chemical concentrations below detection limits may be a common challenge of future studies because it has been cited as one reason why chemicals might be best used in combination with microbial indicators as part of a source tracking toolbox (16). In addition, in studies that compare the abundance of chemicals upstream of treatment plants, at treatment plants, and in treated/untreated wastewater effluent or surface waters, the chemical concentrations follow expected trends of declining after reaching maximum concentrations in effluent samples (e.g. (19,26)). Thus, while the beaches in our study may not be representative of all freshwater and marine coastal beaches in the U.S., other U.S. beaches may share similar challenges.

A second way to address quantitative limitations of this study is to use similar, but improved methods for water sample collection and chemical analysis. Analytical methods used in this study were the first generation of "contaminants of emerging concern" methods developed by USGS, and were intended for screening purposes. Since then, USGS has refined these methods; for example Cahill et al., 2004 (42), which was used to determine 22 pharmaceuticals, has been replaced by Furlong et al. 2014 (63), which is used to determine 110 pharmaceuticals with equal or lower reporting levels. Contemporary analytical methods have greatly expanded numbers of chemicals analyzed with substantially lower reporting levels, which should decrease the amount of left-censored data. Lastly, we acknowledge that the landscape of chemical markers has evolved and expanded extensively since this study was conducted. The chemicals available at the time of our study, which we used for our experimental design, may not have been best suited to our objectives by today's standards. For example, our chemical list did not include artificial sweeteners (e.g. sucralose, acesulfame) that are increasingly attractive as source-tracking markers because they do not readily degrade in the environment or after wastewater treatment (64). We suspect that studies conducted using these newer methods and chemicals would have substantially better

chances of demonstrating the potential for correlation between chemical markers and microbial beach water quality.

The strengths of this study include the size of the cohort, inclusion of both freshwater and marine beach sites, and systematic collection and analysis of over 60 chemical compounds using then-sensitive quantitative methods. The study design allowed us to measure water quality over a wide range of study days and water depths, so we were able to capture varying water quality conditions over the summer months. Additionally, 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. We relied on broadly-defined, self-reported health symptoms as outcomes 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. While it is possible that our outcomes may also have been affected by recall bias, it is unlikely that recall would be differential by varying levels of water quality or chemical exposure.

Overall, the presence of human associated chemical markers in recreational waters may be associated with GI illness among swimmers, but with the exception of two markers, we did not observe consistent associations across fresh and marine beaches. In addition, no markers plausibly modified associations with illness compared to general, non-source specific *Enterococcus* indicators already in use at beach sites. Our findings may have been influenced by low/no abundance of chemical markers and were limited to the target chemicals and analytic tools used at the time of the study. Human-associated markers may also better characterize risk at sites without a known impact from sewage, sites impacted by runoff, or a broader range of fecal contamination. Additional research is needed to support the use of chemical biomarkers to identify sources contributing to fecal pollution of recreational water.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

CI   Confidence interval

FIB	Fecal indicator bacteria
CCE	Calibrator cell equivalents
DEET	*n,n*-diethyl-meta-toluamide
GI	Gastrointestinal
IC	Interaction contrast
IRB	Institutional review board
NEEAR	National epidemiological and environmental assessment of recreational water
QPCR	Quantitative polymerase chain reaction
RD	Risk difference

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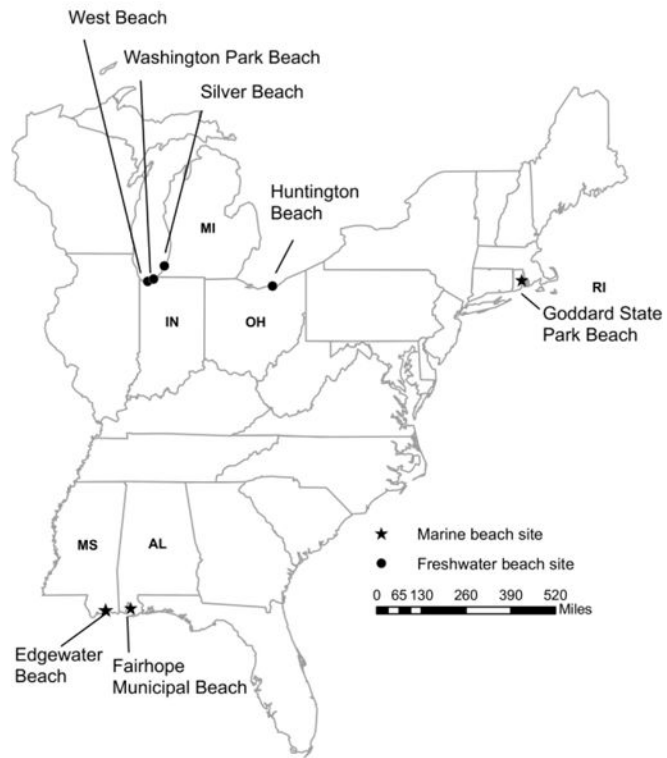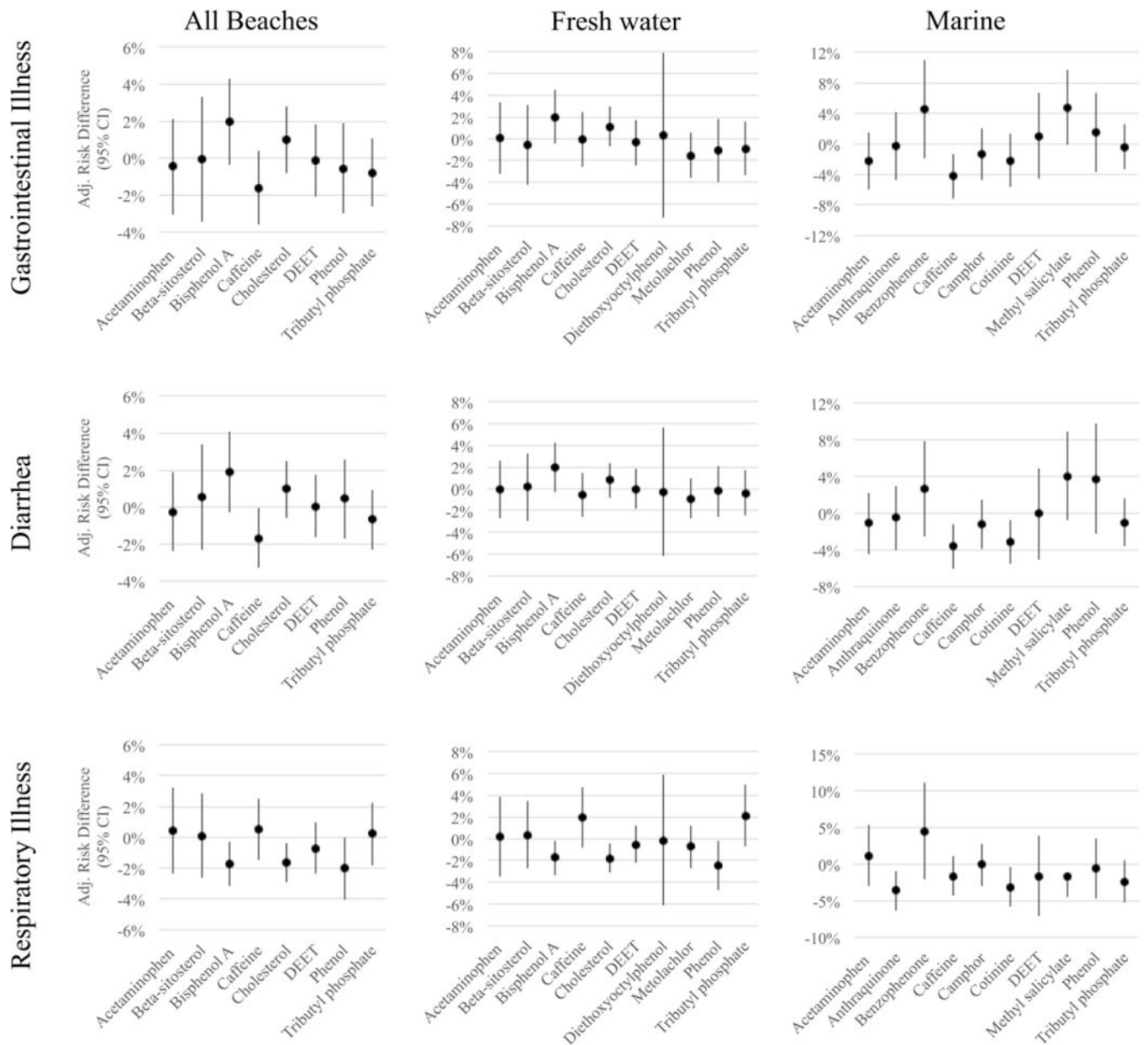
Figure 1.
Freshwater and marine beach sites

Figure 2.
Standardized risk differences (95% CI) for the association between illness and human-associated chemical markers (detected in all daily samples vs. none) among body immersion swimmers in all beaches, freshwater beaches, and marine beaches. CI, confidence interval; DEET, *n,n*-diethyl-meta-toluamide.
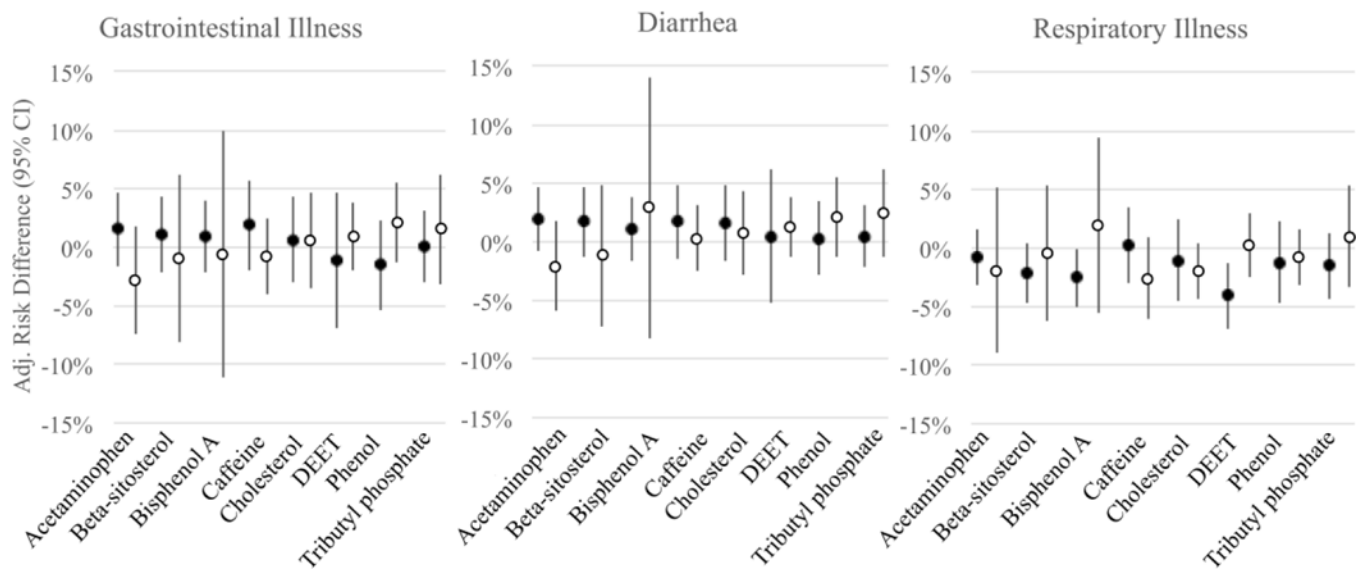
Figure 3.
Association between illness and *Enterococcus* spp. by qPCR modified by chemical markers (detected in all daily samples(○) vs. < all (●)among body immersion swimmers in all beaches. *Enterococcus* spp. dichotomized by qPCR Method 1611   and < EPA guidelines (geometric mean of 470 CCE/100ml for an illness rate of 36/1000). CCE, calibrator cell equivalents; CI, confidence interval; DEET, *n,n*-diethyl-meta-toluamide; qPCR, quantitative polymerase chain reaction.

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Table 1.

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

Chemical	CAS Number	Chemical Group	N samples collected at beach	Chemical samples detected			Samples missing N (%)	Detection Limit [c]	Reporting Limit [c,d]
				N (%)	Min	Max			
Chemicals detected at all 7 beaches									
Acetaminophen [a]	103–90-2	Pharmaceutical	478	86 (18)	0.0005	0.5	3 (1)	0.0086	0.036
Caffeine [a]	58–08-2	Pharmaceutical	478	224 (47)	0.0004	0.3	3 (1)	0.014	0.016
DEET [b]	134–62-3	Household waste	478	335 (73)	0.004	20	20 (4)	0.14	0.5
Phenol [b]	108–95-2	Industrial waste	478	163 (36)	0.08	3	21 (4)	0.11	0.5
Tributyl phosphate [b]	126–73-8	Household waste	478	241 (53)	0.004	0.3	20 (4)	0.1	0.5
Additional chemicals detected at 4 freshwater beaches (Huntington, Silver, Washington Park, West)									
Beta sitosterol [b]	83–46-5	Fecal sterol/stanol	246	37 (16)	0.8	2	20 (8)	2	2
Bisphenol A [b]	80–05-7	Industrial waste	246	81 (36)	0.06	0.7	20 (8)	1	1
Cholesterol [b]	57–88-5	Fecal sterol/stanol	246	116 (51)	0.7	20	20 (8)	0.71	1.5
Diethoxyoctylphenol [b]	N/A	Household waste	246	15 (7)	0.08	0.2	20 (8)	0.37	1
Metolachlor [b]	51218–45-2	Runoff product	246	86 (38)	0.02	0.5	20 (8)	0.08	0.5
Additional chemicals detected at 3 marine beaches (Edgewater, Fairhope, Goddard)									
Anthraquinone [b]	84–65-1	Runoff product	232	74 (32)	0.008	0.09	0 (0)	0.11	0.5
Benzophenone [b]	119–61-9	Household waste	232	51 (22)	0.007	0.1	0 (0)	0.12	0.5
Camphor [b]	76–22-2	Household waste	232	66 (28)	0.006	0.08	0 (0)	0.09	0.5
Cotinine [a]	486–56-6	Pharmaceutical	232	53 (23)	0.002	0.02	0 (0)	0.023	0.023
Diphenhydramine [a]	58–73-1	Pharmaceutical	232	29 (13)	0.0007	0.06	0 (0)	N/A	0.015
Methyl-salicylate [b]	119–36-8	Household waste	232	54 (23)	0.005	0.02	0 (0)	0.08	0.5
Tri-2-chloroethyl phosphate [b]	115–96-8	Household waste	232	8 (3)	0.01	0.04	0 (0)	0.08	0.5
Tri-dichloroisopropyl phosphate [b]	13674–87-8	Household waste	232	29 (13)	0.01	0.09	8 (3)	0.08	0.5

Chemical	CAS Number	Chemical Group	N samples collected at beach	Chemical samples detected			Samples missing N (%)	Detection Limit[c]	Reporting Limit[c,d]
				N (%)	Min	Max			
Triclosan [b]	3380–34-5	Household waste	232	39 (17)	0.02	0.4	0 (0)	0.48	1
Triphenyl phosphate [b]	115–86-6	Industrial waste	232	28 (12)	0.006	0.07	0 (0)	0.06	0.1

DEET, *n,n*-diethyl-meta-toluamide; N/A, not available. All samples collected at 11:00 AM.

[a] Analysis method: liquid chromatography/mass spectrometry.

[b] Analysis method: gas chromatography/ mass spectrometry.

[c] Method detection limits from Zaugg et al. 2002 (43) and Cahill et al. 2004 (42).

[d] Reporting limits from Zaugg et al. 2002 (43) and Glassmeyer et al. 2005 (19). See Childress et al.1999 (65) for further information.