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Ab Initio and in Situ Comparison of Caffeine, Triclosan, and Triclocarban as Indicators of Sewage-Derived Microbes in Surface Waters

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Three organic wastewater compounds (OWCs) were evaluated in theory and practice for their potential to trace sewage-derived microbial contaminants in surface waters. The underlying hypothesis was that hydrophobic OWCs outperform caffeine as a chemical tracer, due to their sorptive association with suspended microorganisms representing particulate organic carbon (POC). Modeling from first principles (ab initio) of OWC sorption to POC under environmental conditions suggested an increasing predictive power: caffeine (0.2% sorbed) < triclosan (9–60%; pH 6–9) < triclocarban (76%). Empirical evidence was obtained via analysis of surface water from three watersheds in a rural-to-urban gradient in Baltimore, MD. Mass spectrometric OWC detections were correlated to microbial plate counts for 40 monitoring sites along 14 streams, including multiple chronic sewage release sites and the local wastewater treatment plant. Consistent with ab initio calculations, correlation analyses of 104 observations for fecal coliforms, enterococci, and *Escherichia coli* in natural surface waters showed that the particle-active antimicrobials triclosan and triclocarban (R^2 range, 0.45–0.55) were indeed superior to caffeine (0.16–0.37) for tracking of microbial indicators. It is concluded that chemical monitoring of microbial risks is more effective when using hydrophobic OWCs such as triclosan and triclocarban in place of, or in conjunction with, the traditional marker caffeine.

Introduction

Leakage of wastewater to surface waters poses a serious threat to both public and ecological health. It is therefore desirable to find rapid, sensitive, and specific markers which can aid in the detection and localization of sewerage leaks for subsequent intervention and mitigation. Potential markers of wastewater leakage to surface water can be classified as microbial, biomolecular, or chemical. Bacteria, coliphages, mammalian viruses, and protozoa typically are enumerated using culturing techniques, including selective plate counts (*1–3*), or biomolecular techniques that target nucleic acids with the polymerase chain reaction or proteins with enzymatic and immunological assays (*3–5*). Organic wastewater compounds (OWCs), including fecal steroids, caffeine, pharmaceuticals, and antimicrobial consumer product additives, are chemical markers that can be tracked by gas or liquid chromatography/mass spectrometry and tandem mass spectrometry (*2, 6, 7*).

Chemical markers offer certain advantages over biological indicators, a major one being the relatively shorter analysis time. Microbial assays relying on culturing techniques typically take from 18 to 96 h (*8, 9*), whereas biomolecular and chemical analyses can be completed in hours or even minutes (*10, 11*). Targeting chemical markers is beneficial since many microbial indicator organisms extant in raw sewage also are common to wildlife and sources other than wastewater (*4, 12*). In addition, chemical markers consistently occur at the highest levels at the source and at relatively lower concentrations in receiving streams (*13, 14*), whereas microbial indicators potentially can multiply in surface waters or become temporally inactive, i.e., viable but nonculturable (*15*). Due to the large number of microbial pathogens contained in sewage and their varying culture conditions and requirements, microbial monitoring typically targets indicator organisms such as fecal coliforms, enterococci, and *Escherichia coli* (*E. coli*) (*9*).

Among the many OWCs employed for source tracking, caffeine is one of the most frequently used chemical indicators. Caffeine typically occurs in sewage at levels of 20–300 μg/L (*16*), and its occurrence in surface waters was observed to correlate with the presence and abundance of various microbial contaminants (*17*). Although the antimicrobial compounds triclosan and triclocarban are among the top 10 OWCs with respect to both occurrence frequency and concentration (*6, 18*), their value for microbial source tracking has not yet been explored in great detail.

When choosing a chemical marker of sewage contamination in surface waters, it would be desirable to find one that associates and migrates selectively with microorganisms suspended in the water column. OWCs exhibiting significant sorption to organic particles, as evidenced by a high octanol–water partitioning coefficient (K_{OW}) and organic carbon partitioning coefficient (K_{OC}), therefore would be expected to outperform other potential markers lacking such preferential partitioning behavior. To explore this hypothesis, we evaluated three of the 10 most common OWCs (*6, 18*), i.e., caffeine, triclosan, and triclocarban, for their ability to indicate microbial contaminants in natural waters.

Experimental Section

Chemicals. Custom synthesis of triclocarban (TCC; 3,4,4′-trichlorocarbanilide; CAS No. 101-20-2) uniformly labeled with carbon-13 in the *p*-chlorophenyl ring was performed by Cambridge Isotope Laboratories Inc. (Andover, MA), which also kindly provided deuterated TCC (TCC-d_7; 99%) as a gift. Ciba Specialty Chemicals (Basel, Switzerland) provided as a gift triclosan (TCS; 5-chloro-2-[2,4-dichlorophenoxy]phenol; CAS No 3380-34-5) uniformly labeled with carbon-13 in the 2,4-dichlorophenoxy ring ($^{13}C_6$-TCS; 98.7%). Anhydrous caffeine (99%; CAS No. 58-08-2) was purchased from Fluka Chemie GmbH (Buchs, Switzerland). Caffeine labeled with

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either one or three carbon-13 containing methyl groups (3-methyl-$^{13}C_3$ moiety [99.3 at. %] and 1,3,7-trimethyl-$^{13}C_3$moiety [99 at. %], respectively) was obtained from CDN Isotopes (Pointe-Claire, Quebec, Canada) or Isotech (Miamisburg, OH), respectively. Solvents of HPLC grade or purer were purchased from Fisher Scientific (Pittsburgh, PA). All other chemicals, including TCC and TCS, were obtained at the highest purity available from Sigma-Aldrich (Milwaukee, WI).

Sampling. Water samples were collected in triplicate using two disposable, precleaned 1 L high-density polyethylene sample bottles for chemical analyses, and one autoclaved reusable polypropylene 1 L bottle for bacteriological assays. Trip blanks consisted of 1 L bottles filled with reagent water (18.2 MΩ resistance) obtained from a Nanopure Diamond UV ultrafiltration water system (Barnstead; Dubuque, IA). All stream samples were collected between October of 2003 and December of 2004. In seven sampling campaigns, river water was taken at various distances up- and downstream from known and suspected sources of sewage contamination along 14 streams in three watersheds straddling Baltimore City and Baltimore County, MD. Sampling sites were dispersed among the following streams in each watershed: in Gwynns Falls watershed (GF), Gwynns Falls, Gwynns Run, Dead Run, and Maiden's Choice Run; in Jones Falls watershed (JF), Jones Falls, Stony Run, and Western Run; in Herring Run watershed (HR), Armistead Creek, Biddison Run, Chinquapin Run, Herring Run, Moores Run, Tiffany Run, and the West Branch of Herring Run. Sites in the GF watershed extend from agricultural, to suburban, to urban/industrial. Sites in the JF and HR watersheds extend from suburban to urban/industrial. Grab samples of wastewater influent also were collected in December of 2005 at the local wastewater treatment plant (WWTP), Baltimore, MD. Upon arrival in the laboratory, samples for chemical analysis were fortified with surrogate standards and frozen at –20 °C until they could be analyzed by mass spectrometry. Surrogate standards ($^{13}C_6$-TCC, $^{13}C_6$-TCS, and either $^{13}C_3$-caffeine or $^{13}C_1$-caffeine) were used as described elsewhere (*19, 20*).

Sample Processing. Target OWCs in natural water samples were determined in both the dissolved and adsorbed phase via solid-phase extraction of the aqueous phase and concurrent organic solvent liquid extraction of the pellet obtained by centrifugation (2000*g* for 20 min), using protocols described in detail elsewhere (*19, 20*). Results are presented as the sum of the aqueous and solid-phase concentrations.

Chemical Analyses. All samples were analyzed for triclocarban and triclosan using liquid chromatography negative electrospray ionization quadrupole mass spectrometry (LC-ESI-MS) and the isotope dilution method as described previously (*18*). Caffeine was tracked in positive ionization mode using polarity switching. The typical retention time for caffeine was 4.0 min, with characteristic mass-to-charge ratios of m/z 195, 196, and 198 for nonlabeled, $^{13}C_1$-, and $^{13}C_3$-labeled caffeine, respectively. To attain enhanced detection limits, samples from the Herring Run watershed were also analyzed for triclocarban by LC triple quadrupole tandem mass spectrometry as described in detail elsewhere (*19*). Nondetect measurements were expressed as half of the limit of detection (LOD) for all mass spectrometric determinations regardless of the instrument used. Quality assurance and quality control protocols for triclosan and triclocarban have been reported previously (*18, 19*). Caffeine was recovered from spiked performance samples at an average recovery rate of 92 ± 21% over a concentration range of 5−150 μg/L. Method and trip blanks carried along in each analytical batch consistently yielded nondetect concentrations.

Bacteriological Assays. Bacteria were enumerated using standard membrane filtration methods. Enterococci and *E. coli* were assayed using U.S. EPA Methods 1600 (*21*) and 1103 (*22*), respectively. Fecal coliforms were enumerated using Standard Method SM 9222 D (*8*).

Partitioning Calculations. Measured and predicted values for the physicochemical characteristics of the three chemicals and the 14 sampled streams were used to predict the partitioning behavior of each chemical under environmentally relevant conditions. Values for the acid ionization constant (pK_A), the pH-dependent *n*-octanol–water distribution ratio (D_{OW}), pH-dependent organic carbon partitioning coefficient (D_{OC}), and the solubility in water of each compound were extracted from the literature or predicted (Supporting Information; Table S1). Organic carbon and pH dependence of partitioning isotherms for three potential chemical markers of sewage leakage to surface water were calculated using eq 1,

$$f_{\text{dissolved}} = \frac{V_W}{V_W + K_D M_S} \tag{1}$$

where $f_{\text{dissolved}}$ is the mass fraction in the aqueous phase, V_w is the volume of water, K_D is the solid-water distribution coefficient, and M_S is the mass of suspended solids. K_D can be approximated as the product of D_{OC} and f_{OC}, where D_{OC} is the pH-dependent organic carbon partitioning coefficient described above, and f_{OC} is the fraction of M_S composed of organic carbon. The product of f_{OC} and M_S can be defined as M_{POC}, the mass of suspended particulate organic carbon, to yield eq 2 (*23*):

$$f_{\text{dissolved}} = \frac{V_W}{V_W + D_{oc} M_{POC}} \tag{2}$$

Data Analysis. Histogram analysis, before and after log transformation, was performed on each set of analytical data. From this analysis it was determined that log-normal distribution best described the data. The chemical and biological data were log-transformed and least-squares regression analyses were performed to calculate coefficients of determination (R^2) for 12 pairwise comparisons of chemical and biological indictor data. A total of four indicator/groups (caffeine, triclosan, triclocarban, and the sum of the latter two) were evaluated against biological data for three bacterial indicators (fecal coliforms, enterococci, and *E. coli*). Correlation analyses were performed with and without inclusion of nondetect values to ensure that similar outcomes result regardless of the data processing strategy chosen.

Results

Ab Initio Analysis. Physicochemical characteristics of the three chemical indicators were used to predict the partitioning of each chemical in the range of environmental conditions extant in the 14 urban streams of the three Baltimore watersheds investigated here (Figure 1). Values for the pK_A, D_{OC}, D_{OW}, and the water solubility of each compound were taken from the literature or calculated (Supporting Information; Table S1). At ≥3.9 g/L, caffeine was found to have a water solubility limit 1000-fold greater than those of the more hydrophobic antimicrobials triclosan and triclocarban. Caffeine and triclocarban were predicted to ionize only in environmentally atypical conditions of pH < 1 and > 12. In contrast, due to its pK_A value of 7.8, triclosan was predicted to traverse the full range of ionization extremes from complete protonation (D_{OC} of 15500 at pH 5.4) to almost full deprotonation (D_{OC} of 600 at pH 9.2) in the pH range extant in Baltimore's streams (pH 5.4–9.2). Monitoring data collected by the local municipality over the course of 2 years revealed that, in addition to substantial variations in pH, the investigated streams also showed a wide range of total suspended solids concentrations (nondetect–255 mg/L TSS). The organic carbon content of particulate matter present in the monitored

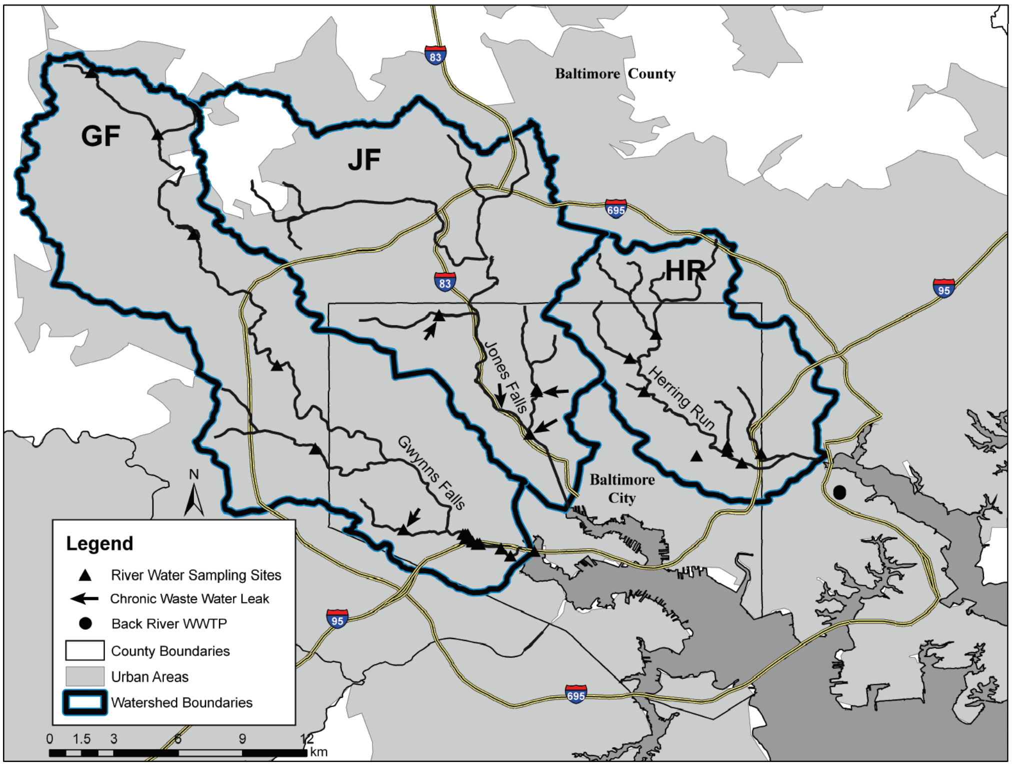

FIGURE 1. Map of 34 sampling sites along 14 streams in three watersheds of Baltimore City and County, Maryland. Also shown are five chronic sources of wastewater leakage to surface water and the wastewater treatment plant (WWTP) monitored in this study.

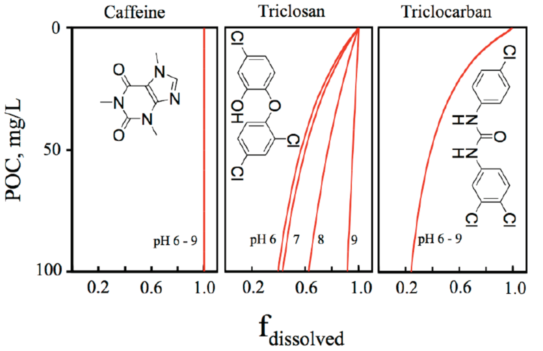

FIGURE 2. Sorption isotherms illustrating the effect of particulate organic carbon (POC) and pH on the partitioning behavior of three organic wastewater contaminants and the relative fraction of their total mass remaining in solution ($f_{\text{dissolved}}$).

streams was estimated from the literature (2.2–35% POC); raw wastewater entering the local sewage treatment plant showed an even broader range of 40–363 mg/L TSS and 0.8–41% POC (Supporting Information; Table S2).

These observed ranges in environmental conditions guided the calculation of pH-dependent partitioning isotherms (Figure 2). Caffeine and triclocarban exhibited constant pH-adjusted K_{OC} values (D_{OC}) between pH 6 and 9, and therefore each plotted as a single line. Triclosan sorption was more complex in that it displayed a pH dependence characteristic of an acidic hydroxyl group; its mass fraction in the dissolved state decreased from 91% (pH 9) to 40% (pH 6) at a POC concentration of 100 mg/L. Caffeine was insensitive to the amount of POC present and remained essentially fully dissolved (99.8%) even at maximum POC levels potentially encountered in the study setting. In contrast, the fraction of triclocarban in the dissolved phase decreased from 100 to 24% as organic carbon levels increased from 0 to 100 mg/L.

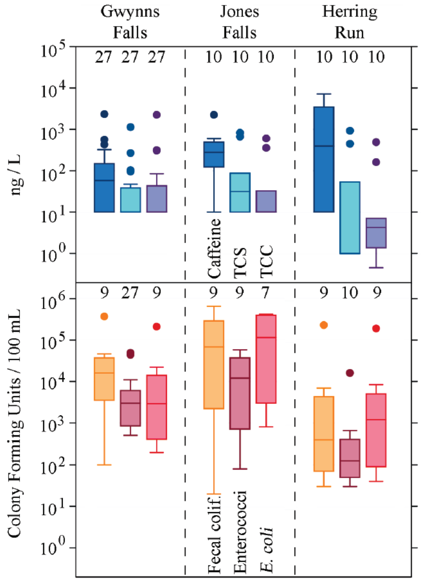

FIGURE 3. Box plots showing the concentrations of caffeine, triclosan (TCS), and triclocarban (TCC) as well as those of microbial indicators in aquatic samples by watershed. Shown are the median (line in box), 25th, and 75th percentile (lower and upper hinges), a line drawn from the upper hinge to the upper adjacent value and from the lower hinge to the lower adjacent value (whiskers), and individual outlier values are shown by circles. The number of observations expressed in each box plot is displayed directly above it.

Chemical Analyses. Surface waters from all three Baltimore watersheds contained detectable amounts of the OWCs of interest (Figure 3). Caffeine was detected in all streams but Tiffany Run and peaked at a concentration of 7110 ng/L in a sample originating from Armistead Creek. Triclosan and triclocarban were found, respectively, in 9 (64%) and 12 (86%) of the 14 streams monitored; maxima for both antimicrobials,

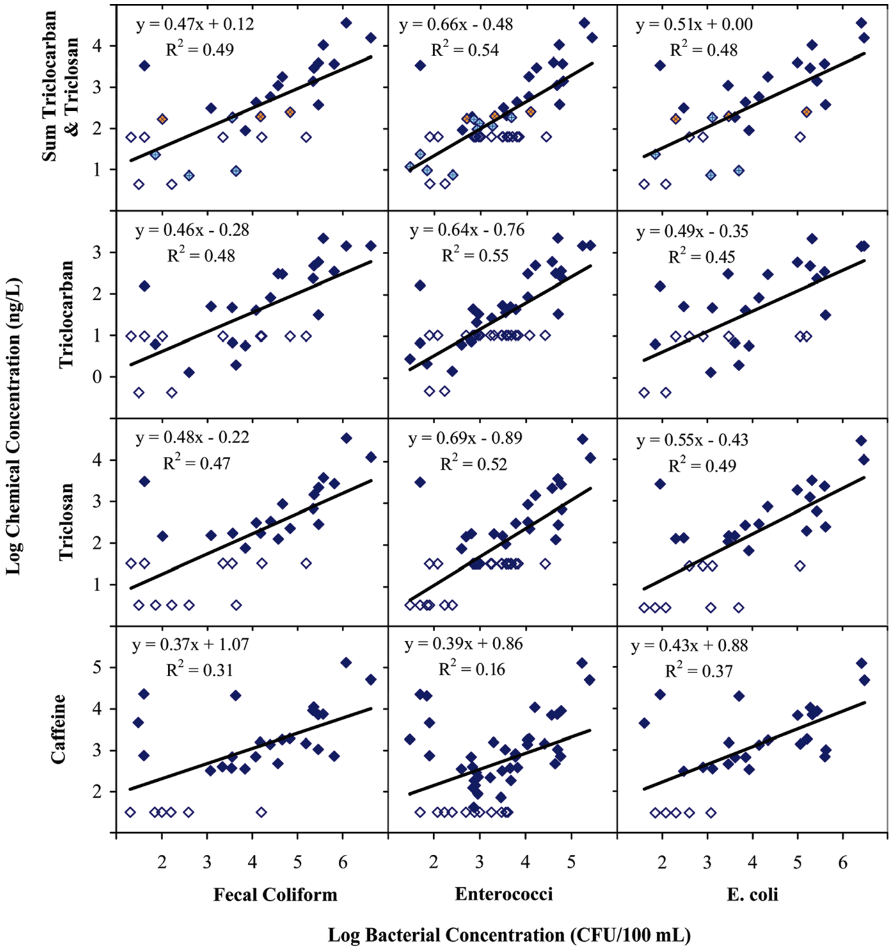

FIGURE 4. Correlation between chemical and bacteriological markers of wastewater leakage to surface water. Chemical detected (filled diamonds), chemical not detected (open diamonds), triclosan not detected (diamonds with crosses), and triclocarban not detected (diamonds with "x"). Nondetect measurements are expressed as half of the limit of detection or LOD, which varied by the method used.

detected in a sample taken from Gwynns Run, were 1130 and 2230 ng/L, respectively. Thus, the concentrations of both antimicrobial compounds were within the range previously reported for a small subset of Baltimore streams (*19, 20*). Raw sewage sampled at the inlet of the local wastewater treatment plant showed concentrations for caffeine of 15700 ng/L and average concentrations for triclosan and triclocarban of 4700 and 6100 ng/L, respectively, as determined from long-term monitoring (*24, 25*). Additional statistics are shown in the box plots presented in Figure 3 and in the Supporting Information.

Bacteriological Analyses. All 14 streams tested positive for each of the three bacteriological indicator organisms examined in this study (Figure 3). Fecal coliforms, enterococci, and *E. coli* were detected in surface water samples at a frequency of 92, 96, and 88%, respectively; the corresponding maximum concentrations of these indicators were 6.5 × 10^5, 5.8 × 10^4, and 4.2 × 10^5 CFU/(100 mL), respectively. Concentrations of fecal coliforms, enterococci, and *E. coli* measured in raw sewage (influent) sampled at the local wastewater treatment plant were 4.2 × 10^6, 2.5 × 10^5, and 3.0 × 10^6 CFU/(100 mL), respectively. Additional information is presented in the Supporting Information (Table S3).

Evaluation of Chemical Indicators. The value of the three OWCs as chemical indicators of microbial risks in surface waters was evaluated in correlation analyses using microbial plate counts as a benchmark. Coefficients of determination (R^2 values) for triclosan and triclocarban were in the range of 0.45−0.55, and similar results were obtained when the combined mass of both antimicrobials was used as a tracking parameter ($R^2 = 0.48-0.54$). By comparison, caffeine was an inferior chemical predictor of bacterial contaminants, as indicated by R^2 values ranging from 0.16 to 0.37. The strongest positive association was observed between triclocarban and enterococci ($R^2 = 0.55$) and the weakest between caffeine and enterococci ($R^2 = 0.16$; Figure 4). Observed trends were consistently found regardless of the approach chosen for treating nondetect values (Table S4).

Discussion

Nationwide, the U.S. EPA reports over 63000 impaired waters, some 13% of which are impacted by pathogens, the largest category of contaminants together with mercury (*26*). The majority of these reported microbial impairments are based either upon levels of frank pathogens or, more commonly, of fecal coliforms, enterococci, and *E. coli*. The bulk of testing for the latter three indicators does not differentiate pathogenic from nonpathogenic strains (*26*). Following release of raw sewage into surface waters during spill events, microbial contaminants typically travel by advection but they also may associate with settleable particulates and leave the water column to become part of the sediment. One report showed microbial sorption to be particularly extensive during storm events, when an average of 40% of fecal indicator bacteria were contained in settleable material (*27*). Another study showed a positive correlation between particle associated bacteria and POC, and a negative correlation between free bacteria and POC (*28*).

Here, the association between chemical and biological markers of wastewater leakage to surface water was explored in greater detail by testing the hypothesis that hydrophobic chemicals showing consistent partitioning behavior at environmental pH are preferable to nonsorptive alternatives when monitoring for biological risks.

Caffeine is a widely used chemical indicator of microbial risks in surface waters (*17, 29–32*). However, since caffeine displays essentially no association with POC via sorption (Figure 2), it cannot track the settleable microbial burden. As microorganisms are removed from the water column by gravity settling, caffeine and other nonsorptive chemical tracers will become separated from the microbial burden of interest. If loss by settling is extensive, as is the case during storm events (*27*), the predictive value of caffeine as a chemical marker will be diminished. False positive results are expected to increase in frequency with increasing distance from the sewage spill site. This is because caffeine will remain in the water column even after contaminating microbes have settled. In contrast, particle-active chemical markers, such as the antimicrobials evaluated here, presumably would not suffer from this limitation and settle to bottom sediments along with the microorganisms.

Indeed, the monitoring data obtained for the three watersheds under study support the selection of sorptive OWCs over the conventionally employed marker caffeine (Figure 4). Coefficients of determination were consistently superior for the two hydrophobic antimicrobials, whose concentrations in natural waters are orders of magnitude below milligrams per liter minimum inhibitory concentrations (*33*) that would impact the viability and survival of target microorganisms. Caffeine and fecal bacteria previously were measured simultaneously in environmental samples, and the associated R^2 values (derived from reported correlation coefficients, R) showed a considerable range from 0.22 to unity (*17, 31, 32*). One report showed favorable R^2 values of 1.0 and 0.97 when comparing concentrations of caffeine to those of fecal coliforms and enterococci, respectively; however, this study was limited to only four sampling events on a single small lake (*31*). Another study featuring two sampling campaigns and six sites yielded an R^2 value of 0.49 when correlating caffeine concentrations to the occurrence of five microbiological indicators, including those used here (*32*). The present study investigated a large number of streams and produced a data set of comparatively greater geographical and temporal diversity. In addition, the simultaneous determination of three chemical tracers and three microbial indicators allowed for a direct and methodologically unbiased comparison of caffeine to its hydrophobic tracer alternatives.

Triclosan consistently outperformed caffeine as a chemical indicator but also has limitations. Most notable is the variable sorptive behavior of triclosan in the ambient pH range (Figure 2). This behavior may help to explain why triclosan overall performed slightly less favorably than triclocarban (Figure 4). Another potential issue concerning the use of triclosan as a chemical marker for sewage spills is the compound's extensive use for purposes other than personal care products destined for disposal into wastewater. Incorporation of triclosan into textiles and plastics of daily use is becoming more prevalent, which opens the possibility of nonsewage specific detections in natural surface waters. In addition, at least 1500 triclosan-containing personal care product formulations are commercially available today (*34*) and some of these may wash off of swimmers and other recreational water users rather than being introduced alongside microbial contamination in leaked sewage.

Triclocarban was identified as a promising chemical marker that performed well in situ, presumably because of its pronounced sorption behavior and insensitivity to pH in the range of interest. Triclocarban also does not have as many uses as triclosan, presumably because of its limited processability in chemical manufacturing, its order of magnitude lower solubility in water (*35*), and a comparatively limited body of toxicological data (*36*).

One potential limitation of using triclosan and triclocarban for chemical monitoring of microbial risks is their environmental persistence, particularly under anaerobic conditions prevailing in aquatic sediments. Whereas both compounds are susceptible to aerobic biodegradation and other destructive processes (*37, 38*), their half-lives in anaerobic soils and sediments is on the order of months to years (*39*). This implies that antimicrobial agents deposited over time in sediments may be released spontaneously during rain storms, boating, dredging, and other activities as a result of sediment suspension. In contrast, caffeine has the more desirable property of disintegrating fairly quickly in ambient waters and not accumulating to any significant extent in aquatic sediments (*16, 40*). The potential for false positives associated with sediment suspension of bound TCC and TCS can be minimized by monitoring these markers concurrently with POC and other indicators of microbial risks for example caffeine, fecal coliforms, enterococci, or *E. coli*.

Ideally, chemical indicators used for monitoring of microbial risks from sewage spills to surface waters should originate exclusively in raw wastewater, occur therein at elevated and relatively constant levels, adsorb to microorganisms, be removed along with suspended microorganisms during sewage treatment, be insensitive to environmental pH changes, and attenuate in natural waters at rates comparable to those of pathogenic microorganisms. While the antimicrobial OWCs evaluated here do not satisfy all of the above requirements, they certainly represent a valid alternative to caffeine when tracking microbial risks. Whereas TCS and TCC are manmade compounds present in domestic wastewater worldwide, caffeine does occur naturally in surface waters in coffee bean producing countries, which can compromise its usefulness for tracking of microbial risks in these locales (*17*). Its lack of sorption to indicator organisms is another limitation. For this reason, chemical monitoring of microbial risks may be more effective when using hydrophobic OWCs in place of, or in conjunction with, the traditionally employed chemical indicator, caffeine.

Acknowledgments

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Supporting Information Available

Summary data for levels of chemicals and indicator organisms in the streams investigated and details of the regression analyses in tabular form. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Montemayor, M.; Valero, F.; Jofre, J.; Lucena, F. Occurrence of Cryptosporidium spp. oocysts in raw and treated sewage and river water in north-eastern Spain. *J. Appl. Microbiol.* **2005**, *99*, 1455–1462.

(2) Blanch, A. R.; Belanche-Muñoz, L.; Bonjoch, X.; Ebdon, J.; Gantzer, C.; Lucena, F.; Ottoson, J.; Kourtis, C.; Iversen, A.; Kühn, I.; Mocé, L.; Muniesa, M.; Schwartzbrod, J.; Skraber, S.; Papageorgiou, G. T.; Taylor, H.; Wallis, J.; Jofre, J. Integrated analysis of established and novel microbial and chemical methods for microbial source tracking. *Appl. Environ. Microbiol.* **2006**, *72*, 5915–5926.

(3) Chapron, C. D.; Ballester, N. A.; Fontaine, J. H.; Frades, C. N.; Margolin, A. B. Detection of astroviruses, enteroviruses, and adenovirus types 40 and 41 in surface waters collected and evaluated by the information collection rule and an integrated cell culture-nested PCR procedure. *Appl. Environ. Microbiol.* **2000**, *66*, 2520–2525.

(4) Straub, T. M.; Quinonez-Diaz, M. D.; Valdez, C. O.; Call, D. R.; Chandler, D. P. Using DNA microarrays to detect multiple pathogen threats in water. *Water Sci. Technol. Water Sup.* **2004**, *4*, 107–114.

(5) Perry, J. D.; James, A. L.; Morris, K. A.; Oliver, M.; Chilvers, K. F.; Reed, R. H.; Gould, F. K. Evaluation of novel fluorogenic substrates for the detection of glycosidases in *Escherichia coli* and *enterococci. J. Appl. Microbiol.* **2006**, *101*, 977–985.

(6) Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999–2000: A national reconnaissance. *Environ. Sci. Technol.* **2002**, *36*, 1202–1211.

(7) Weigel, S.; Kuhlmann, J.; Hühnerfuss, H. Drugs and personal care products as ubiquitous pollutants: Occurrence and distribution of clofibric acid, caffeine and DEET in the North Sea;. *Sci. Total Environ.* **2002**, *295*, 131–141.

(8) *APHA In Standard Methods for the Examination of Water & Wastewater*, 20th ed.; American Public Health Association, 1999.

(9) Noble, R. T.; Weisberg, S. B. A review of technologies for rapid detection of bacteria in recreational waters. *J. Water Health* **2005**, *3*, 381–392.

(10) Stoob, K.; Singer, H. P.; Goetz, C. W.; Ruff, M.; Mueller, S. R. Fully automated online solid phase extraction coupled directly to liquid chromatography-tandem mass spectrometry: Quantification of sulfonamide antibiotics, neutral and acidic pesticides at low concentrations in surface waters. *J. Chromatogr., A* **2005**, *1097*, 138–147.

(11) Nelson, M. A.; Gates, A.; Dodlinger, M.; Hage, D. S. Development of a portable immunoextraction-reversed-phase liquid chromatography system for field studies of herbicide residues. *Anal. Chem.* **2004**, *76*, 805–813.

(12) Seurinck, S.; Deschepper, E.; Deboch, B.; Verstraete, W.; Siciliano, S. Characterization of Escherichia coli isolates from different fecal sources by means of classification tree analysis of fatty acid methyl ester (FAME) profiles. *Environ. Monit. Assess.* **2006**, *114*, 433–445.

(13) Hua, W.; Bennett, E. R.; Letcher, R. J. Triclosan in waste and surface waters from the upper Detroit River by liquid chromatography-electrospray-tandem quadrupole mass spectrometry. *Environ. Int.* **2005**, *31*, 621–630.

(14) Skutlarek, D.; Exner, M.; Färber, H. Perfluorinated surfactants in surface and drinking waters. *Environ. Sci. Pollut. Res.* **2006**, *13*, 299–307.

(15) Federighi, M.; Tholozan, J. L.; Cappelier, J. M.; Tissier, J. P.; Jouve, J. L. Evidence of non-coccoid viable but non-culturable Campylobacter jejuni cells in microcosm water by direct viable count, CTC-DAPI double staining, and scanning electron microscopy;. *Food Microbiol.* **1998**, *15*, 539–550.

(16) Buerge, I. J.; Poiger, T.; Müller, M. D.; Buser, H.-R. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. *Environ. Sci. Technol.* **2003**, *37*, 691–700.

(17) Peeler, K. A.; Opsahl, S. P.; Chanton, J. P. Tracking anthropogenic inputs using caffeine, indicator bacteria, and nutrients in rural freshwater and urban marine systems. *Environ. Sci. Technol.* **2006**, *40*, 7616–7622.

(18) Halden, R. U.; Paull, D. H. Co-occurrence of triclocarban and triclosan in U.S. water resources. *Environ. Sci. Technol.* **2005**, *39*, 1420–1426.

(19) Sapkota, A.; Heidler, J.; Halden, R. U. Detection of triclocarban and two co-contaminating chlorocarbanilides in US aquatic environments using isotope dilution liquid chromatography tandem mass spectrometry. *Environ. Res.* **2007**, *103*, 21–29.

(20) Halden, R. U.; Paull, D. H. Analysis of triclocarban in aquatic samples by liquid chromatography electrospray ionization mass spectrometry. *Environ. Sci. Technol.* **2004**, *38*, 4849–4855.

(21) *Enterococci in water by membrane filtration using membrane-enterococcus indoxyl-β-d-glucoside agar (mei)*; U.S. EPA Method 1600; Office of Water (4303T), U.S. Environmental Protection Agency: Washington, D.C., 2002.

(22) *Escherichia coli (E. coli) in water by membrane filtration using membrane-thermotolerant Escherichia coli agar (mTEC)*; U.S. EPA Method 1103.1; Office of Water (4303T), U.S. Environmental Protection Agency: Washington, D.C., 2002.

(23) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*,2nd ed.; Wiley-Interscience: Hoboken, NJ, 2003.

(24) Heidler, J.; Halden, R. U. Mass balance assessment of triclosan removal during conventional sewage treatment. *Chemosphere* **2007**, *66*, 362–369.

(25) Heidler, J.; Sapkota, A.; Halden, R. U. Partitioning, persistence, and accumulation in digested sludge of the topical antiseptic triclocarban during wastewater treatment. *Environ. Sci. Technol.* **2006**, *40*, 3634–3639.

(26) U.S. EPA. National Section 303(d) List Fact Sheet. Accessed on Sep. 28, 2007: http://oaspub.epa.gov/waters/national_rept.control.

(27) Krometis, L.-A. H.; Characklis, G. W.; Simmons, O. D., III; Dilts, M. J.; Likirdopulos, C. A.; Sobsey, M. D. Intra-storm variability in microbial partitioning and microbial loading rates. *Water Res.* **2007**, *41*, 506–516.

(28) Grossart, H.-P.; Brinkhoff, T.; Martens, T.; Duerselen, C.; Liebezeit, G.; Simon, M. Tidal dynamics of dissolved and particulate matter and bacteria in a tidal flat ecosystem in spring and fall. *Limnol. Oceanogr.* **2004**, *49*, 2212–2222.

(29) Snyder, S. A.; Westerhoff, P.; Yoon, Y.; Sedlak, D. L. Pharmaceuticals, personal care products, and endocrine disruptors in water: Implications for the water industry. *Environ. Eng. Sci.* **2003**, *20*, 449–469.

(30) Scott, T. M.; Rose, J. B.; Jenkins, T. M.; Farrah, S. R.; Lukasik, J. Microbial source tracking: Current methodology and future directions. *Appl. Environ. Microbiol.* **2002**, *68*, 5796–5803.

(31) Sankararamakrishnan, N.; Guo, Q. Chemical tracers as indicator of human fecal coliforms at storm water outfalls. *Environ. Int.* **2005**, *31*, 1133–1140.

(32) Ferreira, A. P. Caffeine as an environmental indicator for assessing urban aquatic ecosystems. *Cad Saúde Pública* **2005**, *21*, 1884–1892.

(33) Walsh, S. E.; Maillard, J.-Y.; Russell, A. D.; Catrenich, C. E.; Charbonneau, D. L.; Bartolo, R. G. Development of bacterial resistance to several biocides and effects on antibiotic susceptibility. *J. Hosp. Infect.* **2003**, *55*, 98–107.

(34) Hileman, B. Regulation antiseptic soaps under Scrutiny FDA to assess whether household use leads to bacterial resistance. *Chem. Eng. News* **2005**, *83*, 14.

(35) Loftsson, T.; Össurardóttir, Í. B.; Thorsteinsson, T.; Duan, M.; Másson, M. Cyclodextrin solubilization of the antibacterial agents triclosan and triclocarban: Effect of ionization and polymers. *J. Inclusion Phenom. Macrocyclic Chem.* **2005**, *52*, 109–117.

(36) Aiello, A. E.; Larson, E. Antibacterial cleaning and hygiene products as an emerging risk factor for antibiotic resistance in the community. *Lancet Infect. Dis.* **2003**, *3*, 501–506.

(37) Gledhill, W. E. Biodegradation of 3,4,4′ trichlorocarbanilide, TCC, in sewage and activated sludge. *Water Res.* **1975**, *9*, 649–654.

(38) Latch, D. E.; Packer, J. L.; Stender, B. L.; VanOverbeke, J.; Arnold, W. A.; McNeill, K. Aqueous photochemistry of triclosan: Formation of 2,4-dichlorophenol, 2,8-dichlorodibenzo-p-dioxin, and oligomerization products. *Environ. Toxicol. Chem.* **2005**, *24*, 517–525.

(39) Ying, G. G.; Yu, X. Y.; Kookana, R. S. Biological degradation of triclocarban and triclosan in a soil under aerobic and anaerobic conditions and comparison with environmental fate modelling; *Environ. Pollut.*, online early, in press; doi: 10.1016/j.envpol.2007.02.013.

(40) Bradley, P. M.; Barber, L. B.; Kolpin, D. W.; McMahon, P. B.; Chapelle, F. H. Biotransformation of caffeine, cotinine, and nicotine in stream sediments: Implications for use as wastewater indicators. *Environ. Toxicol. Chem.* **2007**, *26*, 1116–1121.

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