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January 21, 2005

Dear Sir or Madam:

I would like to bring to your attention a recent study completed by my research group on the environmental fate of the antimicrobial compound triclocarban (CAS 101-20-2; the compound is also known as TCC or 3,4,4'-trichlorocarbanilide). After studying the occurrence of triclocarban in various environments, we concluded that the antimicrobial compound contaminates US water resources nationwide (60% of all streams) at levels as high as 6.75 ppb. Triclocarban concentrations were markedly higher than non-peer reviewed numbers (240 ng/L <30% frequency) currently used by the US EPA for evaluating triclocarban's ecological and human health risks. More details of our study can be found in the attached paper, published online in Environmental Science & Technology on January 21, 2005, and titled, "Co-Occurrence of Triclocarban and Triclosan in U.S. Water Resources." It will appear in print in Environmental Science & Technology later this year.

Triclocarban is currently being reviewed under the HPV Chemical Challenge Program of the U.S. EPA. The information contained in our paper should prove helpful for addressing some of the questions that were left unanswered by the robust summary report provided by the chemical industry. In summary, the concentrations found by our group were 28-fold higher than those reported previously by the TCC consortium.

Please feel free to contact me by phone (410-955-2609) if you have questions or would like to obtain additional information. My research group is currently conducting a nationwide study on the fate of triclocarban and other pharmaceuticals and personal care products in the environment. We will make every effort to provide the collected information to the U.S. EPA in a timely fashion.

Respectfully,  
  
Rolf Halden, PhD, PE, Assistant Professor of Environmental Health Sciences  
Johns Hopkins University Center for Water and Health



# Co-Occurrence of Triclocarban and Triclosan in U.S. Water Resources

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Triclocarban (TCC) and triclosan (TCS) are antimicrobial additives in personal care products. Whereas TCS has been studied extensively, the environmental fate of TCC remains largely unknown. To address this data gap, we performed quantitative structure-activity relationship (QSAR) analyses that suggested a propensity of TCC to persist in various environmental compartments with predicted half-lives ranging from 0.75 days in air to 540 days in sediment. Moreover, concentrations of both antimicrobials were measured in 42 environmental samples from the Greater Baltimore region using a combination of solid-phase extraction, liquid chromatography/mass spectrometry, and isotope dilution. The co-occurrence of TCC and TCS was observed, owing to similar properties, usage, disposal, and environmental half-lives. A linear empirical correlation ( $R^2 = 0.9882$ ) fit the log-log-transformed data from diverse aquatic media and spanned 5 orders of magnitude in concentration. Occurrences of TCC predicted for 85 U.S. streams were statistically indistinguishable from experimental regional data ( $\alpha \leq 0.05$ ). Annual loading of antimicrobials to water resources probably is dominated by activated sludge treatment plants (39-67%), followed by trickling filters (31-54%) and combined and sanitary sewer overflows (2-7% and <0.2%, respectively). Study results suggest that TCC is a previously unrecognized contaminant of U.S. water resources nationwide, likely ranking in the top 10 in occurrence rate and in the top 20 in maximum concentration among 96 organic pollutants considered. The magnitude and frequency of TCC contamination (regional, 6750 ng/L, 68%; predicted nationwide for 1999-2000, 1150 ng/L, 60%) were markedly higher than non-peer-reviewed numbers (240 ng/L, 30%, U.S.) currently used by the U.S. Environmental Protection Agency for evaluating TCC's ecological and human health risks.

## Introduction

The antimicrobial triclocarban (TCC) is a toxic (1), persistent (2), and potentially bioaccumulative (3) polychlorinated binuclear aromatic urea pesticide whose environmental fate remains largely uncertain after almost half a century of mass production and down-the-drain disposal. Since 1957, TCC has been added to detergents, cosmetics, and other personal care products in the United States at levels of 0.5-5 wt % for preventing spoilage and microbial infections (4). Eighty-four percent of all antimicrobial bar soaps sold in the United

States contain TCC (5). Approximately 500 000-1 000 000 lb (227 000-454 000 kg) of TCC are used in the United States every year (4).

Triclocarban is toxic to mammals, interferes with mammalian reproduction (1), and causes methemoglobinemia in humans (6-8). In rats, it triggered a reduction in the rate at which animals conceived, in the number of pups born, as well as in the survival rate and body weight of the offspring (1). In rabbits, TCC was observed to have significant dose-related maternal toxicity, including weight loss, abortion, and death (1). Although no direct evidence of carcinogenicity and teratogenicity was found (1), TCC manufacturers are labeling their product with the European risk phrases R45 and R46, indicating a potential to cause cancer and heritable disease. Of concern is a possible cleavage of the carbon-nitrogen bonds and release of *N*-hydroxylated metabolites, which may function as metabolic precursors of carcinogens (9). Thus far, cleavage of TCC has been observed at elevated pH and temperature only, with the resultant primary aromatic amines causing methemoglobinemia in humans (7, 8). Environmental transformation of TCC also yields mono- and dichlorinated anilines as important breakdown products (2); these are of concern due to their inherent hematotoxicity (10), genotoxicity (10), ecotoxicity (10), and environmental persistence (2). Less severe adverse health effects of TCC exposure include photoallergic reactions (11).

Despite the heavy usage (1, 12) and potentially problematic environmental behavior of TCC (10), few peer-reviewed studies have concentrated on the occurrence and environmental fate of the antimicrobial in American water resources. The sole peer-reviewed investigation exploring the fate of TCC in U.S. wastewater treatment works was laboratory-based and dates back 30 years. In the study, TCC persisted in raw sewage and was mineralized in activated sludge only partially (56%), even after long-term acclimation of the culture (2). No mandatory monitoring programs for TCC exist, and the voluntary monitoring data obtained by the chemical industry are largely inaccessible to the public (listed in refs 4 and 12). The first national reconnaissance of pharmaceuticals, hormones, and other organic wastewater contaminants, conducted by the United States Geological Survey (USGS), did not include TCC among the 95 compounds targeted (13). In 1991, TCC was detected by chance in effluent of two publicly owned New Jersey treatment works (14). At present, no other peer-reviewed environmental exposure data exist for the United States.

This lack of information is hampering the ongoing evaluation of TCC by the United States Environmental Protection Agency (U.S. EPA) under the High Production Volume (HPV) Chemical Challenge Program. For the purpose of assessing potential environmental and human health risks, the Federal agency relies on robust summaries prepared by chemical manufacturers. On the basis of the non-peer-reviewed data compiled in two industry reports (listed in refs 4 and 12), the U.S. EPA has determined that additional information is required to complete an assessment of the biocide with respect to its environmental fate, health effects, and ecological risks (15).

To address the knowledge gap, we performed quantitative structure-activity relationship (QSAR) analyses to estimate the physical-chemical properties of TCC in relation to those of a related HPV chemical, triclosan (TCS), another polychlorinated binuclear aromatic antimicrobial bearing many similarities with TCC. In addition, a monitoring program was initiated in the Greater Baltimore region in Maryland to determine environmental occurrences of TCC. An underlying

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hypothesis of this work was that—as a result of similarities in structure, function, and usage—both antimicrobial compounds share analogous environmental fates and behaviors.

## Experimental Section

**Chemicals.** Triclocarban (CAS Registry No. 101-20-2) was purchased from Aldrich (Milwaukee, WI); it is also known under the following synonyms: 3,4,4'-trichlorocarbanilide; 1-(3',4'-dichlorophenyl)-3-(4'-chlorophenyl)urea; *N*-(4-chlorophenyl)-*N'*-(3,4-dichlorophenyl)urea; *N*-(3,4-dichlorophenyl)-*N'*-(4-chlorophenyl)urea; Caswell No. 874; Cusiter; Cutisan; 4-12-00-01265; A13-26925; BRN 2814890; CCRIS 4880; CP 78416; carbanilide, 3,4,4'-trichloro; 3,4,4'-trichlorodiphenylurea; ENT 26925; EPA Pesticide Chemical Code 027901; Genoface; HSDB 5009; NSC-72005; Procufene; Solubacter; and Trichlocarban. Deuterated triclocarban (TCC-*d*<sub>7</sub>) was a gift from Cambridge Isotope Laboratories Inc. (Andover, MA). Carbon-13 labeled triclosan (<sup>13</sup>C<sub>6</sub>-TCS), containing a uniformly labeled 2,4-dichlorophenoxy ring, was a gift from Ciba Specialty Chemicals (Basel, Switzerland). All analytical solvents (HPLC grade or better) were purchased from Acros Organics (Fairlawn, NJ). All other chemicals, including TCS (CAS Registry No. 3380-34-5), were purchased from the Sigma-Aldrich Co. (Milwaukee, WI) and were of the highest purity available.

**Sampling.** Water samples were collected in duplicate using disposable, precleaned, 1-L plastic bottles. Trip blanks consisted of the same 1-L bottles filled with reagent water (18.2 MΩ resistance) obtained from a Nanopure Diamond Ultrapure water system (Barnstead; Dubuque, IA). The sampling period for this study ranged from the fall of 2002 through the spring of 2004.

Grab samples of raw and finished drinking water were obtained from three drinking water plants in Baltimore: Montebello Filtration Plants 1 and 2 and the Ashburton Treatment Plant. Grab samples of wastewater were collected at the Back River wastewater treatment plant (WWTP), on three occasions over the period of 2002 through 2004. Sampling matrixes included wastewater influent, effluent, and six types of slurries sampled at the following process stages: primary treatment, secondary treatment, gravity sludge thickener tank, gravity belt thickener facility, dissolved air flotation facility, and anaerobic digester. Additional sampling locations included three residential wells in Baltimore County (groundwater samples), and 19 locations along six urban streams in the Greater Baltimore area (Gwynns Falls, Gwynns Run, Jones Falls, Maidens Choice Run, Stoney Run, and Western Run). No measurable precipitation was recorded at the sampling locations in the 12-h period preceding the sampling events. Prior to the collection of residential well and drinking water samples, point-of-use water filters were removed as needed and the faucets were allowed to run for several minutes to avoid capture of stagnant water. All urban stream sampling locations were located upstream of WWTP inputs. Some locations were chosen strategically on the basis of previous reports of wastewater infiltration from leaking sewer systems. All samples were immediately placed on ice, shipped to the laboratory, fortified with isotope-labeled standards, and stored at -20 °C prior to analysis. All analytical data reflect average concentrations of two independent measurements of duplicate samples processed in parallel.

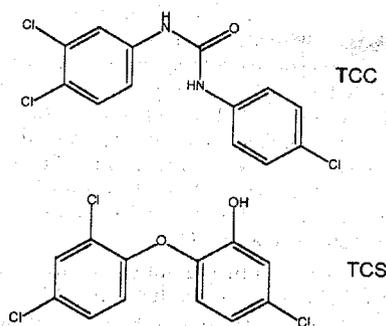
**Sample Preparation.** Water samples were centrifuged at 2000g for 20 min to remove solids. The supernatant was passed through a solid-phase extraction (SPE) (Oasis HLB, 3 cm<sup>3</sup>/60 mg sorbent; Waters Corp., Milford, MA) and eluted with organic solvents (4 mL, 50:50 methanol:acetone containing 10 mM acetic acid). Eluates were dried under a gentle stream of nitrogen, reconstituted (1 mL, 50:50 methanol:acetone), filtered (0.2 μm PTFE; 13 mm syringe filters;

Nalge Nunc Int.; Rochester, NY), reduced to initial eluent strength by dilution with water, and analyzed by liquid chromatography/negative electrospray ionization/mass spectrometry (LC/ESI(-)/MS). Sample particulates harvested by centrifugation were extracted overnight with organic solvents (2 mL, 50:50 methanol:acetone), dried, and processed as described above. Raw sewage (40 mL) was diluted (250 mL final volume) and centrifuged at 10 000g for 20 min. The complete sample preparation and analysis strategy have been described in detail elsewhere (16).

**LC/MS Analysis.** Chromatography was carried out on an Ultra IBD C<sub>18</sub> column (5 μm particle size, 2.1 × 150 mm; Restek Corp., Bellefonte, PA) using a DGU-14A eluent degaser, two LC-10ADvp gradient pumps, and an SCL-10Avp system controller (Shimadzu Corp., Columbia, MD). Sample portions (10–100 μL) were introduced with an autoinjector (Shimadzu SIL-10ADvp) controlled by LCMS Lab Solutions software (v 2.04). An isocratic method (0.2 mL/min; 70% acetonitrile, 30% water, 10 mM acetic acid) was used for samples lacking noticeable turbidity. Compounds were detected and quantified using a quadrupole mass spectrometer (Shimadzu LCMS 2010) in negative ESI mode. The curved desolvation line (CDL) and Q-array were set to 35 and -5 V, respectively. Block and CDL temperatures were 220 and 230 °C, respectively. Nitrogen desolvation gas was flowing at 4.2 L/min. Quantitative analysis was performed by selected ion monitoring (SIM). Positive identification of analytes was based on three criteria: (a) elution within the expected retention time window ( $t_R \pm 0.1$  min); (b) detection of the characteristic molecular ions ( $[M - H]^-$ ) of triclocarban (*m/z* 313), its acetic acid adduct (*m/z* 373), and triclosan (*m/z* 287); and (c) detection at the anticipated intensity of molecular ions containing a minimum of one or more naturally occurring <sup>37</sup>Cl atoms that distinguished these reference ions (*m/z* 315, 317 and 375, 377, respectively, for TCC and its acetic acid adduct, as well as *m/z* 289 and 291 for TCS). Isotope-labeled standards were tracked in the same fashion (TCC-*d*<sub>7</sub>, *m/z* 320, 322, 324; TCC-*d*<sub>7</sub> acetic acid adduct, *m/z* 380, 382, 384; and <sup>13</sup>C<sub>6</sub>-TCS, *m/z* 293, 295, and 297). Samples having noticeable turbidity, such as raw sewage, were analyzed using a linear gradient method (0.2 mL/min; 20 min) using 25–100% acetonitrile amended with 10 mM acetic acid. Quantification was performed using an external, linear calibration and a minimum of seven calibration levels. Incomplete (absolute) recovery of analytes was compensated for by using stable isotopes of TCC (TCC-*d*<sub>7</sub>) and TCS (<sup>13</sup>C<sub>6</sub>-TCS) in conjunction with the isotope dilution method (16). During routine analysis of environmental samples, instrument precision and accuracy were assessed every fourth sample by measuring a 100 ppb quality control standard using a tolerance cutoff value of ±20%. Measurements of TCS were periodically confirmed via analysis by GC/MS. Additional experimental details and quality-assurance and quality-control protocols are described elsewhere (16).

**TCS/TCC Loading to U.S. Surface Waters.** The mass range of TCS and TCC entering U.S. surface waters was estimated from total annual discharge data and from effluent concentration extremes published in the literature and determined experimentally. Mass releases by trickling filter systems were calculated by multiplying the above concentration extremes with published removal efficiencies for TCS (58–86%) (17).

**Computer Modeling.** Physical and chemical properties of antimicrobials were estimated using standard quantitative structure–activity relationship models; analyses were performed on personal computers using the following software: CambridgeSoft ChemOffice, PBT Profiler, ECOSAR, and Kow-WIN. Geographic maps were created using ArcGIS version 8.3.



**FIGURE 1.** The substituted diaryl urea pesticide triclorcarban (TCC) and its chemical cousin triclosan (TCS) share a number of structural features, including two aromatic benzene rings carrying three chlorine substituents.

**Statistical Analyses.** Statistical data analyses were performed using Systat v10.2, freeware "R" v 1.9.0, and SAS v 9.1. For all statistical analyses, chemical concentration data were transformed with the log function, so that the transformed empirical distribution would approximate the normal distribution. The transformed data were fit to various linear regression models, and the linear model presented in the results was selected on the basis of statistical hypotheses tests of the linear parameters and evaluation of residuals. For analysis using the two-sided Wilcoxon rank sum test (with *t*-approximation) and the two-sample, two-tailed *t*-test (with Cochran adjustment for unequal variances), nondetectable values were set equal to half the method detection limit of the analyte. Box plots and statistical parameters of detectable concentrations were calculated according to the method used by Kolpin et al. (13) to allow direct comparison of generated data to those of the first national reconnaissance of pharmaceuticals, hormones, and other organic wastewater contaminants.

## Results and Discussion

**Introductory Comparison of TCC and TCS.** Although they belong to distinct chemical groups, the diaryl urea compound TCC and the diaryl ether TCS share a number of similarities. Both are trichlorinated binuclear aromatics (Figure 1) that are classified as pesticides and used or proposed to be used as over-the-counter topical antimicrobial drugs in humans. Both have been mass-produced for several decades at combined rates exceeding 1 000 000 lb (454 000 kg) per year, and both are added either alone or together, to a wide array of personal care products and consumer items for their antimicrobial properties (see ref 4). At the end of their useful lifespan, both are disposed of down the drain into wastewater.

The underlying hypothesis of the present study was that the behavioral similarities of TCC and TCS extend into their chemical afterlives as environmental contaminants. The aromatic nature and high chlorine content of TCC and TCS (>30 wt %; see Table 1) should impart both a significant resistance to biodegradation and a tendency for environmental persistence. Their limited water solubility—estimated in the low mg/L range as shown in Table 1—paired with considerable lipophilicity (octanol–water partitioning coefficients ( $K_{ow}$ ) approaching  $10^5$ ; Table 1) suggest a potential for bioconcentration and bioaccumulation in the food web (3). On the basis of this parallelism in structure, function, usage, and annual production, one may expect both chemicals to have analogous environmental fates and occurrences. Unfortunately, this hypothesis is not easily testable, since the peer-reviewed literature offers much information on one (TCS), yet very little on the other compound (TCC).

There is a notable scarcity of information on the occurrence and behavior of TCC in the environment. In the past

**TABLE 1.** Selected Physical and Chemical Properties of the Antimicrobial Compounds Triclorcarban and Triclosan Calculated Using Quantitative Structure–Activity Relationship (QSAR) Analyses

property	triclorcarban	triclosan
CAS registry no.	101-20-2	3380-34-5
formula	$C_{13}H_9Cl_3N_2O$	$C_{12}H_8Cl_3O_2$
molecular weight (g/mol)	315.59	289.55
chlorine content (wt %)	36.7	33.7
melting point (°C) <sup>a</sup>	140	180
water solubility (mg/L at 25°C) <sup>a,b</sup>	0.65–1.55	1.97–4.6
$\log_{10} K_{ow}$ (at 25 °C, pH 7) <sup>c</sup>	4.9	4.8

<sup>a</sup> Calculated using PBT Profiler. <sup>b</sup> Calculated using ECOSAR. <sup>c</sup> Calculated using  $K_{ow}$ WIN.

40 years, more than 2000 papers have been published dealing with the adverse effect/toxicity, environmental occurrence, and biological properties of TCS. In contrast, a mere 40 peer-reviewed studies investigated TCC in these categories during a comparatively longer time period (Table 2). The only study concerning the fate of TCC in wastewater treatment processes dates back 30 years (2). Results of these laboratory experiments suggested significant resistance of TCC to the attack of nonacclimated microbial consortia from activated sludge. No information was provided on the removal of TCC during full-scale treatment. Peculiarly, the only published environmental occurrence data on TCC in U.S. wastewater are of semiquantitative nature only and were obtained by happenstance while investigating the use of particle beam liquid chromatography/mass spectrometry for enhanced monitoring of wastewater characteristics (14). Concentrations of TCC detected in effluent samples from three New Jersey wastewater treatment plants varied from nondetectable to 6000 ng/L (14), a result that is difficult to interpret given the considerable range of concentrations and the comparatively small number of samples analyzed.

**Study Representativeness and Mass Balance Calculations.** To obtain a first approximation of the removal efficiency of a typical American wastewater treatment plant, we analyzed grab samples from an activated sludge system serving the Greater Baltimore region. Key operational parameters of the plant are presented in Table 3. Activated sludge treatment was selected because this process is applied to about 75% of U.S. wastewaters (18). The average concentration of TCC in raw wastewater obtained in 2002–2004 was 6700 ng/L, of which greater than 98% was removed—but not necessarily degraded—during treatment (Table 4). These values are essentially identical to the concentration and removal rate of TCS determined in this study (6100 ng/L, 99%; Table 4) and to those determined previously for TCS by other research groups. For example, McAvoy et al. (17) found TCS concentrations in influent wastewater in the range of 3800 to 16 600 ng/L and final effluent concentrations of 240–2700 ng/L, when analyzing flow-adjusted composite samples. The authors calculated an average removal efficiency for TCS during activated sludge treatment of 96%. Similarly, Federle et al. observed 98.5% removal of radiolabeled TCS during activated sludge treatment on a bench scale (19), and Bester observed an average removal efficiency of 95% for TCS in a German sewage treatment plant (20). Overall, excellent agreement was observed between our TCS results and those reported by others. This lends credibility to both the sampling methodology used and the representativeness of the TCC data collected in this study. Since the Baltimore tertiary treatment plant represented the lower range of national TCS influent concentrations and the upper range of removal efficiency for the antimicrobial, all estimates based on this facility must be considered conservative, or low, with respect to mass.

**TABLE 2. Triclocarban and Triclosan Have Received Disparate Scientific Attention as Illustrated by the Number of Papers Contained in the Chemical Abstracts Service (CAS) Database for the Time Period from 1957 to May 2004**

CAS search parameter	triclocarban	triclosan
year of first publication	1957	1964
total publications by CAS registry no.	434	2476
publications in category "adverse effect/toxicity"	12	115
publications in category "occurrence"	13	182
publications in category "biological studies"	15	2032

**TABLE 3. Population Served and Operational Parameters of the Wastewater Treatment Plant Monitored in This Study**

parameter	activated sludge wastewater treatment plant
population served	1 300 000
wastewater influent (ML/d)	600
water usage per capita (L/d)	460
influent BOD <sup>a</sup> (mg/L)	138
effluent BOD (mg/L)	2.1
BOD removal (%)	98.5
influent TSS <sup>b</sup> (mg/L)	46
effluent TSS (mg/L)	1.0
TSS removal (%)	97.8

<sup>a</sup> BOD, biological oxygen demand. <sup>b</sup> TSS, total suspended solids.

On the basis of concentrations of antimicrobials entering the treatment plant, we calculated annual per-capita usage rates of TCC and TCS for the population served in the Greater Baltimore region. Average per-capita usage of TCC and TCS was 1130 and 1030 mg per annum, respectively (Table 4). A national and regional assessment of the antibacterial soap market, conducted in 2001, found no spatial differences in the usage of TCC- and TCS-containing antimicrobial products in the U.S. (5). Using this information, we estimated nationwide rates of TCC and TCS usage in personal care products disposed of into wastewater at >330 000 and >300 000 kg/yr, respectively, by extrapolating from our regional data (Table 4). Our estimate for TCC usage is in excellent agreement with industry numbers, as it falls within the TCC usage range recently reported by the chemical industry to the U.S. EPA (4). Similarly, the calculated mass of TCS was within the reported range (Table 4).

**Loading of Antimicrobials to U.S. Water Resources.** Using literature information and experimental data, we estimated the annual mass of TCC and TCS discharged into U.S. surface waters and drinking water resources (Table 5). Provided that 75% of U.S. wastewaters are treated by systems similar to the Baltimore plant (18), we calculated that activated sludge treatment works nationwide release at least 5800 kg of TCC and 2600–10 400 kg of TCS into U.S. water resources. Thus, activated sludge treatment likely is the single most important input of TCC (39–67%) and TCS (50–56%) into U.S. water resources. Trickling filter treatment is applied to only about 5% of U.S. wastewater (18). Nevertheless, these systems also can represent a significant source of antimicrobials in U.S. surface waters because their removal efficiencies for polychlorinated binuclear aromatics are low, ranging from 58% to 86% for TCS, for example (17). If the observed parallelism between TCC and TCS removal in activated sludge systems extends to trickling filters, the resulting additional inputs of TCC to U.S. surface waters would be on the order of 2700 kg to greater than 8100 kg per year. This represents as much as 31–54% of the total estimated loading for TCC.

Other potentially important pathways for significant environmental release of antimicrobials are leaking sewerage systems, as well as sanitary sewer and combined sewer overflows (SSOs and CSOs). A report to Congress that is

currently being prepared summarizes information on the frequency and magnitude of sewage spills (22). Nationwide, there are approximately 43 000 CSO events per year with a combined discharge volume of 3.2 trillion L. The number of SSO events is estimated at 25 000–89 700 with a total flow volume of 11.4–40.1 billion L/yr. The corresponding loading cannot be estimated with great accuracy because the levels of antimicrobials during high flow events and overflow situations are unknown and, most likely, are subject to significant temporal and spatial variations. However, use of conservative estimates of source strength during overflow events allows one to estimate the relative contribution of CSO and SSO events to the total load of antimicrobials discharged to U.S. surface waters. The relative contribution of sewage spills was estimated by assuming a dilution factor of 10–50-fold for SSOs during dry and wet weather events, respectively, and 20–100-fold for CSO events (Table 5). Accordingly, CSOs are expected to contribute between 2 and 7% of the total loading of the antimicrobials TCC and TCS to water resources (from 420 to >2100 kg/yr), whereas the contribution of SSOs probably is negligible (3–51 kg/yr or <0.2% of the total annual loading).

**Modeling the Co-Occurrence of TCC and TCS.** To evaluate the potential environmental co-occurrence of TCC and TCS in aquatic environments, we obtained 42 grab samples from various sampling locations in the Greater Baltimore area. Analyzed sample matrixes included raw and treated drinking water, groundwater, river water from six urban streams, as well as influent, effluent, and slurries representative of six different process streams of the local wastewater treatment plant (16). Maximum concentrations of TCC and TCS by sample matrix ranged from nondetectable (<3 ng/L) in raw and finished drinking water, to nondetectable (<20 ng/L) in groundwater, to 6750 and 1600 ng/L, respectively, in urban streams (Gwynns Run, MD), to 554 500 and 373 400 ng/L in slurries from the 180 million gallons per day wastewater treatment plant. The results of these measurements are presented in the log–log scatter plot shown in Figure 2. A strong positive linear correlation was observed between TCC and TCS occurrences across all aquatic environments and water types examined in a concentration range spanning 5 orders of magnitude. Regression analysis of the data set resulted in a linear empirical model

$$\log_{10} C_{TCC} = 0.9491 \log_{10} C_{TCS} \quad (1)$$

where  $C_{TCC}$  and  $C_{TCS}$  are the concentrations of triclocarban and triclosan, respectively. The magnitude of the linear parameter for the dependence of  $\log C_{TCC}$  on  $\log C_{TCS}$  [0.9491 (95% confidence interval, 0.9164–0.9819)] indicates a nearly one-to-one correspondence between the concentrations of the two antimicrobials across all aquatic environments examined. The intercept was set to zero because a comparison of residual plots indicated better accuracy of the forced model when predicting low levels of TCC from measured TCS concentrations (data not shown). Regardless of whether the correlations were forced through the origin or not, the coefficients of determination ( $R^2$  values) were close to unity, indicating the excellent predictive power of both models

**TABLE 4. Fate of the Antimicrobials Triclocarban and Triclosan during Activated Sludge Wastewater Treatment in a Plant Serving the Greater Baltimore Area, and Estimated Nationwide Antimicrobial Usage Rates**

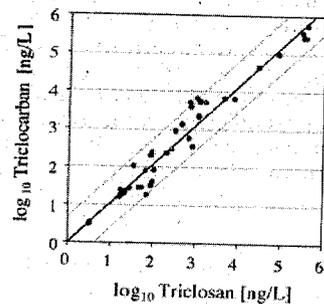
treatment parameters and mass estimates	triclocarban	triclosan
average WWTP <sup>a</sup> influent (ng/L) <sup>b</sup>	6700 ± 100	6100 ± 1600
average WWTP effluent (ng/L) <sup>b</sup>	110 ± 10	35 ± 20
average removal efficiency (%)	>98 <sup>d</sup>	>99 <sup>d</sup>
estimated annual mass entering WWTP (kg/year)	1470	1340
calculated per-capita usage (mg/yr), Baltimore	1130	1030
U.S. usage estimated in this study (kg/yr) <sup>c</sup>	>330000 <sup>d</sup>	>300000 <sup>d</sup>
U.S. usage reported elsewhere (kg/yr)	227000–454000 <sup>e</sup>	170000–970000 <sup>f</sup>

<sup>a</sup> WWTP, wastewater treatment plant. <sup>b</sup> Average concentrations of antimicrobials (± standard deviation) determined via analysis of grab samples taken on three occasions in 2002–2004. <sup>c</sup> Calculated using a U.S. population number of 290 342 000 (2003). <sup>d</sup> Estimates are considered conservative (low in mass) due to input parameters from the Baltimore plant that ranks low in influent concentrations and above average in treatment efficiency for antimicrobials compared to other activated sludge plants (17). <sup>e</sup> Taken from ref 4. <sup>f</sup> Personal communication.

**TABLE 5. Estimated Annual Loading of Antimicrobials to U.S. Surface Waters from Four Different Input Sources Expressed in Units of kg/yr and as a Percentage of the Sum of All Inputs Considered**

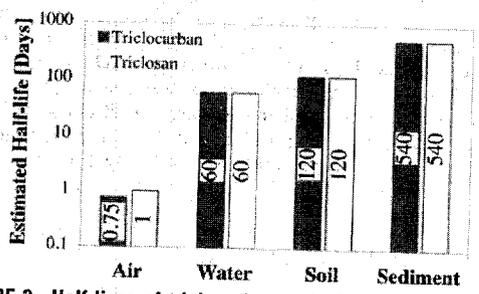
source	triclocarban	triclosan	input parameter ref
activated sludge WWTPs <sup>a,b</sup>	39–67% (>5800)	50–56% (2600–10500)	17, 21
trickling filter systems <sup>b,c</sup>	31–54% (2700 to >8100)	39–47% (2400–7300)	17
combined sewer overflows <sup>d</sup> (CSOs)	2–7% (220 to >1100)	3–5% (200–1000)	22
sanitary sewer overflows <sup>a</sup> (SSOs)	<0.2% (2 to >27)	<0.2% (1–24)	17

<sup>a</sup> WWTP, wastewater treatment plant. <sup>b</sup> Calculated using a U.S. population number of 290 342 000 (2003), a water usage rate of 543 L per capita per day, average removal rates for TCS and TCC of 96% (17) and 98% (this study), respectively, and assuming that 75% of all wastewaters are treated by this process. <sup>c</sup> Trickling filter treatment rate estimated at 5%. <sup>d</sup> Assuming 3.2 × 10<sup>12</sup> L annual flow for CSOs and a dilution factor of 20–100. <sup>e</sup> Assuming (1.14–4.01) × 10<sup>10</sup> L annual flow and a dilution factor of 10–50; the sum of percentages does not yield 100% due to consideration of multiple variables. Experimental input data represent average concentrations of antimicrobials in grab samples taken on three occasions in 2002–2004 at the Back River plant.



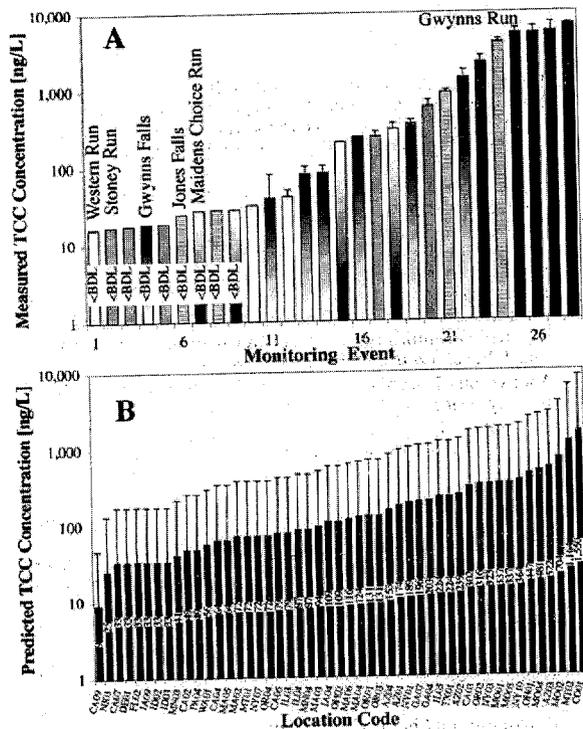
**FIGURE 2. Log–log scatterplot showing experimentally determined co-occurrences of triclocarban and triclosan in 42 environmental samples from the Greater Baltimore region. The linear regression model best fitting the observations took the form:  $\log C_{TCC} = 0.9491 \log C_{TCS}$ , where  $C$  is the concentration of antimicrobial compounds. The corresponding coefficient of determination ( $R^2 = 0.9882$ ) indicated a statistically highly significant linear relationship. Upper and lower prediction intervals of the model (95% PI) are depicted as dashed lines.**

across a large range of water types and concentrations, as indicated by the prediction intervals in Figure 2. The residuals from the linear model passing through the origin had a distribution with a mean (0.028), skewness (–0.44), and kurtosis (–0.08) near zero, which supports an assumption of normality for the model errors. Forcing the intercept through the origin ( $R^2 = 0.9882$ ), implies that both compounds have identical sources and are attenuated and compartmentalized in the environment to the same extent. This assumption is typically reserved for isomers and groups of congeners of polychlorinated dioxins, biphenyls, and other closely related chemicals.



**FIGURE 3. Half-lives of triclocarban and triclosan in air, water, soil, and sediment, estimated using quantitative structure–activity relationship (QSAR) analysis.**

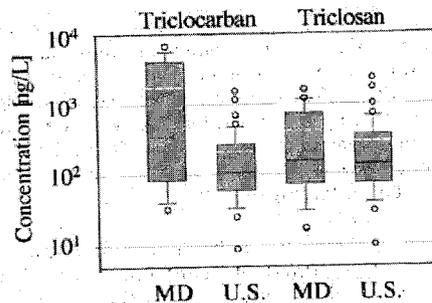
Although TCC and TCS are members of two different groups of chemicals, the prerequisites for use of the forced model may still be satisfied. As discussed earlier, both compounds have identical uses and sources. Our observations at the wastewater treatment plant also showed analogous partitioning and attenuation behaviors of TCC and TCS during activated sludge treatment (see Table 4 and ref 16). In addition to these empirical findings and the congruent estimates of chemical properties presented in Table 1, we used quantitative structure–activity relationship analyses to estimate the sum of physical, chemical, and biological attenuation rates for both antimicrobials in a variety of environmental compartments. The results, summarized in Figure 3, reveal that TCC and TCS are predicted to have essentially identical life spans in all four major environmental compartments, i.e., air, water, soil, and sediment. Estimated half-lives ranged from 0.75 days in air (TCC) to as long as 540 days in sediment for both TCC and TCS (Figure 3). On the basis of this combination of experimental and modeling data, adoption of the model that passes through the origin was



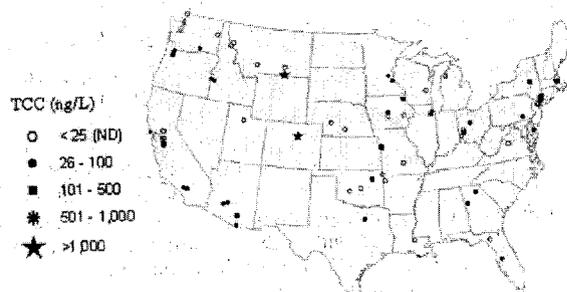
**FIGURE 4.** Bar graphs showing average TCC concentrations determined experimentally in duplicate samples from six urban streams in the state of Maryland (A) and predicted nationwide (B) on the basis of TCS concentrations published by the United States Geological Survey for 85 U.S. streams sampled in 1999–2000 (13). Values at Maryland locations 1–9 were nondetectable and are plotted at the detection limit. Sampling locations are coded by shading patterns; some of the regional TCC concentrations were published previously (16). Nondetectable values (<33 or <41 ng/L) calculated for 36 U.S. streams have been omitted from plot B. Location codes are defined elsewhere (13). Positive error bars indicate the higher of two independent experimental measurements (A) and the 95% upper prediction limit of estimated values (B).

considered both justifiable and judicious, as it provided better accuracy when predicting low levels of TCC likely extant in natural waters following massive dilution of wastewater inputs.

**Nationwide Prediction of TCC Contamination.** Although the first national reconnaissance of pharmaceuticals, hormones, and organic wastewater contaminants conducted by the USGS did not include TCC as a target analyte, this landmark study (13) may serve to shed some light on the frequency and magnitude of TCC contamination in U.S. streams. Since the study provided measurements of total (dissolved and particle-associated) TCS, the empirical model could be applied to estimate qualitatively and quantitatively the environmental occurrence of TCC in 85 U.S. streams nationwide for the years 1999 and 2000 (13). Detectable concentrations of TCC were predicted for 49 of 85 U.S. streams located in 21 of 28 states examined. Estimated concentrations ranged from 9 to 1550 ng/L, with a mean and median of 213 and 109 ng/L, respectively. At a calculated detection frequency of 58%, TCC likely is tied with TCS for fifth place among 96 pharmaceuticals, hormones, and organic wastewater contaminants considered (13). Regional TCC concentrations (determined experimentally) and national estimates (predicted using eq 1) were in good agreement (Figure 4). Frequency, mean, and median values of detectable concentrations of TCC in the Greater Baltimore region were, respectively, 68%, 1765 ng/L, and 356 ng/L. Box plots of



**FIGURE 5.** Box plots of triclocarban and triclosan concentrations determined for the Greater Baltimore region in Maryland (MD) and nationwide (U.S.). Median and mean concentrations are displayed as solid and dashed horizontal lines, respectively. Central locations, scatter, and dispersion of the observations indicate good agreement between experimentally determined regional data and calculated U.S. estimates. A detailed explanation of the statistical parameters shown in the box plot can be found elsewhere (13).



**FIGURE 6.** Geographic map showing the spatial distribution of estimated triclocarban concentrations in 85 U.S. streams in 1999–2000. Values were calculated from triclosan concentrations published in ref 13.

measured and predicted concentrations of both TCC and TCS also showed good agreement between regional observations and national estimates (Figure 5). Finally, a geographic map visualizing the spatial distribution of predicted TCC concentrations showed nationwide contamination of U.S. water resources and no regional concentration clusters (Figure 6).

To test the validity of the empirical model and the assumption of spatial homogeneity of TCC concentrations, experimental regional data and calculated national concentrations were statistically analyzed for differences. Use of the nonparametric Wilcoxon test with a  $t$ -approximation demonstrated that the distribution of TCC occurrences in the MD region were not statistically different from the nationwide distribution ( $\alpha \leq 0.05$ ;  $p = 0.457$ ). The parametric two-sample  $t$ -test with Cochran adjustment for unequal variances gave an equivalent result, with a statistically nonsignificant hypothesis test value of  $p \leq 0.1575$ .

The nationwide predictions made in this study may serve only as a first approximation of the occurrence and fate of TCC in U.S. water resources. Additional studies are required to confirm or refute the modeling results obtained here. Inclusion of TCC in existing water quality monitoring efforts would represent an important first step. In addition, further research is needed to better understand the environmental compartmentalization of TCC and its tendency for bioaccumulation in the food web. Although these and related important questions could not be addressed here, the present investigation did produce irrefutable evidence for the co-occurrence of TCC and TCS over a wide range of concentrations in six urban streams in the Greater Baltimore region. An additional noteworthy observation was that surface water

contamination with antimicrobial compounds is not necessarily the result of incomplete removal from sewage during municipal wastewater treatment, as demonstrated by the detection of TCC and TCS in all six urban streams investigated in this study. Sewage spills and leakage were identified as additional, potentially important inputs of antimicrobials to surface waters, causing TCC and TCS concentrations as high as 6750 and 1600 ng/L, respectively, in the Greater Baltimore area (Figures 4 and 5, Table 5).

Study results demonstrate that TCC has been an overlooked and under-reported toxic contaminant of U.S. water resources for a number of years and possibly for as long as half a century. Indeed, four industry-sponsored studies conducted between 1979 and 1987 (listed in ref 12) revealed low-level pollution of U.S. surface waters to be a national phenomenon—an important but not broadly acknowledged discovery. The magnitude and frequency of TCC contamination, determined in the present study, are reason for concern. According to experimental data and modeling results, TCC has an overall frequency of detection of 60% (55 of 91 streams total, including the detections reported here for MD), projected nationwide median and mean detectable concentrations of 109 and 213 ng/L, respectively, and a maximum concentration of 6750 ng/L. Consequently, TCC is expected to rank in the top 20 in maximum concentration, among 96 pharmaceuticals, hormones, and organic wastewater contaminants considered (13). This puts the antimicrobial on par with caffeine, a known and abundant chemical marker of sewage infiltration (6000 ng/L maximum concentration (13)). Furthermore, with respect to the frequency of detection (13), TCC likely is tied with TCS for fifth place behind coprostanol, cholesterol, *N,N*-diethyltoluamide, and caffeine.

Finally, the data collected in the present study are markedly higher than the more favorable numbers (<30% detection frequency;  $\leq 240$  ng/L maximum concentration (12)) currently used by the U.S. Environmental Protection Agency for evaluating the ecological and human health risks of this high production volume chemical. It is concluded that—after 48 years of production and environmental release—TCC is only beginning to receive the scientific scrutiny typically imparted on persistent polychlorinated aromatics of significant human and animal toxicity.

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