Environmental Science & Technology

Article

pubs.acs.org/est

Occurrence of Triclocarban and Triclosan in an Agro-ecosystem Following Application of Biosolids

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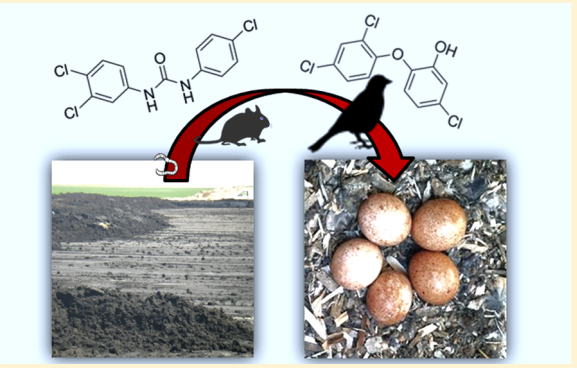
Supporting Information

ABSTRACT: Triclocarban (TCC) and triclosan (TCS), two of the most commonly used antimicrobial compounds, can be introduced into ecosystems by applying wastewater treatment plant biosolids to agricultural fields. Concentrations of TCC and TCS were measured in different trophic levels within a terrestrial food web encompassing land-applied biosolids, soil, earthworms (*Lumbricus*), deer mice (*Peromyscus maniculatus*), and eggs of European starlings (*Sturnus vulgaris*) and American kestrels (*Falco sparverius*) at an experimental site amended with biosolids for the previous 7 years. The samples from this site were compared to the same types of samples from a reference (biosolids-free) agricultural site. Inter-site comparisons showed that concentrations of both antimicrobials were higher on the experimental site in the soil, earthworms, mice (livers), and European starling eggs, but not American kestrel eggs, compared to the control site. Inter-species comparisons on the experimental site indicated significantly higher TCC concentrations in mice (TCC: 12.6−33.3 ng/g) and in starling eggs (TCC: 15.4−31.4 ng/g) than in kestrel eggs (TCC: 3.6 ng/g). Nesting success of kestrels only was significantly lower on the experimental site compared to the reference site due to nest abandonment. This study demonstrates that biosolids-derived TCC and TCS are present throughout the terrestrial food web, including secondary (e.g., starlings) and tertiary (i.e., kestrels) consumers, after repeated, long-term biosolids application.



INTRODUCTION

Triclocarban (TCC) and triclosan (TCS) are two widely used antimicrobial compounds that have been ingredients in personal care products for decades.[1] The accumulation of these antimicrobials in biosolids produced at wastewater treatment plants (i.e., treated sewage sludge), and subsequently environmental systems, raises concern for both humans and wildlife. In the terrestrial environment, TCC and TCS have been observed to accumulate in plants and earthworms inhabiting soils amended with biosolids, and the overuse of antimicrobial products is reported to result in the presence of antibiotic-resistant bacteria in soil.[2−5]

Both TCC and TCS are antimicrobial chemicals introduced into the environment primarily through human activities. When products containing TCC or TCS are used, the antimicrobials wash into sewer systems and enter the wastewater treatment process. TCC and TCS are incompletely removed during such wastewater treatment processes.[6] Much of the removal can be attributed to partitioning of TCC and TCS to carbon-rich biosolids during treatment.[1,4] As a result, relatively high concentrations of TCC (50 000 ± 15 000 ng/g) and TCS (30 000 ± 11 000 ng/g) are frequently detected in biosolids.[7,8]

In the United States, approximately 8 million dry metric tons of biosolids are produced annually, about half of which is applied to land as fertilizer, and the other half is either incinerated or deposited in landfills.[9] Biosolids are classified as treated municipal solid waste that must meet regulatory standards primarily for pathogen and metal content, but are not regulated or monitored for TCC and TCS concentrations.[10] When biosolids containing TCC and TCS are applied to agricultural fields, both antimicrobial compounds

Received: April 13, 2016
Revised: October 28, 2016
Accepted: November 6, 2016
Published: November 7, 2016

DOI: 10.1021/acs.est.6b01834
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

have been detected in the soil.[4] Several studies demonstrate that the fate of TCC and TCS includes accumulation at lower trophic levels in the ecosystems exposed to biosolids, including food crops (e.g., soybean), meadow fescue (*Festuca pratense*), and earthworms (*Lumbricus*).[3,11,12] TCC and TCS have been detected in samples from organisms at higher trophic levels, including aquatic vertebrates, and in the breast milk, plasma, and urine of humans.[13,14]

In addition to concerns raised by the detection of antimicrobials across trophic levels, elevated concentrations of antimicrobials can alter the physiology of animals. Exposure to TCS resulted in a decrease in total serum thyroxine concentrations in Long−Evans female rats (*Rattus norvegicus*) and altered skeletal muscle function in fathead minnows (*Pimephales promelas*).[15,16] Elevated TCC and TCS concentrations have also been shown to be highly toxic to Japanese rice fish (*Oryzias latipes*) during their early life stages and, in the presence of testosterone, can enhance androgenic effects in these fish.[17]

Despite known accumulation in ecosystems and the negative physiological consequences of exposure to TCC and TCS in some organisms, these antimicrobials have not been adequately studied in higher trophic levels in natural systems. For the purposes of this study, we investigated a terrestrial food web encompassing biosolids, soil, earthworms (primary consumer), deer mice (*Peromyscus maniculatus*, secondary consumer), European starlings (*Sturnus vulgaris*, a secondary consumer of invertebrates), and American kestrels (*Falco sparverius*, a tertiary consumer of rodents, small birds, and invertebrates).[18,19] The objectives of this study were to (a) determine the concentrations of the antimicrobials, TCC and TCS, in biosolids, soil, earthworms, deer mice, and eggs of the European starling and the American kestrel collected from a biosolids-amended experimental agricultural site and a reference, biosolids-free agricultural site, and (b) determine if there were correlative relationships between concentrations of TCC and TCS and reproductive end points, specifically egg viability and nesting success, of the two avian species examined. We hypothesized that substrates and organisms at the experimental site would contain higher concentrations of TCC and TCS than those from the reference site due to the application of biosolids. We also hypothesized that kestrels would have higher concentrations of TCC and TCS than starlings because they occupy a higher trophic level than starlings. In addition, we hypothesized that exposure to TCC and TCS would be associated with lower egg viability and lower nesting success by these birds. To our knowledge, this is the first study that focuses on the presence and potential consequences of TCC and TCS in higher trophic levels of a terrestrial food web.

EXPERIMENTAL SECTION

Study Areas. The study area consisted of an experimental site (i.e., biosolids-applied site) and a reference site (i.e., biosolids-free site). These sites were separated by approximately 10 km. The reference site is located near Nampa, Idaho, in Ada and Canyon Counties (43°32′83″ N, 116°27′31″ W, elevation 797 m, Figure S1a,b). This reference site consisted of an area of 85 km^2, including agricultural and residential development, that has never received biosolids according to county records. Within the reference site, we monitored 74 wooden nest boxes (Figure S1b) mounted at approximately 2.5 m high on poles, 20 of which were used by American kestrels and 22 by European starlings, over two field seasons.

The experimental site was a 16 km^2, municipal-owned farm located near Kuna, Idaho, in Ada County (43°23′38″N, 116°17′87″W, elevation 866 m, Figure S1a,c). The experimental site currently uses dewatered and anaerobically digested municipal biosolids from the Ada County Wastewater Treatment Facility (WWTF) as fertilizer for alfalfa, corn, and winter wheat used for livestock feed. The Ada County WWTF treats wastewater primarily by secondary treatment processes from Boise, Idaho (population: 205 671), a city that produces approximately 1.1×10^8 L of wastewater per day. When this study was conducted, the experimental site had a continuous, 7-year history of biosolids application for certain plots within the site. All fields within the experimental site were tested for heavy metals. When concentrations of heavy metals were below county standards, biosolids were applied to those fields. Biosolids were not applied to all fields every year, in response to annual variation in heavy metal content measured in soil. Biosolids were applied at a rate of approximately 3 tons per acre (673 kg/1000 m^2, 0.64-cm-thick layer covering approximately 50% of the soil surface), and that was then incorporated via disc harrow into the soil to a depth of 20 cm. Given the variation in history of biosolids application on the experimental site, we selected and sampled fields receiving biosolids representative of the median historical number of applications for the experimental site. There were 20 nest boxes located on the experimental site that were within 1 km of the plots amended with biosolids. Of the 20 nest boxes, five were used by American kestrels and six were used by European starlings.

Biosolids. A composite biosolids sample, consisting of nine independent samples weighing 225 g each, was collected in March 2012 from one of the two open-air concrete holding areas at the experimental site prior to application of the biosolids to the agricultural fields (Figure S1c). Biosolids holding areas store large volumes of biosolids, and each holding area contained biosolids from the same source. Biosolids sampling involved using a clean shovel to break the thick outer-crust on the biosolids, and then using a hexane-rinsed stainless steel spoon to collect each sample. The collected samples were spaced 2 m apart. Samples were pooled, homogenized to be representative of that applied on the fields, and stored in glass jars sealed with foil-lined plastic lids at −20 °C until analysis. This protocol is in accordance with the regulated collection protocol used by the municipal-owned farm.[20]

Soil. Soil from the experimental site was also sampled in March 2012 prior to application of the biosolids, and it was resampled in May 2012 approximately 2 weeks after the biosolids had been applied and incorporated into the soil. Soil from the reference site was sampled in March 2012, and it was resampled in June 2012 to correspond with the sample collections from the experimental site. Areas where soil was sampled at each site were chosen primarily to maintain a consistent soil type (fine, silty, mixed-mesic, Xerollic Haplargids),[21] and secondarily because they were within 1 km of nest boxes used by breeding American kestrels and European starlings (Figure S1b,c). Three samples within the experimental site (N = 3) or reference site (N = 3) were collected at a depth of 20 cm and approximately 40 m apart (Figure S1b,c).[20] The three samples within each site were subsequently pooled and mixed with a hexane-rinsed stainless steel spoon to create a single sample that was representative of the soil throughout each site. A total of 225 g of each pooled

DOI: 10.1021/acs.est.6b01834
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

sample was retained, placed in a glass jar with a foil-lined plastic lid, and stored at −20 °C prior to analysis.

Earthworms. Earthworms (*Lumbricus*), a common food source for European starlings and deer mice, were collected from the experimental and reference sites in June 2012 after biosolids application had occurred and following a suitable exposure period (Figure S1b,c). We used a composite sample of earthworms as representative of that consumed by the starlings. Approximately 30 g of earthworms were collected from the experimental and reference sites in the same general areas from which soil samples were collected. Earthworms were pooled within each site, brought back to the laboratory, and rinsed with deionized water to remove soil residues from their surfaces. The earthworms were not allowed to void their guts prior to storage and analysis so as to be representative of earthworms consumed by higher trophic organisms. The pooled earthworms were then weighed, placed in glass jars with foil-lined plastic lids, and stored at −20 °C prior to analysis.

Deer Mice. Deer mice, a common food source for American kestrels, were collected in June 2012 (Boise State University IACUC permit no. 006-AC11-005).[19] Kill-traps, located approximately 100 m from the nearest nest box, were used to collect 11 deer mice from the reference site and 28 deer mice from the experimental site. Mice were collected and stored intact in glass jars with foil-lined plastic lids at −20 °C until dissection. Deer mice were thawed, weighed, and sexed before dissection. The liver and skeletal muscles were excised from each individual. Each tissue sample was individually weighed, wrapped in aluminum foil, placed in a glass vial, and stored at −20 °C prior to analysis. Of the 39 mice samples collected, matched liver and muscle tissues from the same 10 mice ($N = 7$ experimental site; $N = 3$ reference site) were analyzed for TCC and TCS concentrations.

Eggs. As it is difficult to determine the order in which eggs have been laid, one egg was selected at random from each complete clutch of ≥3 eggs from randomly selected nests of the starlings and kestrels on the experimental and reference sites during the spring of 2011 and 2012 (Boise State University IACUC permit no. 006-AC11-005). All egg collections occurred during the same time period using the same protocol on both the experimental and reference sites. The eggs were stored at 5 °C until they were prepared for freezing. Of the eggs collected, only eggs collected in 2012 were used for the measurement of TCC and TCS content to coincide with collection of other samples. Ten kestrel eggs (5 per site) and 12 starling eggs (6 per site) were analyzed for TCC and TCS concentrations.

Egg Measurements. Each egg was weighed to the nearest milligram, and the length and breadth were measured to the nearest millimeter using a Reid digital caliper (model no. K309A.W-1230). Eggs were dissected along the equator, and the mass of the egg contents was measured. Egg contents were stored at −60 °C in sterilized glass vials with aluminum foil-lined lids. After these egg measurements were recorded, egg shells were boiled (7 min for American kestrel eggs, 4 min for European starling eggs) in deionized water to remove the inner membrane. Egg shells were allowed to dry, and then egg shell mass was measured. Egg shell thickness was measured using a dial indicator gauge mounted on a bench comparator.[22]

Egg volume of the American kestrel was calculated using length (L) and breadth (B) measurements in the equation:[23]

$$(0.6057 - 0.0018B) \times L(B^2) \quad (1)$$

Egg volume of the European starling was calculated using length (L) and breadth (B) measurements in the equation:[24]

$$(0.035 + 0.530) \times L(B^2) \quad (2)$$

Nest Success. All nests were monitored on a weekly basis, and the numbers of eggs and nestlings were recorded. Nest box occupancy rates by species were calculated and compared between the two study sites. We used the standard nesting success assessment protocol to classify nest success.[25] A nest was considered to be successful when at least one nestling within the brood had reached 80% of the average age of when nestlings fledge from their nest; for American kestrels, this would be when at least one chick within the brood had reached 22 d of age, while for European starlings, it would be 16−17 d of age.

Biosolids, Soil, and Earthworm Extraction of TCC and TCS. Following Kinney et al.,[12,26] biosolids, soil, and earthworm samples were prepared in triplicate for TCC and TCS quantification using pressurized liquid extraction (PLE; Dionex-ASE100, Dionex Corp., Sunnyvale, CA) followed by quantitative analysis by liquid chromatography/mass spectrometry (LC/MS; Shimadzu LCMS 2010A). Method performance data are available in Table S1. TCC and TCS concentrations for earthworms, mice liver and muscle, and bird eggs are reported on a wet weight (ww) basis.

Deer Mouse Tissue Extraction for TCC and TCS. The method for extracting TCC and TCS from liver and muscle tissue was developed to incorporate sample extraction and cleanup into a single pressurized liquid extraction (PLE) step,[27] and the samples were analyzed using isotope dilution LC/MS analysis. For the extraction of liver and muscle tissue, the high-pressure extraction cell (66 mL) was loaded with silica (7 g, 7:1 silica:tissue ratio by mass). Tissue samples were first homogenized with Na_2SO_4. The homogenate tissue sample was then placed on top of the sorbent, and the isotope-labeled internal standards (50 μL each of 2 μg/mL $^{13}C_{13}$-TCC and $^{13}C_{12}$-TCS solutions) were added. Any remaining void volume was filled with ashed Ottawa sand (400 °C, 4 h). TCC and TCS were extracted from the liver homogenate using a 1:1 dichloromethane:ethyl acetate solution. TCC and TCS were extracted from the muscle homogenate using a 3:1:1 dichloromethane:acetonitrile:methanol solution. All tissue homogenates were extracted by PLE at 100 °C and 10 300 kPa for one static cycle at 5 min. The resulting extracts were evaporated to dryness under nitrogen at 70 °C (Labconco RapidVap N2) and then reconstituted with 1 mL of acetonitrile:water (75:25). The samples were stored at 5 °C until analysis by LC/MS. As a measure of quality control, spiked (TCC and TCS fortified ashed sand) and blank (ashed sand only) samples were extracted and included with each set of 10 tissue samples.

Egg Extraction for TCC and TCS. Egg samples were extracted using a modified liquid−liquid extraction method prior to quantification using isotope-dilution LC/MS analysis. Approximately 3 g of egg was homogenized by blender and transferred to an ashed glass centrifuge tube. Isotope-labeled internal standards (50 mL each of $^{13}C_{13}$-TCC, 2 μg/mL^{-1}; $^{13}C_{12}$-TCS, 2 μg mL^{-1}) were added to the homogenized egg sample. TCC and TCS were extracted from the egg homogenate using 6 mL of a 75:25 dichloromethane:methanol solution. The sample−solvent mixture was vigorously mixed by

DOI: 10.1021/acs.est.6b01834
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Table 1. Concentrations of Triclocarban (TCC) and Triclosan (TCS) in Abiotic and Biotic Samples Collected at Different Trophic Levels in 2012 from the Experimental Site Amended with Municipal Biosolids for 7 Years and the Reference Site in 2012

sample	concentration (ng/g ww)[a]	
	TCC	TCS
Biosolids (n = 1)	1250[c]	1230[c]
Soil — Reference Site[b]		
pre-application (n = 1)[b]	ND[c]	ND[c]
post-application (n = 1)[b]	ND[c]	ND[c]
Soil — Experimental Site		
pre-application (n = 1)	14.8[c]	4.4[c]
post-application (n = 1)	27.3[c]	2.7[c]
Earthworms[d]		
reference (n = 1)	ND[c]	5.9[c]
experimental (n = 1)	5.1[c]	42.8[c]
Deer Mice — Liver[d]		
reference (n = 3)	ND	ND
experimental (n = 7)	**17.0** (12.6–33.3) [20.0 ± 4.6]	**3.3** (6.6–10.7) [5.0 ± 1.2][e]
Deer Mice — Muscle[d]		
reference (n = 3)	ND	ND
experimental (n = 7)	ND	ND
European Starling Eggs[d]		
reference (n = 6)	**4.6** (3.6–5.0) [3.9 ± 0.7][e]	**5.8**[f]
experimental (n = 6)	**23.2** (15.4–31.4) [20.4 ± 4.4][e]	**13.7** (9.4–37.9) [16.3 ± 5.1][e]
American Kestrel Eggs[d]		
reference (n = 5)	**3.3**[g]	**4.6** (4.1–5.4) [4.0 ± 0.8][e]
experimental (n = 5)	**3.6**[g]	**4.2** (4.2–13.4) [5.4 ± 2.2][e]

[a]Medians (bold), min–max (in parentheses), and mean ± SEM (in brackets) are presented when appropriate; means and standard errors about the mean (SEM) are presented in Figure 1. ND = not detected. [b]Biosolids were not applied to the reference site. [c]Values represent the average of triplicate measurements of a composite sample from three independent locations. [d]Reported values are on a wet weight basis. [e]For the purposes of statistical analysis, values that were positively detected (correct retention time, ions, and ion ratios) but below the method detection limit (MDL) were assigned half the MDL value (TCC: 1.35 ng/g ww for eggs; TCS: 1.8 ng/g ww for mice liver and 1.65 ng/g ww for eggs). [f]One out of 6 eggs had a detectable level of TCS; the others had nondetectable concentrations. [g]Reference site eggs had 4 out of 5 detections of TCC. Only one was above the MDL. Experimental site eggs had 5 out of 5 detections of TCC. Only one was above the MDL.

hand. After mixing, the sample was centrifuged (5000 rpm for 5 min) to facilitate separation of the solvent and egg. The solvent layer was removed, transferred to ashed evaporation glassware, and evaporated to dryness at 70 °C under a gentle stream of nitrogen (Labconco RapidVap N2). The extract was reconstituted with 6 mL of acetonitrile. To limit the presence of lipids in the final extract, the sample was washed with 3.5 mL of n-hexane. Following addition of the n-hexane, the sample was placed on a vortex mixer for 30 s. The hexane layer was removed and discarded. The extract was again evaporated to dryness at 70 °C under nitrogen and reconstituted with 1 mL of 75:25 acetonitrile:water. As a measure of quality control, spiked ("organic" chicken egg fortified with TCC and TCS) and blank ("organic" chicken egg) samples were prepared and analyzed with every set of 10 egg samples.

Analytical QA/QC. At least one method spike and method blank sample were evaluated for each set of extractions and quantifications.[12,26] No detectable quantitites of TCC and TCS were observed in any of the method blanks analyzed in this study. Isotope-labeled internal standards were added to all samples, spikes, and blanks to correct for any differences in sample volume, as a marker of retention time for TCC and TCS, and to correct for deviations in method recoveries. In addition, the ratio of multiple characteristic ions for each compound and chromatographic retention times were compared to those of authentic standards for compound verification. The method performance for soil, biosolids, and earthworm samples had been previously assessed, including recovery of spiked sample (method and matrix spikes) as well as statistically determined method detection limits (MDLs) for each method.[5,12,26] Method recovery and MDLs for existing methods and those developed as part of this project are included in Table S1.

Liquid Chromatography/Mass Spectrometry Instrumental Analysis. TCC and TCS were quantified by high-performance liquid chromatography coupled with electrospray ionization/quadrupole mass spectrometry (HPLC/ESI/MS; Shimadzu 2010A LCMS, Shimadzu Scientific, Columbia, MD) operated in the negative-ion mode using selected-ion monitoring to improve sensitivity and minimize chemical interferences. Sample components were separated using a Synergi 4 μm hydro-RP 80A 50 mm × 4.6 mm column (Phenomenex).

Biota-to-Soil Accumulation Factors. Biota-to-soil accumulation factors (BSAFs) for TCC and TCS in the organisms included in this study were calculated as the ratio of the lipid-normalized concentration of TCC or TCS in the organism to the carbon-normalized concentration of TCC or TCS in the soil at the experimental site. The total lipid contents in the earthworms and deer mice liver and muscle tissues were determined using a method described previously.[28] Briefly, homogenized samples were extracted using 2:1 (v:v) chloroform:methanol. The extract mixture was centrifuged to promote solvent separation. The chloroform layer was removed

DOI: 10.1021/acs.est.6b01834
Environ. Sci. Technol. XXXX, XXX, XXX–XXX

and filtered through a 0.45 μm syringe filter. The total lipid content was determined gravimetrically. The total lipid content of the starling and kestrel eggs was determined using a method described previously,[29] which is similar to that used for the earthworms and deer mice tissues. Briefly, about 1 g of homogenized egg sample was extracted using 2:1 (v:v) chloroform:methanol overnight at 4 °C. The extract was filtered through a 0.45 μm syringe filter, and 4 mL of a 0.88% NaCl solution was added. Following phase separation, the chloroform layer was collected, and the total lipid content was determined gravimetrically.

Statistical Analysis. Positive detection of TCC and TCS measured in starling eggs and kestrel eggs at concentrations that were less than the MDL was assigned a value of half the MDL (TCC: 1.35 ng/g ww for eggs; TCS: 1.8 ng/g ww for mice liver and 1.65 ng/g ww for eggs) for statistical purposes only. The limitations of this commonly used approach have been recognized;[30] given the small numbers of results in this study, half MDL substitutions were used to provide limited summary statistics estimates. Residuals were analyzed using a Shapiro−Wilks Normality test and were not normally distributed, nor could they be log-transformed. Therefore, Kruskal−Wallis non-parametric ANOVA tests were performed, comparing TCC and TCS concentrations between the reference and experimental sites within each bird species (intra-species comparisons) to identify differences relating to the applications of the biosolids (e.g., comparing TCC concentrations in starling eggs from the reference site to those in starling eggs from the experimental site). Kruskal−Wallis ANOVA tests were also used to compare TCC or TCS concentrations among species on the same site (e.g., kestrel eggs vs starling eggs from the experimental site), to identify possible inter-species differences in exposure to these chemicals. When significant differences were found ($p < 0.05$) among the three species (mice, starlings, kestrels), Wilcoxon *post hoc* comparisons were performed. A contingency table was used to test for site differences in occupancy rates and nesting success of each species in 2011 (both avian species), and repeated for kestrels breeding in 2012. Spearman's rank correlations were used to identify significant correlations between TCC or TCS egg concentrations and egg size parameters collected in 2012. All statistical analyses were performed using SAS 9.4, except for the contingency tables that were analyzed using RStudio (version 2013, R Core Development Team). We report median, mean, range, and standard error of mean (SEM), when appropriate, and statistical significance was considered to be $p \leq 0.05$.

RESULTS AND DISCUSSION

TCC and TCS Concentrations. In general, the application of biosolids to agricultural fields on the experimental site yielded higher concentrations of TCC and/or TCS in soil, earthworms, the liver of deer mice, and starling eggs than the reference site (Table 1); this pattern, however, was not evident with the kestrel eggs. In the current study, the concentrations of TCC (1026−1472 ng/g ww) and TCS (1114−1350 ng/g ww) in the biosolids collected prior to application were generally lower than concentrations reported in most comparable studies where biosolids were applied to agricultural soil.[12,31,32] Although it is unknown if these concentrations were representative of TCC and TCS in the biosolids applied at the experimental site in previous years, Pycke et al.[33] demonstrated little variation in TCC and TCS concentrations in biosolids from by a single WWTF over more than 1 year of sample collection. Correspondingly, the concentrations of TCC (14.8−27.3 ng/g ww) and TCS (2.7−4.4 ng/g ww) in the soil collected from the experimental site for this study were at the lower range of concentrations reported for biosolids-amended soils.[12,31,32] The lower concentrations of TCC and TCS in the biosolids and soils in this study compared to other similar studies may result from differing inputs of antimicrobials and treatment methodologies among WWTFs, differential field application rates of biosolids, conditions of uptake or degradation, and different environmental conditions among studies.

Concentrations of TCS detected in earthworms from the experimental site in this study (5.9−42.8 ng/g ww) were 34-fold lower than concentrations detected in earthworms in a previous study,[12] which likely reflects the lower concentrations of TCS in the biosolids and soils in this study compared to previous research. TCC concentrations in earthworms at the experimental site in this study (5.1 ng/g ww) were 5−26 times lower than TCC concentrations previously reported in *Eisenia fetida* in a laboratory exposure study, which likely reflects lower TCC in the biosolids applied at the experimental site in this study compared to the laboratory exposure study.[4] In this study, the earthworms were not depurated so as to represent the potential exposure to TCC and TCS available to predators that consume earthworms (e.g., starlings). Here, the concentrations of TCS in the earthworms exceeded what was measured in the soil at the experimental site, while the opposite pattern occurred with TCC.

Both TCC (20.0 ± 4.6 ng/g) and TCS (5.0 ± 1.2 ng/g) were detected in the deer mice livers but not their muscle, and only in mice from the experimental site (Table 1). Since TCC and TCS are lipophilic, it is probable that very low amounts of both antimicrobials occurred in the muscle of these mice, but at concentrations below the limits of detection. The lipid content of deer mice liver was greater than that of deer mice muscle tissue (Table S2). There is limited, if any, information available for TCC or TCS concentrations measured in the tissues of free-ranging small mammals, including mice exposed to biosolids. Assuming toxicity thresholds would be similar between free-ranging mice and laboratory rodents, the TCS hepatic concentrations measured in the deer mice during this study (6.6−10.7 ng/g) were likely below the threshold for disrupting thyroid function that occurred in laboratory rats dosed with TCS concentrations of 35.6−300 mg/kg.[15,34] To our knowledge, this is the first study to detect TCC and TCS in wild rodents and suggests that accumulation of these antimicrobials by small mammals is a potential route of exposure to predators foraging on them (e.g., American kestrels). Overall, it is likely that total exposure to TCC and TCS accumulation of these compounds is underestimated in this work because common metabolites/degradation products of TCC and TCS known to be present in biosolids were not included as analytes in this project.[33]

Bioaccumulation of TCC and TCS. In an effort to assess the bioaccumulation of TCC and TCS in the biological samples included in this study, BSAFs were calculated (Table 2). With the exception of the BSAF for TCS in earthworms and starling eggs, all of the BSAFs were below a value of 1. This may suggest that direct exposure to soil was not a major source of TCC and TCS to organisms in this study other than earthworms and possibly starlings. The BSAFs for TCC (0.79) and TCS (67) in earthworms were estimates because

DOI: 10.1021/acs.est.6b01834
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Table 2. Biota-to-Soil Accumulation Factors (BSAFs) for Organisms from the Experimental Site

sample	TCC	TCS
earthworms	0.79[a]	67[a]
deer mouse liver	0.20	0.50
starling eggs	0.25	2.0
kestrel eggs	0.05	0.77

[a]BSAF estimated for earthworms because earthworms were not depurated as part of the experimental design.

the earthworms in this study were not depurated prior to TCC and TCS analysis. However, the concentration reported for TCS in the earthworms was substantially greater than that reported for the soil, which suggests any TCS in the gut contents were a minor component of TCS reported for earthworms. These estimated BSAFs for TCC and TCS in earthworms suggest greater bioavailability of TCS in soils compared to TCC. A comparison of BSAFs for TCC and TCS for the organisms analyzed in this study to other studies is limited. BSAFs for TCC in earthworm *Eisenia fetida* exposed to biosolids-amended soils were previously reported to range from 0.22 to 0.71.[4] The BSAF for TCC in earthworms of 0.79 in this study is similar to the values previously reported. In addition, a comparison of the bioaccumulation factor (BAF; ratio of concentration in the organism to the concentration in the soil) for TCS in earthworms can be made with previously reported values. In this study, the BAF for TCS in earthworms can only be estimated because the earthworms, by design of this study, were not depurated. The estimated BAF for TCS (15.9) is within the range previously reported (BAF = 10.9−40.1) for earthworms in biosolids-amended soils and demonstrates a similar relative uptake of TCS by earthworms at this field site.[35]

Assessing Intra-species Differences in TCC and TCS Concentrations: Avian Eggs. Generally, concentrations of TCC and TCS were detected in the eggs of both avian species collected from the experimental and reference sites; notably, TCS occurred at measurable concentrations in only one starling egg from the reference site, and TCC concentrations were below measurable concentrations in most of the kestrel eggs from both sites (Table 1). The detection of TCC and TCS in the starling and kestrel eggs from the reference site may reflect the mobility of the birds when foraging and/or the general ubiquitous occurrence of these antimicrobials in the environment.

Intra-species site comparisons indicated that the starling eggs from the experimental site had significantly higher concentrations of TCC than those from the reference site (X^2 = 3.7, df = 1, p = 0.054, Figure 1). Although the concentration of TCS in starling eggs at the experimental site exceeds the concentration of TCS detected in a single egg from the reference site by a factor of 1.6−6.5, the difference in concentrations was not statistically significant (p = 0.32, Table 1). The eggs of American kestrels collected from the experimental site had similar concentrations of TCS (p = 0.71), and very low concentration of TCC (p = 1.0), compared to the kestrel eggs from the reference site (Figure 1). The fact that starling eggs, but not kestrel eggs, from the experimental site had measurable and higher TCC and TCS concentrations may be related to differences in feeding strategies and prey choice between the two species. Starlings tend to forage in the same general area for most of the year, including directly in agricultural fields.[34] In contrast, kestrels forage more frequently in fallow land adjacent

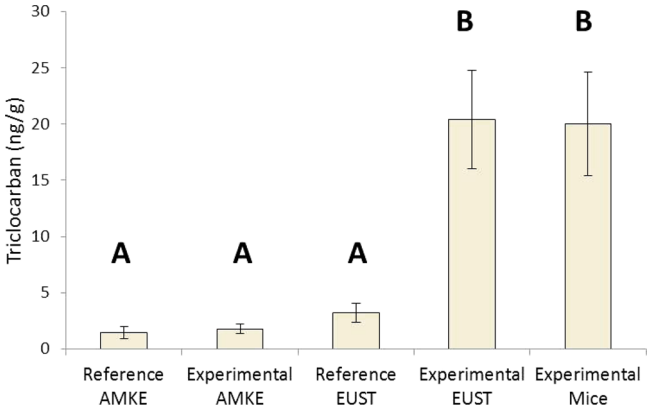


Figure 1. Arithmetic mean concentrations ± standard errors about the means (SEM) of triclocarban (TCC) in American kestrel (AMKE) and European Starling (EUST) eggs and mice tissue (liver) collected in 2012. Different letters depict significant differences between means using Wilcoxon non-parametric test.

to agricultural fields, and their territory size is more variable.[19] Consequently, starlings may more consistently feed in agricultural areas amended with biosolids throughout the year compared to kestrels.

Assessing Inter-species Differences in TCC and TCS concentrations. TCC and TCS concentrations were also compared between the two avian species on the experimental site, and then similarly on the reference site. The concentrations of TCS were similar in the kestrel and starling eggs from both study sites ($p \geq 0.12$). The concentrations of TCC in the kestrel eggs were significantly lower than those in the starling eggs collected on the experimental site (X^2 = 7.54, df =1, p = 0.02, Figure 1) and marginally lower on the reference site (X^2 = 3.68, df =1, p = 0.06). Starlings insert their beaks into the ground to retrieve various invertebrates and other prey,[36] and in so doing, they may simultaneously ingest soil particles containing TCC. In contrast, when feeding or capturing prey, kestrels do not come into direct contact with or ingest soil. That a similar, clear pattern was not observed with TCS may reflect the lower TCS concentrations in the soil at the experimental site compared to TCC. Alternatively, because species can vary in rates of metabolism,[37] it is also possible that kestrels metabolize or eliminate antimicrobials more efficiently than starlings.

The inter-species comparisons were expanded to include mice as well as the starling and kestrel eggs, but this was possible only on the experimental site since TCC and TCS were not detected in mice on the reference site (Figure 1 and Table 1). On the experimental study site, TCS concentrations remained similar among the mice, starling eggs, and kestrel eggs (p = 0.13), while concentrations of TCC differed significantly among the three species overall (X^2 = 7.54, df =2, p = 0.023). The TCC concentrations were similar between the mice and starling eggs (p = 0.669), but significantly higher in both species (mice: X^2 = 6.55, df = 1, p = 0.010; starling eggs: X^2 = 6.55, df = 1, p = 0.010) when compared to the kestrel eggs (Figure 1 and Table 1). In contrast to kestrels, starlings and mice come into direct contact with biosolids-amended soil: starlings through inserting their beaks to capture soil invertebrates, and mice by digging and burrowing in soil. This inter-species difference in direct contact with the soil may explain the greater concentrations of TCC in the mice and starling eggs compared to the kestrel eggs. More research is required to characterize

DOI: 10.1021/acs.est.6b01834
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Table 3. Number of Nests for American Kestrels and European Starlings Breeding on the Experimental Site and Reference Site in 2011 and 2012[a]

species	site	year	unsuccessful nesting attempt	successful nesting attempt	nesting success (%)	mean no. of fledglings per successful nesting attempt
American kestrel	reference	2011	12	6	50	3.2
	experimental	2011	10	1	10	2.0
	reference	2012	12	6	50	3.3
	experimental	2012	6	1	17	2.0
European starling	reference	2011	6	6	100	2.5
	experimental	2011	6	5	83	2.5

[a]A nesting attempt was classified as unsuccessful if a full clutch was observed but no nestlings grew to be 20 days old (the equivalent of 80% of the average age at first flight). A nesting attempt was deemed successful only if at least one nestling reached 20 days or 80% of the average age at first flight. Nesting success information was not collected for European starling nests in 2012.

and determine the relative importance of soil contact and diet as routes of exposure to TCC by vertebrates.

Concentrations of Antimicrobials in Relation to Egg and Reproductive Measures. The results of this study indicate that both bird species were exposed to and accumulated TCC and TCS since both antimicrobials were found in detectable concentrations in their eggs. However, there was no evidence to suggest that the concentrations of TCC or TCS were correlated with the egg size or egg shell thickness variables of either species (all p-values ≥ 0.21, Table S3), which were similar to measurements previously reported for these species.[18,19] Nor was there a significant difference in nest success between experimental and reference sites for starlings ($p = 0.30$) (Figure 1 and Table 3). However, in 2012, nesting success of kestrels was significantly lower on the experimental site (10% and 17% success rates in 2011 and 2012, respectively) compared to that of the kestrels on the reference site (50% success rate, $p = 0.015$, Table 3). The sample size in our study was small, so caution should be used when interpreting these results. Nevertheless, nesting success rates of 10% and 17% at the experimental site were substantially lower than the average rate of 51% determined in a concurrent study of 102 American kestrel pairs conducted in the same county as our study.[38]

A combination of multiple stressors may have contributed to the reduced nesting success of the kestrels observed at the experimental site in our study.[39] The kestrels' exposure to the low concentrations of TCC and TCS observed was unlikely to be a contributing factor, since they were far below known toxic levels for aquatic invertebrates and fish.[40−42] The possible toxicity of TCC and TCS to terrestrial wildlife species remains unknown and warrants further investigation. The reduced rate of nesting success by kestrels on the experimental site was principally due to nest abandonment. The underlying causes of nest abandonment were unclear but could be influenced by exposure to other contaminants that are known to be commonly present in biosolids but not analyzed in this study, disturbance caused by different farming practices, or other experimental site-specific conditions that determine nesting success (e.g. the availability and quality of prey), thus determining foraging behavior.

With reports of antimicrobial concentrations of 30 000 ± 11 000 ng/g dw in biosolids from the mid-Atlantic United States,[8] together with the potential for toxicity of these antimicrobials to invertebrates and vertebrates, it is important to continue to monitor TCC and TCS in biosolids and food webs receiving biosolids.[12−14] This study demonstrates that, following extended application of municipal biosolids containing TCC and TCS to an agricultural field (at concentrations lower than reported in other studies[12,31,32]), concentrations of these two antimicrobials were measured in each trophic level of the terrestrial food web examined. Antimicrobials were detected in soil as well as in primary (earthworms), secondary (deer mice, starlings), and tertiary (kestrels) consumers. Furthermore, the TCC and TCS concentrations in these trophic levels reflect extended application of biosolids to agricultural soils for 7 years: concentrations were higher in biosolids, soil, deer mice livers, and starling eggs at the experimental site than at the reference site. Trophic level differences in concentrations of these two antimicrobials may reflect differences in feeding behavior and strategies between species. The concentrations of TCC and TCS measured in the current study were not correlated with egg parameters of either avian species, although the kestrels demonstrated lower reproductive success on the experimental site. Understanding the toxicokinetics of these and other antimicrobials, and identifying possible effects of TCC and TCS, in wild animals (e.g., mice, birds) requires further investigation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b01834.

Analytical performance parameters and detection limits; Tables S1−S3, listing data describing the egg morphometric measurements of American kestrel eggs and European starling eggs from the experimental and reference sites; Figure S1, showing maps of the study sites (PDF)

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Notes

The authors declare no competing financial interest.

[#]It is with great sadness that we note that our colleague and coauthor, Dr. Alfred M. Dufty, Jr., passed away before the publication of this article.

DOI: 10.1021/acs.est.6b01834
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■ ACKNOWLEDGMENTS

This work was supported by funding from a Colorado State University−Pueblo Seed Grant and funding from the College of Science and Mathematics; support from the U.S. Geological Survey Toxic Substances Hydrology Program, Sigma Xi Grant-In-Aid of Research, Raptor Research Center and the Department of Biological Sciences at Boise State University, and Environment and Climate Change Canada. The use of trade, firm, or brand names in this paper is for identification purposes only and does not constitute endorsement by the authors or the U.S. Geological Survey.

■ REFERENCES

(1) Halden, R.; Paull, D. Co-occurrence of triclocarban and triclosan in U.S. water resources. *Environ. Sci. Technol.* **2005**, *39*, 1420−1426.
(2) Kemper, N. Veterinary antibiotics in the aquatic and terrestrial environment. *Ecol. Indic.* **2008**, *8* (1), 1−13.
(3) Wu, C.; Spongberg, A. L.; Witter, J. D.; Fang, M.; Czajkowski, K. P. Uptake of pharmaceutical and personal care products by soybean plants from soils applied with biosolids and irrigated with contaminated water. *Environ. Sci. Technol.* **2010**, *44*, 6157−6161.
(4) Higgins, C. P.; Paesani, Z. J.; Abbott Chalew, T. E.; Halden, R. U.; Hundal, L. S. Persistence of triclocarban and triclosan in soils after land application of biosolids and bioaccumulation in *Eisenia foetida*. *Environ. Toxicol. Chem.* **2011**, *30*, 556−563.
(5) Kinney, C. A.; Furlong, E. T.; Werner, S. L.; Cahill, J. D. Presence and distribution of wastewater-derived pharmaceuticals in soil irrigated with reclaimed water. *Environ. Toxicol. Chem.* **2006**, *25*, 317−326.
(6) Chalew, T. E.; Halden, R. Environmental exposure of aquatic and terrestrial biota to triclosan and triclocarban. *J. Am. Water Resour. Assoc.* **2009**, *45* (1), 4−13.
(7) Heidler, J.; Sapkota, A.; Halden, R. Partitioning, persistence, and accumulation in digested sludge of the topical antiseptic triclocarban during wastewater treatment. *Environ. Sci. Technol.* **2006**, *40*, 3634−3649.
(8) Heidler, J.; Halden, R. U. Mass balance assessment of triclosan removal during conventional sewage treatment. *Chemosphere* **2007**, *66*, 362−369.
(9) *Emerging Technologies for Biosolid Management*, EPA-832/R-06-005; U.S. Environmental Protection Agency: Washington, DC, 2006; http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P1006DGM.txt.
(10) *Primer for Municipal Wastewater Treatment Systems*, EPA-832/R-04-001; U.S. Environmental Protection Agency: Washington, DC, 2004; https://www.epa.gov/sites/production/files/2015-09/documents/primer.pdf.
(11) Macherius, A.; Eggen, T.; Lorenz, W. G.; Reemtsma, T.; Winkler, U.; Moeder, M. Uptake of galaxolide, tonalide, and triclosan by carrot, barley, and meadow fescue plants. *J. Agric. Food Chem.* **2012**, *60*, 7785−7791.
(12) Kinney, C. A.; Furlong, E. T.; Kolpin, D. W.; Burkhardt, M. R.; Zaugg, S. D.; Werner, S. L.; Bossio, J. P.; Benotti, M. J. Bioaccumulation of pharmaceuticals and other anthropogenic waste indicators in earthworms from agricultural soil amended with biosolids or swine manure. *Environ. Sci. Technol.* **2008**, *42*, 1863−1870.
(13) Fair, P. A.; Lee, H.-B.; Adams, J.; Darling, C.; Pacepavicius, G.; Alaee, M.; Bossart, G. D.; Henry, N.; Muir, D. Occurrence of triclosan in plasma of wild Atlantic bottlenose dolphins (*Tursiops truncates*) and in their environment. *Environ. Pollut.* **2009**, *157* (8-9), 2248−2254.
(14) Adolfsson-Erici, M.; Pettersson, M.; Parkkonen, J.; Sturve, J. Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. *Chemosphere* **2002**, *46*, 1485−1489.
(15) Crofton, K. M.; Paul, K. B.; DeVito, M. J.; Hedge, J. M. Short-term *in vivo* exposure to the water contaminant triclosan: Evidence for disruption of thyroxine. *Environ. Toxicol. Pharmacol.* **2007**, *24*, 194−197.
(16) Fritsch, E. B.; Connon, R. E.; Werner, I.; Davies, R. E.; Beggel, S.; Feng, W.; Pessah, I. N. Triclosan impairs swimming behavior and alters expression of excitation-contraction coupling proteins in fathead minnow (*Pimephales promelas*). *Environ. Sci. Technol.* **2013**, *47*, 2008−2017.
(17) Ishibashi, H.; Matsumura, N.; Hirano, M.; Matsuoka, M.; Shiratsuhki, H.; Ishibashi, Y.; Takao, Y.; Arizono, K. Effects of triclosan on the early life stages and reproduction of medake (*Oryzias latipes*) and induction of hepatic vitellogenin. *Aquat. Toxicol.* **2004**, *67* (2), 167−179.
(18) Cabe, P. R. European Starling (*Sturnusvulgaris*). *The Birds of North America Online*; Poole, A., Ed.; Cornell Lab of Ornithology: Ithaca, NY, 1993; http://bna.birds.cornell.edu/bna/species/048, DOI: 10.2173/bna.48.
(19) Smallwood, J. A.; Bird, D. M. American Kestrel (*Falcosparverius*). *The Birds of North America Online*; Poole, A., Ed.; Cornell Lab of Ornithology: Ithaca, NY, 1993; http://bna.birds.cornell.edu/bna/species/602, DOI: 10.2173/bna.602.
(20) Field Soil Sampling Plan - Twenty Mile South Biosolids Application Site, Boise City Public Works, August 2010.
(21) Natural Resources Conservation Service. Soil Survey Geographic (SSURGO) database for Ada County (ID001) and Canyon County (ID665), 2012; http://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx (accessed Aug 11, 2016].
(22) Kiff, L. F., Sumida, S. Private communication. 2012.
(23) Narushin, V. G. Egg geometry calculation using the measurements of length and breadth. *Poult. Sci.* **2005**, *84*, 482−484.
(24) Ricklefs, R. E. Variation in the size and composition of eggs of the European Starling. *Condor* **1984**, *86*, 1−6.
(25) Steenhof, K.; Newton, I.Assessing nesting success and productivity. In *Raptor Research and Management Techniques*; Bird, D. M., Bildstein, K. L., Barber, D. R., Zimmerman, A., Eds.; Hancock House Pub Ltd.: Surrey, Canada, 2007.
(26) Kinney, C. A.; Furlong, E. T.; Zaugg, S. D.; Burkhardt, M.; Werner, S. L.; et al. Survey of organic wastewater contaminants in biosolids destined for land application. *Environ. Sci. Technol.* **2006**, *40*, 7207−7215.
(27) Subedi, B.; Usenko, S. Enhanced pressurized liquid extraction technique capable of analyzing polychlorobenzo-p-diozins, polychlorodibenzofurans, and polychlorobiphenyls in fish tissue. *J. Chromatogr. A* **2012**, *1238*, 30−37.
(28) Randall, R. C.; Lee, H., II; Ozretich, R. J.; Lake, J. L.; Pruell, R. J. Evaluation of selected lipid methods for normalizing pollutant bioaccumulation. *Environ. Toxicol. Chem.* **1991**, *10*, 1431−1436.
(29) Cherian, G.; Traber, M. G.; Goeger, M. P.; Leonard, S. W. Conjugated linoleic acid and fish oil in laying hen diets: Effects on egg fatty acids, thiobarbituric acid reactive substances, and tocopherols during storage. *Poult. Sci.* **2007**, *86*, 953−958.
(30) Helsel, D. R. Summing nondetects: Incorporating low-level contaminants in risk assessment. *Integr. Environ. Assess. Manage.* **2010**, *6*, 361−366.
(31) Cha, J.; Cupples, A. M. Detection of the antimicrobials triclocarban and triclosan in agricultural soils following land application of municipal biosolids. *Water Res.* **2009**, *43*, 2522−2530.
(32) Xia, K.; Hundal, L. S.; Kumar, K.; Armbrust, K.; Cox, A. E.; Granato, T. C. Triclocarban, triclosan, polybrominated diphenyl ethers, and 4-nonylphenol in biosolids and in soil receiving 33-year biosolids application. *Environ. Toxicol. Chem.* **2010**, *29*, 597−605.
(33) Pycke, B. F.; Roll, I. B.; Brownawell, B. J.; Kinney, C. A.; Furlong, E. T.; Kolpin, D. W.; Halden, R. U. Transformation products and human metabolites of triclocarban and triclosan in sewage sludge across the United States. *Environ. Sci. Technol.* **2014**, *48*, 7881−7890.
(34) Zorrilla, L. M.; Gibson, E. K.; Jeffay, S. C.; Crofton, K. M.; Setzer, W. R.; Cooper, R. L.; Stoker, T. E. The effects of triclosan on puberty and thyroid hormones in male Wistar rats. *Toxicol. Sci.* **2009**, *107*, 56−64.
(35) Kinney, C. A.; Furlong, E. T.; Kolpin, D. W.; Zaugg, S. D.; Burkhardt, M. R.; Bossio, J. P.; Werner, S. L. Earthworms: Diagnostic indicators of wastewater derived anthropogenic organic contaminants

DOI: 10.1021/acs.est.6b01834
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

in terrestrial environments. In *Contaminants of Emerging Concern in the Environment: Ecological and Human Health Considerations*, ACS Symposium Series *1048*; Halden, R. U., Ed.; Oxford University Press: Ontario, Canada, 2011; Chapter 14.
(36) Caccamise, D. F. European starling fidelity to diurnal activity centers: role of foraging substrate quality. *Wilson Bull.* **1991**, *103*, 13−24 (https://sora.unm.edu/sites/default/files/journals/wilson/v103n01/p0013-p0024.pdf).
(37) Boĭko, S. S.; Zherdev, V. P.; Kisliak, N. A.; Piatin, B. M.; Klimova, N. V.; Glan, P. V.; Dvorianinov, A. A. Interspecific differences in Kemantan pharmacokinetics. *Eksp. Klin. Farmakol.* **1993**, *57*, 48−50 (PMID: 7756963).
(38) Heath, J. A. Private communication. 2014.
(39) Sih, A.; Bell, A. M.; Kerby, J. L. Two stressors are far deadlier than one. *Trends. Ecol. Evol.* **2004**, *19*, 274−276.
(40) Lawrence, J. R.; Zhu, B.; Swerhone, G. W.; Roy, J.; Wassenaar, L. I.; Topp, E.; Korber, D. R. Comparative microscale analysis of the effects of triclosan and triclocarban on the structure and function of river biofilm communities. *Sci. Total Environ.* **2009**, *407*, 3307−3316.
(41) Harada, A.; Komori, K.; Nakada, N.; Kitamura, K.; Suzuki, Y. Biological effects of PPCPs on aquatic lives and evaluation of river waters affected by different wastewater treatment levels. *Water Sci. Technol.* **2008**, *58*, 1541−1546.
(42) Ciba. Irgasan DP 300/Irgacare MP: Toxicological and ecological data/Official registration, Brochure no. 2521; Ciba Specialty Chemicals: Basel, Switzerland, 1998.

DOI: 10.1021/acs.est.6b01834
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