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ENVIRONMENTAL EXPOSURE OF AQUATIC AND TERRESTRIAL BIOTA TO TRICLOSAN AND TRICLOCARBAN[1]

Talia E. A. Chalew and Rolf U. Halden[2]

ABSTRACT: The synthetic biocides triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) and triclocarban (3,4,4′-trichlorocarbanilide) are routinely added to a wide array of antimicrobial personal care products and consumer articles. Both compounds can persist in the environment and exhibit toxicity toward a number of biological receptors. Recent reports of toxicological effects in wildlife, human cell cultures, and laboratory animals have heightened the interest in the occurrence of these biocide and related toxic effects. The present study aimed to summarize published environmental concentrations of biocides and contrast them with toxicity threshold values of susceptible organisms. Environmental occurrences and toxicity threshold values span more than six orders of magnitude in concentration. The highest biocide levels, measured in the mid parts-per-million range, were determined to occur in aquatic sediments and in municipal biosolids destined for land application. Crustacea and algae were identified as the most sensitive species, susceptible to adverse effects from biocide exposures in the parts-per-trillion range. An overlap of environmental concentrations and toxicity threshold values was noted for these more sensitive organisms, suggesting potential adverse ecological effects in aquatic environments. Affirmative evidence for this is lacking, however, since studies examining environmental occurrences of biocides vis-à-vis the health and diversity of aquatic species have not yet been conducted.

(KEY TERMS: toxicology; biocides; environmental indicators; pesticides; sustainability; environmental regulations.)

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INTRODUCTION

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol; TCS) and triclocarban (3,4,4′-trichlorocarbanilide; TCC) are popular antimicrobial agents contained in a variety of consumer products of daily use. Whereas TCC is added mostly to antimicrobial soaps, usage of TCS is broader and includes applications in antibacterial mouthwash and toothpaste, as well as in household items, such as plastic cutting boards, sports equipment, textiles, and furniture (Bester, 2003; Sabaliunas *et al.*, 2003; USEPA, 2003). Antimicrobial soap bars typically contain 2% by weight of TCC. Concentrations of TCS in this and other personal care and household products are lower, primarily in the range of 0.1-0.3% by weight (Sabaliunas *et al.*, 2003). Many antimicrobial consumer products

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[2]Respectively, Graduate Student (Chalew), Adjunct Associate Professor (Halden), Center for Water and Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland; and Associate Professor (Halden), Center for Environmental Biotechnology, The Biodesign Institute, Arizona State University, 1001 S. McAllister Avenue, P.O. Box 875701, Tempe, Arizona 85287-5701 (E-Mail/Halden: halden@asu.edu).

are washed down the drain after use and thus become part of domestic wastewater treated in municipal sewage treatment facilities. Usage of antimicrobial and antibacterial products is steadily increasing in both the United States (U.S.) and worldwide (USEPA, 2003), which implies continual release of these compounds into the environment.

The fate of TCS and TCC in the environment is of interest to environmental scientists and regulatory agencies alike. Stimulus for environmental exposure and risk assessments is provided by various reports on increased microbial resistance following exposure to household biocides (Yazdankhah *et al.*, 2006), ecotoxicity to aquatic organisms (Orvos *et al.*, 2002; Reiss *et al.*, 2002), the potential for generation of toxic biocide degradates in the environment (Latch *et al.*, 2005; Sanchez-Prado *et al.*, 2006), and the possibility of adverse human health effects inferred from work with animal models (Chen *et al.*, 2008). In addition to these toxic effects, both biocides have been shown to bioaccumulate in aquatic species, and TCS also has been detected in human milk (Adolfsson-Erici *et al.*, 2002; Coogan *et al.*, 2007). Furthermore, TCS and TCC have been observed to persist in the environment for extended periods of time, particularly under anaerobic conditions (Ying *et al.*, 2007; Miller *et al.*, 2008).

Several aquatic risk assessments have been conducted for TCS (Reiss *et al.*, 2002; Capdevielle *et al.*, 2008) These studies concluded that under most environmental conditions, there was negligible to no risk for aquatic biota based on the exposure scenarios and indicator species examined. Risk assessment and toxicity information for organisms other than aquatic biota are scarce or unavailable.

This paper presents a comparative summary of available environmental concentrations of TCS and TCC and their respective toxicity threshold values for nontarget organisms residing in aquatic and terrestrial environments.

METHODS

Literature Review

The following databases were searched for articles pertaining to biocide occurrences and toxicity information: PubMed (http://www.ncbi.nlm.nih.gov/PubMed/), ISI Web of Science (http://isiwebofknowledge.com/), SciFinder Scholar (http://www.cas.org/products/sfacad/index.html), Scopus (http://www.scopus.com/scopus/home.url), and Scirus (http://www.scirus.com/). Additional searches were performed to compile data on the occurrence and fate of TCS and TCC in soils and sediments, and of ecotoxicity studies helpful in interpreting environmental exposures.

Estimation of Pore-Water Concentrations

Concentrations of TCC and TCS in the pore water of soil and sediment were calculated using standard approaches (Schwarzenbach *et al.*, 2003) by considering the soil-water partitioning coefficient (K_d) relationship of concentration of a given chemical in soil (C_{soil}) divided by its concentration dissolved in the pore water at equilibrium (C_{aq}). Assuming sorption to occur only between the organic chemical and the fraction of organic matter of the soil (f_{oc}), the K_d value can be related to K_{oc}, the organic carbon normalized sorption coefficient as the product of f_{oc} and K_{oc}. These two relationships can be rearranged to solve for the chemical concentration in the pore water, C_{aq}

$$C_{aq} = C_{soil} / f_{oc} \times K_{oc}. \quad (1)$$

K_{oc} values were taken from SciFinder Scholar and adjusted for ambient pH, if applicable, to yield the corresponding pH-dependent K_{oc} value, typically referred to as D_{oc} (Young *et al.*, 2008). Aqueous concentrations were calculated for the pH range of natural surface waters, pH 7-9 (Wells, 2006). The D_{oc} value for TCC in this range remained constant at 31,700. The D_{oc} value for TCS was calculated to equal 13,400 at pH 7, 6,020 at pH 8, and 934 at pH 9. Published ranges of f_{oc} in sludge (2.3, 12.7, and 22.6%) and soil (2, 5, 7, and 33%) were used to estimate the pore water concentrations. The maximum estimates were used to calculate the pore water concentrations in agricultural fields amended with biosolids assuming a maximum mixing ratio of 1:1, which may result from heavy application of biosolids or from incomplete mixing of both materials. Concentrations of TCS and TCC in the soil and sediment were estimated from literature values of sediment concentrations and biosolid concentrations, assuming a mixing ratio of 1:1 (Singer *et al.*, 2002; Bester, 2003; Morales *et al.*, 2005; Heidler *et al.*, 2006; Kinney *et al.*, 2006; Chu and Metcalfe, 2007; Ying and Kookana, 2007; Miller *et al.*, 2008).

RESULTS

Environmental Occurrence of TCS and TCC

Environmental occurrence data for TCS and TCC were separated into wastewater treatment, process streams, aquatic, and terrestrial ecosystems (Figure 1).

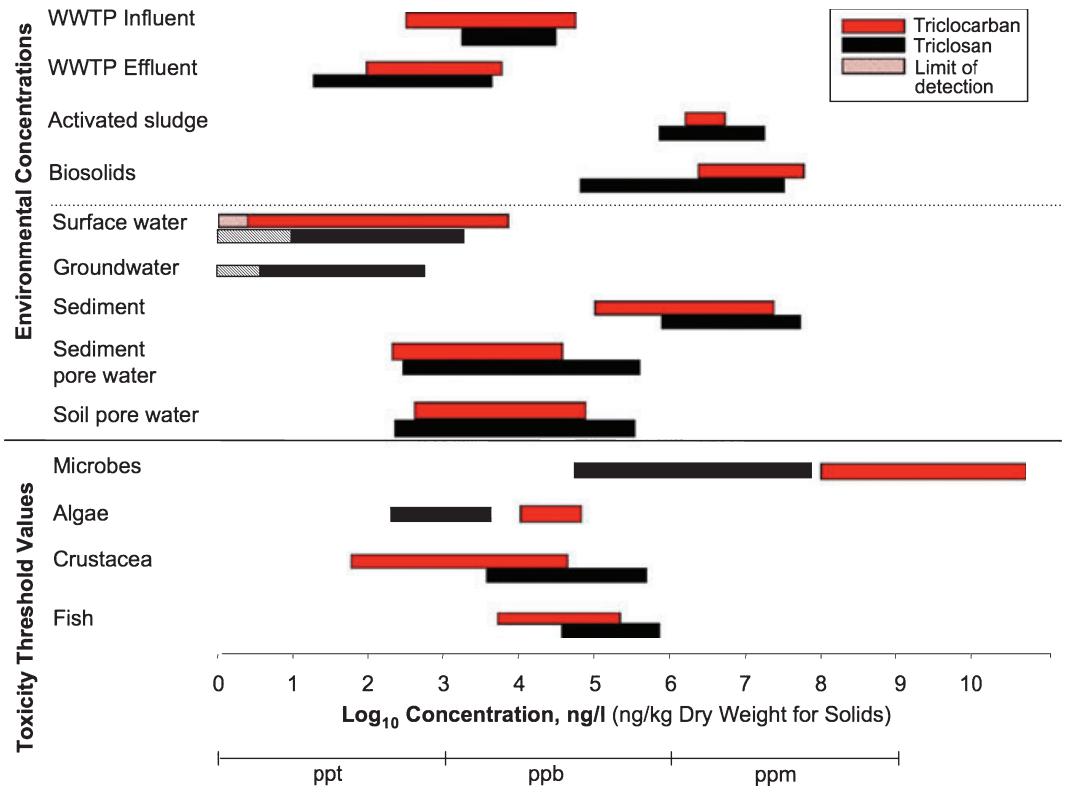

FIGURE 1. Visualization of Published Environmental Occurrence Data for Triclosan and Triclocarban in Comparison to Available Toxicity Threshold Values for Various Indicator Organisms. Concentrations are presented on a logarithmic scale in parts-per-trillion by volume (ng/l) or mass (ng/kg) for liquid and solid matrices, respectively. Concentrations in activated sludge and biosolids are expressed on a dry weight basis. Pore water concentrations were calculated from published environmental monitoring data. Overlap of occurrence data with toxicity threshold values of biota residing in the respective environmental compartment indicate locales of unhealthy conditions allowing for chronic or acute toxic effects. WWTP, wastewater treatment plant.

Biocide concentrations observed in wastewater treatment plant (WWTP) influent ranged from 1.86 to 26.8 μg/l for TCS and from 0.4 to 50 μg/l for TCC (McAvoy *et al.*, 2002; Halden and Paull, 2005; Lishman *et al.*, 2006; Waltman *et al.*, 2006; Heidler and Halden, 2007). Concentrations in activated sludge, customarily reported on a dry weight basis, were shown to be higher and in the range of 580-14,700 and 2,170-4,820 μg/kg for TCS and TCC, respectively (McAvoy *et al.*, 2002; Singer *et al.*, 2002; Chu and Metcalfe, 2007). Biosolids concentrations were shown to range from 90 to 32,900 μg/kg for TCS and from 3,050 to 51,000 μg/kg for TCC (Bester, 2003; Morales *et al.*, 2005; Heidler *et al.*, 2006; Kinney *et al.*, 2006; Chu and Metcalfe, 2007; Ying and Kookana, 2007). Biocide concentrations in WWTP effluent published in the peer-reviewed literature were found to range from 0.027 to 2.7 μg/l for TCS and from 0.1 to 6 μg/l for TCC (Bester, 2003; Kanda *et al.*, 2003; Sabaliunas *et al.*, 2003; Bendz *et al.*, 2005; Halden and Paull, 2005; Thompson *et al.*, 2005).

Concentrations in the natural aquatic environment typically span a range from below the detection limit to maxima of 2.3 μg/l for TCS and 0.25 μg/l for TCC in U.S. surface waters (Kolpin *et al.*, 2002, 2004; Lindstrom *et al.*, 2002; Bendz *et al.*, 2005; Glassmeyer *et al.*, 2005; Sapkota *et al.*, 2007; Zhang *et al.*, 2007), and up to 1.6 and 6.75 μg/l for TCS and TCC, respectively, in streams with known inputs of raw wastewater (Halden and Paull, 2005). Concentrations of TCS in freshwater sediment have been reported to range from 800 to 53,000 μg/kg, and levels of TCC, available only for estuarine sediments, were reported to be in the range of 1,700-24,000 μg/kg (Singer *et al.*, 2002; Morales *et al.*, 2005; Miller *et al.*, 2008).

The literature does not contain information on concentrations of TCS and TCC in the pore water of sediments and soils but these levels were estimated here based on published data, by considering the range of organic carbon levels typically extant in these settings. Assuming a f_{oc} range in sediment of 2.3-22.6% (Ouyang *et al.*, 2006) and pH range of 7-9, estimated sediment pore water concentrations ranged from 0.26 to 382.8 μg/l for TCS and from 0.24 to 32.9 μg/l for TCC. Biocide levels in the pore water of sludge-amended

soils were calculated at 0.201-273.3 μg/l for TCS and 0.292-80.4 μg/l for TCC, assuming a soil f_{oc} range of 2-33% (Jobbagy and Jackson, 2000) and pH range of 7-9. Soils and sediments with lower f_{oc} values yielded higher pore water concentrations. None of the calculated concentrations exceeded the solubility limits of both compounds, which have been reported to be in the range of 1,970-4,600 μg/l for TCS and on the order of 650-1,550 μg/l for TCC at 25°C and neutral pH (Halden and Paull, 2005).

Toxicity Threshold Data

Ecotoxicity data are essential for the interpretation of biocide occurrences in the environment (Table 1). Common measures of toxicity can be divided into acute and chronic effects. Threshold values for acute toxicity in fish have been determined to range from 260 to 440 μg/l for TCS and from 49 to 180 μg/l for TCC, whereas chronic effect thresholds were in the range of 34-290 μg/l for TCS and 5 μg/l for TCC (Consortium, 2002; Orvos *et al.*, 2002; Ishibashi *et al.*, 2004; Tatarazako *et al.*, 2004). Acute toxicity threshold values in crustacea were determined to range from 185 to 390 μg/l for TCS and 1.9 to 40 μg/l for TCC. Chronic toxicity in crustacea was observed at levels as low as 6-182 μg/l for TCS and 0.06-4.7 μg/l for TCC (Consortium, 2002; Orvos *et al.*, 2002; Samosoe-Petersen *et al.*, 2003; Tatarazako *et al.*, 2004). Concentrations toxic to algae also are in the parts-per-billion range, with values of 0.2-2.8 μg/l for TCS and 10-30 μg/l for TCC (Consortium, 2002; Orvos *et al.*, 2002; Reiss *et al.*, 2002; Samosoe-Petersen *et al.*, 2003; Yang *et al.*, 2008). Inhibitory effects on microorganisms were shown to begin at levels ranging from 25 to 80,000 μg/l for TCS and 100,000-40,000,000 μg/l for TCC (Consortium, 2002; Federle *et al.*, 2002; Samosoe-Petersen *et al.*, 2003; Sivaraman *et al.*, 2004; Neumegen *et al.*, 2005; Stasinakis *et al.*, 2007; Farre *et al.*, 2008; Stickler and Jones, 2008). Readers will note that the upper range minimum inhibitory concentrations (MICs) reported are well in excess of published solubility limits for both biocides discussed here.

Additional studies have concentrated on determining and quantifying endocrine disruption, behavioral changes, immuno-toxic effects, bioaccumulation, and growth impairment in frogs, clams, earthworms, and cucumber plants following short and long-term exposure to biocides (Samosoe-Petersen *et al.*, 2003; Smith and Burgett, 2005; Canesi *et al.*, 2007; Chen *et al.*, 2008; Kinney *et al.*, 2008). However, due to the relative paucity of such data, this work was excluded from the analysis provided in this study.

DISCUSSION

Sources

TCS and TCC have experienced increased usage in personal care products, which are transported through the domestic waste stream to WWTPs (USEPA 2003). TCS and TCC are subject to biodegradation during municipal wastewater treatment, which helps to achieve average removal efficiencies in the range of 58-98% (McAvoy *et al.*, 2002; Bester, 2003; Lishman *et al.*, 2006). However, mass balance studies have demonstrated that both biocides also exhibit significant persistence, partitioning and sequestration in biosolids, the by-product of wastewater treatment. Approximately 50 ± 19 and 76 ± 30% of the incoming mass of TCS and TCC, respectively, was observed to persist and become sequestered in biosolids produced by a conventional WWTP employing activated sludge treatment in conjunction with anaerobic biosolid digestion (Heidler *et al.*, 2006; Heidler and Halden, 2007). Thus, important pathways of biocide release into the environment include WWTP effluent discharge into surface waters and the land application of biosolids. Effluent from WWTPs contains a complex mixture of anthropogenic and natural compounds. The biocides TCS and TCC, along with many other compounds, may have multiplicative or synergistic effects on organisms. Toxicity tests on multiple compounds occurring simultaneously are still rare.

Environmental Occurrence

Both TCC and TCS are frequent contaminants of aquatic and terrestrial environments, detected in a concentration range spanning more than six orders of magnitude from parts-per-trillion levels in surface waters to parts-per-million levels in biosolids. Concentrations of both TCS and TCC are highest in biosolids and aquatic sediments, which may be interpreted as the combined result of high usage, significant environmental persistence and strong sorption of both compounds to organic matter. From Figure 1, it is evident that maximum concentrations of TCC often exceed those of TCS; however, occurrence data for TCC in some environmental compartments are still limited, which complicates the interpretation of available information.

Concentrations of TCS and TCC in the environment are subject to attenuation by physical, chemical, and biological processes (Sabaliunas *et al.*, 2003; Morrall *et al.*, 2004). Half-lives are on the order of hours, years, or decades, depending on the environmental compartment and prevailing conditions

TABLE 1. Toxicity Threshold Values of TCS and TCC for Various Target and Nontarget Organisms.

Indicator Organism	Assay	Parameter	Exposure Time	TCS (μg/l)	TCC (μg/l)	References
Fish						
Pimephales promelas	LC_{50}	Acute toxicity	24 h	360	-	Orvos *et al.* (2002)
			48 h	270	-	Orvos *et al.* (2002)
			72 h	270	-	Orvos *et al.* (2002)
			96 h	260	-	Orvos *et al.* (2002)
	NOEC	Hatchability of eggs, growth, and survival	35 days	-	5	Consortium (2002)
Oryzias latipes	LC_{50}	Acute toxicity	96 h	602	-	Ishibashi *et al.* (2004)
	NOEC	Hatchability of eggs, growth, and survival	21 days	156	-	Ishibashi *et al.* (2004)
	IC_{25}	Hatchability of eggs, growth, and survival	9 days	290	-	Tatarazako *et al.* (2004)
Oncorhynchus mykiss	LC_{50}	Acute toxicity	96 h	-	180	Consortium (2002)
	NOEC	Hatchability of eggs, growth, and survival	61 days	34.1	-	Orvos *et al.* (2002)
Lepomis macrochirus	LC_{50}	Acute toxicity	96 h	440	97	Orvos *et al.* (2002), Consortium (2002)
	NOEC	Acute toxicity	96 h	-	49	Consortium (2002)
Salmo gairdneri	NOEC	Acute toxicity	96 h	-	<49	Consortium (2002)
	LC_{50}	Acute toxicity	96 h	-	120	Consortium (2002)
	NOEC	Acute toxicity	96 h	-	<49	Consortium (2002)
	NOEC	Hatchability of eggs, growth, and survival	35 days	-	5	Consortium (2002)
Crustacea						
Daphnia magnia	EC_{50}	Acute toxicity	48 h	390	-	Orvos *et al.* (2002)
	NOEC	Reproduction rate	21 days	40	2.9	Orvos *et al.* (2002), Consortium (2002), Samosoe-Petersen *et al.* (2003)
	LOEC	Reproduction rate	21 days	200	4.7	Orvos *et al.* (2002), Consortium (2002), Samosoe-Petersen *et al.* (2003)
	NOEC	Acute toxicity	48 h	-	5	Consortium (2002)
	EC_{50}	Acute toxicity	48 h	-	ca. 10	Consortium (2002)
	EC_{100}	Acute toxicity	48 h	-	40	Consortium (2002)
	NOEC	Reproduction rate	21 days	-	2.9	Consortium (2002)
	LCEC	Reproduction rate	21 days	-	3.7	Consortium (2002)
Ceriodaphnia sp.	NOEC	Acute toxicity	48 h	-	1.9	Consortium (2002)
	EC_{50}	Acute toxicity	48 h	184.7	ca. 3.1	Orvos *et al.* (2002), Consortium (2002)
	EC_{100}	Acute toxicity	48 h	-	6.3	Consortium (2002)
	NOEC	Chronic toxicity, pH 8.5	7 days	182	-	Orvos *et al.* (2002)
	NOEC	Chronic toxicity, pH 7	7 days	6	-	Orvos *et al.* (2002)
	IC_{25}	Chronic toxicity	7 days	170	-	Tatarazako *et al.* (2004)
	NOEC	Chronic toxicity	21 days	-	2.9	Consortium (2002)
	LOEC	Chronic toxicity	21 days	-	4.7	Consortium (2002)
Mysidopsis bahia	EC_{50}	Acute toxicity	96 h	-	10-13	Consortium (2002)
	NOEC	Reproduction rate	28 days	-	0.06	Consortium (2002)
	LOEC	Reproduction rate	28 days	-	0.13	Consortium (2002)
	EC_{50}	Reproduction rate	28 days	-	0.21	Consortium (2002)
Algae						
Scenedesmus subspicatus	NOEC	Growth	72 h	0.5	-	Orvos *et al.* (2002), Samosoe-Petersen *et al.* (2003), Reiss *et al.* (2002)
			96 h	0.69	-	Orvos *et al.* (2002), Samosoe-Petersen *et al.* (2003), Reiss *et al.* (2002)

TABLE 1. (Continued)

Indicator Organism	Assay	Parameter	Exposure Time	TCS (μg/l)	TCC (μg/l)	References
	EC_{50}	Growth	72 h	2.8	20-30	Orvos *et al.* (2002), Consortium (2002), Samosoe-Petersen *et al.* (2003), Reiss *et al.* (2002)
			96 h	1.4	-	Orvos *et al.* (2002), Samosoe-Petersen *et al.* (2003), Reiss *et al.* (2002)
		Biomass	72 h	0.7	20-30	Orvos *et al.* (2002), Consortium (2002), Samosoe-Petersen *et al.* (2003), Reiss *et al.* (2002)
Selenastrum capricornutum	EC_{25}	Biomass	96 h	2.44	-	Orvos *et al.* (2002)
	IC_{25}	Growth	96 h	3.4	-	Tatarazako *et al.* (2004)
Anabaena fios-aquae	EC_{50}	Growth	96 h	1.6	-	Reiss *et al.* (2002)
	NOEC	Growth	96 h	0.81	-	Reiss *et al.* (2002)
Pseudokirchneriella subcapitata	LOEC	Growth	72 h	0.40	10	Yang *et al.* (2008)
	NOEC	Growth	72 h	0.20	<10	Yang *et al.* (2008)
	IC_{50}	Growth	72 h	0.53	17	Yang *et al.* (2008)
Microorganisms						
Activated sludge-mixed liquor	EC_{50}	Acute toxicity	15 min	2.39×10^5	-	Samosoe-Petersen *et al.* (2003)
			5 days	1,820	-	Neumegen *et al.* (2005)
Nitrifying bacteria	MIC	-	-	500	-	Stasinakis *et al.* (2007)
	NOEC	-	-	750	-	Federle *et al.* (2002)
Escherichia coli	MIC	-	-	300	-	Sivaraman *et al.* (2004)
	MIC	-	18 h	200-80,000		Stickler and Jones (2008)
Staphylococcus aureus	MIC	-	-	25-4,000		Stickler and Jones (2008)
Proteus mirabilis	MIC	-	-	200-60,000		Stickler and Jones (2008)
Vibrio fischeri	EC_{50}	Heterotrophic activity	5 days	38,200	-	Stasinakis *et al.* (2007)
	EC_{50}	Heterotrophic activity	10 days	31,500	-	Stasinakis *et al.* (2007)
	EC_{50}	Ammonia uptake	15 days	9,970	-	Stasinakis *et al.* (2007)
	EC_{50}	Bioluminescence inhibition	15 min	0.28	-	Farre *et al.* (2008)
	LOEC	Bioluminescence inhibition	15 min	0.1	-	Farre *et al.* (2008)
Domestic sludge	HA_{50}	Acute toxicity	15 min	-	4×10^7	Consortium (2002)
	NOEC	ADI	16 days	-	1×10^5	Consortium (2002)
	LOEC	ADI	16 days	-	1×10^5-1×10^6	Consortium (2002)

Notes: ADI, anaerobic digester inhibition; EC_{50}, half-maximal effective concentration; EC_{100}, maximal effective concentration; IC_{25}, growth inhibition of 25% concentration; IC_{50}, growth inhibition of 50% concentration; LC_{50}, median lethal dose; LOEC, lowest observed effects concentration; MIC, minimum inhibitory concentration; NOEC, no-observed effects concentration; TCC, triclocarban; TCS, triclosan.

(Bester, 2005; Halden and Paull, 2005; Ying and Kookana, 2007; Miller *et al.*, 2008). The extent and kinetics of sorption and desorption of TCS and TCC in a natural system are affected by the type of organic carbon present and whether equilibrium has been attained. An increased organic matter content in the system will reduce a compound's availability to biota.

Toxicity Thresholds

Current data identify algae and crustacea as the group of organisms most sensitive to biocide exposures (Figure 1). Fish and crustacea tend to be more sensitive to TCC, whereas algae and microbes are more easily inhibited by TCS. Fish may be affected by environmental biocide exposures in surface waters heavily impacted by raw and treated sewage but biocide concentrations typically present in surface waters show little if any overlap with fish toxicity threshold values.

Algae and crustacea are highly sensitive indicator organisms that may be impacted by biocide occurrences in surface waters receiving raw and treated sewage. TCS has been detected in surface waters at concentrations exceeding the no-observed effects

concentration (NOEC) of algae. Crustacea may be adversely affected by TCC present in effluent dominated streams, in typical surface water conditions, and by TCS and TCC levels extant in sediment pore water. All of the observed TCC sediment concentrations exceed even the highest reported acute toxicity thresholds for crustacea.

Although microorganisms are relatively insensitive to both biocides when compared with nontarget species, MIC threshold values for microbes are exceeded by environmental levels of TCS in activated sludge and biosolids. This latter observation could have important implications for the health and fertility of agricultural soils amended with biosolids.

Acute and Chronic Toxicity

Many of the threshold values shown in Table 1 and Figure 1 correspond to acute exposures and were determined in short-term tests. Chronic threshold levels are lower than the corresponding acute toxicity thresholds and represent long-term, low-level exposures that are more common in the environment. These low exposures may trigger adverse outcomes affecting survivorship and reproduction. Many of the investigated organisms are at the bottom of the food chain; therefore, impacts to their populations, due to either die-off from acute toxic exposures or failure to reproduce successfully as a result of chronic exposures, may lead to adverse consequences throughout the ecosystem and food chain. However, such a scenario at present is entirely speculative, since studies appropriate to probe for this outcome have not yet been conducted.

The lower end of the toxicity threshold in Figure 1 often represents the NOEC or lowest observed effects concentration for the more sensitive species within each category.

Due to the considerable environmental persistence of TCS and TCC, many aquatic and terrestrial biota will be exposed to these biocides for their entire life. The expected lifespan of *Daphnia magnia* can be as short as 29 days, while the mean lifespan is about 39.3 ± 1.3 days (Anderson and Jenkins, 1942). The average life span of *Oryzias latipes* under natural conditions is one year and a few months (Egami *et al.*, 1988). With TCS and TCC releases into the environment being continuous and persistence pronounced, organisms in the natural environment likely are exposed for longer periods of time than those tested even in chronic toxicity assays. Furthermore, since TCS and TCC usage and environmental release have occurred for decades, multiple generations of these organisms already have been exposed at concentrations expected to trigger acute and chronic effects or possibly adaptation. However, studies examining these potential outcomes are lacking.

A measured concentration in the environment does not necessarily accurately reflect the exposure experienced by the resident biota. An overestimation of exposure can result when irreversible sorption of contaminants has taken place and triggered a sequestration of TCS and TCC from biota in the environment. On the other hand, an environmental concentration measured in water may underestimate the true exposure level if a significant amount of the contaminant of concern is sorbed to the organism of interest. In addition, organisms can move between different environmental compartments (e.g., between the water column and the underlying sediment), which can result in temporal exposure, nonequilibrium conditions, and additive or synergistic effects from co-exposure or sequential exposure to different contaminants. Additionally, an organism may reside in different compartments during different life stages, and the effects of exposure may vary by developmental stage. Thus, the actual exposure of an organism may differ from those expected based on environmental measurements and the potential for nonequilibrium adds a dimension of complexity not captured by Figure 1, which assumes equilibrium.

Risk Assessment

A recently completed aquatic risk assessment determined that a chronic predicted no effect concentration (PNEC) for TCS would be 1.55 $\mu g/l$ (Capdevielle *et al.*, 2008). This study was based on a predicted environmental concentration (PEC) for TCS in U.S surface waters of no more than 850 ng/l, resulting from modeling output using a worst-case scenario precluding instream removal of the compound. Given the concentration range of TCS actually found around the U.S., the conclusion of no adverse impact on aquatic communities downstream of WWTP will apply to some but not all situations and locales. Indeed, surface water concentration of TCS in excess of the PEC value have been reported by multiple investigators in multiple locations across the U.S. (Kolpin *et al.*, 2002; Glassmeyer *et al.*, 2005). Thus, actual environmental monitoring data cannot always be reconciled with information and conclusions furnished by environmental modeling and risk assessment.

Furthermore, the recent risk assessment for TCS used a PNEC benchmark value that was based on an unconventional and controversial approach involving species sensitivity distribution (SSD) values (Capdevielle *et al.*, 2008). This approach foregoes conventional use of the PNEC for the most sensitive species, and instead anchors the assessment's

outcome on a value that is protective of 95% of all indicator species examined. However, the premise that an impairment or complete elimination of 5% of the species diversity in a natural environment is an acceptable "no-effect" event is not universally agreed upon. Furthermore, if the SSD approach is rejected and the conventional approach of using the PNEC value of the most sensitive species is used in its place, the outcome of the risk assessment for TCS is easily reversed. A reliable risk assessment for these biocides is further complicated by the fact that exposures to TCS and TCC are usually not independent of each other. Both compounds are known to co-occur in the environment, frequently at similar levels (Halden and Paull, 2005). Thus, it appears prudent to consider the possibility of additive, antagonistic or synergistic effects from exposure to mixtures of the two. The potential co-occurrence of additional wastewater-derived compounds alongside TCS and TCC has been reported and could introduce additional complexity (Kolpin *et al.*, 2002). The presence of these co-contaminants may enhance or reduce the ecotoxicological effects of TCS and TCC.

Additional Exposure Effects

TCS and TCC exposures can lead to bioaccumulation in tissue, which opens a potential pathway for chemical biomagnification up the food chain. If and to what extent TCS and TCC biomagnify in the food web is not well understood. Passive bioaccumulation in algae has been demonstrated but the body burdens detectable in human milk can be interpreted in multiple ways other than biomagnification (Adolfsson-Erici *et al.*, 2002; Coogan *et al.*, 2007). The behavior of transformation products also should be considered. Methyl-TCS (M-TCS), a TCS degradate, was detected in the fat of fish from Sweden at concentrations ranging from <0.1 to 360 ng/g lipid (Balmer *et al.*, 2004). TCS, TCC, and M-TCS were detected in tissues of snails caged for two weeks downstream of a WWTP, in the wastewater effluent stream. Snail TCS bioaccumulation factor (BAF) was 500, TCC BAF was 1,600, and M-TCS BAF was 1,200 (Coogan and La Point, 2008).

As concentrations of TCS and TCC in the environment increase, bacterial strains may adapt by developing resistance (McMurry *et al.*, 1998; Stickler and Jones, 2008). The wide range of MICs reported in Table 1 for some microbial pathogens illustrates this adaptive effect to some degree.

The target organisms (microbial pathogens) of TCS and TCC usage in household settings feature the highest toxicity threshold values of all organisms examined. Yet, they are intentionally exposed to the biocides for only a very short period of time (i.e., a few seconds during hand washing). This observation may help to explain why antimicrobial soaps were concluded to offer no proven benefit over the use of regular soap in consumer households (Aiello *et al.*, 2007). However, once released into the environment, the situation is reversed and the more highly susceptible nontarget organisms experience long-term exposures to these agents.

CONCLUSIONS

This paper provides a summary of environmental occurrence data for TCC and TCS and contrasts them with toxicity thresholds for aquatic and terrestrial organisms. Overall, TCC appears to be equally abundant but more persistent in the environment than TCS. Environmental concentrations in some instances exceed toxic threshold values of susceptible organisms, thus implying acute or chronic exposures to toxic levels of TCS and TCC. Nontarget organisms may be exposed to low levels of these compounds for their entire life, as a combined result of a relatively short lifespan, the considerable environmental half-lives of both biocides, and their continual release into the environment. Available data suggest a notable tolerance to biocide exposures among both beneficial microorganisms partaking in the wastewater treatment process and the human pathogens targeted by antimicrobial product applications.

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