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Triclosan: environmental exposure, toxicity and mechanisms of action

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ABSTRACT: Triclosan [5-chloro-2-(2,4-dichlorophenoxy)phenol; TCS] is a broad spectrum antibacterial agent used in personal care, veterinary, industrial and household products. TCS is commonly detected in aquatic ecosystems, as it is only partially removed during the wastewater treatment process. Sorption, biodegradation and photolytic degradation mitigate the availability of TCS to aquatic biota; however the by-products such as methyltriclosan and other chlorinated phenols may be more resistant to degradation and have higher toxicity than the parent compound. The continuous exposure of aquatic organisms to TCS, coupled with its bioaccumulation potential, have led to detectable levels of the antimicrobial in a number of aquatic species. TCS has been also detected in breast milk, urine and plasma, with levels of TCS in the blood correlating with consumer use patterns of the antimicrobial. Mammalian systemic toxicity studies indicate that TCS is neither acutely toxic, mutagenic, carcinogenic, nor a developmental toxicant. Recently, however, concern has been raised over TCS's potential for endocrine disruption, as the antimicrobial has been shown to disrupt thyroid hormone homeostasis and possibly the reproductive axis. Moreover, there is strong evidence that aquatic species such as algae, invertebrates and certain types of fish are much more sensitive to TCS than mammals. TCS is highly toxic to algae and exerts reproductive and developmental effects in some fish. The potential for endocrine disruption and antibiotic cross-resistance highlights the importance of the judicious use of TCS, whereby the use of TCS should be limited to applications where it has been shown to be effective.

Keywords: triclosan; irgasan; review; levels; exposure; effects; toxicity; aquatic species; mammals; human; endocrine disruption; reproduction

BACKGROUND

Triclosan [5-chloro-2-(2,4-dichlorophenoxy)phenol: TCS], a halogenated phenol, is a nonionic, broad spectrum antimicrobial used throughout North America, Europe and Asia, as an ingredient in disinfectants, soap, detergent, toothpaste, mouthwash, fabric, deodorant, shampoo and plastic additives, in addition to innumerable other personal care, veterinary, industrial and household products. TCS is effective against many types of bacteria and certain types of fungi, preventing bacterial propagation and/or eventually resulting in cell death. It permeates the bacterial cell wall and targets multiple cytoplasmic and membrane sites, including RNA synthesis and the production of marcomolecules (Russell, 2004). TCS also blocks synthesis of fatty acids through inhibition of enoyl reductase, but has no effect on bacterial spores (McMurry *et al.*, 1998; Levy *et al.*, 1999; Russell, 2004). TCS may be classified as a halogenated aromatic hydrocarbon, containing phenol, diphenyl ether and polychlorinated biphenyl functional groups (Ahn *et al.*, 2008). The chemical structure of TCS (Fig. 1), a halogenated biphenyl ether, is similar to polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), bispenol A, dioxins and thyroid hormones (Veldhoen *et al.*, 2006; Crofton *et al.*, 2007; Allmyr *et al.*, 2008), molecules with two aromatic rings.

TCS was invented over 40 years ago and has been used increasingly over the past 25 years (Jones *et al.*, 2000; Russell, 2004). In the period from 1992 to 1999, a majority of the 700 antibacterial products on the market contained TCS as an active ingredient (Schweizer, 2001). TCS is the generic name for the chemical, with brand names including Irgasan DP300, Aquasept, Sapoderm and Ster-Zac. Fibres and other materials that have TCS incorporated into them may be referred to as Ultra-Fresh, Amicor, Microban, Monolith, Bactonix and Sanitized (Adolfsson-Erici *et al.*, 2002). The antimicrobial has the capability to migrate from treated surfaces into foodstuffs. Notwithstanding, the addition of TCS to food coverings and surfaces that are in contact with food during processing is currently being considered (Canosa *et al.*, 2008). However, in March 2010, TCS was removed from the EU list of provisional additives for use in plastic food-contact materials.

Unlike some other organochlorine compounds, the use of TCS is not highly regulated, as the antimicrobial has a low acute toxicity and is generally accepted as well tolerated and safe (Jones *et al.*, 2000; Rodricks *et al.*, 2010). Concentrations of TCS in personal care products are typically in the range of 0.1–0.3% of product weight (Sabaliunas *et al.*, 2003), with significant amounts of the antimicrobial entering wastewater treatment facilities (Table 1). The prevalence of TCS in waterways is likely to increase as consumer demand for antimicrobial products is anticipated to grow. TCS is being increasingly scrutinized after

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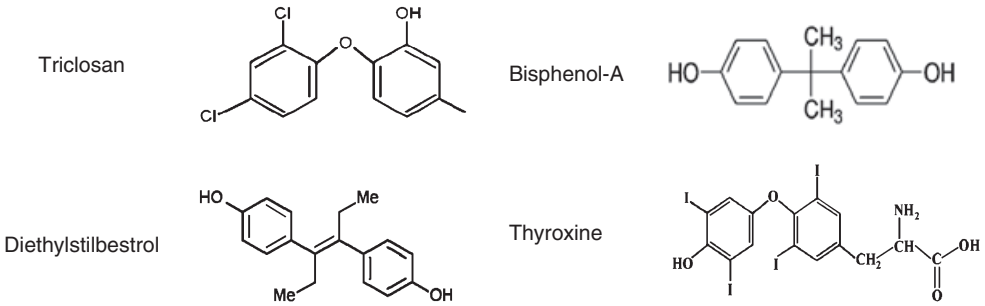

Figure 1. The structural similarity of TCS to Bisphenol A, Diethylstilbestrol, and the thyroid hormone thyroxine.

concerns emerged that the product might be harmful to human health and the environment. TCS has been detected in surface water, sediment, biosolids, soils, aquatic species and humans (Chu and Metcalfe, 2007; Chalew and Halden, 2009; Reiss *et al.*, 2009). Potential health issues surrounding the use of TCS include antibiotic resistance, skin irritations, endocrine disruption, increasing rates of allergies and the formation of carcinogenic by-products (Schweizer, 2001; Adolfsson-Erici *et al.*, 2002; Latch *et al.*, 2003), yet a recent review by Rodricks *et al.* (2010) concluded that exposure to TCS in consumer products is not expected to cause adverse health effects in children or adults who use the products as intended.

Despite the widespread use of TCS, few independently published studies have investigated the emerging health concerns surrounding the use of this antimicrobial and the environmental impact it may have. Previous reviews of TCS have focused primarily on its toxicity in laboratory animals and humans, its fate in the environment, or its link to antibiotic cross-resistance. The objective of this review is to provide a comprehensive review of the literature on TCS, its occurrence in aquatic and terrestrial environments, exposure levels in humans and wildlife, including aquatic species, its toxicity and endocrine disrupting potential.

IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, MANUFACTURE AND USE

Identity, Physical and Chemical Properties

Triclosan (CAS registration number 3380-34-5), is a diphenyl ether and may be referred to as 5-chloro-2-(2,4-dichlorophenoxy) phenol or 2,4,4′-trichloro-2′-hydroxydiphenyl ether. The molecular formula for TCS is $C_{12}H_7Cl_3O_2$ and the chemical has a molecular weight of 289.55. Most commercially obtained grades of TCS are over 99% pure and are available in the solid form as a white to off-white crystalline powder with a barely detectable aromatic odor. TCS is a stable compound with a boiling point between 280-290 °C, and a melting point between 54 and 57 °C. The thermal stability of TCS is why certain manufacturers have chosen the antimicrobial for the incorporation into plastics and fibers. The octanol–water partition coefficient (log K_{ow}) of TCS is 4.76; it is not readily soluble in water (10 mg l^{-1} at 20 °C), although solubility increases as the pH becomes more alkaline. TCS is, however, easily dissolved in a wide array of organic solvents (Bhargava and Leonard, 1996).

In aquatic ecosystems, the majority of TCS exists in the ionized form (Orvos *et al.*, 2002) and it is primarily the un-ionized form that is responsible for the majority of TCS's toxic effects. The half-life of TCS in surface water is approximately 41 min, with most of the parent compound converting to 2,4-dichlorophenol, although degradation rates vary considerably across aquatic ecosystems (Reiss *et al.*, 2002; Lyndall *et al.*, 2010).

Manufacture and Use

During the synthesis of TCS, a chlorinated phenoxyphenol, the potential for contamination with toxic impurities exists. Beck *et al.* (1989) reported trace amounts of lower chlorinated dibenzodioxins and furans in Irgasan DP300, but not in excess of the μg kg^{-1} range, leading to the conclusion that presence of these compounds in TCS is of little concern. Low levels of dioxins and dibenzofurans may be present as unwanted by-products, depending on the quality of the initial materials used to synthesize TCS, as well as manufacturing conditions such as temperature and pressure (Ni *et al.*, 2005). The US EPA considers that TCS may be potentially contaminated with dioxins, with the EU, Canada and the USA having taken initiatives to set standards for maximum permissible levels of impurities in this compound.

Between 1992 and 1999, over 700 antibacterial products, the majority of which contained TCS, entered the consumer market. Personal care products are the most common form of exposure to the antimicrobial, typically at concentrations of 0.1–0.3%, levels which are regulated by the European Community Cosmetic Directive or the US Food and Drug Agency (USFDA) in Europe and the USA, respectively (Sabaliunas *et al.*, 2003; Rodricks *et al.*, 2010). In Sweden, 25% of toothpaste brands contain TCS, which translates into 2 tons of TCS consumption per year. Soaps, deodorants and other personal care products account for another 300 kg of the chemical in Sweden alone (Adolfsson-Erici *et al.*, 2002). On the global front, the production of TCS has now exceeded 1500 tons per year, with Europe being responsible for 350 tons of total production (Singer *et al.*, 2002). As public concern over the transmission of disease is heightened, the use of antimicrobials is anticipated to increase. TCS will continue to be an environmental pollutant that warrants monitoring, especially since its transformation products are not yet fully understood.

ENVIRONMENTAL EXPOSURE

Occurrence in the Aquatic Environment

Triclosan

The antimicrobial TCS is commonly detected in aquatic ecosystems (Table 1; Capdevielle *et al.*, 2008; Chalew and

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Table 1. Concentrations of triclosan (TCS) in the aquatic environment

Medium	Sample description	Location	Concentration of TCS	Reference
Surface water	Natural streams/rivers	USA	ND[a] to 2.3 μg l^{-1}	Kolpin *et al.* (2002); Morrall *et al.* (2004)
		Switzerland	ND to 0.074 μg l^{-1}	Lindström *et al.* (2002)
		Germany	ND to 0.01 μg l^{-1}	Bester (2005)
		Sweden	ND	Bendz *et al.* (2005)
		Australia	0.075 μg l^{-1}	Ying *et al.* (2007)
		Japan	<0.0006–0.059 μg l^{-1}	Nakada *et al.* (2008)
	Streams with inputs of raw wastewater	Switzerland	0.011–0.098 μg l^{-1}	Singer *et al.* (2002)
		USA	1.6 μg l^{-1}	Halden and Paull (2005)
	Estuarine waters	USA	0.0075 μg l^{-1}	Fair *et al.* (2009)
Sediment	Freshwater	Switzerland	53 μg kg^{-1}	Singer *et al.* (2002)
		Spain	ND to 35.7 μg kg^{-1}	Morales *et al.* (2005)
	Estuarine	USA	ND to 800 μg kg^{-1}	Miller *et al.* (2008)
	Marine	Spain	0.27–130.7 μg kg^{-1}	Agüera *et al.* (2003)
Sewage sludge	Activated sludge	USA	0.5–15.6 μg g^{-1}	McAvoy *et al.* (2002)
		Spain	0.4–5.4 μg g^{-1}	Morales *et al.* (2005)
		Germany	1.2 μg g^{-1}	Bester (2003)
		Canada	0.62–1.45 μg g^{-1}	Chu and Metcalfe (2007)
	Biosolids	Australia	90–16 790 μg kg^{-1}	Ying and Kookana (2007)
		USA	10 500–30 000 μg kg^{-1}	Kinney *et al.* (2008); Heidler and Halden (2007)
		Spain	1508 μg kg^{-1}	Morales *et al.* (2005)
		Canada	680–12 500 μg kg^{-1}	Lee and Peart (2002); Chu and Metcalfe (2007)
WWTP influent	In-flowing waste water	USA	2.70–26.80 μg l^{-1}	McAvoy *et al.* (2002); Halden and Paull (2005); Heidler and Halden (2007); Fair *et al.* (2009)
		Canada	0.01–4.01 μg l^{-1}	Lishman *et al.* (2006)
		Germany	1.2 μg l^{-1}	Bester (2003)
		Sweden	0.38 μg l^{-1}	Bendz *et al.* (2005)
		Japan	2.7–11.9 μg l^{-1}	Nakada *et al.* (2010)
WWTP effluent	Treated water	Switzerland	0.042–0.213 μg l^{-1}	Singer *et al.* (2002)
		Germany	0.01–0.6 μg l^{-1}	Bester (2003, 2005)
		Canada	0.01–0.324 μg l^{-1}	Lishman *et al.* (2006)
		USA	0.03–2.7 μg l^{-1}	McAvoy *et al.* (2002); Heidler and Halden (2007); Halden and Paull (2005); Fair *et al.* (2009)
		UK	0.34–3.1 μg l^{-1}	Kanda *et al.* (2003); Sabaliunas *et al.* (2003)
		Australia	0.023–0.434 μg l^{-1}	Ying and Kookana (2007)
		Sweden	0.16 μg l^{-1}	Bendz *et al.* (2005)
		Japan	0.26–0.27 μg l^{-1}	Nakada *et al.* (2010)

[a]ND, not detectable.

Halden, 2009; Lyndall *et al.*, 2010). The majority (96%) of consumer products containing TCS are eventually rinsed down the drain (Reiss *et al.*, 2002) and discharged with wastewater effluent. Although wastewater treatment plants (WWTP) are generally highly effective in removing TCS, a small percentage of the antimicrobial is usually discharged with effluent into receiving waters, usually a river system (Morrall *et al.*, 2004; Nakada *et al.* 2008). The efficiency of TCS removal can be highly variable, with elimination rates ranging from complete removal to 100% ineffective (Kanda *et al.*, 2003; Heidler and Halden, 2007).

The variability in removal rates for TCS is due in part to different treatment processes, as the antimicrobial is readily degraded in aerobic conditions but not under anaerobic conditions (McAvoy *et al.*, 2002). Field measurements from a Swiss WWTP have detailed the elimination process of TCS: 79% was biologically degraded, 15% was sorbed to sludge and 6% left the plant in the final effluent at a concentration of 42 ng l^{-1} (Singer *et al.*, 2002; Table 1). These results are consistent with tests conducted at several WWTPs in Germany, where 4–10% of TCS remained dissolved in out-flowing water (Bester, 2003). Generally, WWTP influent concentrations of the antimicrobial range from 1.86 to 26.8 μg l^{-1}, with effluent concentrations ranging from 0.027 to 2.7 μg l^{-1} (Morrall *et al.*, 2004; Chalew and Halden, 2009; Nakada *et al.* 2010). In the period from 1999 to 2000, the US Geological Survey detected TCS in 57.6% of streams and rivers sampled, at concentrations ranging from below the detection limit up to 2.3 μg l^{-1} (Kolpin *et al.*, 2002). In addition to the incomplete removal from WWTP effluent, the antimicrobial exhibits a tendency to accumulate and persist in biosolids; it is estimated that up to 50% of TCS in WWTP influent will remain in biosolids in WWTPs which utilize activated sludge treatments in combination with anaerobic biosolid digestion (Heidler and Halden, 2007; Chalew and Halden, 2009; Lozano *et al.*, 2010). The TCS removal capacities of various sorbents, including activated charcoal and kaolinite, and the effects of pH, ionic strength and humic acid on the sorptive interactions have been investigated (Behera *et al.* 2010). Organic matter content was a major factor controlling the sorption of TCS. The occurence of TCS and other organic contaminants has been reported in Canadian municipal sewage sludge and biosolids samples (Lee and Peart, 2002; Chu and Metcalfe, 2007; Mackay and Barnthouse, 2010; Table 1). Thus the two main sources of TCS release into the environment are: (1) discharge of WWTP effluent into receiving waters; and (2) land application of biosolids containing residues of the antimicrobial.

A multitude of factors influence TCS concentrations in aquatic systems, including the TCS load in effluent, physical and chemical properties of TCS, characteristics of the aquatic ecosystem (pH, sediment density and organic matter content, water flow and velocity, depth), and even season and intensity of sunlight (Reiss *et al.*, 2002; Trixier *et al.*, 2002; Lyndall *et al.*, 2010). TCS has been measured not only in surface waters, but also in freshwater and estuarine sediment, at concentrations up to 800 μg kg^{-1} (Miller *et al.*, 2008; Chalew and Halden, 2009; Table 1). Monitoring TCS concentrations in surface water is important, as the antimicrobial has demonstrated a propensity for bioaccumulation in aquatic species (Balmer *et al.*, 2004) and can persist in aquatic ecosystems for extended periods of time. The antimicrobial has been measured in 30-year-old sediment from lake Greifensee in Switzerland (Singer *et al.*, 2002). This study provided evidence of the persistence of TCS in sediment and detailed the pattern of use of TCS. TCS concentrations in sediment increased between the early 1960s until the mid-1970s, reflecting steadily increasing patterns of use, then a reverse in this trend was observed from the mid-1970s until the early 1980s, when a new process of wastewater treatment was introduced into most WWTPs. Increases in TCS concentrations occurred again from the early 1980s until the present time. Similar depth–time profiles for TCS spanning last 40 years were reported by Miller *et al.* (2008) for estuarine sediments in the USA. The environmental persistence of TCS in sediment is indicative of the antimicrobial's potential to partition into sediment and resist degradation processes under anaerobic conditions. Buth *et al.* (2009) chronicled the historical pattern of dioxin photoproducts of TCS and its chlorinated derivatives in sediment cores from the Mississippi river. Between 1963 and 2008, TCS levels markedly increased, corresponding to increases in the concentration of several chlorinated derivatives of TCS (CTDs), including dichlorodibenzo-*p*-dioxin (2,8-DCDD), a direct transformation product of the photolysis of TCS. A further source of TCS derived dioxins comes from the solar irradiation of CTDs, leading to the formation of higher level chlorinated dioxins.

Degradation Products of TCS

Methyltriclosan

During the wastewater treatment process, TCS is transformed by biological methylation into methyltriclosan [MTCS; 5-chloro-2-(2,4 dichloropheoxy)anisole; CAS no. 4640-01-1] (Boehmer *et al.*, 2004; Bester, 2005), a more lipophilic compound (K_{ow} 5.2), which is then released into receiving waters. The presence of MTCS in fish has in fact been proposed for use as marker of exposure to WWTP effluent, specifically to lipophilic WWTP contaminants (Balmer *et al.*, 2004). The lipophilicity of MTCS and its resistance to biodegradation processes and photolysis (Lindström *et al.*, 2002) makes this metabolite exhibit a higher degree of environmental persistence than its parent compound.

Dioxins

Over the last decade there has been increasing concern regarding the degradation products of TCS, most notably dioxins, and consequently, the transformation of TCS during manufacturing, incineration and in the aquatic environment. The photolysis of TCS constitutes the principal removal pathway of the antimicrobial in the aquatic environment, with some studies having documented the formation of 2,8-dichlorodibenzodioxin (DCDD) and other dioxin derivatives during the photodegradation of TCS in aqueous solutions (Latch *et al.*, 2003; Mezcua *et al.*, 2004; Lores *et al.*, 2005; Sanchez-Prado *et al.*, 2006; Aranami and Readman, 2007).

There is evidence that the pH of aqueous solutions spiked with TCS influences the formation of dioxin by-products. Latch *et al.* (2003) reported that 1–12% of TCS is converted to DCDD in aqueous solutions buffered at a pH 8 or higher. Considering that the pK_a of TCS is 7.9, it is probable that the dissociated form of TCS is the photoreactive species, potentially explaining why DCDD was not observed in experiments using methanol solutions spiked with TCS (Latch *et al.*, 2003). From this study, it is apparent that, in sunlight-irradiated waters, the conversion of TCS into dioxin by-products is dependent on both the pH and the irradiation wavelength. The findings of Latch *et al.* (2003) were confirmed by Mezcua *et al.* (2004), who were the first to investigate the photodegradation of TCS to dioxins in wastewater samples. The study indicated that 2,7/2,8-dibenzodichloro-*p*-dioxin is indeed a by-product of the photolysis of TCS, in both water and wastewater samples spiked with 8 μg ml^{-1} of the antimicrobial. The degree of photolytic conversion was dependent upon pH and the organic matter content in the sample. Sanchez-Prado *et al.* (2006) were the first to use a solar simulator photoreactor, in conjunction

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with actual contaminated wastewater samples, identifying the formation of 2,8-DCDD and a possible DCDD isomer or dichlorohydroxydibenzofuran independently of sample pH. Aranami and Readman (2007) irradiated freshwater and seawater samples with a low-intensity artificial white light source for a 12 day period. Similar to previous studies, the photodegradation of TCS produced DCDD, in both freshwater and seawater samples, after 3 days of irradiation.

The photochemical conversion of TCS in natural water samples, specifically Mississippi River and Lake Josephine waters, was investigated by Buth *et al.* (2009). The photolysis of the antimicrobial was dependent on speciation, with the phenolate form of TCS being degraded 44–586 times faster than the phenol form. The conversion of chlorinated TCS derivatives into dioxins was substantiated in natural and buffered pure water, with yields of 0.5–2.5%, respectively. The majority of TCS's photolytic transformation products and their kinetics, along with the environmental factors influencing their degradation, have yet to be identified (Aranami and Readman, 2007). Of great importance in quantifying the level of risk to both aquatic environments and humans is determining to what extent and under which environmental conditions the conversion of TCS into toxic by-products occurs.

Chlorophenols

Dioxins are not the only toxic transformation product of TCS that warrants further study. The photochemical transformation of TCS has also been shown to produce 2,4-dichlorophenol and 2,4,6-trichlorophenol, chemicals which the US EPA has flagged as priority pollutants. The generation of chlorophenols from TCS was originally demonstrated by Kanetoshi *et al.* (1987); however, the study used high concentrations of chlorine and TCS, calling into question the environmental relevance of the findings. Later studies validated the finding that chlorophenols are transformation products of TCS, even in the presence of low levels of chlorine or chloramines (Rule *et al.*, 2005; Canosa *et al.*, 2005; Greyshock and Vikesland, 2006). TCS reacted with free chlorine under drinking water conditions and 2,4-dichlorophenol was formed via the ether cleavage of TCS, which then underwent electrophilic substitution to form 2,4,6-trichlorophenol. Consistent with other studies, based on the effect of pH on the formation of TCS by-products, Rule *et al.* (2005) concluded that it was primarily the ionized phenolate form of TCS that reacts with hypochlorous acid. Canosa *et al.* (2005) tested low concentrations of both TCS (ng ml^{-1}) and chlorine (mg l^{-1} and less), and consistently detected 2,4-dichlorophenol and 2,4,6-trichlorophenol in all of the samples analyzed. Even though the molar yields of TCS conversion were <10%, these findings are significant, as it has been demonstrated that these two phenolic by-products are relatively stable over time and potentially toxic (Canosa *et al.*, 2005). The formation of chlorophenols from the degradation of TCS has been confirmed by others (Sanchez-Prado *et al.*, 2006; Latch *et al.*, 2005; Fiss *et al.*, 2007).

Chloroform

There is evidence that, like other phenols, TCS in water or in various consumer products will react with free chlorine or chloramine to produce chloroform and other chlorinated products over a range of pHs (Rule *et al.*, 2005; Greyshock and Vikesland, 2006; Fiss *et al.*, 2007). Rule *et al.* (2005) also assessed the propensity of a dish soap containing TCS to form chloroform when added to chlorinated water. After 5 min, 15 µg l^{-1} of chloroform was produced, with chloroform levels attaining 49 µg l^{-1} after 120 min. Based on the results of this study, while it is unlikely that significant amounts of chloroform are generated from TCS in surface waters, chloroform may be formed during the daily use of household products containing the antimicrobial. The conversion of TCS to chlorinated derivatives is also dependent on temperature, with higher temperatures resulting in increased chloroform yields (Fiss *et al.*, 2007). An exposure model completed by the authors indicated that, under certain conditions, the amount of chloroform produced could be significant, and where chloroform formation is inconsequential, other chlorinated by-products are produced, which may place consumers at an increased risk for adverse health effects.

Exposure to Triclosan and its Degradation Products in Aquatic Organisms

Algae and invertebrates

The incomplete removal of TCS during the wastewater treatment process leads to the continual exposure of aquatic biota in receiving waters, and the accumulation of the antimicrobial and its degradation products in tissues of aquatic organisms (Table 2). Algae, a primary food source for many aquatic species, constitute an important pathway for the accumulation of lipophilic water-borne contaminants, such as TCS (Capdevielle *et al.*, 2008). Coogan *et al.* (2007) sampled the filamentous algae (*Cladophora* spp.) in a receiving stream for the city of Denton (Texas) for TCS and MTCS, measuring 100–150 and 50–89 µg kg^{-1}, respectively. From these measurements, bioaccumulation factors of 1600 and 1100 were estimated for the parent compound and its methylated by-product. The bioaccumulation potential of TCS and MTCS was also determined in freshwater snails (*Helisoma trivolvis*) and again in algae (*Cladophora* spp.), using isotope dilution GC-MS (Coogan and La Point, 2008). Bioaccumulation factors for snail tissue were 500 and 1200 for TCS and MTCS, respectively. The algal bioaccumulation factors were also high, 1400 and 1200, respectively. The occurrence and formation of TCS metabolites was also investigated in estuarine systems. In a study by DeLorenzo *et al.* (2008), adult grass shrimp (*Palaemonetes pugio*) were exposed to 100 µg l^{-1} of TCS and, even though TCS was not measured, they were found to accumulate MTCS after a 14-day exposure period. This finding provides evidence for both the conversion of TCS to MTCS in seawater, and of the bioaccumulation potential of the metabolite in aquatic organisms. Yet, even though MTCS is resistant to biodegradation processes and has demonstrated the ability to persist in the environment for longer periods of time than the parent compound, it has received considerably less attention in the literature. As snails and other aquatic invertebrates depend on algae as a source of nutrients, and considering the ubiquity of TCS in the aquatic environment, it is probable that grazed algal compartments will contain TCS and MTCS, potentially making these compounds available to higher aquatic organisms.

Fish

In addition to invertebrates, TCS and its transformation products have been detected in higher level aquatic organisms, most notably fish (Table 2). Miyazaki *et al.* (1984) was the first to report the presence of MTCS in aquatic biota. Fish and shellfish were collected from the Tama River and Tokyo Bay, and MCTS was identified by GC/MS in all of the freshwater fish samples

Table 2. Concentrations of triclosan (TCS) in aquatic organisms

Organisms	Type of sample	Site description	TCS (μg kg^{-1})	Reference
Algae and invertebrates				
Filamentous algae (*Cladophora* spp.)	Whole organism	Receiving stream for the city of Denton (TX, USA) WWTP	100–150	Coogan *et al.* (2007)
	Whole organism		50–400	Coogan and La Point (2008)
Freshwater snails (*Helisoma trivolvis*)	Muscle		50–300	Coogan and La Point (2008)
Vertebrates				
Rainbow trout (*Oncorhynchus mykiss*)	Bile	Upstream from WWTP, Sweden (caged); downstream 2 km from WWTP (caged)	710 17 000	Adolfsson-Erici *et al.* (2002)
Breams, male (*Abramis brama*)	Bile	River sites (Netherlands)	14 000–80 000	Houtman *et al.* (2004)
	Muscle	River sites (Germany)	0.25–3.4	Boehmer *et al.* (2004)
Pelagic fish	Plasma	Detroit River (USA)	0.75–10	Valters *et al.* (2005)
Atlantic bottlenose dolphins (*Tursiops truncates*)	Plasma	Estuary, South Carolina Estuary, Florida	0.12–0.27 0.025–0.11	Fair *et al.* (2009)
Killer whale (*Orcinus orca*)	Plasma	Vancouver Aquarium Marine Science Centre	9.0	Bennett *et al.* (2009)

(1–38 μg kg^{-1} whole body) and three of the four shellfish samples (3–20 μg kg^{-1}). A well-cited study by Adolfsson-Erici *et al.* (2002) measured TCS levels in rainbow trout (*Oncorhynchus mykiss*) caged in the receiving waters of a WWTP in Sweden, in wild fish living downstream from the plant and in rainbow trout exposed to treated water in tanks. Bile fluid from the fish contained TCS at concentrations ranging from <0.01–0.08 mg kg^{-1} fresh weight in controls and fish sampled at reference sites, and 0.44–120 mg kg^{-1} in fish exposed to sewage water. Houtman *et al.* (2004) also used GC/MS to identify a multitude of xenobiotic compounds, including TCS, in the bile of male bream (*Abramis brama*) living in Dutch surface waters. TCS was detected in two of the three locations sampled, at relatively high concentrations of 14 and 80 μg ml^{-1} of bile. The results of these two studies provide evidence for the accumulation of TCS in the bile of fish. Other European studies have reported TCS and its derivatives in fish tissues. Buser *et al.* (2006) analyzed levels of MTCS in juvenile (1–2 year old) brown trout (*Salmo trutta fario*) from rivers in Northern Switzerland receiving effluent from WWTPs. Concentrations of MTCS in fish were reported between 130 and 2100 ng g^{-1} of lipid weight. Balmer *et al.* (2004) detected MTCS in lake fish in the range 4–370 ng g^{-1}, lower levels compared with those previously measured in fish samples from rivers. This difference is to be expected as concentrations of MTCS should typically be higher in river systems that receive inputs from WWTPs. A large monitoring study on TCS and MTCS was conducted by Boehmer *et al.* (2004) using fish tissues from the German Environmental Specimen Bank. Samples of muscle tissue from breams (*Abramis brama*) from the period 1994–2003 were analyzed for TCS and MTCS. While TCS was only detected in a small number of samples, MTCS was present in all of the muscle samples analyzed. A pattern of increasing MTCS concentrations was observed in bream muscle tissue from the mid 1990s until after 2000, with levels of MTCS increasing from 10 to 14–26 ng g^{-1} of wet weight. TCS concentrations ranged from below the limit of quantification up to 3.4 ng g^{-1}. From their retrospective monitoring data, the authors of the study concluded that MTCS is a persistent pollutant with the potential to accumulate in the muscle tissue of fish.

To date, only a few North American studies have monitored TCS and MTCS levels in freshwater fish (Table 2). Valters *et al.* (2005) detected TCS in the plasma samples of 13 species of fish sampled from the Detroit River, in the range of 750 to >10 000 pg g^{-1} of wet weight. MTCS was also detected in the plasma samples, albeit in much lower concentrations, ranging from 0.4 to 13.4 pg g^{-1} of wet weight. Based on these plasma samples, the authors estimated the body burden of TCS to be 2–67 ng. Leiker *et al.* (2009) identified MTCS in male common carp (*Cyprinus carpio*) from the Las Vegas Bay and in the Las Vegas Wash, Nevada; MTCS was detected in all carp sampled ($n = 29$), with a mean concentration of 520–596 μg kg^{-1} per wet weight basis. The concentrations of MTCS detected in this study were much higher than those documented in previous studies, with the authors, indicating that this might be due to the sediment foraging behavior of carp, which exposes them to higher levels of lipophilic water-borne chemicals than other fish species. TCS and its metabolites have been detected in sediments, both freshwater and marine (Agüera *et al.*, 2003; Miller *et al.*, 2008; Chalew and Halden, 2009). A national pilot study in the USA surveyed the presence of pharmaceuticals and personal care products, including TCS, in fish sampled from five effluent-dominated rivers receiving discharge from WWTPs in large urban centers and a reference river (Ramirez *et al.*, 2009). Although several products, including carbamazepine and norfluoxetine, were detected at ng g^{-1} concentrations in fish tissues, GC/MS analysis revealed only trace amounts of TCS in fillets.

Marine mammals

Fair *et al.* (2009) characterized the occurrence of TCS in the plasma of wild Atlantic bottlenose dolphins (*Tursiops truncates*), a top level predator, and then correlated biological levels with environmental concentrations. This study was the first to document the bioaccumulation of TCS in a marine mammal. Plasma samples were collected from the dolphins in Charleston, South Carolina and Indian River Lagoon, Florida, two southeast USA estuarine sites. TCS measured in estuarine water samples

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ranged from 4.9 to 13.7 ng l^{-1}, averaging 7.5 ng l^{-1}. Plasma concentrations were 0.12–0.27 and 0.025–0.11 ng g^{-1} wet weight, at the two estuaries, respectively. Subsequently, TCS has also been detected at a concentration of 9.0 ng g^{-1} of wet weight in the plasma of a captive killer whale (*Orcinus orca*) fed a diet of herring harvested from the coast of British Columbia (Bennett *et al.*, 2009). These studies further highlight the need to monitor TCS and assess its effects in wild species.

Occurrence of TCS and its Derivatives in the Terrestrial Environment

Although TCS is considered to be primarily a water-borne contaminant, the antimicrobial can, and does, enter the terrestrial environment during the application of sewage sludge to agricultural and/or industrial land (Lozano *et al.*, 2010; Fuchsman *et al.* 2010). Activated sludge concentrations of TCS are typically measured between 580 and 14 700 μg kg^{-1} of dry weight, whereas concentrations in biosolids have been documented in the range of 90–32 900 μg kg^{-1} (Chalew and Halden, 2009; Lozano *et al.*, 2010; Table 1). Studies across three continents examined TCS levels in sewage WWTP sludge and reported similar concentrations of the antimicrobial, with a median concentration of 5000 μg kg^{-1} of dry weight (Reiss *et al.*, 2009). In WWTPs that use activated sludge treatment in combination with anaerobic biosolid digestion, 50 ± 19 of the influent mass of TCS will accumulate and persist in sewage sludge (Chalew and Halden, 2009). Although it is clear that the amendment of agricultural lands with biosolids produced from WWTPs represents a significant pathway for the release of TCS into the terrestrial environment, the environmental impact of land amendment practices that use biosolids from WWTPs has not been assessed.

To predict the effects of TCS in biosolids on the terrestrial environment, it is necessary to understand its fate in soil. Ying *et al.* (2007) investigated the biological degradation of TCS in soil under aerobic and anaerobic conditions. Quantitative structure–activity relationship analyses confirmed findings from previous studies, demonstrating TCS's propensity to sequester in soil and sediment. Laboratory experiments under aerobic conditions showed that TCS had a half-life of 18 days. However, the antimicrobial persisted in anaerobic soil for the entire duration of the 70 day experiment. These findings agree with McAvoy *et al.* (2002), who reported that the bulk of TCS in WWTPs was removed during aerobic sludge digestion, with anaerobic sludge digestion accounting for a very small portion of TCS removal. Thus in both terrestrial and aquatic environments, the biodegradation of TCS occurs primarily under aerobic conditions as the antimicrobial is resistant to anaerobic degradation. Owing to TCS's lipophilic nature, the antimicrobial partitions into sediment and soil, but its transport potential from biosolids into surface runoff has been characterized as low (Sabourin *et al.*, 2009).

Although TCS may not be physically mobile between soil compartments, other processes may transfer TCS from soil to biota. Kinney *et al.* (2008) assessed the potential for organic biosolid- or manure-derived soil contaminants in amended agriculture land to accumulate in biota. Tissue concentrations of TCS in earthworms inhabiting the amended soil reached 2610 μg kg^{-1}, translating into a bioaccumulation factor of 27. Based on the findings of this study, the predation of earthworms by birds and other animals could result in the transfer of TCS up the food chain, although this has not yet been documented.

HUMAN EXPOSURE AND LEVELS

The presence of TCS in human tissues has been documented by a number of studies from populations in Europe, the USA and Australia, which would be expected considering the number of personal care products containing the antimicrobial and the ability of TCS to be absorbed dermally (Queckenberg *et al.*, 2010).

Urine

Measuring the levels of environmental chemicals, such as TCS, in urine represents an important biomonitoring tool for exposure assessment, especially considering that TCS and its metabolites are excreted primarily in urine (Queckenberg *et al.*, 2010). One of the earlier characterizations of the baseline excretion of TCS in urine, along with plasma levels, was published by Sandborgh-Englund *et al.* (2006). Five male and five female subjects with a median age of 28 years were exposed to a single oral dose of TCS, and urine and blood samples were collected before and up to 8 days after exposure. The baseline excretion of TCS was in the range of 0.1–743 μg day^{-1}, and although maximal plasma levels were reached within 1–3 h in all subjects, plasma levels varied considerably, ranging between 0.1 and 8.1 μg l^{-1}. Neither baseline urinary excretion of TCS nor plasma levels were correlated with the use of TCS containing personal care products. The authors provided three possible explanations for these unexpected results: (1) the monitoring of personal hygiene products was not exhaustive; (2) labeling of product contents was not complete; (3) other sources of exposure explain the variable baseline levels. Indeed, other sources of TCS exposure may include sportswear items, shoes, socks and impregnated household items (Adolfsson-Erici *et al.*, 2002). The full range of consumer, industrial and pharmaceutical products that contain TCS needs to be included in future exposure assessment studies in humans.

To further assess the exposure to TCS in a representative sample of the US population, the National Health and Nutrition Examination Survey collected 2517 urine samples and detected TCS in 74.6% of the samples at concentrations of 2.4–3790 μg l^{-1} (Calafat *et al.*, 2008). TCS concentrations varied with age and socio-economic status, but not race/ethnicity or sex. Concentrations were the highest amongst people in their thirties and those with higher household incomes. The high frequency of detection resulting from this study is not surprising, since a significant proportion of personal care products on the market today contain TCS, with personal care products considered the primary route of exposure.

As with other toxicants, including potential endocrine disruptors, childhood exposures are a concern, as it is uncertain how these chemicals may alter growth and development processes. In light of the high potential for exposure to TCS, a better understanding of TCS exposure and levels in children is urgently needed. A pilot study by Wolff *et al.* (2007) collected urine samples from 90 girls between the ages of 6 and 8. Sampling was conducted in a manner that ensured that participants represented four racial groups (Asian, African American, White and Hispanic) and three regional locations (New York City, Cincinnati and the San Francisco Bay area of California). Urinary concentrations of TCS for all sites were 1.6–956.0 μg l^{-1}, with a median concentration of 7.2 μg l^{-1}.

Plasma

TCS was detected in human plasma in a study investigating the body burden of phenolic halogenated compounds (PHC) (Hovander *et al.*, 2002). Ten samples of blood plasma were randomly selected from male donors between the ages of 30 and 40, donated from a blood donor central at a hospital in Stockholm, Sweden. The authors identified TCS as one of more than 100 PHCs present in the plasma of Swedish males. Building on the work of Hovander *et al.* (2002), later studies, including the study of baseline plasma levels and urine excretion of TCS (Sandborgh-Englund *et al.*, 2006, see above), have detected the antimicrobial in humans.

The ubiquitous presence of TCS in the plasma of nursing mothers in Sweden has been documented by Allmyr *et al.* (2006). Plasma concentrations of TCS ranged from 0.010 to 38 ng g^{-1}, and in contrast to the study by Sandborgh-Englund *et al.* (2006), median TCS concentrations in subjects classified as users of products containing TCS were significantly higher than those in the control group, although the antimicrobial was detected in plasma samples from both the exposed and the control group. Of interest is the presence of TCS in the entire study population, indicating that other routes of exposure than personal care products influence plasma concentrations of TCS. Determining the various potential sources of TCS in human tissues warrants further research. As it currently stands, not all countries require TCS to be listed on a product's label, making it difficult to ascertain all the potential sources of exposure to the antimicrobial.

The influence of age, gender and place of residence on plasma concentrations of TCS in the Australian population has been examined by Allmyr *et al.* (2008). In this particular study, place of residence had no effect on serum concentrations of TCS, while age and gender exerted minimal yet significant influence. TCS concentrations were more elevated in males than females, and reached peak concentrations in the group of 31–45-year-old males and females. Owing to the lack of marked differences in plasma concentrations observed in the study, the authors concluded that the exposure of the Australian population to TCS is relatively homogenous. In comparison to data from the Swedish population, serum levels of TCS were 2 times higher in the Australian population, a phenomenon which is most likely due to the fact that the Swedish government strongly discourages consumers from using antibacterial products (Swedish Chemical Agency, 2001).

Dirtu *et al.* (2008) tested the sensitivity of solid-phase extraction and gas chromatography coupled to electron-capture negative-ionization mass spectrometry to detect phenolic compounds, including TCS, in human serum. The method used in the study yielded results that were comparable to previous data collected on the levels of TCS in human fluids. In this study, the median concentration of TCS in Belgian human serum samples ($n = 21$) was 0.52 ng ml^{-1}. As in earlier studies, TCS exhibited a high detection frequency as was evidenced by its presence in all the samples.

Breast Milk

The lipophilicity of TCS ($K_{ow} = 4.76$) coupled with its relative stability in human tissues makes it probable that the antimicrobial will be present in breast milk, with concentrations relating to maternal serum levels and the fat content of the milk (Ito and Lee, 2003). The presence of TCS in the breast milk of Swedish women was first reported by Adolfsson-Erici *et al.* (2002). A second study measuring TCS in breast milk was carried out by Allmyr *et al.* (2006), confirming the findings that were initially reported by Adolfsson-Erici *et al.* (2002). Concentrations of TCS in milk samples were higher in women who used personal care products containing TCS, compared with women who did not, although TCS and/or its metabolites were detected in all the milk samples. Concentrations of TCS ranged from <0.018 to 0.95 ng g^{-1}, which is comparable to the concentrations measured by Adolfsson-Erici *et al.* (2002). The universal presence of TCS in both groups indicates that personal care products are not the only source of human exposure to the antimicrobial. The levels of TCS in the subject's milk were significantly lower than levels measured in their plasma, indicating that infants receive a smaller dose of TCS than what is present in maternal systemic circulation.

Building on the landmark study conducted by Adolfsson-Erici *et al.* (2002) a risk assessment for TCS in human breast milk was conducted by Dayan (2007). The study obtained 62 samples of breast milk from The Mothers Milk Banks in California and Texas. A GC/MS method was used to measure TCS levels in the milk samples, expressing the results as TCS per lipid basis. The results of the study were as follows: no TCS detected in two of the samples, trace amounts present in nine of the samples, with the remaining 51 samples ranging in TCS concentrations from 100 to 2100 μg kg^{-1} lipid. Based on the finding that a 6500-fold margin of safety exists between the levels of human exposure and the highest concentration of TCS that would elicit any adverse effects in human systems, the author concluded that the levels of TCS measured in breast milk do not pose a risk to breastfeeding infants. Interestingly, a recent review on TCS and development of margins of safety for consumer products by Rodricks *et al.* (2010) did not assess the exposure of infants to TCS through breast milk and the associated risks. The prevalence of TCS in human systems warrants further investigation into the bioaccumulation potential and toxicological effects of the antimicrobial, especially during sensitive periods of fetal and neonatal development.

KINETICS AND METABOLISM

Dermal

The percutaneous absorption of TCS from personal care product preparations was first investigated by Black *et al.* (1975). Rat skin was treated with shampoo or aerosol deodorant containing 0.05% (w/v) and 0.1% (w/v) [^{3}H] TCS, respectively. The degree of TCS penetration was calculated from the amount of radioactivity excreted from the animals. Of the total amount of shampoo and aerosol deodorant applied, the bulk of TCS was removed by rinsing, with only small amounts penetrating the skin. Kanetoshi *et al.* (1992) confirmed that, in mice, TCS is absorbed through the skin and is widely distributed throughout the various compartments in the body. Tissue concentrations of TCS peaked at 12 or 18 h and were present in decreasing order in the following tissues: gall bladder, liver, lung, adipose tissue and blood. Moss *et al.* (2000) used a similar approach to characterize the metabolism and kinetics of TCS. Following a dermal application of ^{3}H-labeled TCS to the backs of female rats, the antimicrobial penetrated the dermis within the first hour of the experiment and was subsequently removed from the bloodstream. The primary elimination route

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of radioactivity was through fecal matter, with urinary excretion constituting a secondary removal pathway. In both urine and feces, TCS glucuronide and sulfate were detected, indicating that phase II biotransformation reactions play an essential role in the metabolism of the antimicrobial. Moreover, TCS glucuronide and sulfate were extracted from rat skin *in vivo*, suggesting that the antimicrobial is locally metabolized in skin cells. The amount of TCS that entered systemic circulation over the 24 h period was 21%; 12% of radioactivity was in the feces, 8% in the carcass, 1% in the urine, 30% in the stratum corneum, with 26% remaining on the surface of the skin.

Queckenberg *et al.* (2010) characterized the absorption and pharmacokinetics of TCS after a dermal administration in human subjects. A hydrophobic cream containing 2% TCS was applied to the skin of six Caucasian volunteers. The 12 h exposure period culminated by the subjects taking a shower to eliminate any cream that remained on their skin. Urinary excretion of free and conjugated TCS was measured in intervals up to 168 h post-application. Of the TCS absorbed, the majority of the antimicrobial was excreted within 24 h. The half-life of TCS was calculated to be 10.8 h. This value is consistent with the previous study by Sandborgh-Englund *et al.* (2006), which determined the median half-life of TCS based on urinary excretion to be 11 h, following an oral administration. The total amount of TCS excreted is reflective of the amount absorbed, indicating a limited potential for accumulation in the body, and further reinforcing previous findings that, in humans, urinary excretion is a major elimination route for the antimicrobial.

Subcutaneous

To investigate the kinetics of subcutaneous exposure, female rats were injected with 0.5 ml of [^{3}H] TCS solution in aqueous polyethylene glycol (Black *et al.*, 1975). The animals were housed in individual metabolic cages, and urine and feces were collected for the analysis of radioactivity. Within 4 days of the injection, 89.2% of the dose was recovered, with 33% of TCS recovered in urine. In agreement with other animal studies on the pharmacokinetics of TCS, a greater proportion of radioactivity was eliminated in feces than urine. TCS levels in the blood peaked at 6 h after the administration of the dose, decreasing steadily after this time, and the biological half-life was calculated as 14 h.

Oral

The earliest published kinetic study of orally administered TCS is the study by Tulp *et al.* (1979). A single oral dose of 500 mg kg^{-1} TCS was given to male albino Wistar rats housed in metabolic cages for 7 days. Fecal matter and urine was collected daily and upon termination of the experiment, liver and abdominal fat were collected for analysis. TCS was metabolized primarily through hydroxylation, with scission of the ether bond representing a minor biotransformation pathway. Five hydroxylated metabolites were detected in urine, whereas only three of these metabolites were present in feces. The metabolite 2,4-dichlorophenol was detected in both urine and feces, with 4-chlorocatechol occurring in urine only, both of these metabolites being the product of the scission of the ether bond. In feces, TCS and its metabolites were excreted primarily unconjugated, with significant amounts of the parent compound present in both urine and feces. On completion of the experiment (7 days), TCS was present in both liver and abdominal fat samples, but because the dose of the TCS was so high (500 mg kg^{-1}), no conclusions could be made about its bioaccumulation potential. The authors concluded that the metabolism of TCS is unlikely to yield chlorodibenzo-*p*-dioxins or chlorodibenzofurans.

A later study by Kanetoshi *et al.* (1988) examined the disposition and excretion of TCS and its three chlorinated derivatives in mice. [^{3}H]-TCS and 2′,3,4,4′-tetrachloro-2-hydroxydiphenyl ether, 2′,4,4′,5-tetrachloro-2 hydroxydiphenyl ether and 2′,3,4,4′,5-pentachloro-2-hydroxydiphenyl ether were orally administered to male mice. Radioactivity was primarily distributed in the gall bladder, liver, lung, heart and kidneys. Of the five tissues, TCS was most concentrated in the gall bladder which the authors attributed to biliary excretion via enterohepatic circulation. These results are consistent with other studies that have determined that, in animals, radiolabeled TCS is excreted primarily in feces and secondarily in urine (Siddiqui and Buttar, 1979; Moss *et al.*, 2000). The ^{3}H-labeled TCS was rapidly absorbed and excreted, with calculated half-life of 8 h. At the 24 h mark, the radioactivities of TCS and its three chlorinated derivatives were nearly completely eliminated from all tissues, indicating that TCS and/or its metabolites do not accumulate in the body. Gilbert and Williams (1987) investigated the oral retention and pharmacokinetics of [^{3}H]-TCS in an antimicrobial toothpaste. Twelve healthy male volunteers between the ages of 19 and 37 were recruited for the study and brushed their teeth with 1 g of toothpaste containing 0.02% of [^{3}H]-TCS. The oral retention of TCS was found to be 36.3 ± 1.4%. TCS remained in bacterial plaque for at least 8 h after dosage and in oral mucosa for 3 h. A review of the safety of TCS was conducted by DeSalva *et al.* (1989), evaluating data from multiple sources including pre-clinical and clinical studies, data submitted to the Antimicrobial I OTC Review Panel and unpublished work from the Pharmacology and Toxicology Department of the Colgate-Palmolive company. Humans, dogs, rabbits and rats were used to study the pharmacokinetics of TCS. Routes of administration included oral, dermal and intravenous; pharmacokinetic data on the antimicrobial indicated that, in humans, the kidneys are the main excretory organ responsible for the elimination of TCS, as is evidenced by the proportion of TCS and its conjugates that are concentrated in urine.

The buccal absorption of TCS from 0.03% mouthwash was calculated by Lin (2000). Subjects were given 15 ml of TCS oral mouthrinse or a placebo oral rinse to be used twice daily. Blood and dental plaque samples were collected 4 and 1 h after rinsing, respectively. The average daily retention dose of TCS was 0.660 mg, which translates into 7.33% of the original dose. On average, TCS concentrations in plaque were in the range of 20.5–46.4 μg per gram of plaque collected. The average concentration of TCS in plasma ranged from 74.5 to 94.2 μg ml^{-1}, with peak concentrations attained 2 days following exposure. Plasma levels of TCS returned to baseline concentrations 8 days after the last treatment. More recently, Sandborgh-Englund *et al.* (2006) examined the pharmacokinetic pattern of TCS in humans after a single dose oral administration. Subjects were required to fast overnight and the following morning they were given 13 ml of a 0.03% mouthwash solution, equivalent to a 4 mg oral dose of TCS. Blood and urine levels were monitored prior to exposure and up to 8 days after exposure, and baseline levels of TCS in plasma and urine were

determined for each subject. Plasma concentrations of TCS increased rapidly after dosing, attaining peak levels within 1–3 h, resulting in a terminal plasma half-life of 21 h. In plasma, 30–35% of TCS was present in the unconjugated form. These results are different from those of DeSalva *et al.* (1989), who reported that the entirety of TCS measured in plasma was in the conjugated form (either glucuronide or sulfonate). Unfortunately, the basis for these differences is unknown. Lastly, the cumulative urinary excretion of TCS was 54%, occurring 4 days after exposure, and the calculated urinary excretion half-life was 11 h. The results of this study confirmed again that TCS is rapidly absorbed from the gastrointestinal tract and swiftly eliminated from the body, usually within a 24 h period.

Intravenous

Siddiqui and Buttar (1979) investigated the pharmacokinetics of intravenous and intravaginal injections of TCS in sexually mature virgin Wistar rats. ^{14}C-Triclosan was injected into the femoral vein (5 mg kg^{-1} in polyethylene glycol-400) or the vaginal orifice (5 mg kg^{-1} in corn oil) and liquid scintillation spectrophotometry was used to determine radioactivity. In both treatments, the rate of transfer from plasma to tissues was rapid, probably attributable to the lipophilicity of TCS. The half-life of TCS in the β phase was 8.8 ± 0.6 h and the blood clearance rate was 77.5 ± 11.3 ml kg^{-1} h^{-1} after intravenous injection or intravaginal administration. In the intravenous administration, after 24 h, 18% of TCS was excreted in feces and 9% was eliminated in urine. In contrast, the intravaginal injections resulted in excretions of 26% in feces and 14% in urine.

Phase I and II Enzymes

There is evidence for the ability of TCS to interact with cytochrome P450 enzymes in liver microsomes, although the effects may be dose- and species-dependent. The antimicrobial inhibited *in vitro* methylcholanthrene (MC)- and phenobarbital (PB)-inducible P450-dependent monoxygenases, specifically pentoxyresorufin *O*-dependentylase (PROD) and ethoxyresorufin *O*-deethylase (EROD) activity, competitively or noncompetitively (Hanioka *et al.*, 1996). These results are important since induction of P450 isoforms of the CYP1A or CYP2B subfamily closely relates to toxicity of the antimicrobial. A later study (Hanioka *et al.*, 1997) reported that TCS (Irgasan DP300) induces the P450 isoforms of the CYP2B subfamily. Similar results suggesting that TCS is a phenobarbital-type inducer were reported by Jinno *et al.* (1997), in cultured rat hepatocytes and by Kanetoshi *et al.* (1992), in mice liver microsomes. In contrast, Ishibashi *et al.* (2004) found no evidence that TCS induces EROD and PROD activity in the hepatic microsomes of female medaka, *Oryzias latipes*. This discrepancy may be due to physiological differences between mammals and fish, and/or differences in exposure. Jacobs *et al.* (2005) presented *in vitro* evidence for TCS acting as a ligand, with a moderate affinity for the human pregnane X receptor (hPXR). The hPXR regulates the expression of phase I enzymes such as cytochrome P450 3A4 (CYP3A4), which play an integral role in the biotransformation of approximately 50% of pharmaceuticals (Luo *et al.*, 2002; Jacobs *et al.*, 2005). Compounds that are capable of upregulating the transcription of CYP3A4 enzymes can alter the rate at which pharmaceuticals are metabolized, creating the potential for adverse effects.

TCS, structurally similar to hydroxylated polychlorinated biphenyls, also interacts with phase II enzymes. TCS is both a substrate and inhibitor of sulfonation and glucoronidation in human liver cytosol and microsomes (Wang *et al.*, 2004). The inhibition of sulfonation was noncompetitive, while the inhibition of glucuronidation was competitive. These results confirm earlier evidence of the inhibition of sulfation, specifically that of thyroid hormones, by TCS and other hydroxylated halogenated chemicals (Schuur *et al.*, 1998). TCS sulfonation in polar bear liver, similar to human liver in respect to enzyme affinity, was characterized by Sacco and James (2005). In addition to effects on phase I and phase II enzymes, TCS may have direct effects on mitochondria, impairing function through an uncoupler effect and disrupting mitochondrial membrane fluidity (Newton *et al.*, 2005).

The available evidence indicates that the two most probable routes of exposure to TCS in humans are ingestion and/or percutaneous absorption. Low but detectable levels of TCS have been reported in drinking water (Loraine and Pettigrove, 2006; Servos *et al.* 2007). Blanset *et al.* (2007) estimated the ADI (acceptable daily intake) for TCS at 0.05 mg kg^{-1} per day, and concluded that, based on TCS levels typically measured in drinking water, the risk to human health is minimal. Concentrations of TCS in the blood are directly related to consumer use patterns of the antimicrobial. In humans, after an oral dose of TCS, the antimicrobial is eliminated primarily as conjugated metabolites in urine. In the study by Sandborgh-Englund *et al.* (2006), approximately 70% of the total amount of TCS measured in plasma existed as either sulfate or glucuronide conjugates. TCS interacts with phase I and II enzymes, contributing to its toxicity and endocrine disrupting properties.

TOXICITY

Numerous studies have evaluated the toxicity of TCS in various organisms, including algae, invertebrates, amphibians, fish, birds and mammals. Data from mammalian studies, including humans, has recently been reviewed by Rodricks *et al.* (2010). The following section briefly summarizes those studies and provides a more detailed review of toxicity data from nonmammalian species.

Acute Toxicity

Terrestrial organisms

Several recent studies investigated the terrestrial ecotoxicological effects of TCS. TCS inhibited plant growth (EC_{50} 57–108 mg kg^{-1}) and soil respiration, with some evidence for recovery after 2 days, possibly linked to degradation of TCS (Liu *et al.*, 2009). Waller and Kookana (2009) reported that TCS, at concentrations below 10 mg kg^{-1}, disturbs the nitrogen cycle in some soils. An ecological risk assessment for TCS in the terrestrial environment has been published by Reiss *et al.* (2009). The assessment reviewed available data and found satisfactory margins of safety for terrestrial organisms, including earthworms, plants, and soil microorganisms exposed to TCS in soils amended with sewage sludge, and to birds and mammals exposed indirectly through the consumption of earthworms and fish. However, the number of studies available for the risk assessment was relatively small ($n = 31$), indicating that further investigations of the potential impact of TCS on the terrestrial ecosystems are needed.

Table 3. Effects of triclosan (TCS) on freshwater (FW) and marine (SW) organisms

Test species	Life stage	System type	Route of exposure	Test duration	TCS exposure	Endpoint	Reference
Algae							
Phytoplankton (*Dunaliella tertiolecta*)		SW	Water (static)	Acute (96 h)	3.5 μg l^{-1}	EC_{50} (population density)	DeLorenzo and Fleming (2008)
Green alga (*Selenastrum capricornutum*)		FW	Water (static)	Acute (72 h)	4.7 μg l^{-1}	EC_{50} (growth)	Tatarazako *et al.* (2004)
Green alga (*Scenedesmus subspicatus*)		FW	Water (static)	Acute (96 h)	1.4 μg l^{-1}	EC_{50} (biomass)	Orvos *et al.* (2002)
Alga (*Closterium ehrenbergii*)		FW	Water (static)	Acute (48 h)	620 μg l^{-1};250 μg l^{-1}	EC_{50} genotoxicity	Ciniglia *et al.* (2005)
Blue-green alga (*Anabaena flos-aquae*)		FW	Water (static)	Acute (96 h)	1.6 μg l^{-1}	EC_{50} (biomass)	Orvos *et al.* (2002)
Invertebrates							
Daphnia magna		FW	Water (renewal)	Acute (48 h) 21 days	390 μg l^{-1} 40 μg l^{-1}	EC_{50} NOEC reproduction	Orvos *et al.* (2002)
Ceriodaphnia dubia		FW	Water (renewal)	Acute (48 h) 7 days	240 μg l^{-1} 182 μg l^{-1}	EC_{50} NOEC reproduction	Orvos *et al.* (2002)
		FW	Water (renewal)	6–7 days	220 μg l^{-1}	IC_{50} (growth)	Tatarazako *et al.* (2004)
Chironomus tentans *Hyalella azteca*		FW	Water (renewal)	10 days	400 μg l^{-1} 200 μg l^{-1}	LC_{50}	Dussau *et al.* (2008)
Grass shrimp (*Palaemonetes pugio*)	Embryo Larvae Adult	SW	Water (renewal)	Acute (96 h)	651 μg l^{-1} 154 μg l^{-1} 305 μg l^{-1}	LC_{50} LC_{50} LC_{50}	DeLorenzo *et al.* (2008)
Crustacean (*Thamnocephalus platyurus*)		FW	Water (static)	Acute (24 h)	470 μg l^{-1}	LC_{50}	Kim *et al.* (2009)
Bivalve (*Mytilus galloprovincialis*)	Hemocytes	SW	*In vitro*	Acute (30 min)	1 μM	↓ lysosomal membrane stability	Canesi *et al.* (2007)
	Whole animal	SW	Injection	Acute (24 h)	2.9 ng g^{-1}	Altered hemocyte and digestive gland function	Canesi *et al.* (2007)
Zebra mussel (*Dreisena polymorpha*)	Hemocytes	FW	*In vitro* *In vivo*	Acute (60 min) Acute (96 h)	0.1 μM 1 M	Genotoxicity Genotoxicity	Binelli *et al.* (2009a, 2009b)
Fish	Adult	FW	Water	Acute (96 h)	390 μg l^{-1}	LC_{50}	CIBA (1998)
Rainbow trout (*Oncorhynchus mykiss*)	Embryo	FW	(flow-through)	61 days 35 days	71.3 μg l^{-1}	Delayed swim-up ; ↓ 35 dph survival; erratic swimming, locked jaw	Orvos *et al.* (2002)
Medaka (*Oryzias latipes*)	Fertilized eggs	FW	Water (renewal)	14 days	313 μg l^{-1}	↓ hatching; delayed hatching	Ishibashi *et al.* (2004)
	Larvae (24 h old)			Acute (96 h)	602 μg l^{-1}	LC_{50}	
	Male fish			21 days	20 μg l^{-1}	↑ liver Vtg	
	Fry	FW		Acute (48 h)	350 μg l^{-1}	LC_{50}	Foran *et al.* (2000)
	Eggs	FW		14 days	400 μg l^{-1}	IC_{50} (hatching)	Tatarazako *et al.* (2004)
		SW	*In ovo* injection	1 day post-fertilization	4.2 ng egg^{-1}	EC_{50} (survival)	Nassef *et al.* (2010)

Table 3. *(Continued)*

Test species	Life stage	System type	Route of exposure	Test duration	TCS exposure	Endpoint	Reference
	Larvae	FW	Water (static)	Acute (96 h)	600 μg l^{-1}	LC_{50}	Kim *et al.* (2009)
	Adult	SW	Water (renewal)	Acute (96 h)	1700 μg l^{-1}	LC_{50}	Nassef *et al.* (2009)
Bluegill sunfish (*Lepomis macrochirus*)		FW	Water (renewal)	Acute (96 h)	370 μg l^{-1}	LC_{50}	Orvos *et al.* (2002)
Fathead minnow (*Pimephales promelas*)	Adult	FW	Water (renewal)	Acute (96 h)	260 μg l^{-1}	LC_{50} (at pH 7.5)	Orvos *et al.* (2002)
	Full life cycle	FW	Water (TCS in mixture)		0.1 and 0.3 μg l^{-1} mixture of products	No effects F_o; ↑ larval deformities in F_1	Parrott and Bennie (2009)
Zebrafish (*Danio rerio*)	Eggs	FW	Water (renewal)	9 Days	220 μg l^{-1}	IC_{50} (hatching)	Tatarazako *et al.* (2004)
	Embryo	FW	24-Well microplates	Acute (96 h)	420 μg l^{-1}	LC_{50}; teratogenic effects	Oliveira *et al.* (2009)
	Adult	FW	Semi-static	Acute (96 h)	340 μg l^{-1}	LC_{50}	Oliveira *et al.* (2009)
Amphibians							
Bullfrog (*Rana catesbeiana*)	Tadpoles	FW	Water	Acute (96 h)	0.15 μg l^{-1}	↑hindlimb development, ↓ body weight, disruption of thyroid hormone-associated gene expression	Veldhoen *et al.* (2006)
Xenopus leavis	XTC-2 cells	FW	*In vitro*	Acute (24 h)	0.03 μg l^{-1}	Altered thyroid hormone receptor mRNA expression	Veldhoen *et al.* (2006)
Acris crepitans blanchardii	Larvae	FW	Water	Acute (96 h)	367 μg l^{-1}	LC_{50}	Palenske and Dzialowski (2010)
Bufo woodhousii woodhousii	Stage 30				152 μg l^{-1}		
Rana sphenocephala					562 μg l^{-1}		
Xenopus laevis	Stage 41				343 μg l^{-1}		
Leopard frog (*Rana pipiens*)	Tadpoles	FW	Static (weekly renewal)	24 days	0.23 μg l^{-1} 230 μg l^{-1}	↓activity, loss of startle response, ↓ survivorship	Fraker and Smith (2004)
American toad (*Bufo americanus*)	Tadpoles	FW	Static (weekly renewal)	14 days	230 μg l^{-1}	↑ activity	Smith and Burgett (2005)

EC50, effective concentration; LC50, lethal concentration; NOEC, no observed effect concentration.

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Aquatic organisms

Microorganisms and algae. TCS is primarily a water-borne pollutant, with numerous studies having investigated its toxicity in aquatic organisms (Table 3). The toxicity of TCS to WWTP sludge organisms, algae, daphnids and fish was assessed by Orvos *et al.* (2002). While sludge microorganisms were unaffected by TCS, the aquatic species that appeared most vulnerable to the toxic effects of TCS were algal species such as *Scenedesmus subspicatus*, with a 96 h biomass EC_{50} (median effective concentration) of 1.4 μg l^{-1} and a 96 h no-observed-effect concentration (NOEC) of 0.69 μg l^{-1} (Orvos *et al.*, 2002). Similar evidence regarding algal sensitivity was provided by Tatarazako *et al.* (2004) for *Selenastrum capricornutum*, and by DeLorenzo and Fleming (2008) for a marine phytoplankton, *Dunaliella tertiolecta* (Table 3). The toxic effects of TCS were primarily due to the neutral form of TCS, where sorption and ionization could potentially temper these effects in the aquatic ecosystems. The lowest NOEC for algae, integral components of aquatic food webs, is less than 1 μg l^{-1}, with TCS being measured in the range of 0.2–2.7 μg l^{-1} in US wastewater effluent (Reiss *et al.*, 2002). It is then possible that current levels of TCS in rivers and streams (PEC, predicted environmental concentration) may surpass the NOEC for algae, as indicated by HQ value (Hazard Quotient, PEC divided by NOEC) greater than 1. Coogan *et al.* (2007) measured TCS levels in the algal species, *Cladophora* spp., located downstream from a WWTP. Although TCS concentrations in the water column decreased downstream from the effluent output (0.12–0.06 μg l^{-1}), concentrations of the antimicrobial in algae demonstrated the reverse pattern (100–150 μg l^{-1}), indicating bioaccumulation. It is unclear how the propensity of algae to accumulate TCS affects the species vulnerability to the toxicity of the antimicrobial.

Invertebrates. Aquatic invertebrates also exhibit vulnerability to TCS (Table 3). Short-term (30 min) exposure of hemocytes, the immune cells of bivalve *Mytilus galloprovincialis*, to TCS reduced lysosomal stability and induced the release of lysosomal hydrolytic enzymes (Canesi *et al.*, 2007). Moreover, *in vivo* exposures of the bivalve affected glycolytic enzymes and redox balance in different systems/organs. For *Daphnia magna*, a key invertebrate aquatic species, the 48 h median effective concentration was 390 μg l^{-1} (Orvos *et al.*, 2002). The toxicity of TCS to the midge, *Chironomus tentans*, and the freshwater amphipod, *Hyalella azteca*, was evaluated in a 10-day exposure test (LC_{50} 0.4 and 0.2 mg l^{-1}, respectively). TCS was more toxic than carbamazepine, an anticonvulsant, and atorvastatin, a lipid regulator (Dussault *et al.*, 2008).

Fish. Kim *et al.* (2009) assessed the acute toxicity of TCS in two test species, a freshwater crustacean (*Thamnocephalus platyurus*) and a fish (*Oryzias latipes*). The organisms were exposed to a range of TCS concentrations and the 24 h LC_{50} values were determined by probit analysis. The LC_{50} values of TCS for *T. Platyurus* and *O. Latipes* were 0.47 and 0.60 mg l^{-1}, respectively. The LC_{50} (96 h) value for *O. Latipes* calculated in this study confirms the previous findings of Tatarazako *et al.* (2004), in which an LC_{50} value of 0.40 mg l^{-1} was ascertained for the antimicrobial.

Nassef *et al.* (2010) applied in *ovo* nanoinjection of TCS to medaka embryos and determined 4.2 ng per egg as the EC_{50} value based on survival and embryonic development. Foran *et al.* (2000) examined the acute toxicity of TCS in medaka fry (Table 3). Concentrations of 1 and 500 μg l^{-1} resulted in fry death within 24 h and 3 days, respectively. The LC_{50} (48 h) for medaka fry was calculated to be 352 μg l^{-1}. A 96 h median lethal concentration of 602 μg l^{-1} for medaka fry was reported by Ishibashi *et al.* (2004). Although this value is higher than the LC_{50} (48 h) of 352 μg l^{-1} previously determined by Foran *et al.* (2000), it appears that, during early development, fish are especially vulnerable to the toxic effects of TCS. Nassef *et al.* (2009) used adult Japanese medaka as the test organism in an acute toxicity study and observed concentration- and time-dependent mortality. Test solutions of TCS were 1, 2, 2.4 and 3 mg l^{-1} and the adult fish were exposed to TCS for a period of 96 h. Reported 96 h survival rates were 100% (1 mg l^{-1}), 16.7% (2 mg l^{-1}), 3.3% (2.4 mg l^{-1}) and 0% (3 mg l^{-1}). At higher exposure levels (2.4 and 3 mg l^{-1}), fish displayed abnormal behaviors and experienced a loss of equilibrium. The LC_{50} (96 h) of TCS for adult medaka was 1.7 mg l^{-1}, while the NOEC was estimated at 1.7 μg l^{-1}, which is 12 times higher than the PEC for the antimicrobial. The authors of this study are in agreement with Ishibashi *et al.* (2004), in their conclusion that TCS is highly toxic to fish.

Oliveira *et al.* (2009), in an experiment similar to the one by Nassef *et al.* (2009), studied the acute toxicity of TCS in different life stages of zebrafish (*Danio rerio*). The effect of TCS on mortality and developmental, genetic and enzymatic biomarkers were determined in adult fish and embryo/larvae using the OECD guidelines on Fish Embryo Toxicity. At concentrations above 0.7 mg l^{-1}, TCS exhibited teratogenic effects, delaying embryo development and resulting in mortality within 48 h. The LC_{50} (96 h) of TCS for embryo/larvae was 0.42 mg l^{-1}. The results of the biomarker analysis indicated that TCS increased the activity of cholinesterase (ChE; 0.25 mg l^{-1}), lactate dehydrogenase (LDH; 0.25 mg l^{-1}) and glutathione *S*-transferase (GST; 0.25 and 0.35 mg l^{-1}). Based on the results, concentrations of TCS equal to, or above 0.3 mg l^{-1}, were estimated to constitute a hazard for aquatic ecosystems. Using the OECD Guideline TG 203 in semi-static conditions, the LC_{50} (96 h) value for adult zebrafish was determined as 0.34 mg l^{-1}, a value similar to the LC_{50} (96 h) of 0.42 mg l^{-1} for zebrafish larvae. The acute toxicity of TCS was primarily limited to behavioral effects, none of which were studied in detail. Abnormal behavioral patterns observed during the study included irregular swimming, loss of equilibrium, and anomalous gill movement. In contrast to the embryo/larvae stage, there was no evidence of genotoxicity or changes in enzyme levels of ChE, GST and LDH in adult zebrafish. Although there were differences in species sensitivity to TCS, the range of LC_{50} (96 h) was relatively small (Table 3); the 96 h median lethal concentration values for *Pimephales promelas* and *Lepomis macrochirus* were 260 and 370 μg l^{-1}, respectively (Orvos *et al.*, 2002), while the value for adult zebrafish was 340 μg l^{-1}. During early development, juvenile fish are more sensitive to TCS than adults. In an early life-stage toxicity test with *Oncorhynchus mykiss*, the NOEC and the lowest-observed-effect concentration (LOEC) were 34.1 and 71.3 μg l^{-1}, respectively (Orvos *et al.*, 2002).

Amphibians. The effects of TCS were also investigated in amphibians. There is evidence to indicate that TCS affects behavior and survivorship in tadpoles; however, the effects seem to be species-specific. TCS increased activity levels in American toad tadpoles, *Bufo americanus*, although the effects on survivorship were not concentration-dependent (Smith and

Burgett, 2005). In tadpoles of the northern leopard frog, *Rana pipiens*, ecologically relevant concentrations of TCS decreased activity and survivorship (Fraker and Smith, 2004), indicating that the HQ for this specific endpoint is >1.0. In the latter study, no evidence of an interaction between TCS and caffeine or acetaminophen, pharmaceuticals often co-occuring in WWTP effluents, was detected. Palenske and Dzialowski (2010) assessed the species specific and developmental toxicity of TCS in amphibian larvae for *Acris crepitans blanchardii*, *Bufo woodhousii woodhousii*, *Rana sphenocephala* and *Xenopus laevis*. Bioconcentration factors for *X. laevis*, *B. woodhousii woodhousii* and *R. sphenocephala* were also determined. As is the case with other aquatic species, TCS toxicity was dependent upon larval maturity and amphibian species. *X. laevis* larvae were most vulnerable to TCS during the first two developmental stages. Larval LC_{50} values were reported as follows: 259–664 μg l^{-1} (*X. laevis*), 367 μg l^{-1} (*A. crepitans blanchardii*), 152 μg l^{-1} (*B. woodhousii woodhousii*) and 562 μg l^{-1} (*R. sphenocephala*), with significant differences observed for all three amphibian species (Table 3). In this study, TCS tissue uptake was related to larval species, stage of development and mean mass. Bioconcentration factors ranged from 44 in *X. laevis* up to 740 in *B. woodhousii woodhousii*.

Mammals. The toxicity of TCS has been tested in laboratory rodents, and other mammalian models. An early study by Lyman and Furia (1969), sanctioned by the Geigy Chemical Corporation, provided toxicological data on TCS in rats, concluding that TCS was neither acutely toxic (LD50 oral > 1000 mg kg^{-1}) nor carcinogenic. Later, nephrotoxic effects of orally administered TCS in rats were reported by Chow *et al.* (1977), in a study where the accumulation of *p*-aminohippurate (PAH) was estimated both *in vivo* and *in vitro*, using kidney slices to detect dose-related inhibition. A 1989 review on the safety of TCS was published by DeSalva *et al.* (1989). The majority of data in this review cited unpublished results from reports submitted by Ciba-Geigy Company to the Antimicrobial I OTC Review Panel. Ciba-Geigy Company tested the acute toxicity of TCS on four different species of animals; mouse, rat, rabbit and dog. Based on these studies, TCS was deemed not to be an acute oral toxicant.

One of the more recent studies detailing the acute toxicity of TCS in mammals was conducted by Kanetoshi *et al.* (1992). The study evaluated the acute toxicity and percutaneous absorption of TCS and its chlorinated derivatives in male mice. The results indicated that TCS has a low acute toxicity (LD_{50} > 1 g kg^{-1}), a value which is in agreement with the previous findings of Lyman and Furia (1969). The authors previously reported that Irgasan DP300 is commonly detected in commercial textile products and that an exposure to sodium hypochlorite (domestic bleach) leads to the formation of three different chlorinated derivatives. It is worth noting that the chlorinated derivatives of TCS are significantly more toxic than TCS itself, and as the number of chlorine substitutions in the TCS derivatives increases, their LD_{50} values decrease (Kanetoshi *et al.*, 1992). Dermal contact with textiles that have TCS incorporated into their fibers may expose consumers to chlorinated TCS derivatives. However, Rodricks *et al.* (2010) provided a recent critical review of the current mammalian literature and developed margins of safety for consumer products, concluding that the exposure to TCS in consumer products is not expected to cause adverse health effects in humans.

Current literature suggests that, while TCS is not acutely toxic to mammals, aquatic species such as algae and certain types of fish are highly sensitive to the antimicrobial. The NOEC for fish is in the range of 34.1–200 μg l^{-1} (Orvos *et al.*, 2002; Ishibashi *et al.*, 2004; Capdevielle *et al.*, 2008), a concentration range which exceeds the PECs (0.01–0.14 μg l^{-1}, Table 1) for TCS. The toxicity of TCS is also dependent on the life stage of the organism, with juveniles having a tendency to be more vulnerable to the toxic effects of the antimicrobial.

Subacute/subchronic and Chronic Toxicity

Toxicity data from prolonged exposure studies of aquatic organisms to TCS are relatively scarce (Table 3). Algae were determined to be the most sensitive aquatic organisms in experiments lasting up to 21 days, as has been shown in the acute toxicity studies (Orvos *et al.*, 2002). Significant adverse effects on survival and reproduction were also detected in the invertebrate, *Daphnia magna*. While juvenile rainbow trout were adversely affected by chronic exposures to TCS, fathead minnows were not. Parrott and Bennie (2009) used fathead minnow life-cycle tests to study the sub-lethal effects of environmentally relevant mixtures (ng l^{-1} range) of a personal care product (TCS, up to 115 ng l^{-1}) and six common pharmaceuticals. In comparison to controls, no significant differences in survival, growth, development or reproduction were observed in any of the treatment groups. The only significant effect of the PPCP mixture was observed in the F1 generation; the 100 and 300 ng l^{-1} PPCP mixture but not 1000 ng l^{-1}, produced significant increases in the rates of deformities, including cardiac edema, spinal deformities and yolk-sac edema. The magnitude of the effect was, however, low since only doubling or tripling of the deformity rate compared with controls was observed. The results of the study are noteworthy, as they indicate that the chronic exposure of fathead minnows to an environmentally relevant mixture of seven PPCPs did not affect any of the parameters tested, with the exception of F1 larval deformities, where the impact was relatively minor.

Subacute and chronic toxicity data from mammalian species, including mice, rats, hamsters and baboons, are extensive, and have been reviewed by Rodricks *et al.* (2010). For systemic toxicity, excluding endocrine disruption, the effects of TCS were primarily limited to changes in the liver and kidneys. TCS induced changes in liver weight, liver enzymes and liver hypertrophy, and increased peroxisome size and numbers. In rodent species, renal toxicity was evidenced by inflammation and tubular regeneration.

Genotoxicity

Genotoxicity and mutagenicity studies using classical prokaryotic and eukaryotic systems were reviewed by Rodricks *et al.* (2010). The available evidence led the authors to conclude that TCS is neither genotoxic nor mutagenic. However, there is some evidence to suggest that TCS may be genotoxic in certain types of organisms and/or cell types.

Acute toxicity experiments (96 h exposure) using hemocytes from zebra mussels (*Dreissena polymorpha*) that were exposed to environmentally relevant concentrations of TCS (1, 2 and 3 nM) provided evidence of genotoxicity after only 24 h of exposure (Binelli *et al.*, 2009a). The genotoxicity of TCS in hemocytes was evaluated with the single cell gel electrophoresis (SCGE) assay

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(also known as the comet assay), the micronucleus assay, and the halo test, a measure of the apoptotic frequencies, while cytotoxicity was assessed with the neutral red retention assay. The genetic damage accrued in the hemocytes was significant at all three concentrations of TCS, following a concentration-dependent and time-dependent pattern. The authors of this study concluded that the genotoxicity of TCS in zebra mussels was probably due to a combination of oxidative stress and/or a direct effect on DNA. In a follow-up study (Binelli *et al.*, 2009b), hemolymph from zebra mussels was extracted and then used to investigate TCS's potential for both cytotoxicity and genotoxicity. In this experiment, antimicrobial concentrations of 0.1, 0.15, 0.2 and 0.3 μM caused extensive DNA damage in hemocytes, as indicated by the SCGE assay and the apoptotic frequency. Although the range of concentrations used in this study was very narrow, the results clearly indicated that TCS's genotoxicity increased in a dose-dependent fashion. Based on these two studies, there is compelling evidence to suggest that TCS has genotoxic effects in zebra mussels, both *in vivo* and *in vitro*, although future studies are needed to confirm these findings.

The genotoxicity of TCS has also been evaluated using the comet assay in the algal species *Closterium ehrenbergii* (Ciniglia *et al.*, 2005). Algal cells were exposed to TCS for 96 h, at concentrations in the range 0.125-1 mg l^{-1}. At concentrations of 0.25 mg l^{-1} and greater, the genetic toxicity of TCS was apparent, with the antimicrobial exerting its toxicity in a dose-dependent manner. Complete dissolution of the nucleus was observed at concentrations of 0.5 and 1 mg l^{-1}. Although the results of this study indicate that TCS has genotoxic effects on *C. ehrenbergii*, the concentrations used in the experiment were much higher than those typically observed in surface water (HQ < 1.0), and as such, the results should be interpreted with caution.

The genotoxicity and cytotoxicity of TCS have been tested in animal cell lines. Zuckerbraun *et al.* (1998) demonstrated that TCS is cytotoxic to Smulow–Glickman cells (S-G cells), which are derived from a human gingival epithelial cell line. TCS damaged the integrity of the plasma membrane and induced apoptotic cell death. The effects of TCS on gingival cells are important, as TCS is a common ingredient in a number of oral hygiene products. Jirasripongpun *et al.* (2008) used the comet assay and the apoptosis assay to test the genotoxicity of TCS on two animal cell lines. KB and Vero cell lines were treated with two concentrations of TCS, the 50% inhibition concentration (IC_{50}, 0.034 and 0.036 mM respectively) and the maximum concentration of TCS in personal care products (0.023 mM). In both cell lines, the number of comet cells increased as the concentration and exposure time to TCS increased. Most notably, genetic damage accrued from the exposure to TCS was observed at concentrations in the $IC_{20–30}$ range, following a 5 day exposure period. At levels of TCS that are normally in personal care products, the antimicrobial failed to produce any signs of genotoxicity. TCS is a lipophilic chemical and as such, could potentially accumulate in the body. However, exposure to TCS levels that cause genetic damage is unlikely, considering that pharmacokinetic studies have demonstrated that the antimicrobial is rapidly metabolized and readily eliminated from the body.

Mutagenicity and Carcinogenicity

Reviews of data on TCS's safety by DeSalva *et al.* (1989), Bhargava and Leonard (1996), and more recently by Rodricks *et al.* (2010), included extensive data from mutagenicity assays, many completed by Ciba-Geigy Co. The overwhelming majority of these assays indicated that TCS did not exhibit any mutagenic potential, with the exception of the mammalian spot test. The mammalian spot test was first performed by Fahrig (1978) and resulted in a positive response, but later yielded negative results when repeated by Russell and Montgomery (1980). The study published by Fahrig was criticized, and it has been suggested that the effective dose of TCS used by Fahrig would result in maternal toxicity, thus precluding evaluation in the offspring. It is not clear why these two studies, using the same method, yielded such very different results. Fahrig (1978) used a higher dose of TCS dissolved in HBSS, whereas Russell and Montgomery deemed TCS to be insoluble in HBSS, opting to dissolve the antimicrobial in methanol instead. Montgomery and Russell conjectured that, owing to the limited solubility of TCS in HBSS, the experiment by Fahrig (1978) probably failed to inject any of the dams with TCS, which would explain the limited toxicity observed at the 50 mg kg^{-1} dose, a dose which proved to be highly toxic to embryos in the study by Russell and Montgomery. The majority of researchers seem to accept the findings of the experiment by Russell and Montgomery, as reviews on TCS safety (DeSalva *et al.*, 1989; Bhargava and Leonard, 1996; Rodricks *et al.*, 2010) consistently conclude that TCS is not a mutagen and that personal care products containing the antimicrobial do not pose a risk to human health.

The mutagenic potential of TCS and its photodegradation products were later examined by Onodera *et al.* (1995), in two *Salmonella* strains tested with and without S9 fractions. Any mutagenic effects of TCS went undetected, owing to the high toxicity of the antimicrobial to the test species. Following treatment with photo-irradiation and chlorine, TCS in aqueous solution failed to elicit a mutagenic effect in either of the *Salmonella* strains tested. However, it would seem that the selection of bacteria to test the mutagenicity of TCS is somewhat questionable, considering that TCS is a potent antimicrobial and would be highly toxic to the bacterial test species.

Currently available evidence from studies using classical assay systems indicates that TCS is not genotoxic, mutagenic or carcinogenic. However, there is some evidence that TCS is able to exert genotoxicity in nonmammalian systems, including algae and bivalves. Owing to the limited number of studies addressing the genotoxicity, mutagenicity and carcinogenicity of TCS in nonmammalian systems, it is difficult to conclude with certainty that the antimicrobial does not display a potential for harm.

Reproductive and Developmental Effects

The reproductive and developmental toxicity of TCS was assessed in several aquatic species, under controlled laboratory exposures. Orvos *et al.* (2002) investigated the early life-stage toxicity of TCS to rainbow trout. No statistical differences were observed in mean time to egg hatch among groups exposed to different concentrations of TCS in water, although swim-up behavior was delayed in the 71.3 μg l^{-1} treatment (Table 3). Decreased rates of fry survival were observed in this treatment group. Sublethal effects were also observed during the course of the study, and included a loss of equilibrium, locked jaw, erratic swimming, spinal deformities and reduced activity.

The effects of TCS on early development and reproduction in medaka were studied by Ishibashi *et al.* (2004). In fertilized eggs

Table 4. Endocrine-disrupting effects of triclosan (TCS)

Test species/system	Life stage	Aquatic system	Route of exposure	Test duration	TCS exposure	Effects	Reference
Fish Medaka (*Oryzias latipes*)	Embryos	FW	Water	14 days	100 μg l^{-1}	Weak androgenic (or anti-estrogenic) effect (↑male fin size, slight male bias sex ratio)	Foran *et al.* (2000)
	Male fish	FW	Water	14 days	20 μg l^{-1}	Weak estrogenic activity; ↑Vtg in male fish; activity in yeast assay	Ishibashi *et al.* (2004)
Mosquitofish (*Gambusia affinis*)	Male fish	FW	Water	35 days	101.3 μg l^{-1}	↑vitellogenin, ↓sperm count	Raut and Angus (2010)
Bream (*Abramis brama*)	Bile of male fish	FW	Field sites, Netherlands		No activity up to 0.1 mM	No estrogenic activity detected in ER-CALUX assay	Houtman *et al.* (2004)
Amphibians							
North American bullfrog (*Rana catesbeiana*)	Tadpoles	FW	*In vivo*	18 days	0.15 μg l^{-1}	Disruption of T_3-dependent developmental metamorphosis processes	Veldhoen *et al.* (2006)
South African clawed frog (*Xenopus laevis*)	Tadpoles	FW	*In vivo*	21 days	1.5 μg l^{-1} 0.6–32.4 μg l^{-1}	↓larval growth; no effect on metamorphosis	Fort *et al.* (2010)
	Males	FW	Water; i.p. injection	14 days	20–200 μg l^{-1}; inject 4-400 μg g^{-1} body weight	No effect on Vtg in males; no effects on CYP1A and EROD; ↓Vtg in i.p. injected males	Matsumura *et al.* (2005)
Mammals							
Sheep	Placenta		*In vitro*		0.6 nM	↓Estrogen sulfonation (IC_{50})	James *et al.* (2010)
Rats (Wistar)	Pre-pubescent males		Oral (gavage)	31 days	200 mg kg^{-1} 30 mg kg^{-1}	No effect on timing of puberty; ↓levels of plasma testosterone and T_4	Zorrilla *et al.* (2009)
	Males; isolated Leydig cells		*In vivo* (daily intubation) *In vitro*	60 days 2 h	5–20 mg kg^{-1} 0.01–10 μM	Disruption of LH, FSH, pregnenolone and testosterone synthesis; ↓mRNA expression of StAR and steroidogenic enzymes	Kumar *et al.* (2008, 2009)
Rats (Long–Evans)	Adult female		Oral (gavage)	4 days	100 mg kg^{-1} day^{-1}	↓Plasma T_4	Crofton *et al.* (2007)
	Female weanlings		Oral (gavage)	4 days	300 mg kg^{-1} day^{-1}	↓Plasma T_4 and T_3	Paul *et al.* (2010)
Human	Adults		brush 2/day with TCS tootpaste	14 days	0.3% w/w TCS	No effect on thyroid status	Allmyr *et al.* (2009)

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Cell-based assays				
MCF37 breast cancer cells	*In vitro*	10 μM	Estrogenic and androgenic effects	Gee *et al.* (2008)
2933Y cells (human)	*In vitro*	1.0 and 10 μM	↓Testosterone-induced transcriptional activity	Chen *et al.* (2007)
Cell-based nuclear-receptor-responsive and calcium signaling bioassays (AhR, ER, AR, RyR)	*In vitro*	1–10 μM (for ER- and AR-responsive gene expression; 0.1–10 μM (for RyR response)	Weak AhR activity; antagonistic activity in ER- and AR-dependent gene expression; interaction with RyR1, ↑Ca^{2+} mobilization in skeletal myotubes	Ahn *et al.* (2008)
HuH7 cells (human hepatoma cell line) transfected with human pregnane X receptor (hPXR)	*In vitro*	>10 μM	Activation of hPXR	Jacobs *et al.* (2005)
Induced rat liver microsomes	*In vitro*	3.1 μM (IC_{50})	↓Diiodothyronine (T_2) sulfotransferase activity	Schuur *et al.* (1998)

exposed to 313 μg l^{-1} of TCS, hatchability and time to hatching were significantly decreased and postponed, respectively. A 21 day exposure period to the antimicrobial failed to have any observable effect in the number of eggs produced and fertility, when comparing the control group with the 20, 100, 200 μg l^{-1} TCS treatments. TCS appears to be quite toxic during the early life stages of medaka, and although the metabolite of TCS had weak estrogenic activity, the antimicrobial did not negatively impact the reproductive success of paired medaka or the survivability, growth and sex ratios of the offspring.

In addition to the acute toxicity of TCS in adult fish, Oliveira *et al.* (2009) investigated the teratogenic effects of TCS on zebrafish larvae. The experiment was designed according to the OECD guideline on Fish Embryo Toxicity Test. Zebrafish embryos were exposed to five different concentrations of TCS – 0.1, 0.3, 0.5, 0.7 and 0.9 mg l^{-1} for a 6 day period. The embryos were monitored daily for mortality, developmental parameters, and hatching. Additional larvae were collected for ChE, GST and LDH biomarker analyses. TCS exhibited acute toxicity for embryo/larvae (96 h LC_{50} of 0.42 mg l^{-1}), resulting in delayed hatching, and mortality at 48 h. The teratogenic effects of TCS were observed at concentrations above 0.7 mg l^{-1}. The developmental effects of TCS included delayed otolith formation and eye and body pigmentation, spinal malformations, pericardial edema and undersized larvae. In addition to embryo malformations, biomarker levels were also affected: ChE activity increased in the 25 mg l^{-1} treatment, GST activity increased in both the 0.25 and 0.35 mg l^{-1} treatments, and LDH activity increased in the 0.25 mg l^{-1} treatment. The results of this study indicate that TCS is toxic to zebrafish embryo/larva and negatively impacts hatching, embryonic development, enzyme activities and survival. Based on the sensitivity of the biomarkers analyzed (GST, ChE and LDH), the authors concluded that concentrations of TCS equal to or greater than 0.3 mg l^{-1} constitutes a significant environmental hazard.

Reproductive and teratological studies in rats, mice and rabits carried out by Ciba-Geigy were reviewed by DeSalva *et al.* (1989), Bhargava and Leonard (1996), and Rodricks *et al.* (2010). In the rat study, TCS was administered in the diet. There were no effects on reproductive performance at any of the doses, including the highest dose of 3000 mg kg^{-1}. Effects in the offspring were detected only in pups from mothers fed the highest dose of TCS. In rabbits, TCS was administered by oral intubation to mothers, but no teratogenic effects were observed in the offspring. The reviews concluded that at doses of 150 mg kg^{-1} and higher, TCS is toxic to pregnant rats, but is not an overt teratogen. Similar conclusions regarding reproductive and developmental toxicity of TCS to mammalian species were reached by Rodricks *et al.* (2010). However, a study by Russell and Montgmory (1980), cited mostly for the failure to confirm mutagenicity of TCS in the mouse spot test, did provide some reproductive data for TCS. A single intraperitoneal dose of 25 mg kg^{-1} TCS affected the survival of embryos, significantly reducing litter size. In addition to reduced prenatal survival, an average dose of 3.2 mg kg^{-1} TCS resulted in significant decreases in postnatal survival. Despite the pronounced effects of TCS on survivability, very few externally identifiable abnormalities were observed in newborn mice in the higher dose groups. The study by Russell and Montgomery is one of the few studies to have examined the effects of TCS on mammalian development, as more recent studies have focused primarily on aquatic species.

Endocrine Disruption

The structural similarity of TCS to known estrogenic and androgenic EDCs, including polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and bisphenol A, and to thyroid hormones (Norris and Carr, 2006; Veldhoen *et al.*, 2006; Allmyr *et al.*, 2008) would be predictive, using the structure–activity relationship, of endocrine disruption. Several studies have shown that the antimicrobial has the ability to influence endocrine function in a variety of species (Table 4). This represents a considerable concern, as large amounts of TCS are used on a regular basis, with the antimicrobial having been detected in human plasma (Hovander *et al.*, 2002), breast milk (Adolfsson-Erici *et al.*, 2002), urine (Calafat *et al.*, 2008), and the aquatic environment (Chalew and Halden, 2009).

Thyroid hormones

Investigations of the propensity of TCS to disrupt thyroid homeostasis are based on the structural similarity of the antimicrobial to thyroid hormones. If TCS is indeed capable of perturbing the thyroid axis, the implications for developmental processes could be profound.

The first study to investigate the effects of polyhalogenated aromatic hydrocarbons (PHAHs), including TCS, on the metabolism of thyroid hormones was conducted, to the authors' knowledge, by Schuur *et al.* (1998). The *in vitro* inhibition of diiodothyronine (T_2) sulfotransferase activity was measured using rat liver cytosol (Table 4). After an incubation of the PHAHs with induced liver microsomes, T_2 sulfotransferase inhibiting metabolites were formed. Specifically, the IC_{50} for TCS was 3.1 ± 0.7 μM. The results of this study were the first to indicate that TCS and its metabolites, like other hydroxylated halogenated compounds, are capable of inhibiting *in vitro* sulfation of thyroid hormones. Thyroid hormones are inactivated by sulfation, with sulfation playing a pivotal role in controlling thyroid metabolism during the developmental period (Norris and Carr, 2006). Schuur *et al.* (1998) postulate that fetal exposure to hydroxylated compounds such as TCS could result in the inhibition of thyroid hormone inactivation, with potential consequences to thyroid-mediated developmental processes. Future studies are needed to address the *in vivo* potential of TCS to alter T_2 sulfotransferase activity, and what, if any, effect that would have on the embryo and developing fetus.

To further assess the endocrine disrupting potential of TCS, Veldhoen *et al.* (2006) conducted a study to assess the potential of TCS to alter thyroid-mediated developmental processes in premetamorphic North American bullfrog (*Rana catesbeiana*) tadpoles (Table 4). Tadpoles were immersed in low concentrations of TCS for 4 days, and on day 4 were injected with 3,5,3′-triidothyronine (T_3) or a vehicle control. TCS exposure continued after the injection of T_3, ending on day 6, day 11 or day 18, with tissue samples collected and morphometric measurements made. Pretreatment with TCS concentrations as low as 0.15 μg l^{-1} accelerated metamorphological changes following the administration of T_3. Within 48 h of T_3 treatment, T_3 mediated TRβ mRNA expression in the tadpole tail decreased and levels of PCNA (proliferating nuclear cell antigen) transcript in the brain increased. In the absence of T_3, TCS alone affected thyroid hormone receptor α transcript levels in the brain and resulted in transitory weight loss. The results of this study indicate that environmentally relevant levels of TCS are capable of disrupting developmental processes that are contingent on thyroid hormones in the bullfrog. Fort *et al.* (2010), exposed *Xenopus laevis* (South African clawed frog) larvae to TCS (0.6–32.3 μg l^{-1}) over a 21-day exposure period. Although the authors concluded that TCS did not have an effect on larval development, thyroid histology, plasma thyroxine levels and/or survivorship, the reported data suggest that TCS did have an effect on the postembryonic development of the tadpoles. Moreover, there was a significant difference between the exposed group and the control in the expression of TRβ, which was induced by a magnitude of 1.5 in the 1.5 and 7.2 μg l^{-1} TCS treatments. A reduction in larval growth in the 1.5 μg l^{-1} treatment was also observed. The evidence available from amphibian studies suggests that metamorphosis of amphibians is highly sensitive to TCS and that the HQ value (PEC divided by NOAEC) may be greater than 1.0.

Following the study by Veldhoen *et al.* (2006), Crofton *et al.* (2007) tested the hypothesis that, *in vivo*, TCS exposure influences serum levels of thyroxine (T_4) in rats. Long–Evans females were given TCS (0, 10, 30, 100, 300 or 1000 mg kg^{-1} day^{-1}) via oral gavage for a short-term 4-day dosing schedule. Rats were sacrificed 24 h after the final TCS treatment and serum obtained. TCS doses of 100 mg kg^{-1} day^{-1} and higher decreased serum levels of T_4, with the 30 mg kg^{-1} day^{-1} dose as the NOEL. This study was the first to demonstrate that TCS decreases serum levels of T_4 in female rats. The effects of TCS on pubertal development and thyroid function in the male Wistar rat were investigated by Zorrilla *et al.* (2009). Prepubescent male rats were administered daily doses of 0, 3, 30, 100, 200 or 300 mg kg^{-1} TCS via oral gavage for 31 days. At TCS doses of 30 to 300 mg kg^{-1}, serum levels of T_4 decreased in a dose-dependent fashion. However, observed decreases in serum levels of T_4 only corresponded to decreases in T_3 levels at the 200 mg kg^{-1} dose, and colloid depletion was only observed in thyroid sections of the 300 mg kg^{-1} treatment group. Compared with the controls, no significant differences in serum levels of thyroid-stimulating hormone (TSH) were noted in any of the treatments. To test the hypothesis that TCS decreases circulating T_4 levels by upregulating hepatic metabolism of thyroid hormones, Paul *et al.* (2010) used a 4-day exposure protocol in rats to analyze levels of hepatic enzyme induction (phase I and II enzymes), serum T_4, serum T_3, and TSH. Exposure to TCS caused a decrease in total T_3 and T_4, an upregulation of mRNA expression and an increase in the activity of a number of phase I and phase II enzymes. The results of the study support earlier work, which has demonstrated that TCS-induced hypothyroxinemia is probably due to the induction of hepatic enzymes, which augment the catabolism of T_4. Although it is possible that TCS may have direct effects on the thyroid gland and the production of thyroid hormones, previous studies have found no evidence to indicate this is occurring.

A study by Allmyr *et al.* (2009) is the first to have examined the effect of TCS on thyroid homeostasis in humans. Participants of the study brushed their teeth twice a day, for a period of 14 days, with a commercially available brand of dentifrice, Colgate total, containing 0.3% (w/w) TCS. Blood samples were collected from the participants prior to TCS exposure and the day following the termination of the exposure period. Concentrations of TCS in plasma were significantly higher at the end of the exposure period. However, despite a significant difference between pre- and post-exposure levels of plasma TCS, the increase in TCS had no effect on circulating levels of

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4β-hydroxycholesterol, a cholesterol metabolite used as an indicator of CYP3A4 activity, TSH, free T_4, and free T_3. The study concluded that the regular use of TCS containing toothpaste does not induce CYP3A4 activity or disrupt thyroid homeostasis. Unfortunately, the small study population coupled with the short-term exposure period limits the validity of these findings. Future studies should aim to address the effects of long-term exposure to TCS in human subjects, from multiple exposure pathways, for prolonged periods of time.

Thus far, there has only been one human study (Allmyr *et al.*, 2009) chronicling the effect of TCS on thyroid homeostasis. In this study, TCS did not alter circulating levels of thyroid hormones. In contrast, animal studies have shown that TCS decreases blood levels of T_4, without concomitant changes in TSH concentrations. Environmentally relevant levels of TCS have also been shown to disrupt thyroid-mediated developmental processes in premetamorphic North American bullfrog tadpoles (Veldhoen *et al.*, 2006), and possibly in prometamorphic South African clawed frog tadpoles (Fort *et al.*, 2010). TCS studies on thyroid disruption suggest that the antimicrobial is capable of acting on the thyroid receptor and altering the clearance of thyroid hormones, although future studies are needed to confirm this suspected mechanism of action and whether or not these effects are limited to animals. Given that TCS has been implicated in the disruption of thyroid hormone homeostasis and has been detected in breast milk samples of nursing mothers, the priority of future studies should be to ascertain whether or not TCS exposure can negatively affect fetal and postnatal development. A better understanding of the mechanisms of TCS-mediated thyroid disruption is warranted, in addition to species-specific differences.

Sex hormones

TCS is structurally similar to the anthropogenic estrogens, diethylstilbestrol and bisphenol A, in addition to the anti-estrogen, 2,3,7,8 tetrachloro-*p*-dibenzo-dioxin (Jacobs *et al.*, 2005). Despite these structural similarities, an *in vivo* fish study by Foran *et al.* (2000) suggested that the antimicrobial was weakly androgenic, not estrogenic. In this study, Japanese medakas (*Oryzias latipes*) were exposed to TCS for 14 days, at concentrations of 1, 10, 100 and 500 μg l^{-1} and 1 mg l^{-1}. The effect of TCS on phenotypic sex ratios was determined by inspecting fin size and shape. The antimicrobial had no effect on the sex ratio of exposed fish, although a slight male bias in the 100 μg l^{-1} treatment, and an accompanied difference in fin length between males of different exposure groups, indicated a possible anti-estrogen or a weakly androgenic effect (Table 4).

Ishibashi *et al.* (2004) further investigated the estrogenic potential of TCS. The estrogenic activity of TCS was measured using the induction of hepatic vitellogenin (Vtg) in male medaka, and an *in vitro* yeast two-hybrid assay. Hepatic Vtg levels were increased significantly in males exposed to TCS at 20 and 100 μg l^{-1}, although this was not the case in the 200 μg l^{-1} treatment group. The estrogenic activity of TCS was measured in the yeast two-hybrid assay alone and in the presence of rat S9 liver fractions. Alone, TCS had a weak estrogenic activity, but with the addition of the rat S9 liver treatment, the estrogenic activity of the antimicrobial was increased 2-fold. The results of the study suggest that the metabolite of TCS is a weak estrogen, with the potential of inducing Vtg in male medaka.

Houtman *et al.* (2004) identified TCS at relatively high concentrations in the bile of male breams (*Abramis brama*) collected at river sites in the Netherlands. The estrogenic potencies of TCS and other compounds in the bile were assessed using the ER-CALUX (Estrogen Responsive Chemical Activated Luciferase Gene Expression) assay (Houtman *et al.*, 2007). Estradiol and estrone were the major contributors to estrogenic activity, where TCS concentrations of up to 0.1 mM gave no indication of any estrogenic activity. The authors concluded that the antimicrobial did not contribute significantly to the estrogenic activity measured in the bile of male breams. It is important to keep in mind that key differences between the toxicity of compounds *in vivo* and *in vitro* exist.

The potential of TCS to induce Vtg production and decrease sperm counts, both well-established biomarkers of endocrine disruption, was assessed in male mosquito fish (*Gambusia affinis*) by Raut and Angus (2010). A 35-day exposure period to TCS induced Vtg production and decreased sperm production; moreover, the hepatosomatic index of TCS exposed fish was significantly elevated compared with controls. However, it is important to note that, in this study, endocrine disruption was observed at TCS concentrations approximately 100 times greater than those typically detected in surface water, and as such, it is not known if environmentally relevant concentrations of TCS would produce similar results.

Matsumura *et al.* (2005) investigated the effects of TCS on plasma Vtg levels, testosterone synthesis and hepatic CYP1A and CYP2B activities in male *Xenopus laevis*. Waterborne TCS at environmentally relevant concentrations did not have any estrogenic effects, while male frogs treated with intraperitoneal injections of TCS at 4–400 μg g^{-1} body weight had lower plasma Vtg and testosterone levels than the control group. Hepatic CYP1A and CYP2B activity, as measured by EROD or PROD, was not significantly different from the controls. The authors hypothesized that the observed decrease in plasma Vtg may be partially explained by the (anti)estrogenic effects of TCS in male *X. laevis*.

The evidence provided by fish and amphibian models suggests that TCS has endocrine disrupting activity, however the number of studies with these species is limited. The research efforts to assess endocrine disruption in mammalian models are more extensive. A potential explanation for TCS's ability to act as an endocrine disruptor comes from evidence that the antimicrobial activates the hPXR. This receptor is stimulated by a wide array of environmental chemicals and is responsible for inducing enzymes that metabolize steroids and detoxify xenobiotics (Jacobs *et al.*, 2005). In the study by Jacobs *et al.* (2005), the human hepatoma cell line (HuH7) was used to quantify PXR activity. Cells were exposed to concentrations of TCS from 0.01 to 10 μM and the capacity of TCS to induce PXR activity was expressed as the percentage of the positive control. At 46.2%, TCS proved to be a moderate inducer of hPXR activity. In contrast to the other compounds tested, TCS was the only one to show concomitant increases in percentage maximum induction, with doses above 10 μM.

Subsequently, Chen *et al.* (2007) tested the *in vitro* (anti) androgenic effect of TCS on testosterone induced transcriptional activity, in a cell line lacking essential steroid metabolizing enzymes. These cells (2933Y) are highly sensitive to endogenous steroids, in addition to anthropogenically sourced endocrine disruptors. At TCS concentrations of 1.0 and 10 μM, testosterone-induced transcriptional activity was reduced by 38.8 and 92%, respectively. In the absence of testosterone, TCS did not exhibit any androgenic activity, at concentrations up to 10 μM. A second study to test the

in vitro endocrine disrupting effects of TCS was done by Gee *et al.* (2008). This study examined both the estrogenic and androgenic activity of TCS in breast cancer cells. At environmentally relevant concentrations, TCS was capable of producing both estrogenic and androgenic effects. TCS displaced radiolabeled estradiol from estrogen receptors of MCF7 human breast cancer cells, whilst also inhibiting testosterone from binding to the rat androgen receptor.

The endocrine disrupting potential of TCS was further investigated by Ahn *et al.* (2008) using *in vitro* cell-based and nuclear-receptor–responsive bioassays for aryl hydrocarbon (AhR), estrogen (ER), androgen (AR) and ryanodine receptors. The results of the cell- based AhR-mediated bioassay demonstrated that TCS is an AhR inducer, a receptor which has been implicated in various toxic and biological responses. In both the cell-based ER- and AR-mediated bioassays, TCS acted antagonistically, but was a powerful disruptor of Ca^{2+} regulation. The authors concluded that the results of their study provided sufficient reason to be concerned about the antimicrobial's neurotoxic potential. These results are further supported by previous studies that have shown that TCS alters thyroid homeostasis (Veldhoen *et al.*, 2006). Moreover, there is evidence that TCS can also influence endocrine function indirectly, through effects on the metabolism of key hormones, including the thyroid hormones. The effects of TCS on EROD, PROD, UDP-GT and sulfotransferase enzymes, all of which play a role in the metabolism and clearance of hormones from the body, have been reported (Hanioka *et al.*, 1997; Jinno *et al.*, 1997; Kanetoshi *et al.*, 1992; Schuur *et al.*, 1998). In addition, several *in vivo* studies have shown that TCS has endocrine disrupting effects.

Further evidence for the (anti)androgenic effect of TCS was provided by Kumar and colleagues, who sought to describe the targets of TCS endocrine disruption, in addition to the mechanism(s) of action. An earlier study with Leydig cells exposed to TCS *in vitro* (Kumar *et al.*, 2008), was followed by a whole animal study (Kumar *et al.*, 2009). Male rats were dosed with 5, 10 or 20 mg kg^{-1} of TCS per kg of body weight per day. Rats were treated with TCS once a day for a period of 60 days. RT-PCR analysis indicated that TCS decreased mRNA levels for testicular steroidogenic acute regulatory (StAR) protein, cytochrome $P450_{scc}$, cytochrome $P450_{C17}$, 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD) and AR. The translation of testicular StAR and AR protein was also disrupted by the antimicrobial. Decreases in serum levels of lutenizing hormone (LH), follicle stimulating hormone (FSH), cholesterol, pregnenolone and testosterone were observed. Histopathological analysis of the testes and sex accessory glands were indicative of widespread malformations. TCS-induced decreases in testosterone and spermatogenesis were likely the result of decreases in serum levels of LH and FSH, thereby implicating the pituitary–gonadal axis, at various levels, as a target for endocrine disruption.

The reproductive effects of TCS on testosterone-dependent endpoints in rats were not as clearly evident in a study by Zorrilla *et al.* (2009), which sought to assess the effect of TCS on puberty, as well as thyroid hormones (see section above) in male Wistar rats. The antimicrobial had no effect on the growth or onset of preputial separation. A significant difference in serum testosterone levels was observed in the 200 mg kg^{-1} treatment, but not in the 300 mg kg^{-1} treatment. In addition, the age of pubertal onset and the development of androgen-dependent reproductive tissues did not differ significantly between the experimental and control groups.

James *et al.* (2010) tested the vulnerability of the placenta to the endocrine-disrupting effects of TCS. TCS proved to be a powerful inhibitor of estradiol sulfonation in the placental tissue of sheep, with IC_{50} of 0.6 nM TCS. As the majority of estrogen secreted by the placenta is sulfoconjugated and estrogen sulfonation has been linked to pregnancy loss (Tong *et al.*, 2005), TCS could potentially have a negative impact on the fetal environment and pregnancy maintenance. Environmental and/or household exposure to TCS in humans can lead to blood levels in the low nanomolar range (Allmyr *et al.* 2006, 2008). The possibility then exists for placental levels of TCS to reach concentrations high enough to interfere with placental estrogen metabolism.

There is good evidence for the endocrine disrupting effects of TCS, although it remains unclear as to whether TCS has (anti) estrogenic effects, (anti)androgenic effects, or both. Foran *et al.* (2000) concluded that, based on changes in fin length and slightly skewed sex ratios in medaka fish, TCS is a weak androgen. A later study by Ishibashi *et al.* (2004) reported that a TCS metabolite induced Vtg production in male medaka, suggesting estrogenic activity. Evidence from amphibian studies supported the role of TCS as an anti-estrogenic chemical. Several *in vitro* studies have demonstrated the potential for TCS to act as an anti-estrogen and/or anti-androgen (Chen *et al.*, 2007; Gee *et al.*, 2008; Ahn *et al.*, 2008). Studies with male rats (Zorrilla *et al.*, 2009; Kumar *et al.*, 2009) have shown that TCS decreases serum levels of testosterone and the activity of several important steroidogenic enzymes. Lastly, TCS is a powerful inhibitor of estrogen sulfonation in sheep placental tissue, and as such, could have deleterious effects on the ability of female mammals to maintain a full-term pregnancy. Because TCS has been shown to have estrogenic and androgenic activity at environmentally relevant levels, there is sufficient reason to be concerned about the impact of the antimicrobial on aquatic ecosystems and human health.

EFFICACY AND ANTIBACTERIAL RESISTANCE

Efficacy

The efficacy of TCS-containing consumer products has recently been called into question, as several studies have reported that the antimicrobial is no more effective than regular soap.

Health-care settings

The efficacy and safety of TCS in health-care settings was reviewed by Jones *et al.* (2000). The popularity of TCS in health-care settings has long endured the test of time, as the antimicrobial has proven its immediate, broad-spectrum antimicrobial effects, in addition to the fact that TCS elicits neither dermal irritation nor photosensitization effects. Interestingly, cell culture experiments have even shown that the antimicrobial has anti-inflammatory, anti-allergic, anti-asthmatic activity and can even protect against cell damage. All of the aforementioned characteristics are very important for ensuring antimicrobial acceptance and compliance in health-care settings.

The antimicrobial effects of TCS have been established for concentrations of 0.2–2.0%. TCS formulations of 1% have been proven effective for managing antibiotic-resistant *Staphylococcus*

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aureus outbreaks in health-care settings, in the form of either handwash solutions or bathing antiseptics (Jones *et al.*, 2000). TCS is often favored over other antimicrobial products due to its mild nature, helping to increase handwashing compliance in health-care workers. According to the authors, a 1% TCS formulation is preferable for securing high rates of handwashing compliance and reducing nosocomial infections in high-risk, high-frequency handwashing health-care settings (see Jones *et al.*, 2000). It is worth reinforcing that, while the use of TCS formulations appears to be beneficial in high-risk, high-frequency handwashing health-care settings, these benefits do not necessarily translate into the domestic domain.

Personal care products

As previously mentioned, a staggering number of personal care products contain TCS, at varying concentrations. As TCS is not subject to stringent government regulation, concentrations of TCS in consumer products can vary substantially, although they generally remain in the range of 0.1–0.45% (w/v) (Aiello *et al.*, 2007). A review on the efficacy of TCS-containing hand soaps, with efficacy defined as antibacterial activity above and beyond that of plain hand soap, revealed that, at concentrations typically present in antibacterial soaps, TCS was not superior for reducing bacterial counts on the hands or decreasing the prevalence of infectious diseases (Aiello *et al.*, 2007). In studies that showed TCS reduced bacterial populations on the hands (Bhargava and Leonard, 1996), longer handwashes and/or high concentrations of the antimicrobial were used, accounting for the higher efficacies recorded. Owing to the lack of data supporting the efficacy of TCS-containing antimicrobial soaps, the use of these products seems unnecessary in light of concerns about the potential for the selection of antibiotic cross-resistance and impacts on the aquatic environment.

Despite a lack of data on the efficacy of TCS in antibacterial soaps, there is a growing body of evidence to suggest that, in oral care formulations, TCS and TCS/copolymers deliver effective antibacterial protection by causing bacterial lysis. TCS is the most commonly used antibacterial agent in oral care formulations (van den Broek *et al.*, 2008). According to a review on the management of halitosis by van den Broek *et al.* (2008), a dentifrice formulated with 0.3% TCS, 2.0% of a copolymer of polyvinyl methyl ether maleic acid and 0.243% sodium fluoride significantly reduced the incidence of organoleptic sores and hydrogen sulfide-releasing bacteria. Moreover, the antimicrobial and anti-inflammatory properties of TCS have been proven to reduce plaque and gingivitis, in addition to slowing the progression of periodontal disease (Rosling *et al.*, 1997; Cullinan *et al.*, 2003; Gunsolley, 2006; Rautemaa *et al.*, 2007). The long-term use of antimicrobials is, however, worrisome, as resistance is always a lingering concern, although there is no data to suggest that TCS-resistant strains of pathogens have emerged alongside the incorporation of the antimicrobial into oral care formulations (Rosling *et al.*, 1997; Cullinan *et al.*, 2003; Rautemaa *et al.*, 2007). Based on the evidence from several review studies, the weighing of the risks and benefits of TCS in oral care formulations strongly favors the use of the antimicrobial in dentifrice and mouthwashes (Sreenivasan and Gaffar, 2002; Gilbert *et al.*, 2007).

Plastics and other materials

The number of applications for TCS has expanded immensely over the years, with the antimicrobial now being impregnated in a number of different materials, ranging from medical devices to athletic clothing to meat packaging, in the hopes of providing the user with long-lasting antibacterial protection. The extent to which TCS is able to prevent the proliferation of bacterial populations in many of these products has not been adequately established. Ensuring that TCS is consistently released, at concentrations that are high enough to limit bacterial growth, remains a significant challenge for manufacturers.

The use of TCS in textiles has been banned in Europe, owing to concerns of antibiotic resistance and the generation of toxic by-products, primarily 2,8-dichlorodibenzo-*p*-dioxin. However, manufacturers in North America continue this practice on a widescale basis. The premise of this manufacturing process is that TCS will migrate to the surface of the textile, providing extended antimicrobial release throughout the lifespan of the fiber (Gao and Cranston, 2008). Despite these claims of antibacterial protection, experimental evidence to support the practice of impregnating clothing fibers with TCS remains limited.

There is no doubt that manufacturers are feeling increased pressure from consumers to create products that provide extended antibacterial protection. The development of Microban© products, an innovative technology that enables TCS to be incorporated into virtually any type of plastic material (either directly into the plastic or as a coating with a second type of film), while allowing the antimicrobial to accumulate on the surface to inhibit bacterial growth, has garnered the attention of the food packaging industry (Vermeiren *et al.*, 2002). It has been thought that, by incorporating antimicrobial agents such as TCS, into food packaging, the shelf-life and safety of the product(s) would be ameliorated. Although this technology seems very promising, several *in vitro* studies have demonstrated that TCS-incorporated products do little to prevent the growth of bacteria in commercial applications, probably due interactions between TCS and food particles (Cutter, 1999; Vermeiren *et al.*, 2002).

The effectiveness of TCS incorporated plastic has been investigated by Cutter (1999). In plate overlay assays, TCS demonstrated antibacterial activity, but when the plastic was vacuum-packaged and refrigerated, bacterial growth was not sufficiently reduced. The authors attributed the failure of the TCS polymer to inhibit bacterial growth to the interactions between TCS and adipose deposits in meat. The antimicrobial efficacy of a polymer coated in TCS, for the purpose of packaging perishable foods, has also been evaluated in a study by Chung *et al.* (2003). The applicability of the study is limited, in that only one type of bacteria was used, *Enterococcus faecalis*, in addition to the fact that incubations were carried out at one temperature only, 30 °C. The results of the study specify that only minute amounts of the antimicrobial are released from the polymer, although one must consider that the minimum inhibitory concentration for TCS is fairly low. Despite only small amounts of TCS being released, bacterial inhibition by the TCS coating was clearly seen in both the agar diffusion test and a liquid culture test, although neither test was performed at refrigeration temperature. Plate overlay assays at refrigeration temperature, with different bacterial strains, are needed to confirm the antimicrobial efficacy of TCS in food packaging.

More recently, Camilloto *et al.* (2009) evaluated the antimicrobial efficacy of three different concentrations (0, 2000 and 4000 mg kg^{-1}) of a TCS active film in the preservation of sliced ham. The activity of the film was tested both *in vitro* and in

sliced ham inoculated with *E. coli and S. aureus*. In the *in vitro* experiment, film efficiency was measured by the diameter of the inhibition halos around the TCS containing film in agar plates that had been inoculated with *E. coli*, *S. aureus*, *L. innocua*, *S. choleraesius* or *P. aureginosa*. The film only showed inhibition effects against *E. coli* and *S. aureus*. Correspondingly, in the sliced ham packaged with the TCS-containing film, a reduction of 1.5 logarithmic cycles for *E. coli* and *S. aureus* was observed after 12 days of storage at 7 ± 2 °C. The efficacy of TCS containing films in this particular study are suggestive of a potential for the use of the antimicrobial in food packaging, as the film controlled the proliferation of certain types of bacteria that cause food borne illnesses.

Studies on the efficacy of TCS-incorporated film have been far from conclusive, perhaps in part due to methodological variations. The efficacy of these films is probably dependent on the type of polymer material, the method of TCS incorporation and production, the concentration of antimicrobial used and also the bacterial strains and conditions used (Camilloto *et al.*, 2009). Testing antimicrobial films at refrigeration temperatures, with a variety of meat products, is paramount, since this type of packaging is generally used with refrigerated foods and fatty acids from meat are thought to decrease the efficacy of TCS containing films (Cutter, 1999).

Medical devices

Medical devices are often a site of bacterial proliferation, which can cause debilitating infections and even death. Bacterial adhesion to medical devices is a serious medical problem that places a significant strain on the health-care system. A proposed solution to this problem has been the incorporation of antibacterial agents into medical polymers. The antibacterial potency of TCS incorporated into plasma-modified medical polyethylene (PE) and bulk PVC has been investigated (Zhang *et al.*, 2006; Ji and Zhang, 2009). The results of these two studies demonstrated that TCS-incorporated bulk PVC significantly reduced bio-film on polymer surfaces and that plasma-modified PE with TCS provided adequate antibacterial protection. However, these studies were laboratory-based, and it has been previously shown in antibiotic cross-resistance studies that the effects observed in the laboratory setting often do not translate to the 'real world'. Further studies are needed to confirm that TCS-impregnated medical polymers are genuinely able to reduce bacterial adhesion and proliferation.

The results of the studies by Zhang *et al.* (2006) and Ji and Zhang (2009) do not agree with previous studies on the antibacterial efficacy of TCS incorporated polymers. Junker and Hay (2004) compared biofilm populations on ABS plastic impregnated with or without TCS, after 1–3 weeks of exposure to drinking water. A lack of measurable differences in bacterial populations between TCS-impregnated and control plastic was observed, a phenomenon which can be explained by the fact that only a minute amount of TCS actually migrated from the plastic.

Previously, Kalyon and Olgun (2001) investigated the antibacterial efficacy of TCS-incorporated polymers, reporting that TCS was only capable of inhibiting bacterial growth for a limited period of time, after which bacterial growth flourished. The authors suggested that the majority of TCS was not available to bacteria, as was evidenced by the fact that the amount of TCS incorporated into the polymer was much higher than the minimum inhibitory concentration (MIC) for the bacterial species. The findings of this study are in concordance with those of Imazato *et al.* (1995), who studied the efficacy of dental polymers containing TCS. The TCS incorporated polymer composites reduced bacterial proliferation for 12 h, but once the 24 h mark had been reached, the number of bacteria on the surface of the control and the TCS-incorporated composite were virtually the same.

Despite the fact that TCS-impregnated polymers are commercially available in a wide range of products, few studies have been able to document their efficacy. With the noteworthy exception of oral care products, the antimicrobial efficacy of numerous TCS-containing products has yet to be validated. There is an urgent need to critically evaluate the false sense of security that the mass marketing of antimicrobial products creates, considering that the injudicious use of antimicrobials may lead to antibiotic cross-resistance and should be avoided.

Antibiotic Cross-resistance

Traditionally, TCS was thought not to be implicated in antimicrobial resistance because of its broad-spectrum antibacterial properties and multiple bacterial targets. However, this understanding has been called into question, as several studies have demonstrated the potential for TCS to target a specific bacterial enzyme, enoyl-acyl carrier protein reductase (McMurry *et al.*, 1998). Indeed, laboratory studies have shown that TCS-resistant bacteria can be cultured with relative ease, and it has been suggested that TCS-resistant bacteria may be the result of mutations in and/or the over-production of enoyl reductase, changes in the membrane permeability, or efflux (Russell, 2004). However, it is important to remember that at higher concentrations, biocides, like TCS, have widespread targets. It is usually only at lower concentrations, not typically in-use concentrations, that biocides become more selective in their targets (Russell, 2003, 2004).

The fact that TCS can target a specific enzyme could potentially be a public health issue in the future, as antimicrobials are not intended to target any particular cellular constituents in bacteria (Jones *et al.*, 2000). Instead, it is antibiotics that exert their destructive powers by targeting specific cellular components of bacteria. Unfortunately, it is the case that these cellular targets regularly undergo mutations, subsequently rendering the antibiotic ineffective. Once it was discovered that TCS had the ability to target a specific enzyme in bacteria, researchers began speculating that the antimicrobial may have the potential to prime bacteria for antibiotic cross-resistance.

A review by Russell (2004) examined the link between TCS and antibiotic resistance. Typical in-use concentrations of TCS generally far exceed MICs for most bacterial species, with the exception of *P. aeruginosa*. A TCS-susceptible mutant of *P. aeruginosa*, when exposed to TCS, activates an efflux pump that decreases the susceptibility of the bacterial strain to ciprofloxacin (Russell, 2003). However, later studies have failed to implicate TCS in ciprofloxacin resistance in the clinical setting. Nor is there any evidence to suggest that TCS is linked to antibiotic resistance in *S. aureus*. Moreover, comprehensive surveys on the use of TCS in the home have failed to correlate the antimicrobial with antibiotic resistance (Cole *et al.*, 2003).

A later review by Yazdankhah *et al.* (2006) concluded that, although cross-resistance between TCS and other clinically relevant antimicrobials has been documented for *E. coli* and

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Salmonella spp. in the laboratory setting, these findings have yet to be confirmed in clinical environments. However, the authors of the study draw attention to the fact that very few studies have been done on clinical isolates, suggesting that future studies on this topic may yet expose such a link. There is also a large knowledge gap in the literature concerning the impact of TCS use on commensal bacteria, which have the potential to transfer resistance to bacterial strains that are known to be human pathogens. Future studies should address the effect of TCS on bacteria under typical conditions in community and health-care settings, and the relationship between TCS use and microflora.

Interestingly, some researchers have suggested that, although TCS resistance has been associated with antibiotic cross-resistance in the laboratory setting, these findings do not carry over to clinical environments (Russell 2003, 2004; Gilbert *et al.*, 2007; Aiello *et al.*, 2007). This discrepancy is not necessarily surprising, as laboratory tests on TCS resistance have used predominantly pure cultures, in nutrient rich environments, both of which are not representative of real-world conditions (Gilbert *et al.*, 2007). As it currently stands, there is a lack of clinical evidence to suggest that the use of TCS has led to the propagation of antibiotic resistant staphylococci, antibiotic-resistant Gram-negative bacteria or isoniazid-resistant *M. tuberculosis*, although some researchers continue to speculate that this type of cross-resistance is just over the horizon (Russell, 2003; Aiello *et al.*, 2007).

Conclusions about TCS and a lack of antibiotic cross-resistance in a variety of environments must be drawn with a considerable degree of trepidation, as studies examining the issue have not provided enough evidence to assuage fears that the use of the antimicrobial is devoid of risk. Based on the fact that antibiotic cross-resistance has been demonstrated in the laboratory setting, further studies are needed to confirm that, in clinical, household and community settings, the judicious use of TCS does not, and will not, lead to antibiotic resistance. It would then seem logical to suggest that gratuitous use of TCS should be all but eliminated, whilst allowing for the continuation of its important clinical functions. The use of TCS should be limited to applications where it has been demonstrated to be effective, which includes health-care settings and in oral hygiene formulations (Gilbert and McBain, 2002; Cozad and Jones, 2003; Russell, 2004). Currently, the Canadian Medical Association is calling for a ban on antibacterial products because of concerns that these products may actually promote bacterial growth. Previously, many European countries, such as Sweden, have actively discouraged consumers from using antimicrobial products.

CONCLUSIONS AND FURTHER RESEARCH

Based on the existing literature, the judicious use of TCS should be considered safe, and although antibacterial cross-resistance has been found in the laboratory setting, there is no evidence to suggest that it is occurring in clinical and/or household environments. However, there are a number of different issues surrounding the use of TCS that warrant further research: environmental by-products, bioaccumulation potential, toxicity to aquatic organisms, endocrine disrupting effects, and the potential for TCS to prime bacteria for antibiotic resistance. These issues are of importance for the safeguarding of human health, aquatic ecosystems and the environment.

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