FATE, TRANSPORT, AND RISK ASSESSMENT OF BIOSOLIDS-BORNE TRICLOSAN (TCS)

By

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This dissertation is dedicated to my family

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LIST OF ABBREVIATIONS

BAF-QSAR	Bioaccumulation Factor-Quantitative Structure-Activity Relationship
BASL4	Biosolids amended soil level four model
bw	Body weight
ECOSAR	Ecological Structure Activity Relationships
HPLC	High Performance Liquid Chromatography
LC	Lethal concentration
LC/MS	Liquid Chromatography Mass Spectrometry
LD	Lethal dose
LOAEC	Lowest Observed Adverse Effect Concentration
LOD	Limit of Detection
LOEC	Lowest Observed Effect Concentration
LOQ	Limit of Quantitation
MWRDGC	Metropolitan Water Reclamation District of Greater Chicago
NOAEC	No Observed Adverse Effect Concentration
NOEC	No Observed Effect Concentration
OPPTS	Office of Prevention, Pesticides and Toxic Substances
PBT	Persistence, Bioaccumulation and Toxicity
QSAR	Quantity Structure Activity Relationship
QSPR	Quantitative Structure Property Relationship
RfD	Reference dose
TNSSS	Targeted National Sewage Sludge Survey
USEPA	United States Environmental Protection Agency
wk	Week
WWTPs	Wastewater treatment plants

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Triclosan (TCS) is an antimicrobial compound used in many personal care products, and is a common constituent of domestic wastewater. Removal rates of TCS in wastewater treatment plants are typically >95% and most of the TCS partitions (accumulates) in sludge solids. Sludge processed to produce biosolids is often land applied, and can transfer TCS to agricultural soils. The objectives of this research were to evaluate the environmental fate and ecological effects of biosolids-borne TCS following biosolids land application. As previously published TCS risk assessments were based on the data derived from models or unpublished sources, we used measured TCS concentrations, properties, fate, and transport data to conduct a more realistic risk assessment. The TCS concentrations measured in representative biosolids were 1 to 40 mg kg^{-1} (mean = 18 ± 12 mg kg^{-1}). Water solubility measured at neutral pH was 9 mg L^{-1}, and increased to nearly 800 mg L^{-1} at two pH units above the pK_a of ~8. The log K_d of TCS in biosolids was 4.15 ± 0.03 and log K_{oc} was 4.68 ± 0.07. A TCS biodegradation study determined a primary degradation half-life of ~100 d and a degradation product (Methyl TCS) was identified. Biosolids TCS concentrations of ≤105

mg kg^{-1} did not affect the earthworm survival, suggesting an estimated lethal TCS concentration (LC_{50}) >105 mg kg^{-1}. Bioaccumulation factors (BAF) in earthworms were 4.3 to 12 depending on soil texture. Investigation of TCS effects on microbial processes suggested that biosolids TCS concentrations ≤500 mg kg^{-1} had no significant effect on the microbially-mediated processes. Plant bioaccumulation of TCS was minimal in radish (BAF = 0.004) and bahia grass leaves (BAF = <0.001), but greater in lettuce leaves (BAF = 0.04) and radish roots (BAF = 0.43). No significant leaching of TCS occurred beyond the biosolids incorporation depth of 0 to 2.5 cm in amended soil. A preliminary (multi-pathway, multi-target) risk assessment identified American woodcock as the most sensitive species. A tier-2 assessment, utilizing less conservative estimates, suggested minimal risk of biosolids-borne TCS to human and environmental health, when biosolids are land applied in a sustainable manner.

CHAPTER 1
INTRODUCTION AND PROJECT OBJECTIVES

Background

Chemicals from domestic, municipal, industrial, or agricultural sources that are present in the environment, but not commonly monitored are termed emerging contaminants (ECs). The term “emerging” does not infer that the chemicals are new, but that the interest of scientific community in the chemicals is recent (Aga, 2009). Such chemicals have been recently detected in a variety of matrices including surface water, groundwater, sediments, and biosolids as a result of improved analytical capabilities. One important group of ECs includes the pharmaceuticals and personal care products (PPCPs). The primary route of PPCPs entry into the environment is through human use. With expanding population and increased human use of PPCPs, the appropriate treatment and disposal of these chemicals after use is becoming a cause of concern. Wastewater produced by domestic use enters and is treated in wastewater treatment plants (WWTPs). The products of the WWTPs are reclaimed liquid (effluent) and solid (sludge). The liquid is typically discharged to surface waters and the solid, after processing to reduce water content and pathogens, is called biosolids and is often suitable for land application. Biosolids can contain a variety of ECs (such as PPCPs), due to stability and sorption of ECs to organic rather than aqueous fraction during the WWTP processing.

Options for biosolids disposal include land application, incineration and landfill disposal. The nutrient-rich and organic nature of biosolids makes it a valuable resource for land application to improve soil fertility and is currently considered the most suitable way of biosolids disposal (Epstein, 2002). Despite the benefits of land application,

careful monitoring is essential to ensure the safe and sustainable reuse of biosolids. The USEPA conducted four surveys over the years (1982-2009) to identify contaminants in biosolids for possible regulatory action. One purpose of the most recent survey (USEPA, 2009a) was to obtain information on the presence and levels of certain contaminants of emerging concern (such as PPCPs). Triclosan (TCS) was included among several hundred (n = 145) other chemicals such as polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), antibiotics, drugs, hormones and steroids. The survey results suggested that land application of biosolids can transfer several of the chemicals, including TCS, to the terrestrial environment in sizeable amounts.

Triclosan [TCS, Irgasan DP 300 (trade name); 5-chloro-2-(2,4-dichloro phenoxy) phenol, CAS 3380-34-5] is an antimicrobial compound active against gram-positive, gram-negative bacteria, fungi and viruses. Triclosan is added to a variety of personal care products, including soaps, detergents, and cosmetics for its sanitizing properties (Heidler and Halden, 2007). Effectiveness of TCS as an antimicrobial agent is attributed to inhibition of the enzyme enoyl-acyl carrier protein reductase that is involved in bacterial lipid biosynthesis (Levy et al., 1999; Heath and Rock, 2000). Brausch et al. (2010) suggested that algal sensitivity to TCS is due to the disruption of lipid biosynthesis, membrane destablization (Lyrge et al., 2003; Franz et al., 2008) and uncoupling of phosphorylation (Newton et al., 2005). The typical range of TCS concentrations in personal care products is 0.1 to 0.3% (w/w), which is sufficient to inhibit the activity of bacteria, molds, and yeasts (McAvoy et al., 2002).

Effects of TCS human exposure through personal care products are well studied. Acceptance of wide usage of TCS containing personal care products is based on the reports of no adverse effects of TCS on humans, except skin irritation and dermatitis (Dayan, 2007). However, the use of TCS containing products for household purposes and skin-care was implicated in the occurrence of TCS in human breast milk (<20-300 µg kg lipid wt^{-1}) (Adolfsson-Erici et al., 2002; Ye et al., 2005), human urine (2.4-3790 µg L^{-1}) (Calafat et al., 2008), and blood (0.010 to 38 ng g^{-1}) (Allmyr et al., 2006). Dayan (2007) conducted a human risk assessment in children exposed to TCS-containing milk. Even conservative estimates (highest reported TCS concentration in consumed milk) of exposure represented minimal risk to children from the TCS present in the milk (Dayan, 2007). Other adverse effects that can impact humans have been suggested. Wang et al. (2004) reported that TCS can act as a selective inhibitor of sulfotransferase and glucuronosyl transferase enzymes with an inhibitory concentration (IC50) range of 430 to 2152 µg L^{-1} in the human liver. The two enzymes catalyze the conjugation of xenobiotics, increasing their solubility, and promoting excretion from the human bodies. James et al. (2010) suggested that TCS (concentration <0.03 µg L^{-1}) might endanger pregnancy in sheep by reducing the total placental estrogen secretion in target tissues critical for the maintenance of pregnancy. A threshold of toxicological concern of 30 µg kg^{-1} d^{-1} was estimated to represent the highest dose to which a person could be exposed over a lifetime with no expected adverse health effect (EPHC, 2008). Several risk assessments conducted based on the toxicological, phramacokinetic and clinical data suggested limited toxicity of TCS from personal care products (Rodricks et al.,

2010; Barbolt, 2002; Gilbert and McBain, 2002; Moss et al., 2000). Thus, human effects are deemed inconsequential, and are not directly studied here.

Following use, much of the unused TCS becomes a component of domestic wastewater. The products of WWTPs are liquids (effluents) and solids (sludge). Processed sludge forms biosolids, which may then be land applied. Despite monitoring data demonstrating significant TCS removal (98 ± 1%, Heidler and Halden, 2007) in activated sludge (consists of aerobic conditions promoting biological processes) systems, the relative roles of biodegradation and sorption in TCS removal from influents are not conclusively documented (Heidler and Halden, 2007; McAvoy et al., 2002; Sabaliunas et al., 2003; Singer et al., 2002). Bester (2003) reported 30% TCS sorption on sludge, whereas Heidler and Halden (2007) estimated that 50 ± 19% of TCS accumulated in the sludge and 48 ± 19% was biotransformed in a full scale activated sludge treatment unit.

The 2002 USGS survey of 139 streams expected to be influenced by WWTPs effluent detected TCS in over 60% of the streams at a median concentration of 0.14 μg L^{-1} and a maximum of 2.3 μg L^{-1} (Kolpin et al., 2002). Triclosan was one of the five most frequently detected contaminants, behind widely recognized contaminants such as coprostanol, cholesterol, N, N-diethyl toluamide, and caffeine. Recently, Brausch and Rand (2010) reported the detection of TCS in 57% of the surface water (rivers, streams) samples with a median concentration ~0.05 μg L^{-1}. Benotti et al. (2009) surveyed 19 drinking water treatment plants where the source water was impacted by wastewater and reported TCS median concentration of 0.003 μg L^{-1} (detected in six plants) in source water, and 0.0012 μg L^{-1} (detected in one plant) in finished drinking water.

Detection of TCS in aqueous systems is of concern because TCS can photo-degrade to dioxins (Latch et al., 2005), and the binuclear aromatic ring of TCS (Figure 1-1) is also present in dioxins. Halden and Paull (2005) and Heidler and Halden (2007) opined that the structural similarity suggests that TCS may persist and bioaccumulate in a manner similar to dioxins. Adverse effects of TCS in various aquatic species are illustrated in Table 1-1. A species sensitivity distribution (SSD) diagram is another approach to identify the aquatic risk of TCS. Capdevielle et al. (2008) conducted a risk assessment of TCS in freshwater environments using the SSD approach. The toxicity distribution was constructed based on chronic toxicity data for several (n=14) aquatic species, and represents a more realistic threshold of toxicity values (as it considered multiple species) as compared to no effect concentration approach based on a single most sensitive species (e.g., data in Table 1-1). Ratios of the predicted environmental concentration (PEC) to the predicted no effect concentration (PNEC) ratios were <1 for most aquatic populations, suggesting low risks to even the most sensitive aquatic species (Capdevielle et al., 2008). Thus, the risk to sensitive species is low even at the highest likely exposure, which typically occurs immediately downstream of WWTPs. Capdevielle et al. (2008) opined that, at current TCS usage, TCS is not expected to adversely impact aquatic species. Although the TCS aquatic effects are important based on the toxicity data (Table 1-1), the Capdevielle et al. (2008) study results suggested minimal aquatic TCS effects. Therefore, the focus of the present study is on the occurence of TCS in soils impacted by land-applied biosolids.

In the US, WWTPs generate approximately 7 million Mg of biosolids each year (USGS, 2008), of which 63% (National Research Council, 2002) is land applied.

Biosolids TCS concentrations have been reported in several recent studies (Langdon et al., 2011; McClellan and Halden, 2010; Cha and Cupples, 2009; Xia et al., 2009; Heilder and Halden, 2009; USEPA, 2009a) and suggest a representative concentration range of 10 to 20 mg kg^{-1} in typical biosolids. Reported TCS concentrations in the extensive (78 WWTPs) 2009 Targeted National Sewage Sludge Survey (TNSSS) (USEPA, 2009a) were 0.4 to 133 mg kg^{-1} with an overall mean of 16 ± 65 mg kg^{-1} (including statistical outliers). The treatment plants selected for the survey received secondary treatment or better, but the final products included some materials that were not processed to meet land application standards with respect to disinfection and chemical removal usually obtained by tertiary treatment (USEPA, 2009a). Assuming a TCS concentration of 16 mg kg^{-1} (mean from TNSSS) in activated sludge, an estimated 1.5-6.5×10^4 kg $year^{-1}$ TCS is land applied nationwide. The mass estimate would differ depending on the actual concentration of TCS in the biosolids, but the practice of biosolids land application clearly represents a mechanism for introducing substantial amounts of TCS into the environment. Reports of TCS presence in earthworms (Kinney et al., 2008), algae, snails (Coogan et al., 2007; 2008) and in dolphin's plasma (0.18 ng g wet wt^{-1}; Fair et al., 2009) highlight the ability of TCS to accumulate in a variety of organisms. Additionally, some worry that the TCS present in the environment could contribute to the spread of antibacterial resistance and could threaten human drug therapy (Birosova and Mikulasova, 2009; Pycke et al., 2010; Rooklidge, 2004; McMurry et al., 1998).

Little is known about the risk of biosolids-borne TCS that is land applied. Risk quantification of a chemical requires information on the toxicity of the chemical, as well as exposure. The presence of chemical in the environment is not a hazard unless

humans or other organisms are exposed to concentrations sufficient to induce an adverse effect. Further, the adverse effect will vary with exposure times, and depend on whether an exposure is chronic or acute especially if a chemical has endocrine effects. The environmental fate and transport of biosolids-borne TCS is particularly important to identify the relevant exposed populations and to conduct meaningful environmental and human health risk assessments.

Various studies characterized components of TCS behavior in non biosolids amended (un-amended) soils, but few studies focused on biosolids-amended soils. The average half-life of TCS estimated from studies (Kwon et al., 2010; Lozano et al., 2010; Higgins et al., 2011; Walters et al., 2011) conducted in amended soils was generally >100 d, i.e., TCS was deemed persistent. Further, various studies (e.g. Miyazaki et al., 1984; McAvoy et al., 2002; Lindstrom et al., 2002, Coogan et al., 2007; 2008) suggested that TCS was methylated to Me-TCS in effluents, biosolids, fish bodies, algae, and snails. However, it is uncertain if Me-TCS is formed in biosolids-amended soils, and if it persists or is further mineralized to CO_2. Limited data available for Me-TCS partitioning suggest that Me-TCS is more hydrophobic (log K_{ow} = 5.2) than TCS (log K_{ow} = 4.8) (Boehmer et al., 2004), and likely to be less bioavailable than TCS. Earthworm toxicity data in un-amended soils suggest minimal toxicity of TCS to earthworms (Higgins et al., 2011). The bioaccumulation data were variable in earthworms grown in TCS spiked un-amended soil, and in biosolids-amended soil, creating a need for a definitive assessment. Waller and Kookana (2009) and Butler et al. (2011) suggested inhibitory effects of TCS on some microbially-mediated reactions and on microbial community structure in soil (no biosolids). Triclosan may behave

differently in biosolids-amended soil due to reduced bioavailability of TCS, and needs further investigation.

Plant TCS accumulation was reported (Wu et al., 2010) in a single crop (soybean, *Glycine max*) grown in a biosolids-amended soil, but the study was confounded by the use of biosolids with extremely low solids content (19 g L^{-1} vs avg of 300 g L^{-1}). A chemical's transport potential and bioavailability is likely influenced by the type of biosolids applied to the soils (Edwards et al., 2009). Model estimated leaching potential of TCS was minimal in amended soils (Cha and Cupples, 2010). Another antimicrobial chemical, [i.e., triclocarban, (TCC)] was minimally phytoaccumulated and leached (Snyder et al., 2011) in a biosolids-amended soil. The same study reported formation of TCC bound (non-extractable) residues. We speculated that because TCS has a reported range of low water solubility values (1.9-17 mg L^{-1}) and because the partitioning coefficient of TCS (log K_{oc} = 4.3) is even greater than TCC (log K_{oc} = 3.88) (Agyin-Birikorang et al., 2010), TCS should tend to partition onto soils and sediments. Fuhr et al. (1998) defined bound residues as "compounds in soil, plant, or animal which persist in the matrix in the form of the parent substance or its metabolite(s) after extractions. The extraction method must not substantially change the compounds themselves or the structure of the matrix". Due to high partitioning coefficient of TCS (log K_d = 2.3), it is expected to form bound residues and have minimal potential of leaching and phytoaccumulation in biosolids-amended soils, as with TCC. Several ecological risk assessments were conducted recently (Reiss et al., 2009; Fuchsman et al., 2010; Langdon et al., 2010) that addressed the risk of TCS from biosolids-amended soils. Langdon et al. (2010) quantified the risk to aquatic organisms from surface runoff

and leaching of TCS from biosolids-amended soils and found some hazard to the most sensitive aquatic species; but, acknowledged that the risk might have been overestimated. Both Reiss et al. (2009) and Fuschman et al. (2010) suggested minimal risk to terrestrial organisms from biosolids-borne TCS, but most of the data utilized in the assessments were derived from models or extracted from unpublished sources. Thus, a characterization of biosolids-borne TCS fate, transport and risk assessment based on measured data is necessary.

Based on the available literature for TCS, appropriate hypotheses for the project were formulated as follows:

- Biosolids-borne TCS and its metabolites are persistent (half-life >60d) in the environment.
- Biosolids-borne TCS forms bound residues of limited bio- and environmental-lability.
- Exposure to biosolids-borne TCS poses minimal risk to soil micro and macro organisms.
- Biosolids-borne TCS has minimal mobility (leaching tendency) and phytoavailability.
- Biosolids-borne TCS poses minimal risk to human and environmental health.

The ultimate goal of our study was to perform a human and ecological health risk assessment of biosolids-borne TCS. The following intermediate objectives were designed to test the first four hypotheses and to obtain data required to fulfill the ultimate objective.

Objective 1: Quantify TCS Concentrations in Biosolids

The largest database of biosolids TCS concentrations is the 2009 TNSSS (USEPA, 2009a), representing 78 WWTPs across the U.S. Reported TCS concentrations range from 0.4 to 133 mg kg^{-1} (mean = 16 ± 65 mg kg^{-1}, including statistical outliers). The large variability in the TNSSS data likely represents inclusion of

some unique WWTPs producing sewage sludge with exceptionally high (minimally processed) and low (probably processed by tertiary treatment) product concentrations of TCS. Our objective was to determine the TCS concentration in additional biosolids. Biosolids analyzed in our study included some from the TNSSS (USEPA, 2009a), and some from WWTPs managed by Metropolitan Water Reclamation District of Greater Chicago (MWRDGC). The biosolids from MWRDGC were chosen as the District serves a large metropolitan area and because the MWRDGC biosolids TCS concentrations were expected to be modest. The low initial concentrations were particularly useful for our studies as we tested the effect of variable (spiked) TCS concentrations on various microbial phenomena (microbial respiration, nitrification and ammonification) (Chapter 2).

Objective 2: Determine/Verify Basic Physico-Chemical Properties of TCS

Chemical's water solubility and partitioning coefficients (K_{ow}, K_d and K_{oc}) are critical to characterizing the fate and transport of environmental contaminants. Solubility and partitioning behavior relate to a chemical's availability for degradation, leaching, plant uptake and bioaccumulation in organisms. Conflicting TCS solubility data exist in the literature, making accurate predictions of environmental fate and transport problematic. The water solubility of TCS was measured at various pH values as TCS is a weak acid and is expected to be more soluble at pH values greater than its acid dissociation constant (pK_a). In addition, the partitioning coefficient of TCS in various biosolids was determined (Chapter 3). Agyin-Birikorang et al. (2010) measured the partitioning coefficients (K_d and K_{oc}) of TCS in biosolids and biosolids-amended soils. But, the coefficients of TCS inherent to biosolids have not been determined. The inherent coefficients are likely to be greater than the coefficients in spiked systems as

inherent TCS is expected to be thoroughly incorporated and evenly distributed within the organic component of the biosolids.

Objective 3: Determine the Degradation (Persistence) of Biosolids-Borne TCS

Knowledge of a chemical's persistence is fundamental to estimating the chemical's fate and transport in the environment. Further, chemicals may degrade to form metabolites that may be more hydrophilic or lipophilic than the parent and behave differently. Persistence of TCS in aerobic soils and sediments is reasonably well studied, but TCS degradation in biosolids-amended soils is less studied. A few studies reported degradation of TCS in amended soils, but the half-lives were based on the loss of extractable TCS with time, and may not be accurate as loss of extractable TCS may simply represent the conversion of extractable TCS to non-extractable (bound residue) forms. We utilized biosolids spiked with ^{14}C-TCS to determine the degradation (mineralization/ primary degradation) potential of TCS in two biosolids-amended soils, identified the metabolites and assessed the bound residue formation (Chapter 4).

Objective 4: Determine the Impacts of Biosolids-Borne TCS to Soil Organisms

Accurate estimates of TCS toxicity and bioaccumulation are critical to conduct ecological and human health risk assessments. A few researchers [e.g., Kinney et al. (2008); Higgins et al. (2011)] have directly addressed the effect of biosolids-borne TCS on terrestrial organisms. However, the results were confounded either by the detection of significant amounts of TCS in the control soil or by the lack of sufficient number of replicates. We conducted a laboratory study (USEPA, 1996b) using spiked biosolids to investigate the toxicity and bioaccumulation of biosolids-borne TCS to earthworms. Further, long-term bioavailability of TCS, was estimated by utilizing earthworms recently

collected from a field soil amended with biosolids two years earlier at a high application rate (Chapter 5).

Objective 5: Determine the Toxicity of Biosolids-Borne TCS on Microbial Reactions

Triclosan can affect the rate of microbial reactions like respiration and nitrification in un-amended soil systems (Waller and Kookana, 2009; Butler et al., 2011). To our knowledge, however, potential impacts of biosolids-borne TCS on soil micro-organisms or microbially-mediated reactions have not been published. We examined the impacts of biosolids-borne TCS on microbial processes using USEPA (1996c) methods in two soils with contrasting physico-chemical properties. Further, changes in microbial counts and community structure due to biosolids-borne TCS are not known, and may affect ecosystem processes (such as nutrient recycling) and the effectiveness of microbial invasions (such as growth of pathogens) (Garland, 1997). We utilized several sets of biosolids-amended soils to perform bacterial counts and to quantify changes in microbial community structure. A direct bacterial count method (Matsunaga et al., 1995) and biolog ECO plates were utilized to quantify the effects of biosolids-borne TCS (Chapter 6).

Objective 6: Quantify the Phytoavailability of Biosolids-Borne TCS

Literature provides evidence of plant accumulation of polar and non-polar organic compounds. A few studies of TCS toxicity and accumulation were conducted in soil-less cultures (Herklotz et al., 2010), un-amended soils (no biosolids) (Liu et al., 2009) and saturated systems (wetlands) (Stevens et al., 2009) that suggested the possibility of TCS plant accumulation from land-applied biosolids. Because chemical toxicity and bioaccumulation can vary with the species tested (Duarte-Davidson and Jones, 1996),

we utilized four plant species representing monocotyledons (monocots), dicotyledons (dicots), above-ground (leaves), and below-ground (roots) biomass as well as grasses. A field-equilibrated soil previously amended with a high biosolids application rate was utilized as the growth media for the plants (Chapter 7).

Objective 7: Quantify the Leaching Potential of Biosolids-Borne TCS

Despite the occurrence of TCS in biosolids, and frequency of biosolids land application, little is known about the mobility of TCS in biosolids-amended soils. The partition coefficient of TCS (log K_{oc} = 4.26; Agyin-Birikorang et al., 2010) measured in amended soils suggests that TCS has a propensity to partition onto soils and sediments in the environment. Our study explored the mobility of TCS in a biosolids-amended soil subjected to repeated irrigations (Chapter 8).

Ultimate Objective: Risk Assessment of Biosolids-Borne TCS

Several ecological risk assessments were conducted recently (Reiss et al., 2009; Fuchsman et al., 2010; Langdon et al., 2010) but most of the data used in the assessments were derived from models or extracted from unpublished sources.Thus, a characterization of biosolids-borne TCS behavior, and a risk assessment based on measured data are necessary.

Studies mentioned in each of the intermediate objectives are described in Chapters 2 to 8. Each intermediate objective is presented as a separate chapter in the dissertation, and each chapter has specific hypotheses and objectives. Data accumulated from our studies and from published sources were used to identify various pathways of TCS exposure from biosolids land-application practices, and to perform an integrated human and ecological risk assessment of biosolids-borne TCS applied to

soils (Chapter 9). Suggestions for the future direction of biosolids-borne TCS work are included.

Table 1-1. Toxicity end-points of TCS for some aquatic species

Toxic effect	Species	Toxicity endpoint	Reference
Endocrine disruption	Activation of the human pregnane X receptor	>2870 µg L^{-1}	Jacobs et al. (2005)
Toxic effects	Rainbow trout (*Oncorhynchus mykiss*)	NOAEL[a] of 71.3 µg L^{-1}	Orvos et al. (2002)
Toxic effects	*Daphnia magna*	EC50[b] of 390 µgL^{-1}	Orvos et al. (2002)
Toxic effects	Amphibian larvae (*Xenopus laevis*)	LC_{50}[c] of 259 µgL^{-1}	Palenske et al. (2010)
Gene expression	American bullfrog (*Rana catesbiana*)	0.15-1.4 µg L^{-1}	Veldhoen et al. (2006)
Growth	Freshwater microalga (*Pseudokirchneriella subcapitata*)	72-h IC50[d] of 0.53 µg L^{-1}	Yang et al. (2008)
Genotoxic and cytotoxic effects	Zebra mussel hemocytes	0.692 ngL^{-1}	Binelli et al. (2009)

[a] no-observable-adverse-effect-level, [b] effective concentration, [c] lethal concentration, [d] inhibitory concentration

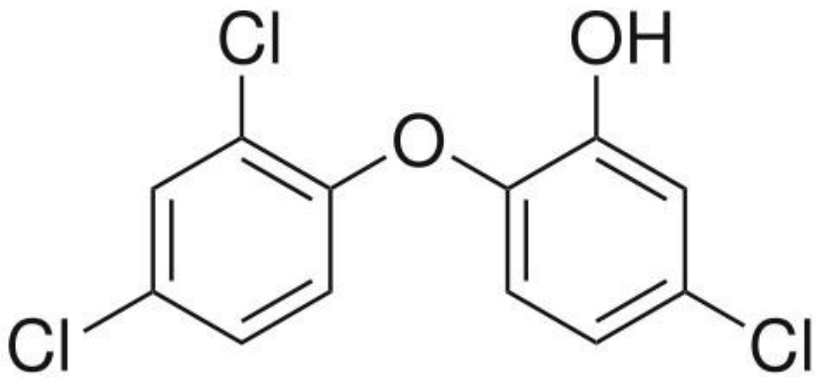


Figure 1-1. Chemical structure of Triclosan [TCS; 5-chloro-2-(2,4-dichloro phenoxy) phenol, CAS 3380-34-5]

CHAPTER 2
BIOSOLIDS-BORNE TCS CONCENTRATIONS

Background

Numerous biosolids TCS concentrations are reported in the literature. The mean TCS concentration of 12.6 ± 3.8 mg kg^{-1} was reported for the composited biosolids archived from the 2001 Targeted National Sewage Sludge Survey (TNSSS) (McClellan and Halden, 2010). The largest database of biosolids TCS concentrations is the 2009 TNSSS report (USEPA, 2009a), and reports a TCS concentration range of 0.4 to133 mg kg^{-1} (mean=16 ± 65 mg kg^{-1}, including statistical outliers) representing 78 WWTPs sampled in the survey. The WWTPs selected for the survey received secondary treatment or better, but the final products included some materials not processed to meet land application standards (USEPA, 2009a). Some of the WWTPs in the survey were likely not as efficient in removing TCS. Thus, the large variability in the TNSSS data might represent some unique WWTPs producing sewage sludge with exceptionally high (minimally processed) and low product concentrations (probably processed by tertiary treatment) of TCS. Biosolids analyzed in our study included some from the TNSSS (USEPA, 2009a), and some from WWTPs managed by Metropolitan Water Reclamation District of Greater Chicago (MWRDGC). Our objective was to determine the TCS concentration in representative biosolids including the biosolids utilized in our experiments.

Material and Methods

Fifteen biosolids were analyzed to determine the TCS concentrations as described in Heidler et al. (2006). Four biosolids samples were collected by the MWRDGC personnel and promptly shipped to the laboratory on ice packs. The samples

were frozen (-20°C) until analysis. We also utilized biosolids collected for a previous triclocarban project (biosolids also utilized in the TNSSS by USEPA, 2009) which were frozen at -20°C until analysis. Briefly, the biosolids samples were thawed, lyophilized, and 1 g (dry wt. equivalent) samples were extracted twice with 20 mL of methanol (MeOH) + acetone (50:50, v/v) in triplicate. The samples were shaken for 18 h on a platform shaker, followed by sonication (2 h) (Branson 2210, Danbury, CT; temp. 40°C, 60 sonications min^{-1}). The extract was centrifuged at 800 x g, dried under N_2 gas and reconstituted in 50:50 MeOH: Milli-Q water. The samples were then fortified with TCS-d7 internal standard and analyzed by Liquid Chromatography Mass Spectrometry (LC/MS) (TSQ Quantum, Thermo Scientific, Williston, VT, USA). Chromatography was performed on Phenomenex Luna C18 column (3 µm particle size, 2×100 mm; Phenomenex, Inc., Torrance, CA) with sample injection volume of 5 µL min^{-1}. The mobile phase consisted of water: MeOH with 1mM ammonium acetate at a flow rate of 300 µL min^{-1}. The gradient consisted of 25:75 water: MeOH (held for 1 min), increasing to 0:100 (water : MeOH) held for 5 min. and decreasing back to 25:75 water: MeOH (over 0.5 min). Mass spectrometry was performed using selective ion monitoring in negative ionization mode. Linear calibration consisted of eight standard levels (0-500 ng g^{-1}). Detection was based on a characteristic molecular ion TCS (m/z 287) as well as TCS with the naturally occurring isotope ^{37}Cl (m/z 289, 291). The TCS concentrations were corrected for the TCS recoveries (recovery = 64 ± 5%) determined by spiking known ^{14}C-TCS activities in the biosolids. The samples ran for 12 min with an average retention time of 8 min. The limit of detection (LOD) was 0.40 ng g^{-1} and limit of quantitation (LOQ) was 1.3 ng g^{-1}. The LOD and LOQ values were calculated as 3-fold

and 10-fold, respectively, the standard deviation in the signal from multiple runs of the lowest calibration standard (Signal/Noise >10) (USEPA, 1984). The details of detection limits and recoveries are provided in the Appendix C.

Results and Discussion

The measured TCS concentrations in 15 biosolids were 0.40 to 40 mg kg^{-1} with an average of 18 ± 12 mg kg^{-1} and a median concentration of 21 mg kg^{-1} (Table 2-1). The average and median concentrations are consistent with the mean 2009 TNSSS (USEPA, 2009a) value (16 ± 65 mg kg^{-1}), and the mean (12.6 ± 3.8 mg kg^{-1}) of composited samples archived from the 2001 TNSSS (McClellan and Halden, 2010). The majority of the biosolids analyzed in our study were anaerobically digested, but differences in TCS concentrations could have occurred due to differences in digestion periods (time), inputs, or dewatering methods (air-dried vs cake) (Table 2-1). The measured unpublished and published biosolids TCS concentrations are summarized in Figure 2-1. The Figure 2-1 illustrates variability in TCS concentrations (0.4-133 mg kg^{-1}) in a variety of biosolids including the ones from our study and other studies from U.S, Canada, Australia, Spain and Germany. A typical representative range of biosolids TCS concentration appears to be ~10 to 20 mg kg^{-1} (Figure 2-1), consistent with the mean TCS concentration (16 ± 65 mg kg^{-1}) reported in the 2009 TNSSS (USEPA, 2009a). Xia et al. (2010) found significantly greater median TCS concentrations in un-composted (9.6 mg kg^{-1}) biosolids than in composted (1.2 mg kg^{-1}) biosolids obtained from 16 WWTPs located in Georgia, South Carolina, Colorado, Illinois, and California. They also observed greater TCS concentrations in biosolids obtained from WWTPs serving residential areas than industrial areas (Xia et al., 2010). Xia et al. (2010) results suggest

that TCS concentrations can vary widely, but the concentrations are consistent with the wide range of TCS concentrations reported in the 2009 TNSSS and in our study.

Of particular interest were the biosolids (anaerobically digested) obtained from MWRDGC, as the District serves a large metropolitan area and much of the biosolids is land applied. The TCS concentrations averaged 6 mg kg^{-1} in air-dried and 7 mg kg^{-1} in centrifuge cake samples of Stickney plant biosolids. Calumet plant samples had average TCS concentrations of 0.4 ± 0.0 mg kg^{-1} in air-dried and 4.7 ± 0.1 mg kg^{-1} in centrifuge cake samples. Our values for the Stickney plant are consistent with data for the TCS concentration (6.4 ± 0.3 mg kg^{-1}) of composited three year samples (dewatered) reported by Higgins et al. (2011). Triclosan concentrations in all MWRDGC materials were less than the nationwide representative concentration range (~10 to 20 mg kg^{-1}) (Figure 2-1). If the samples of biosolids furnished to us or the concentrations reported for composited samples (Higgins et al., 2011) are truly representative of the products produced year-round, the TCS concentrations in biosolids produced by major Chicago WWTPs can be regarded as modest to low. The concentrations are much smaller than values of 16 ± 65 mg TCS kg^{-1} (USEPA, 2009a) and 30 ± 11 mg TCS kg^{-1} (Heidler and Halden, 2007), suggesting that the initial environmental and human health concerns expressed by Heidler and Halden (2007) based on an assumed TCS concentration of 30 mg kg^{-1} may be over-estimated. The lower concentrations of TCS in biosolids from Stickney and Calumet WWTPs, however, do not necessarily mean minimal risk to human or the environmental health. Concerns about biosolids-borne persistence, environmental fate, phytoavailability and mobility require quantification, as outlined in the later chapters.

Table 2-1. Triclosan (TCS) concentrations in fifteen biosolids (n = 3) obtained from wastewater treatment plants across the U.S.

Biosolids ‡	Identification †	Treatment Process	TCS content mg kg^{-1}
UNKB		Anaerobic digestion	33 ± 0.7
UNKC		Anaerobic digestion (33 d)	21 ± 0.8
UNKD		Anaerobic digestion	40 ± 3
UNKE		Anaerobic digestion	22 ± 0.4
UNKF		Anaerobic digestion	20 ± 1
UNKG		Anaerobic digestion	31 ± 0.6
UNKH		Anaerobic digestion	25 ± 1
UNKI		Anaerobic digestion	1 ± 0.1
UNKJ		Unknown	22 ± 0.4
UNKK		Unknown	23 ± 1
UNKL		Unknown	11 ± 2
CHBC	Stickney water reclamation plant, Chicago	Anaerobic digestion (air-dried)	6
CLBC	Stickney water reclamation plant, Chicago	Anaerobic digestion (centrifuged cake)	7 ± 0.3
CHAD	Calumet water reclamation plant, Chicago	Anaerobic digestion (air- dried)	0.4 ± 0.0
CHCC	Calumet water reclamation plant, Chicago	Anaerobic digestion (centrifuged cake)	4.7 ± 0.1
Overall average			18 ± 12

† Empty boxes represent the treatment plants whose locations are unknown

‡ Acronyms are given for identification purposes only

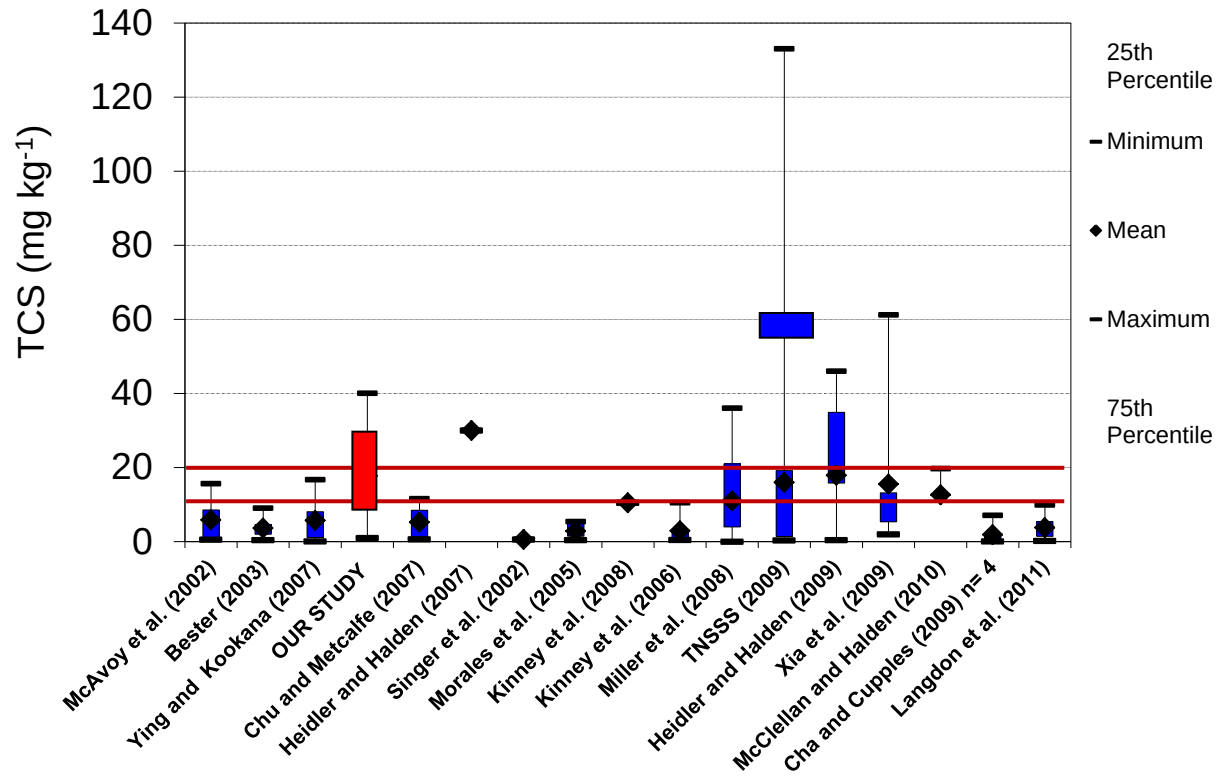


Figure 2-1. Representative biosolids TCS concentrations collected from various sources. The n represents the number of biosolids samples. For McClellan and Halden (2010) n = 110 representing composite biosolids from 94 wastewater treatment plants.

Represents the 95th percentile value for TCS concentration in the 2009 TNSSS (USEPA, 2009a)
Represent our qualitative assessment of the range of typical, representative TCS concentrations in biosolids.

CHAPTER 3
BASIC PHYSICO-CHEMICAL PROPERTIES OF TCS

Background

Chemical solubility and partitioning coefficients (K_{ow}, K_d and K_{oc}) are important factors for characterizing the fate and transport of environmental contaminants. Relationships between solubility, K_{ow} and K_{oc} are often (log-log) linear, but the specific relationship varies with the class of compounds (Lyman, 1990). The relationships can be used to calculate K_{oc} from the available K_{ow} and solubility data. In general, adsorption of a contaminant is directly related to the partitioning coefficient and inversely related to contaminant solubility (Comfort et al., 1994).

Water solubility is an important chemical property that strongly influences a compound's environmental transport, bioavailability, and degradation rate (Delgado, 2002). Conflicting TCS solubility data exist in the literature, making accurate predictions of environmental fate and transport of TCS problematic. Solubility values calculated from the Ecological Structure Activity Relationships (ECOSAR) model and PBT (Persistence, Bioaccumulation and Toxicity) Profiler vary from 1.97-4.6 mg L^{-1} (Halden and Paull, 2005) (Table 3-1). Actual solubility measurements vary from 10 mg L^{-1} (Ciba Speciality Chemicals, 2001a) to 17 mg L^{-1} (MITI, 1992). The variability in the reported and the modeled values of solubility requires resolution. The pK_a value of TCS is reported as 8.14 (Jakel, 1990) and TCS water solubility is expected to vary significantly with pH because the weak acid dissociates to a more soluble form as pH approaches and exceeds the pK_a of TCS.

The octanol-water partition coefficient (K_{ow}) is the ratio of the concentration of a chemical in octanol and in water at equilibrium at a specified temperature. Octanol is an

organic solvent regarded as a surrogate for hydrophobic lipids. The K_{ow} value is used in many environmental models, e.g., Bioaccumulation Factor-Quantitative Structure-Activity Relationship (BAF-QSAR) and Quantitative Structure Property Relationship (QSPR) to estimate the fate of chemicals in the environment. The K_{ow} also provides an estimate of a chemical's potential to bioaccumulate and bioconcentrate in the food-web (Dimitrov et al., 2003).

The log octanol/water partition (K_{ow}) coefficient of TCS is calculated to be 4.8 (at 25°C, pH 7) using KowWIN model (Halden and Paull, 2005) (Table 3-1). The model estimates log K_{ow} values of organic chemicals using an atom/fragment contribution method developed by Syracuse Research Corporation. Experimentally determined values of TCS are similar: log K_{ow}, 4.76 (Lei and Snyder, 2007; Wezel and Jager, 2002), and 4.70 (Ying et al., 2007) (Table 3-1). Although the collective data are in close agreement, the pH values at which the K_{ow} measurements were made are not known and K_{ow} is expected to decrease at pH ≥ pK_a values. We calculated TCS dissociation and log K_{ow} values as a function of pH using an equation that estimates the log K_{ow} of chlorophenols with varying pH (Nowosielski and Fein, 1998). Calculations suggest decreases in log K_{ow} of TCS at pH values above pK_a. The K_{ow} vs pH curve (Figure 3-1) closely resembles an acid dissociation curve, but is offset to higher pH values. Log K_{ow} values are minimally affected at pH≤pK_a, but a significant decrease at greater pH values. The predicted log K_{ow} becomes pH-independent at pH ≥ pK_a+3, with a value 2.9 log units less than normally assessed (Figure 3-1). A decrease in log K_{ow} values at higher pH predicts lower concentrations partitioned to biosolids and greater concentration in the aqueous phase during sewage treatment involving lime stabilization

(pH = 12). However, such high pH values are not common in most conventional sewage treatment or biosolids-amended soil systems.

The consistency of log K_{ow} literature values (4.8 and 4.76) and the negligible change in log K_{ow} values predicted at pH values (<7) representative of most sewage treatment systems (or amended soils) convinced us that it was unnecessary to conduct measurement of K_{ow} at different pH values. However, variation of TCS solubility with pH has not been addressed. Therefore, we measured the TCS solubility at pH = pK_a (8.14) and 2 units above (10.14) and below (6.14) the pK_a.

Mobility of TCS in biosolids-amended soil determines the potential for soil and groundwater contamination. The relatively high log K_{ow} (4.8) of TCS suggests extensive retention in (or on) biosolids and limited transport in soil systems, but, mobility may vary with the soil pH. Recent data obtained for a sandy loam and a silt loam soil (no added biosolids) using spiked TCS concentrations of 0.2, 0.5,1 and 2 mg L^{-1}, suggest relatively low values of log K_d of ~2.4 and log K_{oc} of ~2.3 (Karnjanapiboonwong et al., 2008). Other estimated and measured values of log K_{oc} reported are much greater: 4.26 (estimated, Ying et al. 2007) and 4.6 (measured, Heim, 1997), and a log K_d of 4.3 (measured Heim, 1997). The partitioning coefficients of added TCS in biosolids (K_d and K_{oc}) or in biosolids-amended soils have not been determined.

Alternatively, K_d and K_{oc} can be measured directly in biosolids ("inherent K_d and K_{oc}") (Carballa et al., 2008). Triclosan entering WWTPs is present throughout the entire biosolids production process. The compound "ages" as an inherent component of the solid fraction from the time TCS enters the waste stream to eventual biosolids land-application. As such, biosolids-borne TCS is expected to be thoroughly incorporated

and evenly distributed within the organic component of the biosolids. A spike, alternatively, is added to the exterior of the matrix and allowed to react after the biosolids are produced. A TCS spike is not expected to sorb as completely or uniformly as an inherent component of biosolids within the time frame of a typical partitioning experiment (<24 h). A K_d value estimated using a spiked matrix could then underestimate the true solid-phase partitioning of hydrophobic compounds, such as TCS. Inherent K_d values were measured for a similar compound TCC (Triclocarban) in several biosolids and differed from spiked K_d values (Snyder, 2009). We measured the inherent K_d and K_{oc} values of TCS in various biosolids.

Material and Methods

Solubility

Water solubility was measured according to the EPA standard method guideline of Office of Prevention, Pesticides and Toxic Substances (OPPTS) 830.7840 (USEPA, 1996a). The OPPTS is now renamed to Office of Chemical Safety and Pollution Prevention (OCSPP) but the guideline remains identified as OPPTS, and is used throughout the dissertation. The EPA guidance describes three major methods (shake-flask, generator column, column elution) for the solubility measurement depending on the expected solubility of the compound. The shake flask method is intended for compounds with solubilities >10 mg L^{-1}, whereas the column elution method is intended for compounds with solubilities ≤10 mg L^{-1}. The literature contains variable TCS solubility values, but measured values are reportedly >10 mg L^{-1}. Thus, the simple shake flask method was employed in our study. Solubility measurements were performed at pH values two units above and below the pK_a as well at pH = pK_a. A preliminary test was performed to estimate the quantity of TCS necessary to saturate

the desired volume of water. Approximately 5× the quantity of material determined in the preliminary test was weighed into nine Teflon tubes (3 replicates for each pH value) with stoppers. Water (30 mL) was added to the 40 mL tubes and the pH of the water was adjusted to 6.14, 8.14 and 10.14. The pH adjustment was performed by adding acid (HCl) or base (KOH) to attain target pH values. The tubes were then agitated at room temperature (20-25°C). Additional monitoring of pH was conducted every 12 h during the initial 24 h agitation and at each 24 h after wards. At sampling times of 1, 2 and 3 d the tubes were removed and 1mL of the aliquot was collected The 1 mL of water removed was replaced by adding 1 mL additional water to maintain constant volume of the solution. The removed aliquot was centrifuged (8000 × g) for 10 min and the TCS concentration in the clear aqueous sample was measured. Samples were quantified on LC/MS (Thermo scientific discovery max TSQ Quantum) in negative ionization mode. Chromatography was carried out on C18 column (5 µm particle size, 2.1 x100 mm; Phenomenex). Mobile phase consisted of water: MeOH with 1mM ammonium acetate at a flow rate of 300 µL min^{-1}. The gradient consisted of 25:75 water: MeOH (held for 1 min), increasing to 0:100 (water : MeOH), held for 5 min. and decreasing back to 25:75 water: MeOH (up to 12 min). Linear calibration consisted of eight standard levels (0-500 ng g^{-1}). The TCS quantification was performed by the isotope dilution method using the $^{13}C_{12}$ internal standard with a run time of 12 min. The ion monitoring was performed at m/z values of 287 for TCS and 293 for $^{13}C_{12}$ TCS. The m/z of 289, 291, 295 and 297 were used as qualifying ions due to the presence of naturally occurring ^{37}Cl atoms in the organic molecules.The limit of detection (LOD) was 0.4 ng mL^{-1} and the limit of quantitation (LOQ) was 1 ng mL^{-1}. The LOD and LOQ values were calculated as 3-fold

and 10-fold, respectively, the standard deviation in the signal from multiple runs of the lowest calibration standard (Signal/Noise >10) (USEPA, 1984). The details of detection limits and recoveries are provided in the Appendix C.

Partitioning Coefficients (K_d and K_{oc})

The K_d value of TCS was previously measured (Agyin-Birikorang et al., 2010) according to the EPA standard method guideline for soil and sediment (OPPTS 835.1220) (USEPA, 2007). The measurement was performed in biosolids, soils and biosolids-amended soils. Details are described by Agyin-Birikorang et al. (2010). Briefly, 0.8 mL of the stock solution (1.29×10^5 dpm ^{14}C-TCS mL^{-1}) was utilized to prepare a working solution (250 mL) in 0.01 *M* $CaCl_2$. The batch experiment involved addition of 2.5 mL of the ^{14}C-TCS working solution to triplicate 0.5 g (oven-dry equivalent) samples of the biosolids (1:5; g solid: mL solution) + Control samples containing TCS spiked $CaCl_2$ (no biosolids). Ten biosolids used in TCS concentration determination (Chapter 2) were used in the partitioning study along with 5 additional biosolids received as a part of the USEPA TNSSS (USEPA, 2009a). The samples and controls were analyzed for ^{14}C-TCS throughout the experiment to monitor the stability of the TCS concentration in the solution phase. No, or minimal, degradation was expected during the 24 h study given the estimated half-life of TCS in biosolids-amended soils (100 d) (Chapter 4). Replicated blank samples, containing 0.5 g of biosolids and 2.5 mL of 0.01 *M* TCS free $CaCl_2$, were also included. Samples were equilibrated on an end-over-end shaker for 24 h (based on a preliminary kinetic study), and subsequently centrifuged (8000×*g*) for 10 min at constant temperature (24 ± 2°C). Supernatant aliquots (1mL) were added to 10 mL of Ecoscint A scintillation cocktail (National Diagnostics, Georgia) in 20 mL glass scintillation vials. Radioactivity was determined via liquid scintillation counting with

background correction against blanks (^{14}C-free 0.01 M $CaCl_2$). Controls (no biosolids) were utilized for determining the initial concentration (C_0). The amount of TCS partitioning onto the biosolids solid phase was calculated as a percentage of ^{14}C-TCS initial activity added and the change in activity in the solution phase over time. Partition coefficients (K_d) were estimated for TCS adsorption on seventeen biosolids as:

$$K_d = \frac{\text{Activity of TCS adsorbed/kg biosolids}}{\text{Activity of TCS in solution/ L solution}}$$

Seven biosolids were utilized to measure "inherent" log K_d and K_{oc} values. The concept of inherent K_d and K_{oc} has been previously utilized for determining the coefficients in pharmaceuticals and musk fragrances (Carballa et al., 2008). Biosolids with known biosolids treatment process, and high TCS concentrations (≥20mg kg^{-1}, Chapter 2, Table 2-1) were selected to improve the probability of detecting TCS in the aqueous phase by LC/MS (limited due to LOQ of 1 ng mL^{-1}). The whole biosolids (cake) was extracted with an organic extractant (methanol, acetone mixture) to determine the total TCS concentration (sorbed + aqueous) as described in Chapter 2 (Table 2-1). A separate sample of biosolids (cake) was then centrifuged to obtain a sample of the presumed equilibrium solution and analyzed for TCS. The concentration of TCS sorbed was obtained by subtracting the mass of TCS in supernatant from the total TCS. Estimation of K_d ("inherent K_d") was obtained by taking the ratio of the sorbed TCS to the supernatant TCS. The mean K_d and K_{oc} values were calculated based on actual measurement of five biosolids supernatant TCS concentrations and on two biosolids concentrations estimated from the LOQ of the instrument.

Results and Discussion

Measured TCS water solubilities were 9 mg L^{-1} (pH = 6.14), 27 mg L^{-1} (pH = pK_a= 8.14) and 800 mg L^{-1} (pH = 10.14) (Table 3-2). The overall 90-fold increase in the solubility resulted from dissociation of the neutral acid to anionic species. Increased solubility at higher pH portends greater concentration in the aqueous phase during sewage treatment processes utilizing lime stabilization and in high pH soils. Extremely high pH levels are not common in most sewage treatment systems or in most soils, except some sodic soils. The solubility of TCS measured at pH 6.14 (i.e., 9 mg L^{-1}) appears reasonable for predicting the fate and transport of TCS in many soils. Thus, typical biosolids applied to typical, near-neutral soils would be expected to demonstrate a TCS water solubility of 9 mg L^{-1}, which is similar to reported measured values of 10 and 17 mg L^{-1} (Ciba Specialty Chemicals, 2001; MITI, 1992), but greater than calculated values of 1.97 to 4.6 mg L^{-1} obtained using Quantity Structure Activity Relationship (QSAR) and Estimation Programs Interface (EPI Suite v3.10) (Halden and Paull, 2005, Ying et al., 2007). The models underestimated the solubility of TCS and thus, the log K_{ow} values were expected to be overestimated by the model. However, the measured and model estimated log K_{ow} values (4.8 and 4.76) were relatively consistent The unexpected relationship between TCS solubility and log K_{ow} values highlights the importance of measured data collected by standardized methodology.

Mean log K_d values ± standard error (S.E.) were 3.76 ± 0.04 and log K_{oc} values were 4.30 ± 0.03 in TCS spiked biosolids (Table 3-3). The log K_{oc} values were similar to estimated values in soil of 4.26 (Ying et al., 2007), but slightly lower than measured value of 4.6 reported by Heim (1997). The log K_d and K_{oc} values suggest relatively strong sorption of TCS to the biosolids and low mobility in acid and circum neutral

(pH=6.5-7.5) soils. Also, the same biosolids were utilized to measure K_d and K_{oc} directly from biosolids (unspiked or "inherent K_d and K_{oc}"). The mean (n=7) inherent log K_d was 4.15 ± 0.03, and log K_{oc} was 4.68 ± 0.07. Based on the limited number of biosolids analyzed, the differences were not statistically significant, but the inherent log K_d and K_{oc} values tend to be greater than the spiked log K_d and K_{oc} values (Table 3-3). The log K_d values determined for biosolids (4.15 ± 0.03) was greater than those determined for soils (2.25 ± 0.26) and biosolids-amended soils (2.31 ± 0.19) (Agyin-Birikorang et al., 2010). The difference in the coefficients was attributed to the difference in organic carbon among the various matrices. Following normalization to organic carbon, the coefficients (K_{oc}) determined in the soils, biosolids and biosolids-amended soils were not significantly different and averaged 4.26 ± 0.31 (Agyin-Birikorang et al., 2010). Thus, a specific or narrow range of TCS partitioning coefficient (K_{oc}) can serve as a first approximation to describe the behavior of TCS in soils or other matrices. The K_{oc} values measured in our study and reported in other studies suggest significant adsorption of TCS to the soils and or biosolids. Thus, the mobility of TCS in biosolids-amended soils is expected to be restricted and the extent of retardation to be highly dependent on the organic carbon content of the amended soils and pH in case of calcareous soils.

Table 3-1. Physico-chemical Properties of TCS reported in the literature.

Property	Value	References	Comments
Molecular Weight (g mol^{-1})	289.55	Formula ($C_{12}H_9Cl_3O_2$)	CAS Registry no (3380-34-5)
Solubility (mg L^{-1})	1.97-4.6	(Halden and Paull,2005)	Estimated
	10	(Ciba Speciality Chemicals, 2001a)	
	17	(MITI, 2002)	Measured
log K_{ow}	4.8	(Halden and Paull, 2005)	Estimated Kow WIN
	4.70	(Ying et al., 2007)	Estimated PBT Profiler
	4.76	(Lei and Snyder, 2007)	Measured
	4.76	(Wezel and Jager, 2002)	Measured
log K_{oc}	4.26	(Ying et al., 2007)	Soils (1.3% OC) PBT profiler,
	4.6	(Heim, 1997)	Measured
pK_a	8.14	(Jakel,1990)	
Vapor Pressure (mm Hg at 20^0C)	4×10^{-6}	(Ciba Specialty Chemicals, 2001b)	Non –Volatile
Henry's Law Constant (atm-m^3 $mole^{-1}$ at 25^0C)	4.99×10^{-9}	(Meylan and Howard, 1991)	Estimated
Half-life (d)	120(Soils) 540(Sediment)	(Ying et al., 2007)	Estimated PBT Profiler, USEPA Level III fugacity model
	60 (Soils) 240 (Sediment)		Measured
	18-58 persistent	(Ying et al., 2007), (Wu et al., 2009)	Aerobic, anaerobic soils
	540		
	15-35	(Miller et al., 2008)	Sediments
	12-15	(Ciba Speciality Chemicals, 2001a)	Soils
	107	(Xu et al., 2009)	Soils
	56-107	(Lozano et al., 2010)	Biosolids-amended soils
		(Kwon et al, 2010)	Biosolids- amended soils

.

Table 3-2. Water solubility (mg L^{-1}) of TCS at various pH values

pH	Time (h)				Average
	24	48	72	96	
	mg L^{-1}				
6.14	8.5 ± 0.20	8.9 ± 0.47	9.7 ± 0.67	8.4 ± 0.30	8.9 ± 0.26
8.14	14 ± 0.71	30 ±1.0	26 ± 1.8	26 ± 1.6	26 ± 0.00
10.14	n/d ‡	n/d ‡	n/d ‡	n/d ‡	791†

‡n/d is not determined
† Not an average (single sample)

Table 3-3. Mean log partition coefficients (K_d and K_{oc}) (n=3) ± standard error (S.E) for TCS on spiked (Agyin-Birikorang et al., 2010) and unspiked (inherent) biosolids (our study).

	Spiked K_d, K_{oc} on biosolids				Inherent K_d, K_{oc} (Our study)	
Biosolids	Treatment process	log K_d	Fraction Organic carbon	log K_{oc}	log K_d	log K_{oc}
UNKB	Anaerobic digestion	3.68 ± 0.02	0.38	4.19 ± 0.02	3.99 ± 0.01	4.51±0.00
UNKC	Anaerobic digestion	3.79 ± 0.02	0.37	4.28 ± 0.02	3.88 ± 0.04	4.30±0.14
UNKD	Anaerobic digestion	3.74 ± 0.01	0.31	4.35 ± 0.01	4.78†±0.00	5.35†±0.07
UNKE	Anaerobic digestion	3.61 ± 0.03	0.32	4.09 ± 0.07	4.51†±0.01	5.01†±0.00
UNKF	Anaerobic digestion	3.62 ± 0.04	0.21	4.35 ± 0.04	3.85±0.03	4.60±0.03
UNKG	Anaerobic digestion	3.76 ± 0.02	0.42	4.21 ± 0.04	4.17±0.08	4.55±0.18
UNKH	Anaerobic digestion	3.77 ± 0.01	0.30	4.35 ± 0.01	3.88±0.06	4.44±0.06
UNKI	Anaerobic digestion	3.63 ± 0.04	0.24	4.30 ± 0.04		
UNKK	Unknown	3.75 ± 0.01	0.28	4.35 ± 0.01		
GRU		3.90 ± 0.02	0.38	4.31 ± 0.02		
OSBC	Anaerobic digestion	3.93 ± 0.01	0.42	4.37 ± 0.01		
ORBC-BL	Untreated (before lime stabilization)	3.85 ± 0.03	0.41	4.26 ± 0.05		
ORBC-AL	Lime stabilization (following lime addition)	3.68 ± 0.03	0.34	4.22 ± 0.07		
RCKF	Anaerobic digestion	3.85 ± 0.01	0.39	4.31 ± 0.01		
CFBC	Anaerobic digestion	3.95 ± 0.01	0.41	4.38 ± 0.03		
CALC	Anaerobic digestion	3.70 ± 0.03	0.28	4.36 ± 0.03		
CHCC	Anaerobic digestion	3.67 ± 0.02	0.28	4.35 ± 0.02		
Average		3.76 ± 0.04		4.30 ± 0.03	4.15 ± 0.03	4.68 ± 0.07

†Estimated using the ½ LOQ (Limit of quantitation) of LC/MS

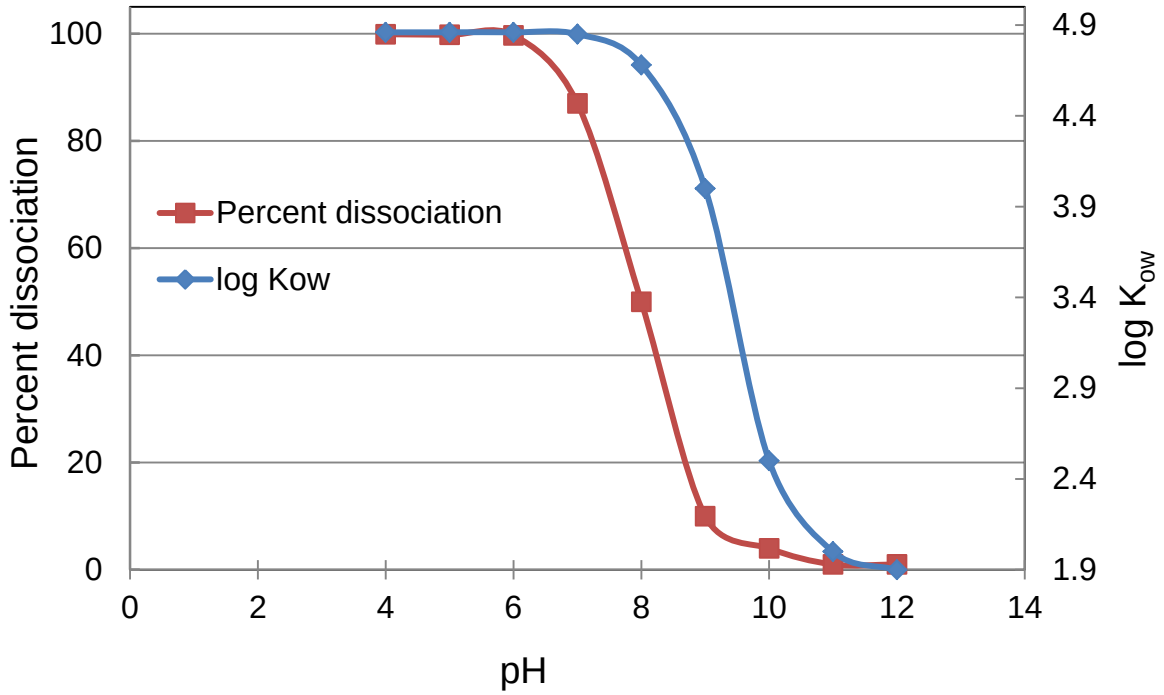


Figure 3-1. Calculated octanol-water partitioning coefficient (log K_{ow}) curve and dissociation diagram for TCS.

CHAPTER 4
BIODEGRADATION OF BIOSOLIDS-BORNE TCS

Background

The antimicrobial triclosan (TCS) is a common constituent of domestic wastewater and >50% of TCS mass entering the WWTPs partitions into biosolids (Langdon et al., 2011; McClellan and Halden, 2010; Cha and Cupples, 2009; Xia et al., 2009; Heilder and Halden, 2009; USEPA, 2009a). Land application of biosolids transfers TCS to soils where its fate and transport depends on TCS persistence in the terrestrial environment. Biodegradation half-lives for TCS in aerobic soils have been estimated using fugacity-based models like Persistence, Bioaccumulation, and Toxicity (PBT) Profiler (USEPA) ($t_{1/2}$ = 60-120 d) and measured [$t_{1/2}$ = 18 d (Ying et al., 2007) and 2 to 13 d (Kwon et al., 2010)] (Table 3-1). Miller et al. (2008) suggested long-term persistence of TCS in anaerobic sediments (40 years), compared to the estimated (PBT) half-life of 540 d under anaerobic soil conditions. Persistence of TCS in aerobic soils and sediments is reasonably well studied, but TCS degradation in biosolids-amended soils is less studied. Degradation studies in biosolids-amended soils are important, as TCS preferentially partitions into biosolids potentially reducing TCS bioavailability to degrading organisms.

Lozano et al. (2010) estimated a TCS half-life of 107 d in a biosolids-amended soil (unknown soil texture)**,** while Kwon et al. (2010) reported measured half-lives ranging from 56 to 107 d in two biosolids-amended loam soils [fine loam, pH:7.8, coarse loam, pH:4.7]. Recently, a mesocosm study conducted in Maryland resulted in an estimated first order half-life of 182-193 d (Walters et al., 2010). Higgins et al. (2011) reported a smaller half-life (42 d) in a biosolids-amended field soil (silty clay loam).

However, Higgins et al. (2011) half-life estimate was approximate, as the authors acknowledged that the study did not intend to calculate degradation. Kwon et al. (2010), Lozano et al. (2010), Walters et al. (2010), and Higgins et al. (2011) reported loss of extractable TCS with time, but did not distinguish between compound loss due to TCS mineralization [conversion to carbon dioxide (CO_2)] or primary degradation (formation of metabolite from the parent compound). Loss of extractable TCS may represent the conversion of initially extractable TCS to non-extractable (bound) residues with time. Snyder et al. (2010) reported the formation of triclocarban (TCC) bound (non-extractable) residues in biosolids-amended soils. We speculated that because TCS has a reported low water solubility value (1.9-9 mg L^{-1}) and because the partitioning coefficient of TCS (log K_{oc} = 4.3) is even greater than TCC (log K_{oc} = 3.88) (Agyin-Birikorang et al., 2010), TCS should tend to partition into organic C in soils and sediments and form bound residues as well. An accurate measurement of TCS half-life should include the determination of TCS mineralization, or primary degradation, identification of metabolites, and demonstration of mass balance.

Transformation by biological methylation forms methyl ether derivatives that are usually more lipophilic than the parent compounds (Valo and Salkinoja-Salonen, 1986; Neilson et al., 1983). Tulp et al. (1979) reported that TCS degrades by hydroxylation of benzene ring and cleavage of the ether bond forming dichlorophenols in urine samples. Hundt et al. (2000) showed that TCS microbial degradation can form Methyl-TCS (Me-TCS), dichlorophenols, and conjugated metabolites where carbohydrates are connected to the hydroxyl group of TCS. Miyazaki et al. (1984) reported Me-TCS in fish, but it was unclear whether the methylation occurred in the surface water or in the fish body.

However, a later study (Balmer et al., 2004) confirmed the presence of Me-TCS in surface water as well as fish bodies. Lindstrom et al. (2002) reported that Me-TCS formed in wastewater effluents and surface waters through biological methylation. Evidence of TCS methylation was also obtained from the presence of the anisole in semi-permeable membrane devices (SPMDs). Lindstrom et al. (2002) opined that the presence of only Me-TCS (no TCS) in SPMDs might represent significant bioaccumulation potential of Me-TCS. Further, McAvoy et al. (2002) detected Me-TCS (0.13-0.45 μg g^{-1}) in sludge and aerobically digested biosolids obtained from two WWTPs in Ohio. Xia (2010) reported that TCS biomethylated to a small quantity (~9%) of Me-TCS in a biosolids-amended soil (sandy loam) utilized for a TCS leaching study. Thus, we might expect formation of Me-TCS under aerobic conditions when biosolids-borne TCS is added to the soils. However, it is uncertain if the formed Me-TCS persists or further mineralizes to CO_2. Limited data for Me-TCS partitioning suggest that Me-TCS is more hydrophobic (log K_{ow} = 5.2) than TCS (log K_{ow} = 4.8) (Boehmer et al., 2004), and therefore, Me-TCS is likely to be less bioavailable than TCS and more likely to form bound residues.

We hypothesize that biosolids-borne TCS and its metabolites are persistent in the environment. The objective of our study was to determine the degradation (mineralization/primary degradation) potential of TCS in two biosolids-amended soils. Specifically, we sought to (i) identify the TCS metabolites (ii) determine the mineralization and/or primary degradation half-lives, and (iii) assess the extent of bound residue formation. We conducted a biodegradation study in soils using ^{14}C-TCS spiked biosolids under biotic and inhibited biotic aerobic conditions to determine the

degradation rates of TCS in biosolids-amended soils. Aerobic conditions were chosen as biosolids-borne TCS is expected to undergo primarily aerobic degradation under the typical conditions of surface applied and incorporated biosolids. The biodegradation study was conducted according to the United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) Harmonized Fate, Transport and Transformation Test Guidelines; Soil Biodegradation (835.3300) (USEPA, 1998).

Material and Methods

Chemicals, Biosolids and Soils

Radiolabeled ^{14}C–TCS uniformly on the chlorophenol ring (specific activity - 48 mCi $mmol^{-1}$ and 99% purity) was custom synthesized by Tjaden Biosciences (Burlington, IA). Ecoscint A liquid scintillation cocktail was purchased from National Diagnostics (Atlanta, GA) and the Me-TCS standard from Wellington laboratories (Shawnee Mission, KS). All other chemicals and solvents were purchased from Fisher Scientific (Atlanta, GA). An anaerobically digested biosolids (CHCC, solids: 320 g kg^{-1}) was collected from a domestic WWTP in Illinois. The biosolids contained 5 mg kg^{-1} of TCS and non-detectable (<0.7 mg kg^{-1}) Me-TCS (Chapter 2). Two soils, the Immokalee fine sand (IFS) (sandy, siliceous, hyperthermic Arenic Alaquods) and the Ashkum silty clay loam (ASL) (fine, mixed, superactive, mesic Typic Endoaquolls) were collected from sites with no known history of receiving land-applied biosolids or sludge. Select physico-chemical properties of soils and the biosolids are presented in Table 4-1.

Biodegradation Study Design

The biodegradation study consisted of 146 glass, round-bottom, 30 mL glass centrifuge tubes (2 soils × 1 biosolids × 1 rate × 4 replicates × 8 sampling periods × 2

treatments (biotic/inhibited) + 18 controls). The first set of controls consisted of 1 biosolids × no spike of ^{14}C–TCS × 2 soils × 2 treatments (biotic/inhibited) × 4 replicates (Table 4-2). The absence of evolved $^{14}CO_2$ in controls would confirm no cross-contamination in the system. The second set of controls consisted of ^{14}C–TCS spiked into autoclaved water. Evolved $^{14}CO_2$ will indicate soil-independent abiotic TCS degradation (e.g., photolysis). Treatments were prepared by first weighing CHCC biosolids (0.10 g dry wt. equivalent) in centrifuge tubes. A sub-stock of ^{14}C (1.3×10^6 dpm ^{14}C-TCS g^{-1}) was prepared in 1mL methanol (MeOH) and dissolved in 200 mL of deionized distilled (DDI) water. One mL of sub-stock TCS was spiked onto the CHCC biosolids sample and allowed to equilibrate for 24 h at room temperature. Equilibrium between spiked TCS and the biosolids was assumed to have occurred in 24 h as suggested by Agyin-Birikorang et al. (2010) who reported TCS sorption equilibrium within 24 h of incubation. The spiked biosolids was mixed with 10 g (dry wt.) of two soils to simulate a realistic field application rate (22 Mg ha^{-1} or ~10 tons $acre^{-1}$ equivalent). The biosolids-amended soils were vortexed for 30 s to promote uniform mixing of the spiked biosolids and the soil sample.

The radioisotope spiking increased the effective biosolids-borne TCS concentration to 40 mg TCS kg^{-1}, and the final nominal TCS concentration in the amended soil was 0.4 mg TCS kg^{-1} dry weight. Treated samples were aerobically incubated under biotic or inhibited conditions, and soil water contents were maintained at field capacity (i.e. 100 g kg^{-1} for IFS and 300 g kg^{-1} for the ASL soils) throughout the experiment. Inhibited treatments quantified the effects of non-microbial reactions on TCS degradation and were facilitated by adding 1200 mg kg^{-1} of 0.1% sodium azide

(Fischer et al., 2005). Biosolids-amended soil samples were weighed at each of the 8 sampling times, and DDI was added to the samples when the difference in weights between initial (week 0) and subsequent samplings was >5%.

The biodegradation study setup was adapted from Snyder (2009) and is shown in Figure 4-1. Briefly, each centrifuge tube was connected to a series of three glass trapping vials (base traps) containing 5 mL of 0.2 M potassium hydroxide (KOH). The base traps collected $^{14}CO_2$ (representing ^{14}C-TCS mineralization) and CO_2 (representing microbial respiration). A pump provided samples with humidified air stripped of CO_2 through a series of containers containing, sequentially, 1 M KOH, CO_2 free DDI water (prepared by boiling water for extended time), soda lime [Calcium hydroxide ($Ca(OH)_2$)>80%, KOH<3%, sodium hydroxide (NaOH)<2%, Ethyl violet<1%], 1 M KOH, and CO_2 free DDI water. The first glass vial remained empty to prevent backflow into the centrifuge tube if the pump failed. The samples were incubated at ~23°C in the hood to avoid possible fugitive radiolabel vapor escape into the room.

Base Trap Analysis and Soil Sample Extraction

Once a week, base traps at position-1 were removed, the remaining traps were moved forward, and a fresh trap added to the newly open position-3. The sampling schedule included soil sample removal at 0, 2, 4, 6, 8, 10, 12, 14 and 18 weeks. Four samples of each amended soil treatment were periodically removed (sacrificial sampling) and sequentially extracted by sonicating (Branson 2210, Danbury, CT; temp. 40°C, 60 Hz min^{-1}) for 1h with 20 mL each of DDI water (twice), MeOH (twice), and 1 M NaOH (once) and finally centrifuged (~800×g for 60 min) to isolate residual soil. A subsample (1g, dry wt.) of the residual soil was combusted at 900°C in a Harvey Oxidizer, Model OX-500 (Tappan, NY). Another subsample of residual soil was

extracted with a mixture of MeOH+acetone (twice) (50:50 v/v). In addition, all the base traps attached to the samples (three for each sample) were removed at each final sampling time. Aliquots (~500 µL) of the supernatants removed from various extracts and base traps were mixed with 15 mL Ecoscint A and stored for 24 h. A liquid scintillation counter (LSC) Model LS 6500 (Beckman Coulter, Irvine, CA, USA) was utilized to quantify ^{14}C (5 min counts). Evolved $^{14}CO_2$ from the oxidizer was also trapped in 15 mL cocktail (Harvey, Tappan, NY) and the radioactivity quantified by LSC.

Removed base traps were also analyzed for total CO_2 (Anderson, 1982) to assess possible effects of added ^{14}C-TCS on overall microbial respiration in biotic treatments. Adverse effects were considered possible because of the relatively high total TCS concentration (40 mg kg^{-1}) in biosolids spiked with ^{14}C-TCS.

Sequential Extraction Scheme

The sequential extraction scheme was modified from a previous study on triclocarban (TCC) biodegradation (Snyder et al., 2010) and represents the extraction of fractions of TCS of assumed varying availabilities (lability) in the soil. The explanation of the sequential extraction scheme is provided in Appendix A. We termed ^{14}C in the water and MeOH extracts as labile, and ^{14}C in NaOH, MeOH+acetone extracts and the combustible fractions as non-labile (bound). The non-labile fractions included: humic-associated (NaOH), loosely sorbed (MeOH+acetone), and bound (combustible) fractions of TCS. The lability assignments are operationally defined (and somewhat arbitrary), but consistent with extraction schemes used by others (Semple et al., 2003; Hickman and Reid, 2005; Heidler et al., 2006; Snyder et al., 2010).

Radiological-Thin-Layer-Chromatography (RAD-TLC) for Extract Speciation

At each sampling time, aliquots of extracts from the first MeOH and the first MeOH+acetone extractions were separately air-dried using an aquarium pump and reconstituted in ~30 µL MeOH. Thin layer chromatography (TLC) plates were used to identify ^{14}C-labeled moieties (parent and metabolites) from the various extracts. The plates were spotted with reconstituted MeOH extract and developed in a chamber saturated with solvent vapors of mobile phase. The mobile phase consisted of Chloroform+Formic acid (99:1, v/v). The TLC plates were either glass backed Partisil LK5D, silica gel 150 Aº, 20×20 cm (Piscataway, NJ) for performing RAD-TLC, or glass backed 20×10 cm silica gel 60F_{254} plates (E. Merck, Darmstadt, Germany) for fluorescent TLC (used for metabolite identification). Fluorescent TLC plates were analyzed by hand-held U-V light (Model UVG-54; San Gabriel, CA), and RAD-TLC plates were analyzed by RAD-TLC instant imager (PerkinElmer Life and Analytical Sciences, Waltham, MA). We initially intended to perform RAD-TLC analysis on water and NaOH extracts as well, but the analyses were not possible due to insufficient amounts of radioactivity in the two fractions for peak quantification. The limit of quantitation (LOQ) of all species (parent and metabolites) using RAD-TLC imager was ~1000 dpm, which represented ~8% of the total ^{14}C added in the system.

The relative amounts of the TCS and the metabolite were measured at each sampling time in the two extracts. The relative proportion of ^{14}C-TCS and metabolite in the water fraction was assumed to be same as in the MeOH fraction and the proportion in NaOH and bound fractions were assumed to be same as in MeOH+acetone fraction. The assumptions were made because the LOQ of the RAD-TLC imager was greater than the quantity of ^{14}C in the water and NaOH extracts. Based on the aforementioned

assumptions, concentrations of TCS and metabolite were estimated for all the extracts and the data were utilized to estimate the primary degradation half-life of TCS to the metabolite.

Statistical Analysis

Carbon dioxide data were analyzed using the general linear model (PROC GLM) of the SAS software, version 9.1 (SAS institute, 2002). Values of CO_2 evolved over time were compared among the various treatments using the multiple slope comparison option of the regression procedure (SAS institute, 2002).

Results and Discussion

Mass Balance and Mineralization of ^{14}C-TCS

Percent total recoveries of ^{14}C in the IFS soil treatments varied from 86 to 103%, with an average of 92.8% for biotic (Table 4-3), and 93.4% for inhibited treatments (Table 4-4). Total recoveries in the ASL soil treatments were 100 to 109% with an average of 104% for biotic (Table 4-5), and 105% for the inhibited treatments (Table 4-6). As the average recoveries in our system were >90%, we did not expect that TCS volatilized as a gas and escaped the system at any time during the experiment. The percent total recoveries obtained in the biodegradation study were deemed acceptable, but to simplify the discussion of ^{14}C-TCS partitioning into various fractions, the data were normalized (Figures 4-2, 4-3) by dividing ^{14}C in each fraction by the total ^{14}C recovered from each soil at each sampling time. In addition, the Figures 4-2 and 4-3 include the ^{14}C associated with the MeOH+acetone extraction of the combustible (bound) fraction.

Mineralization of ^{14}C-TCS to $^{14}CO_2$ in the two soils (IFS and ASL) was minimal (<0.5%) through the study period (18 week) (Figures 4-2, 4-3). The different percentage

of TCS mineralization in the either soil is within the variable total recoveries (range: 86-103%) in the two soil treatments, and is not considered significant. Al-Rajab et al. (2009) also reported minimal (<1%) TCS mineralization in a 42-d degradation study of TCS spiked to dewatered biosolids that was then mixed with soil [sandy loam; Organic matter (OM): 37 g kg^{-1}]. Further, the two controls did not detect any $^{14}CO_2$ suggesting no cross-contamination or abiotic TCS degradation in the system.

In the IFS soil biotic treatment (Figure 4-2a), >90% of spiked ^{14}C-TCS was water +MeOH extractable (labile) at week 0, but extractability decreased to ~35% at 18 week. The large recovery in the labile fraction at week 0, suggests initial reversible sorption of ^{14}C-TCS to the solid phase. The percentages of radioactivity in the combustible (bound) and NaOH extractable fractions at week 0 were 2% and 0.1%, respectively, but increased to nearly 40% in the bound, and 20% in NaOH extractable fractions (week 18). Radioactivity in the water fraction remained fairly constant at 2-4%, much lower than the radioactivity in other fractions. The MeOH+acetone extractable ^{14}C increased from 1% at week 0 to nearly 10% by week 18. Thus, over the 18 week study period , the IFS biotic treatment data revealed a decrease in water+MeOH extractability (TCS lability) with time, and concomitant increases in the non-labile (NaOH, MeOH+acetone extractable and bound) fractions. Similar trends of greater percent extractability in the labile fraction and low percentages in non-labile fractions occurred in the inhibited treatment of IFS soil (Figure 4-2b), but the radioactivity in the bound fraction did not increase over time as in biotic treatments. The lack of increasing trend over time in the inhibited treatment suggests microbially-mediated TCS partitioning into the non-labile fraction.

Similarly, the data for the ASL soil biotic treatment revealed a trend of reduced extractability of labile ^{14}C-TCS fraction from week 0 (40%) through week 18 (7%) (Figure 4-3a). About 60% of the added ^{14}C in biotic and inhibited treatments was immediately associated with the non-labile (NaOH, MeOH+acetone, bound) fractions, suggesting limited TCS lability. The bound ^{14}C in the ASL biotic treatment increased from week 0 (~30%) to ≥40% as early as week 4 (Figure 4-3a), whereas the conversion to the bound fraction was slower in the inhibited treatment, reaching >40% at week 18 (Figure 4-3b). Faster partitioning of ^{14}C-TCS into the non-labile fraction in the biotic treatment again suggests microbially-mediated alteration of TCS that hastened the conversion of labile fraction to the non-labile bound fraction.

Recoveries of ^{14}C-TCS in the labile fraction were consistently greater in the IFS (Figures 4-2a and b) than in the ASL soil treatments (Figure 4-3a and b). The greater clay and organic carbon (OC) contents in the ASL soil (340 g kg^{-1} clay, 34 g kg^{-1} OC) than in the IFS soil (<10 g kg^{-1}clay, 11 g kg^{-1} OC) likely promoted stronger interaction of TCS with the ASL soil and reduced TCS lability. At week 0, 30% of the ^{14}C-TCS partitioned into bound fraction (non-labile) in the ASL soil, whereas the bound fraction was 2-3% in IFS soil, suggesting an interactive effect of soil texture and OC content on the partitioning of ^{14}C-TCS into labile and non-labile fractions. Agyin-Birikorang et al. (2010) conducted a sorption/desorption study on the same soils used herein and reported that log K_d in the IFS soil (1.87 ± 0.21) was significantly smaller than in the ASL soil (2.64 ± 0.19), suggesting texture and OC content effects on TCS partitioning into sorbed and aqueous phases.

Total Carbon Dioxide (CO_2) Analyses

The TCS spiked ASL soil biotic treatment had the greatest CO_2 evolution rate (Figure 4-4b), and the CO_2 evolution rate of the unspiked ASL soil biotic treatment was similar to the spiked treatment until week 13. The similarity was not surprising as the soil microbes were likely utilizing more abundant C sources (biosolids or soil OC) rather than the small amount of added TCS. In the IFS soil, biotic treatments exhibited the greatest CO_2 evolution rates followed by IFS unspiked biotic, IFS inhibited biotic, IFS unspiked inhibited biotic and the spiked water control (Figure 4-4a). The trend is expected, as the micro-organisms will be actively growing in biotic, but less so in the inhibited biotic treatments. The smaller rates of CO_2 evolution from the unspiked IFS soil biotic treatment, as compared to the unspiked ASL soil biotic treatment, may represent differences in microbial community size, or the relative abundance of available C sources in the IFS (11 g kg^{-1} OC) and ASL soils (34 g kg^{-1} OC) (Table 4-1). Despite the addition of sodium azide in the inhibited biotic treatments at week 0, microbial activity continued, but at a reduced rate compared to the biotic treatments. Cumulative CO_2 evolved from spiked water-only control (no soil) was the least, as compared to all other treatments, which confirmed the effectiveness of CO_2 scrubbing system.

The IFS soil data revealed significant differences ($p<0.05$) among all treatments, except between the spiked inhibited biotic and the unspiked inhibited biotic treatment, and between the unspiked inhibited biotic and the spiked water control (Figure 4-4a). The ASL soil data revealed no significant differences between the biotic and unspiked biotic treatments, nor between the inhibited biotic and unspiked inhibited biotic treatments (Figure 4-4b). However, there were significant differences among the biotic, inhibited biotic and the spiked water control (soil less) in the ASL soil.

Cumulative CO_2 increased through week 18 in both biotic soil treatments, suggesting normal microbial activity throughout the biodegradation experiment (Figure 4-4). Thus, the biosolids-amended soil TCS concentration (0.40 mg kg^{-1}) used in our experiment did not significantly affect CO_2 evolution at any time. The results are in accordance with a study on a similar antimicrobial (TCC) where the CO_2 evolution rates were unaffected by the addition of TCC at concentration range of 0.24-7 mg kg^{-1} in biosolids-amended Florida Immokalee sand (Snyder et al., 2011). Waller and Kookana (2009) spiked a sandy soil (clay: 100 g kg^{-1}, OC: 8.5 g kg^{-1}) and a clayey soil (clay: 480 g kg^{-1}, OC: 18.5 g kg^{-1}) with 0, 1, 5, 10, 50, and 100 mg TCS kg^{-1} soil, and monitored changes in substrate (glucose) induced respiration in soils (no biosolids). There were no TCS concentration effects on microbial respiration up to 100 mg TCS kg^{-1} soil. Butler et al. (2011) examined basal and substrate induced respiration in three soils (no biosolids) - sandy loam (OC-17 g kg^{-1}), clay (OC-27 g kg^{-1}) and loamy sand (OC-23 g kg^{-1}) spiked with a range of TCS concentrations (0-1000 mg kg^{-1}). Results suggested some inhibition of respiration 2-4 days after spiking at TCS concentrations >10 mg kg^{-1}, but respiration recovered to the control level by day 6 and overall, there was no effect of TCS spiking.

Metabolite Identification

Each RAD-TLC analysis (up to week 2) of MeOH and MeOH+acetone extracts of both soils and treatments revealed a single major peak and an overall “fingerprint” corresponding to a ^{14}C-TCS standard (Figure 4-5a). There were no peaks suggesting TCS metabolites up to week 2. At week 3, an additional peak (peak 3) appeared in the chromatogram of biotic treatments (Figure 4-5b). The additional peak appeared farther from TCS on the TLC plate and was demonstrated to be Me-TCS. Methyl-TCS is a

reported metabolite of TCS (Figure 4-6), with a log K_{ow} of 5.2 (vs log K_{ow} = 4.8 for TCS) (Boehmer et al., 2004), and is formed by the methylation of hydroxyl group of TCS. The peak could not be conclusively identified with RAD-TLC due of unavailability of a ^{14}C-Me-TCS standard. Rather, the identification was performed using fluorescent chromatography and a cold Me-TCS standard. Chromatography resulted in distinct bands under UV light of Me-TCS that aligned with the cold Me-TCS standard. Further confirmation was performed by comparing retention factors (R_f). The R_f values measured in samples taken at various sampling times were 0.6 to 0.65 for TCS and from 0.72 to 0.75 for Me-TCS. The relative R_f (R_f for TCS/ R_f for Me-TCS) were 0.83 to 0.86. Based on co-migration with an authentic standard, the metabolite was identified as Me-TCS. Analyses of extracts through week 18 revealed continued presence of Me-TCS in biotic treatments for both soils, but no metabolites in inhibited treatments.

The formation of Me-TCS in the present study corresponds well with the previous studies suggesting Me-TCS formation in a variety of matrices (Coogan et al., 2007, 2008; Miyazaki et al., 1984; Lindstrom et al., 2002; McAvoy et al., 2002; Bester, 2005). Methyl TCS bioaccumulated in algae and snails when exposed to effluent containing Me-TCS in the concentration range of 50 to 400 ng L^{-1} (Coogan et al., 2007, 2008). Hundt et al. (2000) found Me-TCS as one of the microbial degradation metabolites in fungus (*T.Versicolor*) cell cultures incubated with TCS. Poulsen and Bester (2010) detected Me-TCS (up to 70 ng g^{-1}) in sewage sludge composted under thermophilic conditions. The bacterial species *Rhodococcus* (strain CG-1, CP-2), *Mycobacterium* (CG-2) and *Actinomycetes* methylated several chlorophenols (Haggblom et al., 1988; Neilson et al., 1983). Triclosan belongs to the class of chlorophenols and may be

methylated by a similar mechanism. An unpublished report (Christensen, 1994) estimated that up to 70-80% of TCS degraded to Me-TCS in a 64 d study conducted on three sludge amended soils (Arkansas silt loam, Kansas loam and Wisconsin sandy loam). Unfortunately, details of the experimental design of this unpublished study were not available. In contrast to the above studies, Xia (2010) reported that TCS biomethylated to only a small quantity (~9%) of Me-TCS after 101 d of biosolids-amended soil (pH = 5.7, sandy loam) incubation in a TCS leaching study. The present study is apparently the first published study that suggests Me-TCS as a major biodegradation metabolite of TCS in biosolids-amended soils.

The mass balance for ^{14}C-TCS for both soils indicated that the total radioactivity decreased in MeOH extracts and increased in the combustible (bound residue) fraction with time. The increase of ^{14}C in the bound fraction did not affect the relative peak heights of TCS and Me-TCS in MeOH extracts in the RAD-TLC analysis. The peak for Me-TCS and percent radioactivity associated with the peak was always smaller than the corresponding values for TCS. However, bound residue characterization (MeOH: acetone extract) suggested an increase in peak height of Me-TCS and no change in TCS peak height with time, indicating Me-TCS formation and partitioning of both TCS and Me-TCS to the bound fraction. The increase in peak height of Me-TCS with time suggests preferential partitioning of Me-TCS over TCS in the bound fraction.

Half-life (Persistence) Determination

The inhibited biotic treatments produced no detectable metabolites, and there was minimal TCS mineralization (<1%) during the 18 week incubation study. Half-life determination was not possible, and TCS would be regarded as persistent (half-life >>120 d) in inhibited biotic soils.

Biotic treatments in both soils also had minimal mineralization (<0.5%) of added TCS. Our study suggests the appearance of a metabolite in the biotic treatment in both soils. The relative proportions of TCS and Me-TCS (of the total ^{14}C measured) in biotic treatments were utilized to estimate the primary degradation half-life of TCS (Figure. 4-7a, b).

The ratios of the two compounds were different in the two soils. In the IFS extracts, ~2-3% of Me-TCS appeared at week 3, and increased to 10% at week 6, and stayed at ~10% for the rest of the study (Figure. 4-7a). In the ASL soil, Me-TCS also appeared at week 3 (~15%), and increased to 60% at week 12. After 18 week, 80% of ^{14}C was detected as Me-TCS and 20% as TCS (Figure. 4-7b). The proportions of TCS and Me-TCS and the variations with time were fitted using zero and first order models (Table 4-7). The zero order model fitted better (R^2 = 0.95-0.97) to the ASL soil data than the first order model (0.76-0.91), and thus, the zero order model was utilized to estimate the time taken for 50% of TCS to disappear and 50% of Me-TCS to appear in the soils. The estimated half-lives were 11 week (77 d) for ASL soil (Figure 4-7b), and >18 week (>126 d) for the IFS soil (Figure. 4-7a). Our half-life estimations are in contrast to expectation based on the lability estimations, as the greater labile or bioavailable fraction was detected in the IFS soil. Thus, it appears that the operationally-defined extraction scheme described herein may not accurately predict the degradation potential of TCS. However, our results were consistent with another study (Kwon et al., 2010) that reported faster TCS transformation in a fine loam soil than a coarse loam soil. Kwon et al. (2010) attributed the difference to the greater microbial population in the fine textured soil (9×10^6 CFU g^{-1} dry soil) than in the to the coarse textured soil

(5×10^6 CFU g^{-1} dry soil). Half-lives determined herein are similar to the TCS disappearance (loss of extractable TCS) half-life of 107 d (Lozano et al., 2010) determined for a field soil (unknown soil texture) amended with a range of biosolids application rates (9-25 Mg ha^{-1}) (inherent TCS-15.8 mg kg^{-1} biosolids). Our estimates are also consistent with a TCS disappearance (loss of extractable TCS) half-life of 50 to 106 d measured in a laboratory incubation of biosolids-amended fine loam and coarse loam soil (Kwon et al., 2010). In contrast, Higgins et al. (2011) reported a smaller half-life (42 d) in a biosolids-amended field soil (silty clay loam). However, the half-life was approximate, as the study was not adequately designed to quantitatively assess degradation.

The loss of extractable TCS as the criterion for TCS degradation used in the above studies is apparently useful for approximating the half-life of TCS, but provides no information on the degradation metabolites. A half-life range of ~77 to >126 d, average ~100 d would be a reasonable first approximation of TCS persistence in typical biosolids-amended soils. We partially accept our hypothesis that TCS is persistent in biosolids-amended soil, as a chemical is termed persistent if the half-life is >60 d (USEPA, 1999). The persistence of metabolite (Me-TCS) was not quantified in the present study and should be a topic of future research.

Our half-life estimation included assumptions that the proportions of TCS and Me-TCS in the water are same as in the MeOH fraction, and the proportions in MeOH+acetone are same as in the other non-labile fractions. But, the relative proportions of TCS and Me-TCS could vary among the various fractions and thus, our estimate of half-lives could be in error. However, the consistency of half-life values

determined in the present study and other studies that used loss of extractable TCS as the criteria of persistence leads us to believe that our assumptions were justifiable.

Comparison of TCS Persistence in Amended, Un-Amended, and Field Soils

Reported degradation rates of TCS in soils not amended with biosolids are typically much greater than the rates measured in biosolids-amended soils. Ying et al. (2007) conducted a biodegradation study in a loam soil (pH 7.4; 13 g kg^{-1} OC) where TCS (1 mg kg^{-1}) was spiked in the soil and incubated under aerobic and anaerobic conditions for 70 d. The half-life, quantified via loss of extractable TCS over time, was 18 d under aerobic conditions, and TCS was reported to be persistent under anaerobic conditions. A similar laboratory study was conducted by Kwon et al. (2010) with two soils incubated for 100 d. The soils included a fine loam (pH 7.8; 18 g kg^{-1}) and a coarse loam (pH 4.7; 6.5 g kg^{-1}) spiked with TCS (1 mg kg^{-1}), either directly or as a part of biosolids. The half-life estimated (loss of extractable TCS) in the unamended soils was 2 to 13 d, but the addition of biosolids and/or soil sterilization significantly retarded degradation (Kwon et al., 2010). The reported half-lives were 50 to108 d in biosolids-amended treatments and 51 to 60 d in unamended sterilized soil.

Al-Rajab et al. (2009) compared TCS mineralization rates in soil (sandy loam; OM: 37 g kg^{-1}) alone, and soil amended with either liquid or dewatered biosolids. Spiked TCS mineralized more (~5%) in un-amended soil and soil amended with liquid biosolids (~17%) than in soil amended with dewatered biosolids (~1%). The greater mineralization in the liquid biosolids treatment was attributed to biosolids stimulation of TCS degrading micro-organisms due to readily available OC in liquid biosolids. The slower degradation in the soil amended with dewatered biosolids was attributed either to the sorption of TCS to the biosolids in forms that are not readily available to degrading

organisms, or to the preference of the soil microbes for biosolids, rather than TCS, as a carbon source. The assumption of sorption reducing bioavailability in the dewatered biosolids was also consistent with the zero-order mineralization kinetics determined for the dewatered biosolids treatments, as compared to the approximately first order kinetics for soil-alone and the liquid biosolids (Al Rajab et al., 2009). Wu et al. (2009) conducted a study involving TCS spiked (2 mg kg^{-1}) in two soils (sandy loam and a silty clay), with and without biosolids, and incubated under aerobic conditions. In contrast to the previously mentioned studies (Ying et al., 2007; Al-Rajab et al., 2009; Kwon et al., 2010), Wu et al. (2009) found no significant affect of biosolids on TCS degradation, despite increased TCS sorption; half-lives were 20 to 58 d, irrespective of the presence of biosolids. Results of most of the published studies (Ying et al., 2007; Al-Rajab et al., 2009; Kwon et al., 2010), except Wu et al. (2009), agree with our assessment that the persistence of biosolids-borne TCS (cake or dewatered) is considerably greater than the persistence of TCS in un-amended soils.

Persistence of biosolids-borne TCS was estimated in laboratory incubations here, but persistence is expected to vary under field conditions. Al-Rajab et al. (2009) opined that TCS degradation rates can vary with temperature, soil moisture conditions, and the degree of biosolids incorporation in soils. Field amended soils, though dominantly aerobic, can contain anaerobic micro-sites (especially inside biosolids clumps) that could hinder microbial degradation process. Field soil temperatures and moisture contents are also expected to be much more variable and extreme than in well-controlled laboratory incubations. Field degradation rates can be expected to lie between the degradation rates determined in aerobic laboratory conditions and those in

anaerobic sediments, especially when the fields are even periodically anaerobic or under drought for a long time. Cha and Cupples (2009) described a field study where biosolids were applied on ten sites between 2003 and 2007, and Xia et al. (2010) investigated a site where biosolids had been applied for 33 years. Both studies estimated the TCS concentrations based on the biosolids loading rates and approximate biosolids TCS concentrations. The measured TCS soil concentrations in the two studies [Cha and Cupples (2009); Xia et al. (2010)] were much smaller than the expected concentrations suggesting significant degradation, but half-lives were not estimated in the either study. Lozano et al. (2010) suggested TCS degradation half-lives ($t_{1/2}$ = 107d) rates from field data (unknown soil texture), similar to our laboratory determination ($t_{1/2}$ = 100d). Lozano et al. (2010) suggested a degradation model based on the field data that could be applicable for laboratory and field conditions. There are a variety of conditions that can affect the TCS degradation under field conditions, but based on our data and the available literature, we conclude that laboratory estimations successfully approximate persistence under field conditions.

Table 4-1. Selected physico-chemical properties of the soils and biosolids used in the study

	Sand	Silt	Clay	Organic carbon	Water holding capacity	pH (1:1)
	——————————g kg^{-1}——————————					
Immokalee fine sand (IFS)	990	<10	<10	11	100	4.5
Ashkum silty clay loam (ASL)	240	420	340	34	300	6.6
Anaerobically digested biosolids (CHCC)	n/d‡	n/d‡	n/d‡	250	750	8.0

‡n/d is not determined

Table 4-2. Biodegradation experiment treatments

	Soil	Spike	Treatment	Replicates
Samples	Immokalee	1.3 x 10^6 dpm	Biotic	32
	Immokalee	1.3 x 10^6 dpm	Inhibited biotic	32
	Ashkum	1.3 x 10^6 dpm	Biotic	32
	Ashkum	1.3 x 10^6 dpm	Inhibited biotic	32
Controls				
	Immokalee	No spike	Biotic	4
	Immokalee	No spike	Inhibited biotic	4
	Ashkum	No spike	Biotic	4
	Ashkum	No spike	Inhibited biotic	4
	Soil-less	1.3 x 10^6 dpm	Autoclaved water	2
Total				146

Table 4-3. Percent recoveries ± standard errors of ^{14}C-TCS in IFS (Immokalee fine sand) biotic soil treatment [week (wk) 0-18].

	Time (weeks) Recovery (%)							
Fraction	wk0	wk2	wk4	wk6	wk9	wk12	wk15	wk18
H_20	3.7±0.1	3.5±0.5	2.2±0.1	2.5±0.3	2.2±0.2	2.0±0.2	4.6±0.3	6.4±0.4
MeOH	88±1.7	79±4.3	63±3.3	62±4.5	46±4.6	45±4.4	35±2.3	33±0.4
NaOH	0.1±0.1	6.2±4.9	12±1.9	11±0.5	14±1.3	15±1.5	18±1.7	18±0.9
$^{14}CO_2$	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.0	0.2±0.0	0.4±0.0	0.5±0.0
Combustion	2.0±0.2	5.4±0.4	9.4±1.6	14±0.8	29±3.0	31±2.2	33±1.5	45±5.8
Total	94±3.2	95±7.2	86±1.7	91±3.5	91±1.5	96±5.3	91±3.0	102±5
Overall average total recovery (wk0-18)					92.8±5.9			

Table 4-4. Percent recoveries ± standard errors of ^{14}C-TCS in IFS (Immokalee fine sand) inhibited soil treatment [week (wk) 0-18].

	Time (weeks) Recovery (%)							
Fraction	wk0	wk2	wk4	wk6	wk9	wk12	wk15	wk18
H_20	3.4±0.4	4.3±1.2	4.2±0.8	4.8±0.3	4.0±0.4	5.2±0.4	8±0.8	7.5±1.7
MeOH	87±3.9	71±2.8	76±2.9	67±0.8	73±4.0	74±2.7	63±5.7	72±4.4
NaOH	1.1±0.1	8.4±1.6	2.3±0.8	7.2±0.8	4.7±0.5	7.0±0.7	23±3.4	6.7±1.5
$^{14}CO_2$	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1	0.1±0.0	0.4±0.0	0.4±0.0
Combustion	2.9±0.4	11±1.5	3.6±0.5	11±1.8	7.7±1.3	5.6±0.8	8.5±0.8	7.4±0.9
Total	95±3.2	94±1.7	86±1.8	91±1.9	91±2.2	92±1.4	103±6.7	94±6.0
Overall average total recovery (wk0-18)					93.4±5.6			

Table 4-5. Percent recoveries ± standard errors of ^{14}C-TCS in ASL (Ashkum silty clay loam) biotic soil treatment [week (wk) 0-18].

	Time (weeks) Recovery (%)							
Fraction	wk0	wk2	wk4	wk6	wk9	wk12	wk15	wk18
H_20	0.5±0.0	0.2±0.0	0.1±0.1	0.1±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.4±0.0
MeOH	41±6.4	25±4.0	19±2.3	20±2.8	11±0.5	10±1.3	15±1.1	6.7±2.9
NaOH	1.2±1.5	7.5±3.9	5.5±0.7	5±0.7	3.1±0.3	3.7±0.4	4.6±0.4	2.5±0.8
$^{14}CO_2$	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.3±0.0	0.3±0.0
Combustion	65±5.1	71±5.1	82±4.3	82±6.2	86±9.5	87±9.9	85±5.0	90±3.7
Total	108±9.2	103±7.2	106±4.7	106±6.2	100±9.1	101±8.4	105±6.0	100±5.6
Overall average total recovery (wk0-18)					104±3.2			

Table 4-6. Percent recoveries ± standard errors of ^{14}C-TCS in ASL (Ashkum silty clay loam) inhibited soil treatment [week (wk) 0-18].

	Time (weeks) Recovery (%)							
Fraction	wk0	wk2	wk4	wk6	wk9	wk12	wk15	wk18
H_20	0.5±0.0	0.3±0.0	0.2±0.0	0.2±0.0	0.3±0.03	0.4±0.0	0.5±0.1	0.3±0.0
MeOH	40±3.0	26±7.4	23±4.0	20±2.9	25±2.5	29±2.8	21±1.7	7.3±1.3
NaOH	2.3±0.2	5.8±2.8	10±1.5	6.4±0.6	10±0.9	11±0.9	18±1.7	6.3±1.8
$^{14}CO_2$	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.0	0.2±0.2
Combustion	61±3.0	75±7.0	74±6.0	76±8.2	69±5.9	59±5.9	65±3.6	95±6.2
Total	104±5.5	106±7.8	106±4.9	103±7.4	104±6.4	99±6.5	105±4.7	109±6.7
Overall average total recovery (wk0-18)					104±3.2			

Table 4-7. Rate constants (k) and regression coefficients (R^2) obtained for the biodegradation data according to zero and first order models.

		Zero order		First order	
		k (µg g^{-1} wk^{-1})	R^2	k (wk^{-1})	R^2
TCS					
	IFS soil	-0.835	0.77	-0.004	0.78
	ASL soil	-4.452	0.97	-0.036	0.91
Me-TCS					
	IFS soil	0.823	0.74	0.059	0.70
	ASL soil	4.460	0.95	0.089	0.76

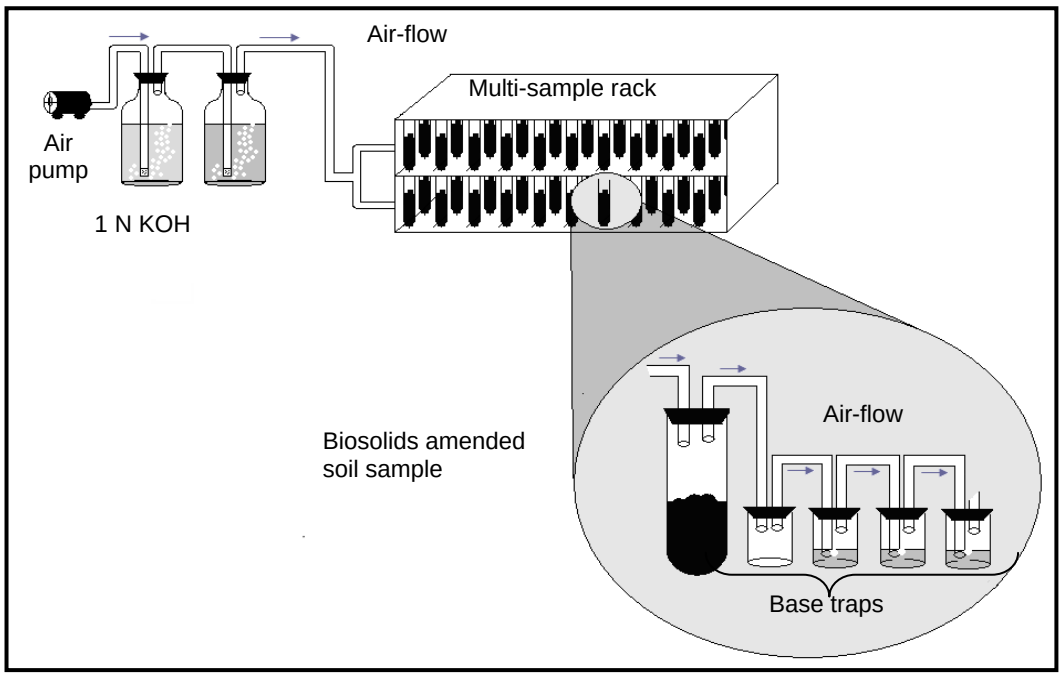
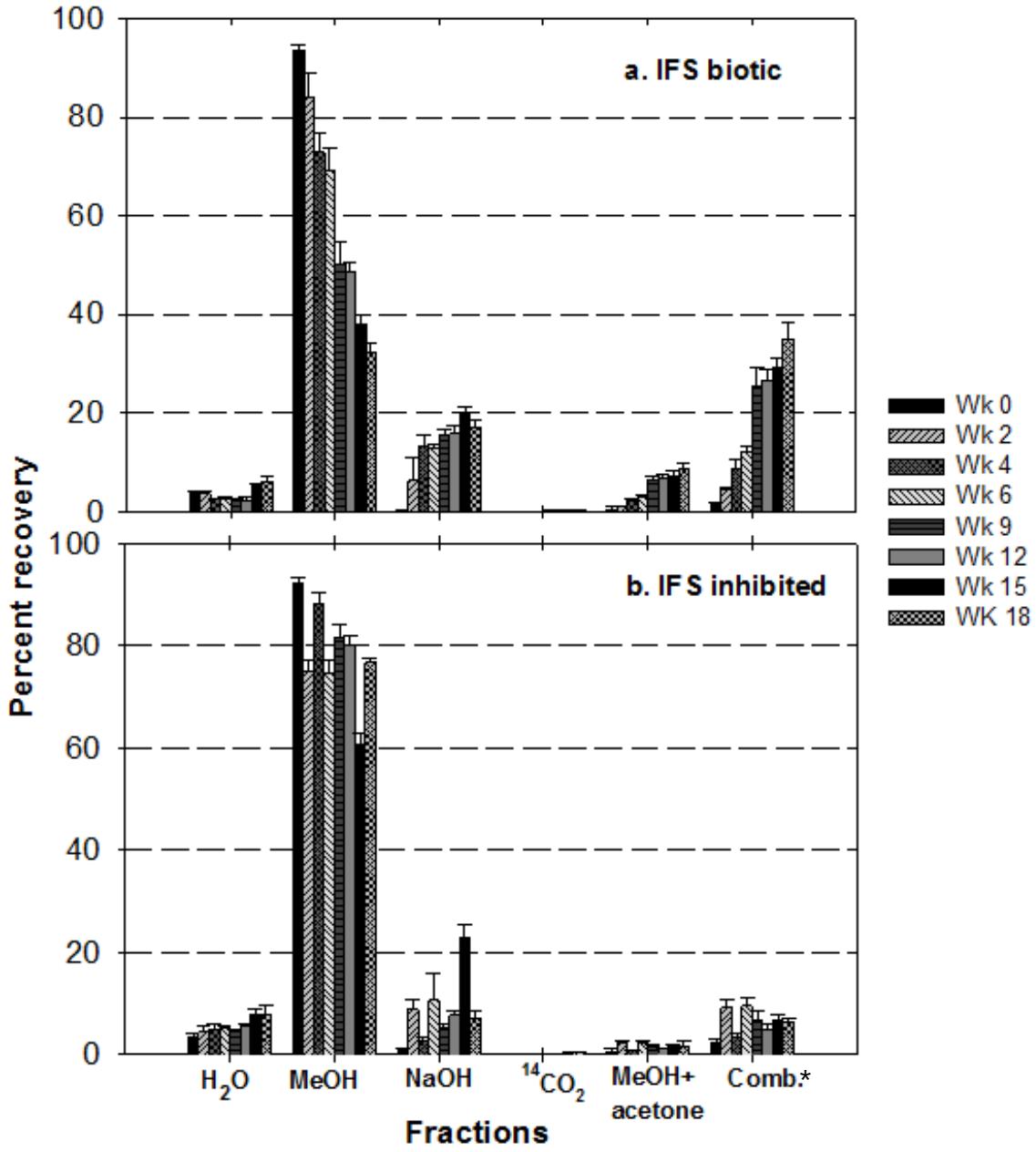
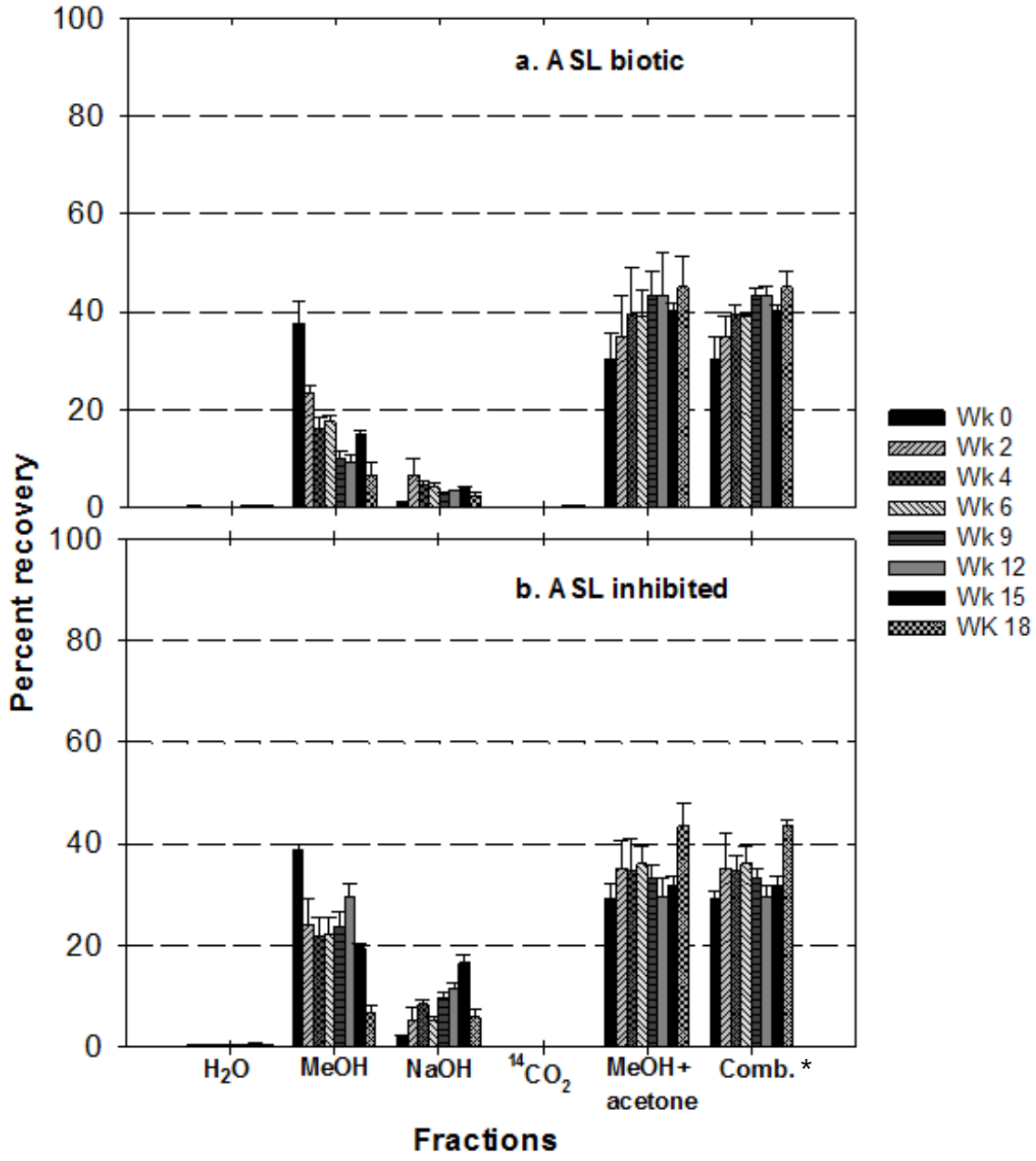


Figure 4-1. Schematic of the biodegradation experimental design (Adapted from Snyder, 2009)



*Combustible (bound) fraction

Figure 4-2. Mean percent recoveries (n=4) of ^{14}C-TCS in various fractions from biosolids-amended Immokalee fine sand (IFS) (a) biotic and (b) inhibited treatments (wk0-18) normalized for total ^{14}C-TCS detected in each treatment. Error bars represent one standard deviation of the mean



*Combustible (bound) fraction

Figure 4-3. Mean percent recoveries (n=4) of ^{14}C-TCS in various fractions from biosolids-amended Ashkum silty clay loam (ASL) (a) biotic and (b) inhibited treatments [week (wk) 0-18] normalized for total ^{14}C-TCS detected in each treatment. Error bars represent one standard deviation of the mean.

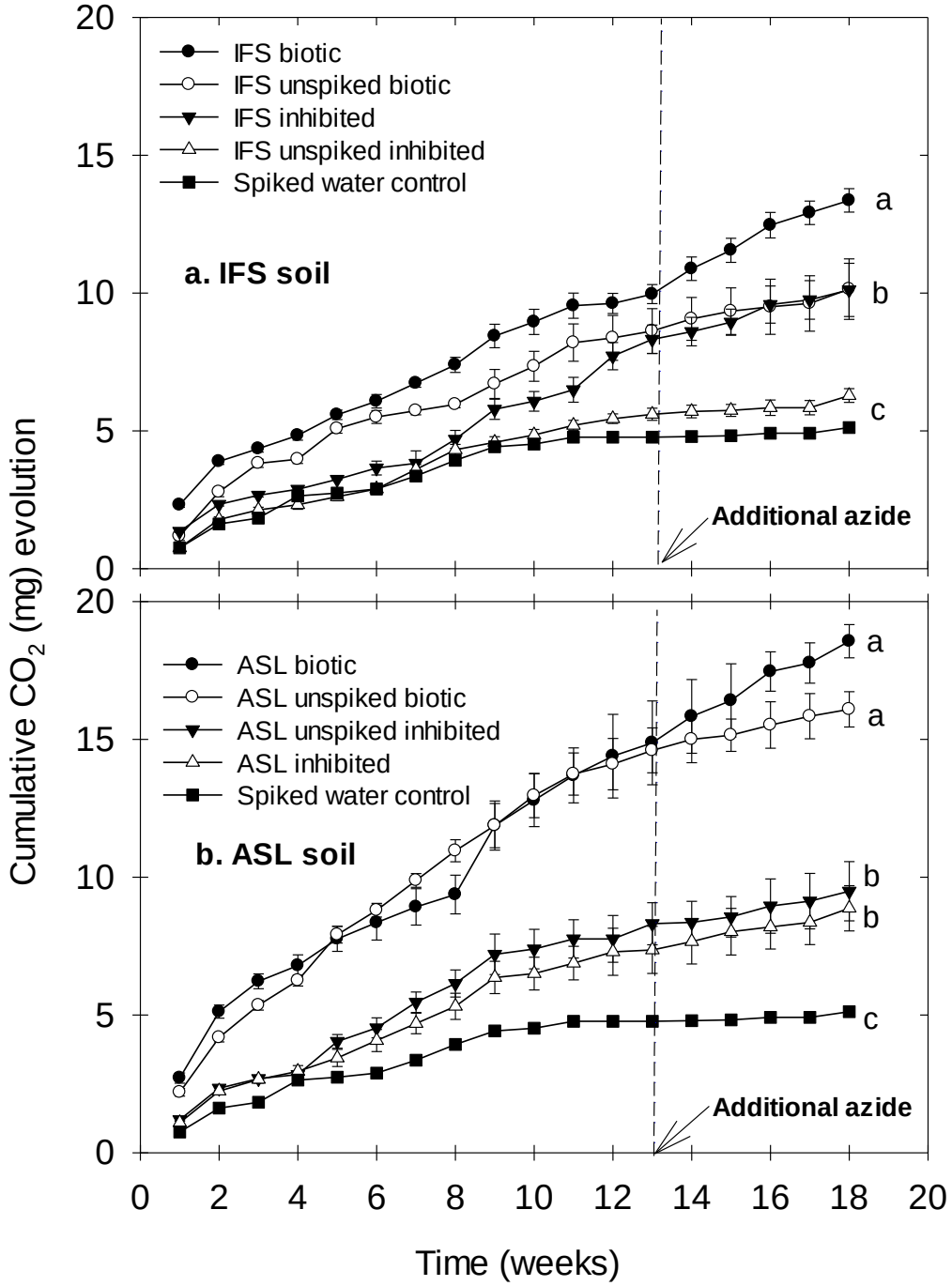


Figure 4-4. Mean cumulative CO_2 production ± standard error bars from biosolids-amended (a) Immokalee fine sand (IFS) (b) Ashkum silty clay loam (ASL) soil treatments over 18 weeks (same letters represent no statistical difference among treatments).

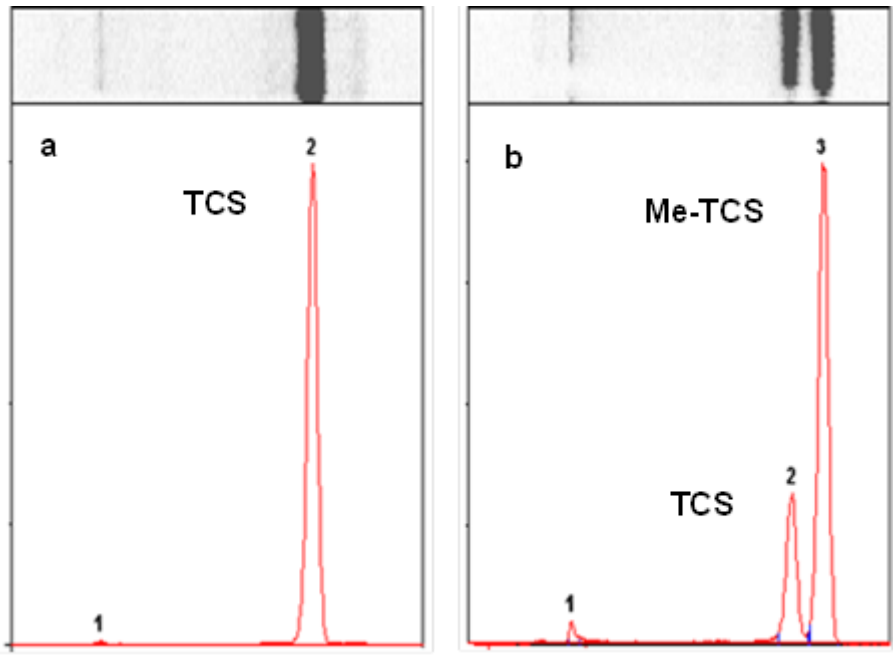


Figure 4-5. Typical RAD-TLC chromatograph (bottom) and a representative "fingerprint" (top) for (a) week 0 extracts, (b) week 3-18 extracts for Immokalee fine sand (IFS) and Ashkum silty clay loam (ASL) soils. Peak 1 represent impurities, peak 2 represents TCS and peak 3 represents Me-TCS.

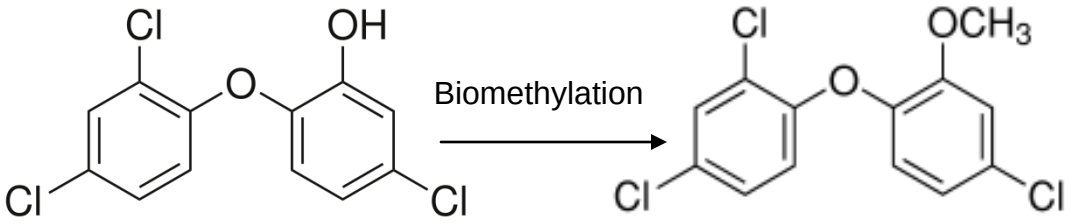


Figure 4-6. Chemical structure of Triclosan [TCS; 5-chloro-2-(2,4-dichloro phenoxy) phenol], and Methyl triclosan [Me-TCS; 2,4-dichloro-1-(4-chloro-2-methoxyphenoxy) benzene].

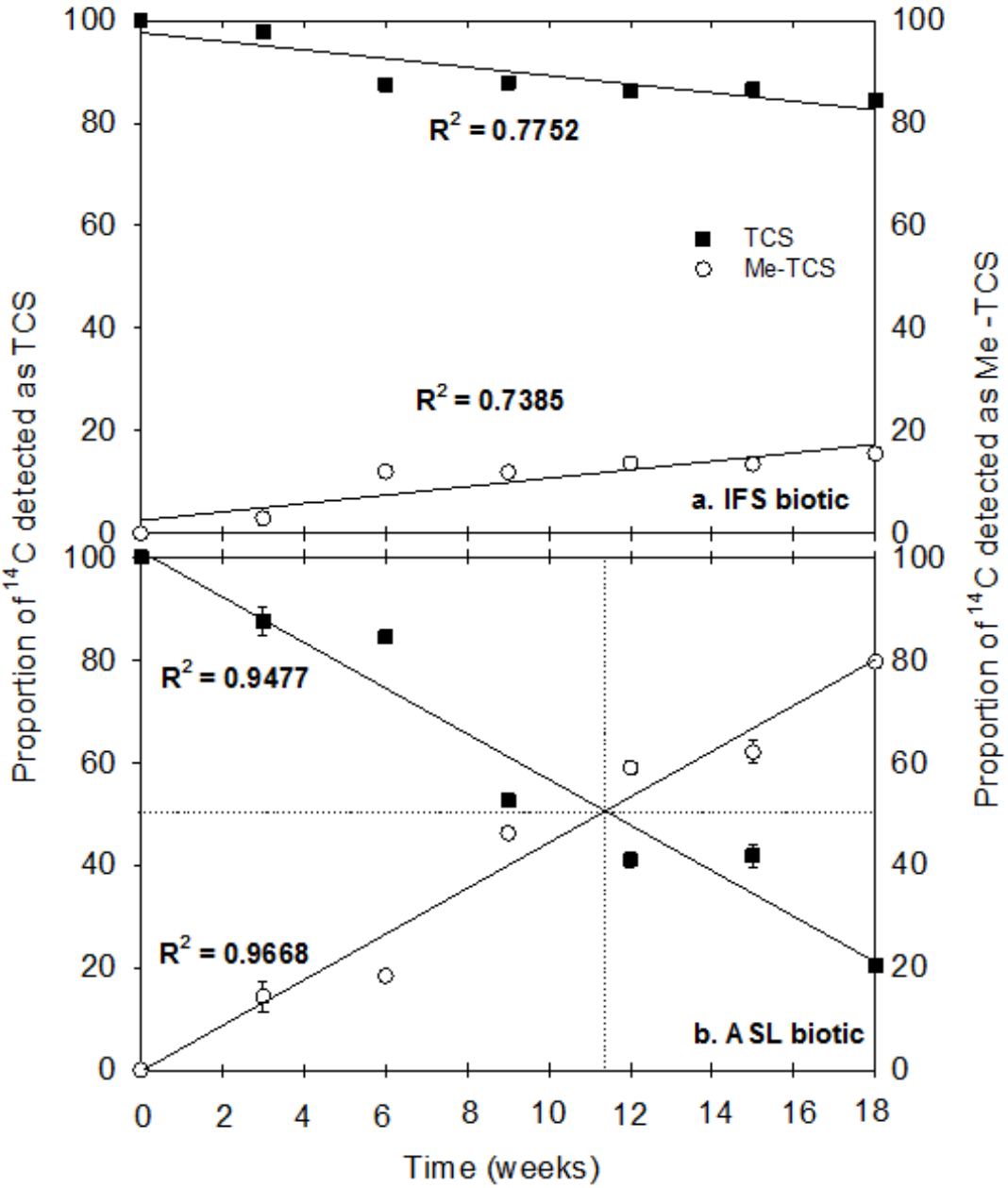


Figure 4-7. Primary degradation half-life (d) estimated using a zero-order model from the proportions of ^{14}C-detected as TCS and Me-TCS for biosolids-amended (a) Immokalee fine sand (IFS) biotic (b) Ashkum silty clay loam (ASL) biotic soil treatments. The intersection of dotted lines in (b) represents our estimation of degradation half-life.

CHAPTER 5
IMPACTS OF BIOSOLIDS-BORNE TCS ON SOIL DWELLING ORGANISMS

Background

Toxicity of a chemical depends on the chemical's bioavailability in the environment, rather than total chemical concentration in a soil (Alexander, 1999), as the latter often fails to predict the toxicity or bioaccumulation in an organism accurately. Further, a chemical's bioavailability and extractability tends to decrease as contact time between the chemical and the soil increases (Alexander et al., 2000) making assessment of bioavailability via determination of total concentration problematic. Bioavailability assessments are performed using toxicity tests (Singh and Ward, 2004). The tests generally involve exposure of test organism to the chemical of interest at varying concentrations, and subsequent monitoring of biological end-points (e.g. mortality, reproduction, growth, and behavioral changes). Effects on reproduction, growth, and development are the most useful data for conducting a risk assessment. However, only mortality data [e.g. lethal dose (LD 50) or lethal concentration (LC 50)] are measured for majority of chemicals (Singh and Ward, 2004). These data may not be sufficient to protect ecological health, but are critical in conducting screening level risk assessments in the absence of chronic toxicity or reproduction effect data.

Triclosan is a constituent of various personal care products and is commonly detected in biosolids (Chapter 2). Concern for TCS effects arise when biosolids are land applied. A TCS degradation half-life of ~100 d, and the appearance of a metabolite [Methyl-TCS (Me-TCS)] (Chapter 4) suggest that both TCS and Me-TCS could exist in soils for extended times and have the potential to adversely affect or bioaccumulate in soil dwelling organisms. Bioaccumulation studies conducted on aquatic species suggest

that algae and snails are good candidates for assessing chemical's distribution in the environment and conducting aquatic risk assessments. Coogan et al. (2007, 2008) reported bioaccumulation of TCS and the degradation metabolite (Me-TCS) in algae and snails grown in surface water and streams affected by wastewater effluent. The measured bioaccmumulation factors (BAFs) were ≥1000, which suggest rapid TCS accumulation in the organisms in the aquatic environments. However, TCS bioavailability is likely to be smaller in soils because TCS has hydrophobic properties and tends to partition to the solid fraction of soils and sediments.

Earthworms are appropriate model organisms for estimating chemical's toxicity and bioavailability in soils. Earthworms live in close contact with soil, have thin permeable cuticles, and the majority of the worm diet consists of soil (Suter et al., 2000). Further, earthworms are exposed to chemicals through both ingestion and dermal contact, and represent a significant portion of diet of many vertebrates (Suter et al., 2000). Thus, earthworms are often used as surrogates for other soil-dwelling organisms for conducting ecological risk assessments.

Samsoe-Petersen et al. (2003) found no negative effect of TCS on earthworm survival at a concentration ≤1,026 mg kg^{-1} in an artificial soil. Lin et al. (2010) suggested no adverse effect on the earthworm survival at TCS soil concentrations ≤100 mg kg^{-1}. Kinney et al. (2008) screened 70+ organic compounds in earthworms collected 31 and 156 d post application (18 Mg ha^{-1}) of biosolids to soil. The data suggested TCS accumulation in earthworms with BAFs ranging from 10.8 to 27. The authors hypothesized the mobilization of TCS directly from biosolids (solid phase) to the earthworms. The study results were confounded by the detection of significant TCS

(833 µg kg^{-1}) in the control (no amendments for last 7 years) as well as in biosolids-amended soil. Kinney et al. (2008), however, concluded that anthropogenic exposure to chemicals like TCS is widespread, bioaccumulation is likely, and urged further quantification. Reiss et al. (2009) conducted a terrestrial risk assessment for TCS and addressed the exposure to TCS experienced by earthworms (no BAFs reported), terrestrial plants and soil organisms from biosolids-amended soil as well as secondary exposure to birds and mammals. Lin et al. (2010) conducted a toxicity test on earthworms (*Eisenia fetida)* exposed to TCS spiked in soil (no biosolids). The study examined the activity of enzymes such as catalase, superoxide dismutase to assess TCS toxicity. Results suggested that TCS (>1 mg kg $soil^{-1}$) is genotoxic and caused oxidative stress in earthworms. Unfortunately, Lin et al. (2010) reported no bioaccmulation data. Higgins et al. (2011) examined TCS bioaccumulation in earthworms grown in field soils collected from Illinois previously amended with biosolids. The bioaccumulation results were variable with no clear relationship between TCS exposure levels and concentration in the earthworms. The authors acknowledged that the bioaccumulation was estimated based on a few samples (no replicates), and the internal inconsistency of the data led to inconclusive results.

Internally consistent earthworm toxicity data for un-amended soils (Lin et al., 2010; Samsoe-Peterson et al., 2003) generally suggest minimal toxicity to earthworm health. However, bioaccumulation data were variable in earthworms grown in TCS spiked un-amended soil and biosolids-amended soil. Accurate estimates of biosolids-borne TCS toxicity and bioaccumulation are critical to accurately assessing ecological health risk.

We hypothesize that:

- Biosolids-borne TCS is not toxic to earthworms but can accumulate in their tissues.
- The extent of accumulation in earthworms varies with soil TCS concentration, and conditions in which earthworms are grown (laboratory vs field).

The objective was to investigate the toxicity and bioaccumulation of biosolids-borne TCS to earthworms. The laboratory study was conducted following the Office of Prevention, Pesticides and Toxic Substances (OPPTS) Earthworm Sub-chronic Toxicity Test guideline (Guideline 850.6200) (USEPA, 1996b). The guideline requires a range-finding and a definitive test, and prescribes direct addition of the chemical of interest to a natural soil. The protocol was modified herein to deliver TCS as a component of biosolids in an effort to better mimic the primary mechanism of TCS transfer to the soil through biosolids. To estimate the longer-term bioavailability of TCS, we also analyzed earthworms recently collected from a field soil previously amended with biosolids and a representative control site.

Material and Methods

Chemicals, Biosolids and Soils

Triclosan (CAS No. 101-20-2; >99.9% purity) standard was purchased from United States Pharmacopeia (USP) (Maryland, USA). Internal standard ($^{13}C_{12}$-TCS), pyridine, bis (trimethylsilyl) trifluoroacetamide (BSTFA) +1% trimethylchloro silane (TMCS) were obtained from Sigma-Aldrich (St. Louis, MO). Methyl-TCS standard was purchased from Wellington Laboratories (Shawnee Mission, KS). Potassium chloride and solvents [methanol (MeOH), acetone] of HPLC grade or greater were purchased from Sigma Aldrich (St. Louis, MO), JT Baker (Phillipsburg, NJ) or Fisher Scientific (Atlanta, GA). Anaerobically digested cake biosolids (identification code: CHCC) was

collected from a domestic WWTP in Illinois; this sample had an inherent TCS concentration of 5 mg kg^{-1} (Chapter 2). Two soils, an Immokalee fine sand (IFS) (sandy, siliceous, hyperthermic Arenic Alaquods), and the Ashkum silty clay loam (ASL) (fine, mixed, superactive, mesic Typic Endoaquolls) were collected from sites with no known history of receiving land-applied biosolids or sludge. An artificial soil (68% silica sand, 20% kaolin clay, 10% sphagnum peat moss, 2% calcium carbonate; all by weight.) was prepared by mixing the various ingredients in the laboratory as prescribed by OPPTS guidelines (USEPA, 1996b) and utilized in the earthworm toxicity test along with the two natural soils. The earthworms were purchased from Carolina biologicals (NC). Prior to use, the earthworms were grown in moist peat moss (growing medium) and fed with worm food (Magic products, WI) each day for 3 weeks.

Range-Finding Toxicity Test Design

A range-finding test was conducted to identify the appropriate range of biosolids-borne TCS concentrations for a subsequent definitive toxicity assessment. Two-gram samples of oven-dry CHCC biosolids (TCS = 5 mg kg^{-1}) were spiked with 0, 10, 100, 1000, or 10,000 mg TCS kg^{-1} biosolids using MeOH as the carrier solvent, and were subsequently dried, re-wetted, and equilibrated for 48 h. Treatment spikes were in addition to the biosolids inherent TCS concentration, so the nominal final concentrations were 5, 15, 105, 1005, and 10,005 mg TCS kg^{-1} biosolids. The EPA guideline prescribes the use of an artificial soil to avoid synergistic effects with unknown chemicals present in natural soils. We also included two natural soils to estimate TCS toxicity in real world scenarios.

Biosolids were amended to 200 g (dry wt.) of the artificial, IFS and ASL soils at a 22 Mg ha^{-1} equivalent rate in 800 mL glass Mason jars. The major physico-chemical

properties of the soils and biosolids used in the toxicity and bioaccumulation study are described in Table 5-1. Earthworms were hand picked from the growing medium and washed with deionized water before use in the toxicity test. The soils were brought to field capacity (artificial, 35%; IFS, 10%; ASL, 30% by wt.) and ten *Eisenia fetida* earthworms were added to each sample. Also included were un-amended soil control to quantify potential effects of biosolids addition, and carrier solvent-free biosolids-amended soil control (no additional TCS spike) to quantify carrier solvent effect. Lids were placed loosely on top of the incubation jars to reduce moisture loss and to prevent earthworm escape. Dead earthworms at the soil surface were counted and removed as necessary each day, and the number of living earthworms were tallied each week for four weeks (28 d). Replicates are not required by the OPPTS range-finding guideline, nor are extraneous food sources. The USEPA guideline also prescribes that the test results become unacceptable if ≥20% earthworm die-off occurs in the control.

Definitive Toxicity Test Design

Results of the range-finding test suggested a narrower range of TCS concentrations for the definitive test with the IFS soil. Thus, the test included biosolids spiked at concentrations of 2.5, 5, 10, 50, and 100 mg additional TCS kg^{-1} biosolids. The test procedure was same as the range-finding test except that the definitive test included four replicates for each treatment. Accounting for the inherent TCS concentrattion of 5 mg kg^{-1}, the nominal final concentrations were 5, 7.5, 10, 15, 55 and 105 mg kg^{-1} biosolids. Also included were the soil-only control and the solvent-free biosolids-amended soil control. Statistical analyses were performed with SAS software, version 9.1 (SAS Institute, 2002) using the Tukey's studentized range test (HSD) to assess the treatment effect.

Earthworm Bioaccumulation Test

Design for the laboratory study

An earthworm TCS bioaccumulation test was run concurrently with the definitive earthworm toxicity test. The test included both IFS and ASL soils to quantify TCS earthworm accumulation in soils of different textures. The TCS treatments included a lower concentration range (as determined by the range-finding test) as live earthworms were required for quantifying chemical accumulation. Two-gram samples of CHCC biosolids were spiked with 0, 2.5, 5, 10, 50, and 100 mg additional TCS kg^{-1} biosolids and amended to the IFS and ASL soils, in quadruplicate as in the definitive toxicity test. The nominal final concentrations were 5, 7.5, 10, 15, 55 and 105 mg TCS kg^{-1} biosolids. Surviving earthworms at the end of week 4 (28 d) were removed, counted, washed, and weighed. The earthworms were allowed to depurate for 24h in petridishes lined with moistened filter paper (Banks et al., 2006), and were subsequently frozen until analyses. We recognize that the birds and worm-eating animals feed on non-depurated earthworms, but depuration is prescribed by the OPPTS procedure (USEPA, 1996b). Depuration allows earthworms to excrete TCS-contaminated soil or organic matter remaining in the gut, so that the measured TCS concentration in earthworms reflects accumulation in the tissue, rather than TCS sorbed to gut contents.

Bioaccumulation in field soils

Longer-term bioavailability of TCS was estimated by utilizing earthworms and corresponding soil samples collected at a field site in Illinois.The site received a single application of CHCC biosolids (application rate of 228 Mg ha^{-1}) in 2008 and the earthworms were collected in 2010. Earthworms were also collected from an adjacent control site (18 m apart; same soil texture) that had no history of biosolids application.

The earthworms were collected utilizing the hot mustard extraction method described in Lawrence and Bowers (2002). Briefly, a mustard (allyl isothio cyanate) solution is applied to the soil surface which encourages earthworm emergence to escape the mustard's irritant properties. The method was efficient in collecting a consistent number (≥20 from each location) of earthworms and did not require digging or hand-sorting. The collected worms were kept in wet peat moss in aerated bags and promptly shipped to the laboratory under ice packs. Upon arrival, earthworms were cleaned with DDI water to remove peat moss residue, kept in petridishes with wet filter paper and allowed to depurate for 24 hours.

Sample extraction and derivatization for earthworm bioaccumulation

A sample extraction technique was modeled after those described by Higgins et al. (2009) and Snyder et al. (2011) with few modifications. Laboratory collected frozen earthworms were thawed, and fresh earthworms from the field were transferred to aluminum weigh boats, dried at 50°C to a constant weight, and ground. The dried and ground tissue (0.5-1 g) was loaded into 25 mL glass centrifuge tubes. A solvent mixture (10 mL) of MeOH+acetone (50:50, v/v) was added to each centrifuge tube. The extraction was performed on a platform shaker for 18 h, followed by 60 min of sonication (Branson 2210, Danbury, CT; temp. 40°C, 60 sonications min^{-1}) in a water bath. Suspensions were centrifuged at 800 x g, and the supernatant was transferred to 20 mL glass scintillation vials. The extraction procedure was performed twice and the extracts were combined and dried under a gentle nitrogen stream. The extracts were reconstituted in 1 mL of MeOH and transferred to 2 mL microcentrifuge tubes. The microcentrifuge tubes were then centrifuged at 18,000 × g for 30 min, supernatant removed and transferred to 1 mL GC vials, $^{13}C_{12}$-TCS was added, and the mixture dried

under a mild N_2 stream. We included solvent controls (containing no TCS) that were subjected to the same extraction procedure to confirm that TCS was not introduced during the extraction. The derivatization was performed according to the method by Shareef et al. (2006) with slight modification. Briefly, the dried contents were reconstituted in a mixture of 4:1 of derivatization agent BSTFA +1% TMCS and the solvent (pyridine), vortexed for 10 s, and heated in a dry bath for 1h. The samples were then transferred to fresh GC vials with glass inserts and Teflon lined caps. Fresh earthworms (untreated) were also depurated, frozen, thawed and spiked with known concentrations of TCS, $^{13}C_{12}$-TCS and Me-TCS. The spiked samples were incubated for 24 h to allow carrier solvent evaporation. Earthworms containing spiked chemicals were extracted with the same solvent mixture of MeOH+acetone (as described above) to determine percent recoveries which were >90% for spiked TCS and Me-TCS.

Instrument analyses and quantification

The samples obtained after the derivatization step (as described above) were analyzed by splitless injection (5 µL) on the Varian 4000 Gas Chromatograph (GC) equipped with Restek Rxi-5Sil column coupled with Varian 4000 MS/MS. The GC column was initially held at 100^{o}C for 1 min, and then increased to 310^{o}C at a rate of 10^{o}C min^{-1} with no final hold time. Helium was used as a carrier gas. The ion trap, manifold and transfer line temperatures were 200, 80 and 270^{o}C, respectively, and the ionization source was internal/electron ionization. Data acquisition monitored two fragment ions for each compound. The ion masses were m/z of 345/347 for TCS trimethylsilylether, 302/304 for TCS-OMe, and internal standard $^{13}C_{12}$ TCS-trimethylsilylether mass was monitored at 357/359. The samples ran for 22 minutes with an average retention time of 14.8 min for all compounds. For quantification, an 8-point

internal calibration curve was generated in the TCS concentration range of 1-1000 ng g^{-1} with an average R^2 >0.999. The LOD was 0.22 µg g^{-1} and LOQ was 0.7 µg g^{-1} for both TCS and Me-TCS in the earthworm tissue. The LOD and LOQ values were calculated as 3-fold and 10-fold, respectively, the standard deviation in the signal from multiple runs of the lowest calibration standard (Signal/Noise >10) (USEPA, 1984). The details of detection limits and recoveries are provided in the Appendix C.

Results and Discussion

Range-Finding Toxicity Test

Biosolids TCS concentrations ≤10,005 mg kg^{-1} had minimal adverse effect on earthworm survival in the ASL and artificial soils. In the ASL soil, all the earthworms in all the treatments survived at the end of week 4, except a 10% die-off at a concentration of 1005 mg kg^{-1} (Figure 5-1b). Similarly, in the artificial soil, TCS concentration did not adversely affect the worm survival, except for a 20% die-off at a concentration of 105 mg kg^{-1} and 10% die-off at 5 mg kg^{-1} (solvent-free) (Figure 5-1c). We attributed the death of 20% of the earthworms (2 earthworms out of 10) to natural causes (such as lack of sufficient food or stress), due to lack of adverse effect at greater (>105 mg kg^{-1}) concentrations. The data suggest that TCS concentration ≤10,005 mg kg^{-1} biosolids (equivalent to an amended soil concentration of 100 mg kg^{-1}) have no adverse effect on earthworm survival in the ASL and the artificial soils.

Earthworms in the IFS soil were adversely affected by TCS addition. By week 4, nearly 100% mortality occured at TCS concentrations ≥5 mg kg^{-1} biosolids (Figure 5-1a). There was a 25% die-off at week 4 even in the soil-only control (no biosolids). Due to the >20% die-off in the control, the experimental results were unacceptable. The die-off could be attributed to the lack of sufficient food for the worm survival without

biosolids, as no extraneous food source was supplied. The earthworm survival differed in the TCS treatments and the solvent-free control (biosolids-amended) had a 100% die-off by week 4. We tentatively attributed the die-off in the solvent-free control to the absence of solvent residues acting as a food source to the earthworms. No further investigation for the causes of the die-off was performed as it was a range-finding test. Definitive conclusions and calculation of LC_{50} values can only be performed in the definitive study, which includes replicates. However, the EPA guidelines do not require a definitive test if the LC_{50} value is greater than the highest concentration tested (i.e. 10,005 mg kg^{-1} biosolids). Thus, due to the lack of adverse effects in the ASL and artificial soils, only the IFS soil was utilized in the definitive toxicity test.

Definitive Toxicity Test (IFS Soil)

The mean earthworm survival was >90% in all the treatments as well as in soil-only control, except at concentrations of 10 and 15 mg TCS kg^{-1} biosolids (Figure 5-2). Unlike in the range-finding test, the soil-only control (no biosolids) appeared to provide sufficient nutrients for earthworm survival up to week 4. There were no adverse TCS treatment, biosolids addition, or carrier solvent addition effects on the survival of the earthworms. The earthworm survival was slightly affected in the concentration range of 10 to 105 mg TCS kg^{-1} biosolids, but there was no statistical difference among the various treatments due to the large standard deviations (Figure 5-2). Thus, no adverse effect was observed on the earthworm survival up to a TCS biosolids concentration of 105 mg kg^{-1}. Adverse effects were not anticipated due to Me-TCS exposure, as a suggested degradation half-life of TCS is at least 77 d (Chapter 4), so significant Me-TCS formation was not expected in the short length (28 d) of our present study. A definitive earthworm LC_{50} value cannot be calculated from our study because there was

no significant adverse effect up to the maximum tested concentration. The estimated LC_{50} value in the IFS soil is >105 mg TCS kg^{-1} biosolids. If the biosolids LC_{50} value is ~100 mg TCS kg^{-1}, the application of biosolids at 22 Mg ha^{-1} followed by incorporation at 15 cm depth (~100 fold dilution of the TCS concentration), results in LC_{50} of ~1 mg TCS kg^{-1} soil. Similarly, the range-finding test cannot be used to calculate an LC_{50} value as no replicates were involved; however, a rough approximation yields an LC_{50} value of >10,005 mg TCS kg^{-1} biosolids or >100 mg TCS kg^{-1} soil for the artificial and the ASL soils.

The ecological structure activity relationship (ECOSAR) program (USEPA, 2009b) is a model based on the structure-activity relationship concept, and predicts the aquatic toxicity of a chemical. The toxicity is estimated based on the similarity of a chemical's structure to other chemicals for which the aquatic toxicity has been previously measured. Extrapolation from aquatic to terrestrial toxicity may not be accurate (Hartnik et al., 2008), but for the sake of comparison, ECOSAR was utilized to calculate LC_{50} value for terrestrial species. Most calculations in the ECOSAR program are based on the chemical's K_{ow} and do not consider soil/sediment parameters where the organism is growing. The earthworm 14-d LC_{50} value predicted by the ECOSAR program is ~22 mg TCS kg^{-1} soil. Our estimated LC_{50} value in IFS soil (~1 mg kg^{-1}) was much smaller, and the LC_{50} value in the other two soils (>100 mg kg^{-1}) was considerably greater than the value calculated by the ECOSAR. Thus, the ECOSAR underestimates the toxicity in one (IFS) soil and over-estimates the toxicity in the other two (artificial and ASL) soils. The model estimated LC_{50} values should be used with caution.

Our toxicity data in the artificial and the ASL soil are consistent with a short-term (2 week) Danish study (Samsoe-Petersen et al., 2003) in which there was no negative effect of TCS on earthworm survival at a concentration ≤1,026 mg kg^{-1} in a similar artificial soil. A 14-d TCS exposure study (Lin et al., 2010) was conducted in a soil similar in texture to ASL soil, but with lower OC content (pH = 8.1, OC = 12.8 g kg^{-1}). Data suggested no adverse effect on the earthworm survival at TCS soil concentrations ≤100 mg kg^{-1}. Although our results were in agreement with the published studies, the LC_{50} values determined in our short-term study are best regarded as estimates, due to lack of adverse effects at the highest concentration tested. Future investigations could include long-term TCS impact studies or studies using greater TCS concentrations although greater concentrations are environmentally unrealistic. Further, toxicity effects of major metabolite of TCS (Me-TCS) were not assessed in our study and should be addressed.

Bioaccumulation Laboratory Study

The measured TCS concentrations accumulated by earthworms incubated in two soils amended with biosolids-borne TCS are presented in Tables 5-2 and 5-3. The earthworm tissue concentrations were utilized to calculate BAF values for each soil. Degradation study results (Chapter 4) suggested the formation of Me-TCS in the biosolids-amended soils ($t_{1/2}$ ~77d). We might expect formation of Me-TCS in amended soils and/or biosolids and possible subsequent accumulation in earthworms. However, given the short length of the earthworm bioaccumulation study (28 d) and a suggested half-life of at least 77 d (Chapter 4), we did not expect significant Me-TCS formation from the added TCS. In addition, the Me-TCS concentration in the biosolids used in this study was below the LOQ (0.7 mg kg^{-1}) of the instrument. Thus, as expected, we did not

detect Me-TCS in earthworms exposed to the incubated amended soils or Me-TCS inherent to the biosolids.

Uptake assessment occured through the calculation of bioaccumulation factors (BAFs) expressed as the ratio of TCS concentration in the earthworm tissue to the TCS concentration in the soil in which the worm was exposed to TCS. The average BAF values in the two soils, irrespective of the spiked TCS concentration, were 6.5 ± 0.84 for the IFS soil (Table 5-2) and 12 ± 3.08 for the ASL soil (Table 5-3). The average values were significantly different ($p<0.05$) from each other. The difference in BAFs in the two soils might reflect differences in physico-chemical properties of the two soils. The soils differed in native soil organic carbon (OC) contents (11 g kg^{-1} for IFS soil and ~34 g kg^{-1} for ASL soil, Table 5-1), suggesting more accumulation in soil with greater OC content. Application of biosolids with an OC content of 250 g kg^{-1} at a rate of 22 Mg ha^{-1} adds an additional 2.7 g kg^{-1} of OC to both soils, but the total OC in the amended IFS soil (13.7 g kg^{-1}) was still smaller than the amended ASL soil (36.7 g kg^{-1}). The trend is possibly due to earthworm's preferential feeding on the soil with greater OC content, and ingesting both OC and TCS that were associated with the biosolids. Our results were consistent with a bioaccumulation study (Snyder et al., 2011) conducted on a similar antimicrobial compound triclocarban (TCC). The measured BAF value was greater in the silty clay loam (20 ± 2.1) than in the sand (18 ± 3.5), but the results were not significantly different. Luo et al. (2008) opined that besides the OC content, the bioaccumulation may also vary with the chemical tested, and perhaps with the composition of organic matter.

The BAFs obtained in our study were generally not a function of spiked TCS concentration. In the IFS soil, the BAFs were not significantly different in various TCS treatments. Some concentration dependent accumulation was observed in the ASL soil, with significantly greater accumulation at a concentration of 0.10 mg TCS kg^{-1} soil, however, there was no significant difference in accumulation among other TCS treatments. Kinney et al. (2008) determined earthworm TCS BAFs of 10.8 and 27 for a soil (sandy clay loam; OC-12 g kg^{-1}) amended with biosolids either 31 or 156 d previously. The inherent TCS concentration of biosolids applied was 10.5 mg kg^{-1}. Earthworm samples were collected from a soybean field site receiving biosolids as a fertilizer for the first time. The range of BAFs obtained in our study (6.5-12.7) is within the range reported by Kinney et al. (2008), despite the shorter (28 d) exposure time used in our study. Further, we used Biosolids-Amended Soil Level IV (BASL4) model (Webster and Mackay, 2007) to estimate earthworm bioaccumulation. The program utilizes the concept of fugacity, and models chemical distribution in soil by assuming that the medium in which organisms are growing could be either at equilibrium, steady-state, or non-steady state. In our study, earthworms were exposed to soil in closed jars, so we can assume a steady-state condition for modeling purposes. The model parameters were selected based on the physiochemical properties of TCS and soil type where the organisms were grown. BASL4 assumes that the properties of invertebrate and mammals are representative of earthworms and shrews, respectively (Hendriks et al., 1995; Armitage, 2004). The BASL4 predicted average BAF value for IFS soil (5.0 ± 0.0) was reasonably close to the measured value (6.5 ± 0.84) (Table 5-2). For the ASL

soil, the predicted average BAF value (2.2 ± 0.0) was much less than the measured value (12 ± 3.1) (Table 5-3).

The partitioning theory for traditional hydrophobic organic contaminants (HOC) is commonly used to predict the partitioning of chemicals to invertebrates from sediments (Higgins et al., 2009). The bioaccumulation potential is calculated by using the lipid normalized worm and organic carbon normalized soil chemical concentrations. The HOC theory predicts a biota-sediment accumulation factor (BSAF) of approximately 1.6 for nonmetabolized organic compounds if the log K_{ow} of a compound is less than 6 (Morrison et al., 1996).

The BSAF values were estimated in our study as:

$$BSAF_{oc,\ lip} = \frac{C_{org,\ dry\ wt}/f_{oc}}{C_{soil,\ dry\ wt}/f_{lip}} \qquad (5\text{-}1)$$

The f_{lip} (fraction of lipid) in the earthworm is 0.032 ± 0.01 (Higgins et al., 2010) and f_{oc} (fraction of organic carbon) is 0.011 for IFS soil and 0.034 for ASL soil (Table 5-1). The estimated BSAF values were 1.0 ± 0.27 for the IFS soil and 9.63 ± 3.29 for the ASL soil. A lower than expected BSAF for the ASL soil may suggest either the metabolism of TCS or decreased TCS bioavailability. Alternatively, the difference likely suggests that HOC theory does not accurately predict the TCS accumulation in earthworms. Higgins et al. (2011) suggested that bioaccumulation of a similar chemical (TCC) was somewhat consistent with the HOC theory; but, some reduction in TCC bioavailability was observed.

The HOC theory assumes equilibrium between the soil solid and soil water phases, which is not always true. Further, the expected BSAF value is ~ 1.6 when the earthworms acquire the chemical only from the soil solution. Soil dwelling earthworms

can accumulate TCS from the soil solution, direct ingestion of soil or biosolids and from direct partitioning of biosolids-borne TCS to the worm tissue (Kinney et al., 2008). Higgins et al. (2011) examined the bioaccumulation of TCS in earthworms grown in field soils previously amended with biosolids. The range of BSAF values was 0.31 to 1.2, and there was no dependence of bioaccumulation on TCS exposure levels. After adjusting the BSAF values from Higgins et al. (2011) to dry weight of soil and earthworms, the estimated BAF values were 2 to 2.3 in the sand and 1.3 in the silty clay loam. The BAF values estimated using Higgins et al. (2011) BSAF data were smaller than the range of BAF values obtained in our study (6.5-12.7). The difference in values may be attributed to the lack of replicates in the Higgins et al. (2011) study that minimizes the significance of their data.

The TCS concentration in earthworm tissue can also be calculated using a mechanistic approach proposed by Jager (1998) that assumes hydrophobic partitioning between the soil pore-water and the earthworm tissue. The bioconcentration factor in that case can be calculated as:

$$BCF_{earthworm} = \frac{0.84 + 0.01 * K_{ow}}{f_{oc} * K_{oc}} \qquad (5\text{-}2)$$

Inserting the *foc* (fraction of soil organic carbon) value of 0.011 (IFS) and 0.034 (ASL), a log K_{ow} of 4.8 and log K_{oc} in biosolids-amended soils of 4.26 (Agyin-Birikorang et al., 2010), yields a bioconcentration factor of 1.85 for IFS soil and 4.30 for ASL soil. The values underestimate the potential for bioaccumulation reported in the literature and our measured values. Soil pore-water earthworm models have been criticized (Suter et al., 2000) for potentially underestimating the contaminant uptake in cases where absorption via gut is the primary mechanism (e.g. earthworms).

Models (BASL4 and partitioning) appear to underestimate the bioaccumulation potential of TCS by earthworms, as the models assume bioaccumulation to occur only from soil pore-water. Earthworm bioaccumulation of TCS also does not appear to follow HOC theory. Thus, the various estimates should be used with caution.

Bioaccumulation Field Test

The average TCS concentration measured in the earthworms collected in a field equilibrated soil was 4.3 ± 1.9 mg kg^{-1} corresponding to a BAF value of 4.3 ± 0.7 (Table 5-4). Grab soil samples collected from the same area where the earthworms were collected averaged 0.99 mg TCS kg^{-1}amended soil. The BAF value in the earthworms collected from the three locations within the amended soil were 2.68 to 5.93. The TCS concentrations in the earthworm tissues were variable among the three locations, ideally representing varying activity patterns in the soil. Earthworms occupy a range of soil depths depending on the season, species and life history stage (Bouche and Gardner, 1984) and may be exposed to varying amounts of chemical. Triclosan concentrations were non-detect in earthworms and soil collected from a representative control site.

The texture of the field soil was similar to the ASL soil used in our laboratory study, but the average BAF value in the field collected earthworms was significantly smaller ($p<0.05$) than the average values in laboratory study. Even when we compare the average BAF obtained in the field soil with the BAF in the similar ASL laboratory treatment (1 mg TCS kg^{-1} soil, Table 5-3), the BAF was significantly greater in the laboratory study (12 ± 3.1) than in the field soil (4.3 ± 1.9). The difference may reflect greater TCS availability under laboratory conditions, as TCS was spiked to the laboratory study soils as opposed to inherent TCS in field soils; the inherent log K_d

values tend to be greater than the spiked log K_d values (Chapter 3). We also speculated that high rate of biosolids-borne TCS applied in the field conditions, though dominantly aerobic, may experience anaerobic micro-sites (especially inside biosolids clumps) that could hinder the chemical's movement and bioavailability and, hence, lower the accumulation in earthworms. The BAF values (2.68-12.7) (laboratory and field) in the present study was smaller than the BAF values (10.8-27) reported by Kinney et al. (2008). The implications of earthworm bioaccumulation to TCS ecological risk assessment are unknown, but Snyder (2009) estimated that the earthworm–predator pathway was limiting in a biosolids-borne TCC risk assessment. The risk estimation of biosolids-borne TCS to earthworms and other organisms are critical to identify potential pathways of concern.

Biosolids-borne TCS accumulates in, but is not toxic to, earthworms (hypothesis 1). We partially accept the second hypothesis also, as the earthworm accumulation varied significantly between laboratory and field conditions, but the accumulation did not vary with TCS concentration in biosolids. Implications of TCS accumulation in earthworms are assessed via a risk estimation of biosolids-borne TCS (Chapter 9).

Table 5-1. Major physico-chemical properties of the soils and biosolids utilized in the present study

Soil	Texture	Organic carbon	pH (1:1)
		g kg^{-1}	
Immokalee fine sand (IFS)	Sand	11	4.5
Ashkum silty clay loam (ASL)	Silty clay loam	34	6.6
Artificial soil	Peat moss-10% Ca carbonate- 2%	48	7.7
Biosolids (CHCC)	n/d‡	250	8.0
Field control landscaping	Clay loam	40	7.8
Field amended landscaping	Silty clay loam	80	7.1

‡n/d is not determined

Table 5-2. Measured [average; n = 4 and standard error (SE)] TCS concentrations (mg kg^{-1}) and bioaccumulation factors (BAFs) in earthworms grown in the Immokalee fine sand (IFS) (same letters represent no statistical difference among treatments).

Nominal spiked soil concentration	Measured earthworm tissue concentration	Mean BAFs† dry wt.	Calculated BAF (BASL4)
——————mg kg^{-1}——————			
0	<LOQ ‡	<7	5.0
0.025	0.5±0.0	7.3±1.9 a	5.0
0.05	0.5±0.1	4.9±1.4 a	5.0
0.10	0.6±0.1	4.3±1.4 a	5.0
0.5	4.9±0.5	8.8±0.9 a	5.0
1	7.3±1.0	7.3±1.0 a	5.0
Average		6.5±0.8	5.0±0.0

Table 5-3. Measured (average; n = 4 and SE) TCS concentrations (mg kg^{-1}) and bioaccumulation factors (BAFs) in earthworms grown in the Ashkum silty clay loam soil (ASL) (same letters represent no statistical difference among treatments).

Nominal spiked soil concentration	Measured earthworm tissue concentration	Mean BAFs† dry wt.	Calculated BAF (BASL4)
——————mg kg^{-1}——————			
0	<LOQ‡	<14	2.2
0.025	<LOQ‡	<4.7	2.2
0.05	1.1±0.1	7.4±2.3 a	2.2
0.10	1.6±0.3	18±1.3 b	2.2
1	12.6±0.7	12±0.7 a	2.2
Average		12±3.1 §	2.2±0.0

† The BAF values were calculated by accounting for the total TCS concentration (5 mg kg^{-1} of inherent biosolids and the spiked)

‡<LOQ is not quantifiable (Signal/Noise >10). The limit of quantitation of TCS was 0.7 ug g^{-1} for the earthworm tissue. The concentrations < LOQ were estimated as ½ the LOQ of the instrument.

§ The average BAFs excluded the samples where TCS concentrations in earthworms are <LOQ

Table 5-4. Measured TCS concentrations (mg kg^{-1}) and bioaccumulation factors (BAFs) in the earthworms collected from the field equilibrated biosolids-amended landscaping soil (same letters represent no statistical difference among treatments).

	Inherent amended soil concentration	Measured earthworm tissue concentration	BAFs dry wt.
	——————mg kg^{-1}——————		
Location 1	0.99	2.6±0.2	2.7±0.2 a
Location 2	0.99	4.4±2.5	4.4±2.5 a
Location 3	0.99	5.9±0.9	5.9±0.9 a
	Overall average	4.3±1.9	4.3±0.7

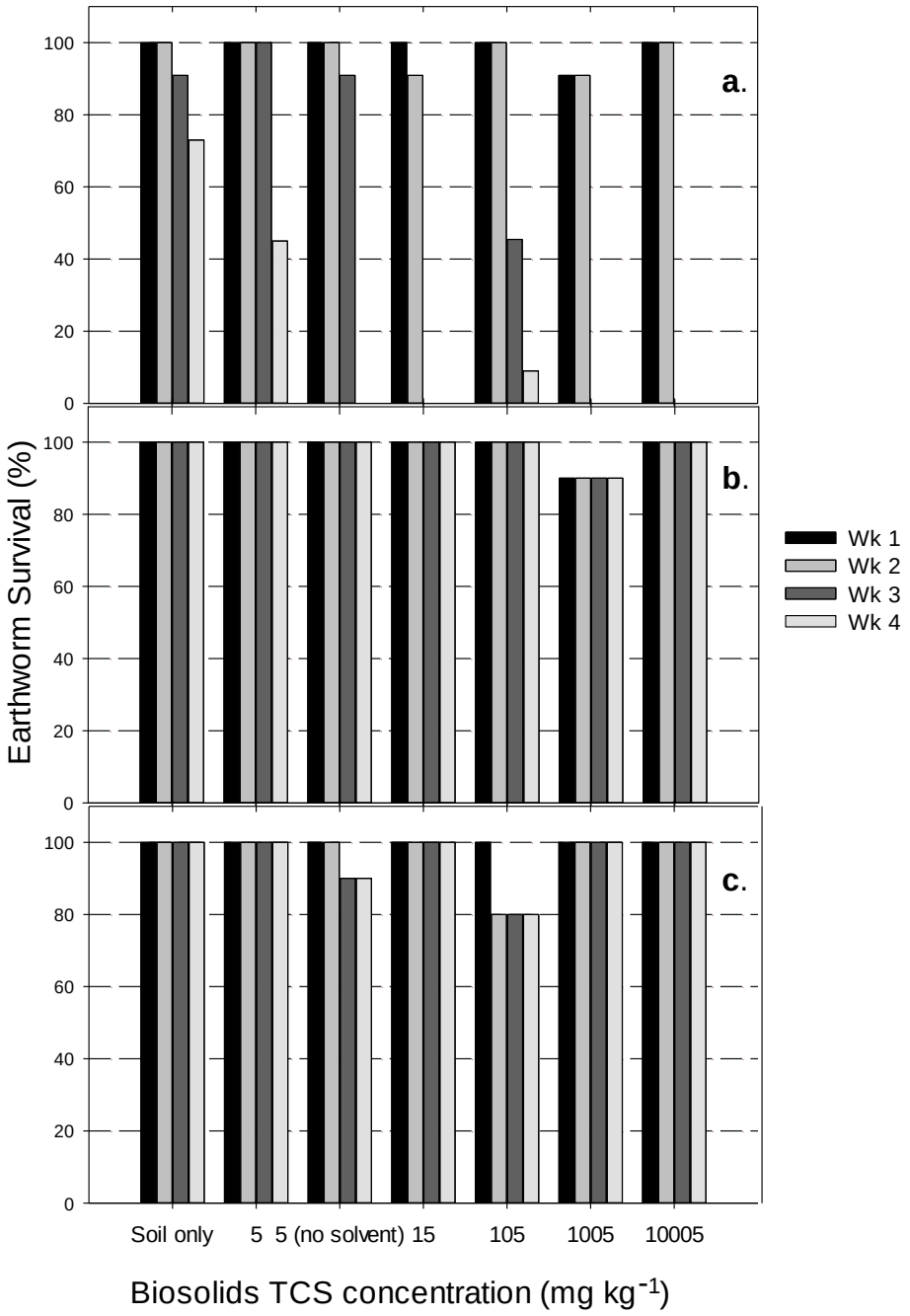


Figure 5-1. The earthworm survival (%) as affected by the biosolids-borne TCS concentration and the duration of earthworm exposure (weeks) in the biosolids-amended (a) Immokalee fine sand (IFS), (b) Ashkum silty clay loam (ASL), and (c) Artificial soils, range-finding test

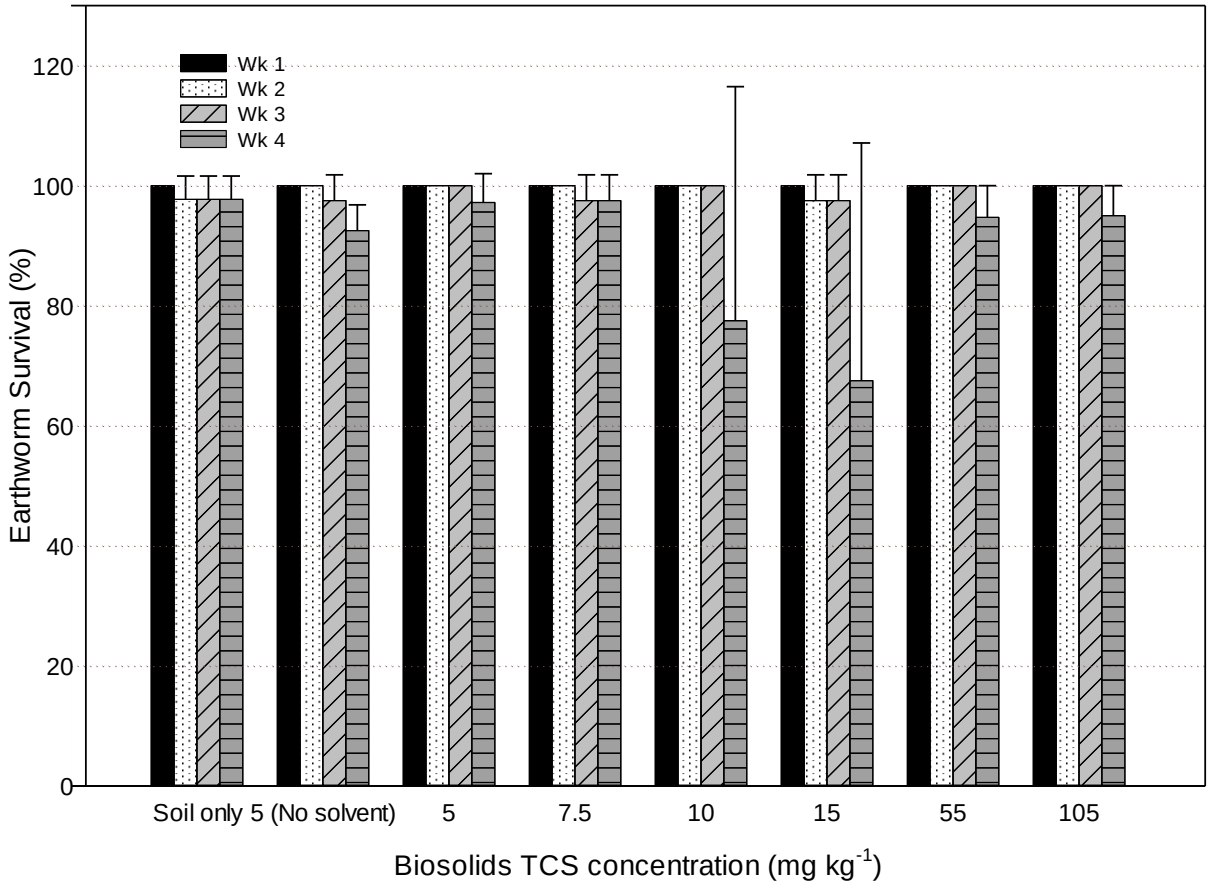


Figure 5-2. The mean earthworm survival (%) (n=4) ± standard deviation as affected by the biosolids-borne TCS concentration and the duration of earthworm exposure in (weeks) in biosolids-amended Immokalee fine sand (IFS), definitive toxicity test.

CHAPTER 6
BIOSOLIDS-BORNE TCS EFFECTS ON SOIL MICROBES

Background

The nutrient and organic rich solid product of wastewater treatment plants (WWTPs) is called biosolids. Land application of biosolids recycles the nutrients to accelerate plant growth and supply abundant carbon for sustainable agriculture. In the U.S. WWTPs generate approximately 7 million Mg of biosolids each year (USGS, 2008), 63% of which is land applied (NRC, 2002). Land application is considered the most suitable means of biosolids disposal/use (Epstein, 2002).

Besides acting as a nutrient boost for agriculture, biosolids addition changes diversity, richness, and structure of plant, animal and microbial communities (USEPA, 1991). Microbial community changes may affect ecosystem processes (such as nutrient recycling) and the effectiveness of microbial invasions (such as growth of pathogens) (Garland, 1997). Dennis and Fresquez (1989) suggested that biosolids (22-90 Mg ha^{-1}) application improved soil fertility by changing the soil microbial composition. Garcia-Gil et al. (2004) found that microbial biomass, respiration, and enzymatic activities increased following biosolids (36 Mg ha^{-1}) application. Sullivan et al. (2006a) analyzed the long-term (12 years) impacts of biosolids application on microbial communities using biolog plate analysis that compared substrate utilization by microbial communities in control and amended soils. Analysis indicated quicker substrate utilization by communities in amended than in control soil plots, suggesting that biosolids addition changed the microbial community structure (Sullivan et al., 2006a). Similarly, Zerzghi et al. (2010a) observed that long-term (20 years) biosolids application enhanced the activity and density of microbes and changed the microbial community composition.

Along with the microbial community changes, biosolids addition may alter soil microbial diversity and microbial processes. Soil microbial diversity is critical in maintaining soil processes such as decomposition of organic matter and nutrient cycling (Garbeva et al., 2004). Zerzghi et al. (2010b) examined the microbial diversity in soils following biosolids (8-72 Mg ha^{-1}) applied each year for 20 years. The results suggested no adverse effect of biosolids application on the microbial diversity. The diversity in amended soil either remained the same or increased following biosolids addition. Rojas-Oropeza et al. (2010) amended a saline-sodic sand with biosolids (28-115 Mg ha^{-1} soil), and observed increased microbial diversity and rates of microbial processes (ammonification and nitrification). Further, biosolids addition elevated microbial respiration (CO_2 production) rates in soils (Barbarick et al., 2004; Sullivan et al., 2006a) even 6 to 12 years following the biosolids amendment. Holt et al. (2010) observed increased N mineralization (conversion of organic N to NH_4-N) due to organic N addition through biosolids application. Dennis and Fresquez (1989) reported that biosolids application increased most of the microbial populations but, fungal diversity decreased initially. Consistently, Kourtev et al. (2003) found that bacteria dominate in fertile (e.g., biosolids applied) soils and the fungi occur more frequently in infertile ecosystems. Thus, the biosolids application may, or may not, alter microbial diversity, but may change individual microbial species and their activities (e.g. N-cycling, respiration) (Lawlora et al., 2000).

Besides supplying nutrients and carbon to soil, biosolids add a variety of organic contaminants. A recent Targeted National Sewage Sludge Survey (TNSSS, USEPA, 2009a) found various contaminants of emerging concern in the biosolids. Triclosan

(TCS) was included among several hundred chemicals such as polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), antibiotics, drugs, hormones and steroids. Triclosan is an antimicrobial compound frequently added in liquid soaps, detergents and household products, which lead to transfer of TCS to WWTPs, and then partition to biosolids. The TNSSS results suggested that biosolids land application can transfer several chemicals, including TCS, to the soil environment. Previous studies suggested changes in microbial community diversity, structure, and microbially-mediated reactions following biosolids (unknown TCS concentrations) addition, but the effects of TCS have not been quantified.

Previous studies (Hansen et al., 2001; Ingerslev et al., 2001; Schmitt et al., 2004) suggest adverse effects of antibiotics on the number of soil micro-organisms and microbial community structure. Similar to antibiotics, TCS (an anti-microbial) may also affect soil micro-organisms. Studies (Liu et al., 2009; Waller and Kookana, 2007) conducted in soils (no biosolids) suggest TCS effects on microbial diversity and structure. Liu et al. (2008) evaluated the effects of TCS spiked in soil (no biosolids) on the soil microbial number and functional diversity. The results suggested that soil TCS concentrations (>10 mg kg^{-1}) increased carbon source utilization, but had no effect on the microbial population numbers and diversity. Waller and Kookana (2009) found increased carbon utilization at spiked TCS concentration <50 mg kg^{-1} soil, and suggested microbial structure changes. Svenningsen et al. (2011) suggested a decrease in cultivable microbial numbers when a sewage-drain field soil was exposed to a TCS (4 mg kg^{-1} soil). Further, the same study suggested increased persistence of ibuprofen and alkylbenzene sulfonate in the presence of TCS (>0.16 mg kg^{-1} soil) in the

same soil. The only study of TCS spiked in biosolids-amended soil is preliminary work by Young (2011, unpublished, personal communication), and suggested adverse effects on the overall community composition. Thus, definitive effects of biosolids-borne TCS on microbial community structure changes need quantification. Further, changes in microbial count (number) following TCS addition have not been reported.

Similar to affects of biosolids addition, biosolids-borne TCS may affect microbially-mediated reactions. Zhao (2006) suggested that TCS inhibited nitrification via competitive inhibition of the enzyme ammonia monooxygenase (AMO) in *Nitrosomonas europaea,* and the inhibition occurs in both sludge and soils (Stasinakis et al., 2007). In a laboratory study, McBain et al. (2004) reported occurrence of TCS resistant strains of *E. coli*, however, the study found no TCS-resistant ammonia oxidizers even after chronic TCS exposure. Waller and Kookana (2009) measured inhibitory effects of TCS concentration (50-500 mg kg^{-1} soil) on nitrification, respiration and enzyme activity in Australian soils (un-amended, no biosolids). Butler et al. (2011) evaluated basal and substrate induced respiration following TCS spiking (0-1000 mg kg^{-1}) in un-amended soils (sandy loam, loamy sand, and clay). Results suggest that TCS inhibited initial respiration in the unacclimated soils, but following the respiking, TCS acted as a C source and stimulated respiration, at least at TCS concentration <100 mg TCS kg^{-1} soil.

Overall, the literature suggests that microbes and microbially-mediated reactions can be altered by biosolids addition or TCS spiked in to soils. However, biosolids-borne TCS may have a reduced bioavailability and its effect may be different from TCS spiked

directly in the soil. We hypothesize that biosolids-borne TCS has no adverse effect on soil micro-organisms or microbial-mediated reactions. Objectives of our study were to:

- Determine the impacts of biosolids-borne TCS on microbial processes.
- Determine the microbial community structure changes in soils following the addition of biosolids-borne TCS.
- Determine the changes in microbial count (number) in soils following the addition of biosolids-borne TCS.

Effects on microbial processes were assessed according to the United States Environmental Protection Agency (USEPA) Office of Prevention, Pesticides, and Toxic Substances (OPPTS) Soil Microbial Community Toxicity Test (Guideline 850.5100) (USEPA, 1996c). The guideline focuses on the effect of added chemical on the microbial processes of ammonification, nitrification, and respiration. The guideline requires a range-finding and a definitive test, and prescribes direct addition of the chemical of interest to a natural soil. The protocol was modified herein to deliver TCS as a component of biosolids in an effort to better simulate the primary mechanism of TCS transfer to the soil in land-applied biosolids.

Microbial community structure changes were assessed by the community-level physiological profiling (CLPP) technique (Schmitt et al., 2004), utilizing biolog plates. The bacterial count (number) was assessed by direct counting (Matsunaga et al., 1995).

Material and Methods

Chemicals, Biosolids, and Soils

Triclosan (CAS No. 101-20-2; >99.9% purity) standard was purchased from United States Pharmacopeia (USP) (Maryland, USA). Methanol (MeOH), phenol phthalein indicator, potassium chloride (KCl), potassium hydroxide (KOH), sodium hydroxide (NaOH), barium chloride ($BaCl_2$), hydrochloric acid (HCl), nitrate (NO_3^-) and

ammonium (NH_4^+) standards were purchased from Sigma Aldrich (St. Louis, MO) or Fisher Scientific (Atlanta, GA). Anaerobically digested biosolids (solid content = 320 g kg^{-1}) (identification code: CHCC) was collected from a domestic WWTP plant in Illinois and had an inherent TCS concentration of 5 mg kg^{-1} (Chapter 2). Two soils, an Immokalee fine sand (IFS) (sandy, siliceous, hyperthermic Arenic Alaquods) [organic carbon (OC): 11 g kg^{-1}], and the Ashkum silty clay loam (ASL) (fine, mixed, superactive, mesic Typic Endoaquolls) (OC: 34 g kg^{-1}) were collected from sites with no known history of receiving land-applied biosolids or sludge and utilized in microbial toxicity test.

Bacterial count and microbial community structure analysis included three sets of soil samples. One set consisted of the control soils (no biosolids) and the soils (lowest and highest spiked TCS concentration treatments) from the microbial toxicity test. The second set of samples consisted of the field landscaping control (no biosolids) (clay loam, OC: 40 g kg^{-1}), and the landscaping soil field-equilibrated for two years following amendment with 228 Mg ha^{-1} biosolids (silty clay loam, OC: 84 g kg^{-1}, TCS concentration: ~1 mg kg^{-1} amended soil). The landscaping soils were collected from Illinois fields in 2010, air-dried and promptly sent to our laboratory. A third set of samples were obtained from Will (WL, silty clay loam) and Kankakee (KK, fine sand) Counties (Illinois) field research plots. The selected WL and KK soils were the controls (no biosolids), and the soil amended with 118 Mg ha^{-1} biosolids in 2006 (WL TRT), and 155 Mg ha^{-1} biosolids in 2007 (KK TRT); soils were sampled in 2008. The WL and KK samples (both control and treatment) were air-dried and stored at room temperature. The soils were shipped to our laboratory in 2010 and utilized as such. Collectively, the

three sets of samples offered a wide array of soil textures, biosolids loading rates, TCS soil concentrations and time since last biosolids amendment.

Microbial Toxicity (Range-Finding) Test Design

The microbial toxicity test included a range-finding and a definitive test. The range-finding test was conducted to identify the appropriate range of biosolids-borne TCS concentrations for a subsequent definitive toxicity assessment. One gram samples of oven-dry CHCC biosolids (Inherent TCS-5 mg kg^{-1}) were spiked with 0, 10, 100, 1000, or 10,000 mg TCS kg^{-1} biosolids using MeOH as the carrier solvent and were subsequently dried, re-wetted, and equilibrated for 48 h in 300 mL glass Mason jars. Treatment spikes were added in addition to the inherent TCS concentrations, so the final nominal concentrations were 5, 15, 105, 1005, and 10,005 mg TCS kg^{-1} biosolids. Biosolids were amended to 100 g (dry wt.) of IFS and ASL soils at an equivalent rate of 22 Mg ha^{-1}, and amended soils brought to field capacity (10% for IFS and 30% for ASL by wt.). A soil-only control, a carrier solvent-free biosolids-amended soil control (~5 mg TCS kg^{-1} biosolids), and an empty jar (to confirm efficacy of the CO_2 scrubber system) were also included in the unreplicated study.

The jars were aerated with CO_2-free humidified air at approximately 22°C. Incoming air was stripped of CO_2 and humidified by pumping ambient air first through 2 *M* KOH, followed by CO_2-free water, a column of soda lime chips ($Ca(OH)_2$>80%, KOH< 3%, NaOH< 2%, Ethyl violet<1%), additional 2 *M* KOH, and once more through CO_2-free water. Carbon dioxide evolved from each treatment jar was taken as a measure of microbial respiration, and was collected in a series of two base traps each containing 100 mL of 0.15 *M* KOH.

Microbial Toxicity (Definitive) Test Design

In response to adverse effects observed in the ASL and IFS soils, a concentration range of 0 to 1000 mg TCS kg^{-1} biosolids was selected for the definitive toxicity test. Biosolids were spiked with 5, 10, 50, 100 and 500 mg TCS kg^{-1} biosolids in the ASL soil. An additional treatment of 1000 mg TCS kg^{-1} biosolids was included in the IFS soil. Treatment spikes were added in addition to the inherent TCS concentration, so the nominal final TCS biosolids concentrations were 5, 10, 15, 55, 105, 505 and 1005 mg TCS kg^{-1}. Three replicates were prepared for each treatment and the control, and for each sampling period.

Sample Preparation and Analyses for Microbial Toxicity Test

Samples were removed from the aeration unit and analyzed in the same manner in both the range-finding and definitive tests. Base traps (containing KOH) connected to the treatment jars were removed and replaced with fresh KOH on days 5, 14, and 28, and analyzed for CO_2 (Anderson, 1982). A solution of $BaCl_2$ was used to first precipitate the carbonates (representing trapped CO_2) in a known volume of base, and subsequently centrifuged at ~2,000×g for 10 min. One mL of the supernatant was then transferred to a glass 20 mL scintillation vial, treated with phenolphthalein indicator, and titrated to pH of 8 to 9 with 0.1 *M* HCl. The unused KOH remaining in the base trap was used to calculate the moles of KOH neutralized by evolved CO_2.

Also on days 0, 5 and 28, subsets (10 g dry wt.) of amended soil were sampled and shaken with 100 mL 1 *M* KCl to extract $N0_3^-$-$N0_2^-$-N and NH_4-N (Bremner, 1996). Extracts were filtered (0.45 µm) and analyzed for NO_3^--NO_2^--N (USEPA, 1993a, Method 353.2) and NH_4-N (USEPA, 1993b, Method 350.1) to assess TCS impacts on nitrification and ammonification, respectively. Method 353.2 accomplishes NO_3^-

reduction by passing soil extract through a copper-cadmium coil, and the resultant total NO_2^- is reacted with multiple reagents to form an azo dye for colorimetric analysis. Results were reported as mg $N0_3^-$-$N0_2^-$N per kg soil or biosolids-amended soil. Method EPA 350.1 results in conversion of extracted NH_4^+ to NH_3, which then reacts with phenol to yield indophenol for colorimetric analysis. Results are reported as mg NH_4-N per kg soil or biosolids-amended soil. A rapid flow analyzer (Alpkem, VA) was utilized for NO_3^--NO_2^--N analysis, and AQ2 discrete analyzer (SEAL, WI) was utilized for the NH_4-N analysis.

Soil samples from control and highest TCS spiked treatments, (IFS-500 and ASL-500) utilized in the definitive test were subjected to molecular (DNA) analysis. The analysis included DNA isolation and amplification. The DNA was extracted from the soils (0.25 g wet weight) using MoBio PowerSoil® DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA) according to the manufacturer instructions, and stored at -20°C until use. Polymerase chain reaction (PCR) amplification of bacterial amoB gene was performed using primers *amoA*1F (5'-GGGGTTTCTACTGGTGGT-3') and *amoA*2R (5'-CCCCTCKGSAAAGCCTTCTTC-3') (Rotthauwe et al., 1997) and GoTaq Green Master Mix (Promega, Madison, WI). The reaction mixture contained 25 µl Master Mix, 0.5 µM of each primer, 2 µl of a ten-fold DNA dilute, and distilled water to make a total volume of 50 µl. The PCR was conducted with a BioRad iCycler thermal cycler (Hercules, CA) under the following thermo-cycling conditions: initial enzyme activation and denaturation at 95°C for 15 min, 35 cycles of 95°C for 30 sec, 55°C for 45 sec, and 72°C for 45 sec, with a final extension step at 72°C for 7 min. From the PCR reaction, a 491bp fragment of AOB gene was amplified.

The PCR amplification of amoA was conducted using primers A19F (5'-ATG GTC TGG CT (AT) AGA CG-3') and 643R (5'-TCC CAC TT (AT) GAC CA (AG) GCG GCC ATC CA-3') (Leininger et al., 2006), which produce a 624bp amplicon. The same PCR mixture used for amoB was used for amoA, except for the primers. The thermo-cycle consisted of the denaturation of 15 min at 95°C, followed by 30 sec at 95°C, 45 sec at 55°C, and 45 sec extension at 72°C for 30 cycles, and a final extension of 72°C for 7min. The PCR products were analyzed by electrophoresis through 1.5% Tris-acetate-EDTA (TAE) agarose gels, and visualized by staining ethidium bromide and exposure under UV light.

Microbial Community Structure Test Design

Microbial community structure changes were assessed with the community-level physiological profiling (CLPP) technique (Schmitt et al., 2004), utilizing microtiter biolog plates. Prefilled microtiter plates (ECO microplate; Biolog, Hayward, CA) contained triplicates of 31 organic substrates such as sugars, and amino acids, as well as a tetrazolium dye. Microbial growth on the substrates is assessed from the color development in tetrazolium dye contained in the wells (Rutgers et al., 1998). Tetrazolium dye can be reduced to a soluble puple formazan product by live microbial cells. The amount of formazan is measured spectrophotometically at 590 nm. Quantity of color development or formazan product is directly proportional to the number of living and respiring cells (Cory et al., 1991).

Subsamples (10 g dry wt.) of soil were extracted with 100 mL phosphate buffer, prepared by mixing 61.5 mL of 1*M* dipotassium hydrogen phosphate ($K_2HPO_4.3H_2O$) and 38.5 mL of 1*M* potassium dihydrogen phosphate (KH_2PO_4). The suspension was shaken for 20 min on a reciprocating shaker, and then centrifuged for 10 min at 8000×g.

Supernatants were transferred into micro-centrifuge tubes, immediately frozen under liquid nitrogen, and stored in the freezer until analysis. A nine-fold dilution (estimated in a preliminary study as being sufficient to contain appropriate number of bacteria) was performed on the extracts using the phosphate buffer, and 100 µL of each diluted extract was transferred to biolog plate wells. The plates were covered with aluminum foil and stored in the dark at 20 to 25°C. Temporal analysis of plates included measuring the absorbance of the whole plate at the wavelength of 590 nm every day for 10 d and every other day for the next 20 d on the Bio-TekFL600 micro plate reader (MTX labs, VA). The color development (absorbance) in the plate wells represented the extent of substrate utilization by the microbes, representing growth.

The absorbance was separately measured for each substrate, but the substrates were later grouped into categories (Table 6-1): acids, amino acids, polymers, amines, carbohydrates, and miscellaneous based on the classification by Garland and Mills (1991). The substrate absorbance was calculated by subtracting the absorbance of water controls measured at corresponding times.

Extraction for Bacterial Count

The total bacterial count was estimated by direct counting using a fluorescein isothiocyanate (FITC) stain (Matsunaga et al., 1995). Five grams of soil (dry wt.) was shaken vigorously with 45 mL of 0.1% agar solution. After 30s, a 10 µL of aliquot was spread evenly onto a pre-marked 1 cm^2 area on a slide. The slide was kept on a warmer to heat fix the bacteria. One drop of FITC stain was added on the slide and left to dry for 3 min. Excess stain was washed off with 0.5 M sodium carbonate-bicarbonate (Na_2CO_3-$NaHCO_3$) buffer, and the washed slide mounted with glycerol (pH = 9.6) before putting the cover slide. Bacteria were counted within four microscopic fields at 400×

using an Optiphot Biological UV Microscope (Nikon, NY), and the mean counts recorded. The mean counts represent only viable bacteria, as the technique fails to stain the dead bacteria.

Statistical Analysis

Statistical analysis was performed using SAS software, version 9.1 (SAS institute, 2002). The Tukey's studentized range test was used to assess statistical differences at each sampling period across treatments. Regression analysis with the ESTIMATE procedure assessed the statistical differences between sampling periods within treatments.

Results and Discussion

Microbial Toxicity (Range-Finding) Test

Respiration measures the overall activity of a microbial community. The substrate (biosolids) induced respiration was quantified following OPPTS guideline using CO_2 evolution as a measure of microbial respiration. Biosolids addition increased microbial respiration (CO_2 evolution) over time, but some respiration inhibition occurred due to TCS spiking (Figure 6-1). At 28 d, biosolids TCS spiking caused a reduction of ~27% (at TCS concentration=1005 mg kg^{-1}), and ~42% (at TCS concentration=10,005 mg kg^{-1}) in total CO_2 evolution in the IFS treatments (Figure 6-1a). For ASL treatments, biosolids TCS (concentration=10,005 mg kg^{-1}) caused ~10% reduction in total CO_2 evolution relative to the unspiked biosolids-amended control (Figure 6-1b). Further, biosolids addition increased the NH_4-N production in both soils. Up to day 5, TCS spiking did not inhibit the NH_4-N production (Figure 6-2), and at the study termination (28d), TCS inhibition of NH_4-N only occurred at a TCS concentration of 10,005 mg kg^{-1} biosolids in the IFS soil (Figure 6-2a). Biosolids or TCS addition did not affect the production of $N0_3^-$

-$N0_2^-$-N in the IFS soil (Figure 6-3a), but the ASL soil behaved differently. Biosolids TCS concentrations ≥15 mg kg^{-1} reduced the $N0_3^-$-$N0_2^-$-N production by 75% as compared to unspiked biosolids-amended control (Figure 6-3b).

Microbial Toxicity (Definitive) Test

Effect on respiration rates

Biosolids TCS concentrations ≤1005 mg kg^{-1} in the IFS soil, and ≤505 mg kg^{-1} in ASL soil, did not affect cumulative respiration at any sampling time. Addition of biosolids significantly increased the total CO_2 production in both soils (Figure 6-4a, b), except for one treatment (15 mg TCS kg^{-1} biosolids) in the IFS soil (Figure 6-4b). The cumulative CO_2 evolution in the 15 mg TCS kg^{-1} treatment was significantly smaller than the other TCS treatments, and likely represented an unexplained analytical error. The only effect on microbial respiration was due to biosolids addition, likely attributable to increased substrate induced respiration following the addition of labile organic carbon.

Our results agree with the results from previous studies (Barbarick et al., 2004; Sullivan et al., 2006a). Barbarick et al. (2004) found increased microbial respiration rates in shrubland and grassland soils amended with biosolids (30-40 Mg ha^{-1}) 6 years prior. The increased respiration was attributed to the availability of additional carbon substrates furnished by the biosolids (Barbarick et al., 2004). Sullivan et al. (2006a) positively correlated biosolids addition and CO_2 evolution in semi-arid rangeland soil (sandy loam, pH=6.2). The CO_2 production was enhanced, and the metabolism of the microbial community elevated, even 12 years following biosolids application.

Our CO_2 evolution data suggest that TCS concentrations ≤505mg kg^{-1} biosolids do not adversely affect respiration rates, similar to studies assessing the effects of TCS spiked in to soils (Butler et al., 2011; Waller and Kookana, 2009). Waller and Kookana

(2009) found no negative effect on substrate induced respiration at spiked TCS concentrations <50 mg kg^{-1} soil (equivalent to our 5000 mg TCS kg^{-1} biosolids treatment). Butler et al. (2011) assessed basal and substrate induced respiration changes following TCS spiking in un-amended soils. Three soils- sandy loam [organic carbon (OC)-17 g kg^{-1}], clay (OC-27 g kg^{-1}) and loamy sand (OC-23 g kg^{-1}) were spiked with a range of TCS concentrations (0-1000 mg kg^{-1}). Results suggest respiration inhibition at TCS concentration >10 mg kg^{-1} soil (equivalent to our >1000 mg kg^{-1} biosolids treatment) within 6 h from the initial TCS spiking, but the inhibition disappeared after 2 to 4 d. Respiking of TCS to the same soil, caused an initial microbial stimulation of respiration up to day 4, but the respiration rates returned to the baseline by day 6. Authors reported that TCS inhibited initial respiration rates (6h) in the unacclimated soils, but following the respiking, TCS acted as a C source and stimulated respiration at least at the low TCS concentrations (<100 mg kg^{-1}) (Butler et al., 2011) Thus, the stimulation of microbial respiration observed in our study could be a combined effect of TCS and biosolids serving as C sources, especially at TCS concentration ≤10 mg kg^{-1} amended soil.

Spiked TCS concentrations did not significantly affect total CO_2 evolved, and the results were consistent with our range-finding test, and previous two studies conducted in spiked soils (no biosolids) (Waller and Kookana, 2009; Butler et al., 2011). Our study suggested that biosolids addition, however, stimulated microbial respiration.

Effect on nitrogen cycle

Ashkum silty clay loam (ASL) soil- A TCS concentration ≤505 mg kg^{-1} biosolids did not significantly affect the NH_4-N production in biosolids-amended soil or the control at 5d (Figure 6-5a). At day 28, NH_4-N production surprisingly declined in all treatments

independent of biosolids or TCS additions so there was no significant effect of biosolids or TCS concentration on NH_4-N production (Figure 6-5a). The abrupt decrease in NH_4-N production from day 5 to 28 cannot be easily explained because of a gap between the sampling times. Consistent with our study, a 28 d incubation study (Snyder et al., 2011) revealed insignificant differences in NH_4-N production at triclocarban concentrations up to ~717 mg kg^{-1} biosolids following biosolids addition to a silty loam soil.

Biosolids addition significantly decreased the $N0_3^-$-$N0_2^-$-N production at day 5 (Figure 6-5b). However, $N0_3^-$-$N0_2^-$-N production in spiked biosolids treatments was not significantly different than the un-spiked biosolids-amended control (Inherent TCS=5 mg kg^{-1}). At day 28, $N0_3^-$-$N0_2^-$-N concentrations increased (as compared to day 5), resulting in no overall TCS or biosolids addition effect on $N0_3^-$-$N0_2^-$-N production at biosolids TCS concentration of ≤505 mg kg^{-1}.

Given reports (Zhao, 2006; Stasinakis et al., 2007) that TCS inhibits the nitrification process in sludge and soils, we compared the relationship between the ammonification and nitrification processes in our study. Accumulation of NH_4-N at day 5 coincided with a decrease in $N0_3^-$-$N0_2^-$-N production that might suggest TCS inhibition of nitrification. However, between days 5 and 28, unknown changes in the study conditions appeared to disturb the microbial community structure (or conditions), which overcame any TCS inhibition of nitrification and caused a rapid conversion of NH_4-N to $N0_3^-$-$N0_2^-$-N.

Immokalee fine sand (IFS) soil- A similar study conducted with the IFS soil included an additional sampling period of 14d to avoid the unexplainable changes observed in ASL soil data interpretation due to long sampling intervals.

Ammonium production increased following biosolids addition (Figure 6-6a). At day 5, a TCS biosolids concentration of 1005 mg kg^{-1} caused the greatest NH_4-N production, but TCS addition effect was not significant. All the biosolids-amended treatments increased NH_4-N production at day 5, except the treatment with 505 mg TCS kg^{-1} biosolids, where the effect did not appear until day 14 (Figure 6-6a). At day 5, the biosolids or TCS additions did not affect $N0_3^-$-$N0_2^-$N production. At day 28, biosolids addition caused a significant reduction in $N0_3^-$-$N0_2^-$-N concentrations (Figure 6-6a, b).

Holt et al. (2010) investigated the production of NH_4-N from a soil (unknown texture) amended with biosolids (18 Mg ha^{-1}). The results suggested that diazotrophs fixed more N following biosolids addition up to 42 d, but the stimulation disappeared at 84 d causing no difference in NH_4-N production in control and amended soils. Authors concluded that biosolids amendment added organic N causing rapid NH_4-N production, and that inherent biosolids-borne chemicals (e.g., PPCPs, unknown concentrations) did not adversely affect N cycling. Similarly, Barbarick et al. (2006) attributed elevated N mineralization rates in soil to addition of organic N through biosolids application.

Waller and Kookana (2009) monitored changes in N mineralization in sand (OC: 8.5 g kg^{-1}) and clay (OC: 18.5 g kg^{-1}) soils spiked with 0, 1, 5, 10, 50, and 100 mg TCS kg^{-1} soil. Nitrification process was not adversely affected at TCS concentrations ranging from 5 to 50 mg kg^{-1} soil (corresponds to equivalent concentrations of 500 to 5000 mg kg^{-1} biosolids).

As with CO_2 evolution and NH_4-N production, the $N0_3^-$-$N0_2^-$-N production data at study termination (28 d) suggest significant biosolids addition effects, but no TCS treatment effect, up to 1005 mg TCS kg^{-1} biosolids in the IFS soil.

Bacterial DNA analysis

Zhao (2006) reported that TCS inhibited ammonia monooxygenase (AMO), the first enzyme in the two step nitrification process. The AMO is encoded by two genes from Archeal amoA and bacterial amoA. Archeal amoA dominates in most environments (Leininger et al., 2006). In a preliminary study, we measured the relative concentrations of the two forms of bacterial DNA from IFS control (soil-only), 5 (only biosolids inherent TCS), and 505 mg TCS kg^{-1} biosolids (spiked TCS) treatments. There were no significant differences in archeal amoA concentrations among the treatments but high variability made the final results inconclusive. The preliminary data suggest minimal effects of biosolids-borne TCS addition on the archeal amoA that is likely to be involved in N-cycling at least in one soil. The inconclusive results with bacterial amoA due to TCS spiking requires further investigation perhaps in various soils.

Collectively, the soil microbial toxicity test data suggest no TCS spiking effects on respiration, nitrification or ammonification up to a TCS biosolids concentration of 505 mg kg^{-1} amended to the ASL soil, and up to 1005 mg kg^{-1} amended to the IFS soil. The differences in NH_4-N and NO_3^--NO_2^--N production between the two soils may reflect differences in the availability of TCS, or other C containing substrates in the two soils (Table 5-1). Triclosan availability could differ in the two soils due to less TCS retention in the IFS soil (log K_d = 1.87 ± 0.21), than in the ASL soil (log K_d = 2.64 ± 0.19) (Agyin-Birikorang et al., 2010). Waller and Kookana (2009) monitored changes in N mineralization in two soils and suggested that the TCS concentration required to affect nitrification was ten times greater in the clay soil (50 mg TCS kg^{-1}) than in the sand (5 mg TCS kg^{-1}).

Thus, TCS concentrations of 25 to 50-fold greater (500 mg kg^{-1}) than normally found (~10 to 20 mg kg^{-1}) in most land-applied biosolids did not cause microbial toxicity, at least using microbial respiration and nitrogen cycling as indicators. The effects of long-term biosolids applications are known. Our study and other published studies (Waller and Kookana, 2009; Butler et al., 2011) are short-term and may not represent the effects of long-term biosolids-borne TCS application.

Microbial Community Structure Analysis

The average well color development (AWCD) in various substrates is illustrated in Figure 6-7. Data obtained for each substrate were combined into the general category of carboxylic acids, amino acids, polymers, amines, carbohydrates, and miscellaneous. At study termination (day 40), the well color development varied, suggesting different utilization of the six types of carbon substrates in different soils (Figure 6-7).

Polymers were utilized most readily, carbohydrates were utilized least readily, and the remaining substrate groups were moderately utilized. Addition of biosolids or TCS did not appear to increase substrate utilization as compared to the control, except in IFS soil where the addition of biosolids alone (IFS-0) inhibited the utilization of most groups of substrates, except the polymers. The addition of biosolids spiked with TCS in the IFS soil (IFS-500) resulted in similar color development as in the control (IFS control). High (landscaping) and multiple (WL and KK) loads of biosolids and associated TCS did not increase substrate utilization as compared to the respective controls. Thus, neither inhibition nor stimulation of carbon substrate utilization occurred due to TCS or biosolids addition, suggesting minimal disturbance of the microbial community structure at least with AWCD as an indicator. Results are at odds with the assumption that

microbial community structures would vary with soil texture or biosolids and TCS addition.

Young (2011, unpublished, personal communication) suggested adverse effects of TCS spiked in biosolids on the overall community composition using biolog plates and PCA (principle component analysis). However, a direct comparison and evaluation of the two studies was not possible due different quantification methods. Additional investigation of our data should involve the quantification of the overall well color development for all substrates in each treatment and plotting the various microbial communities using a PCA method.

Various studies (Barbarick et al., 2004; Garcia-Gil et al., 2004; Sullivan et al., 2006b) suggested shifts in soil microbial community structure due to the addition of biosolids alone. Thus, the changes in community structure observed in Young (2011) study may simply be a biosolids effect. In fact, he observed a greater effect of biosolids as compared to TCS concentration effects.

Bacterial Counts

The average bacterial counts were greater in the ASL, landscaping and WL soil control than in the IFS and KK control soils, but the differences were not significant. The trends may be a texture effect, because ASL and landscaping soils (soil collected from an area utilizing for landscaping purposes in Illinois) are silty clay loams, whereas, the IFS and KK are sands. Addition of biosolids increased the bacterial counts in all soils as compared to the control soils (Table 6-2). Biosolids addition likely provided organic carbon for enhancing the bacterial growth.

The ASL soil treated with TCS spiked biosolids (ASL-500) had significantly greater bacterial counts than the corresponding IFS treatments (IFS-500) ($p<0.05$).

Biosolids application rates were same in the two soils, but greater bacterial number enhancement occured in the ASL soil spiked with TCS concentration (ASL-500), which is likely a TCS addition effect in the ASL soil. The landscaping biosolids-amended treatment (landscaping TRT) had significantly greater ($p<0.0001$) bacterial counts than the landscaping control soil, or the biosolids-amended ASL, IFS, WL and KK soils which may have occurred following the application of high loads (228 Mg ha^{-1}) of biosolids. Addition of TCS significantly increased bacterial counts in the ASL-500 treatment as well as in the WL amended treatment (WL TRT) compared to the soil control (ASL control and WL control) and the biosolids-amended unspiked treatments (ASL-0).

There is no evidence of bacterial growth inhibition following high (landscaping TRT) or multiple (WL TRT, KK TRT) biosolids applications or high TCS concentrations (990 ug kg^{-1}) in amended soil. The bacterial counts were greater in biosolids-amended silty clay loam soil (ASL) than in amended sand (IFS), suggesting a texture effect. Zerzghi et al. (2010a) evaluated the effect of 20 years of biosolids land application (8-24 Mg $ha^{-1}year^{-1}$) on the microbial population and activity in soil (southwestern desert soil). Direct counts suggested differences in total microbial counts between the control and amended soils, but the difference was not significant. The authors concluded that even long-term (20 years) biosolids applications, resulting in high total biosolids loads (160-480 Mg ha^{-1}) did not affect microbial numbers of bacteria, actinomycetes and fungi.

We accept our hypothesis that biosolids-borne TCS has no adverse effect on soil micro-organisms, using microbially-mediated processes, community structure, and bacterial counts as indicators.

Table 6-1. The grouping of the various substrates in the biolog ECO plates (Garland and Mills, 1991).

Acids	Amino acids	Polymers	Amines
Galactonic acid	Arginine	Tween 40	Pheny ethyl amine
Galacturonic acid	Asparagine	Tween 80	Glucosamine
2-hydroxy benzoic acid	Phenylalanine	Glycogen	Putresoine
4-hydroxy benzoic acid	Serine		
Itaconic acid	Threonine		
Ketobutryic acid	Glutamic acid		
Malic acid			
Pyruvic acid	**Carbohydrates**	**Miscellaneous**	
Glucosaminic acid	Cellobiose	Erthristol	
	Lactose	Mannitol	
	Methyl glucosidase	Cyclodextrin	
	D-xylose	Glucose-1-phosphate	
		Glycerol phosphate	

Table 6-2. Average bacterial count (number) in soils with varying biosolids application rates, TCS concentrations and textures (same letters represent no significant difference among treatments).

Treatment	Biosolids rates (Mg ha^{-1})	TCS concentration (µg kg^{-1})	Texture	Average ± standard deviation of count of bacteria per g of soil
Landscaping control	na‡	nd†	Clay loam	$2.5*10^8 \pm 1.1*10^8$ b
Landscaping TRT	228 (one time)	990	Silty clay loam	$1.0*10^9 \pm 2.0*10^8$ a
ASL control	na‡	nd†	Silty clay loam	$3.4*10^8 \pm 2.3*10^8$ b
ASL-0	22	5	Silty clay loam	$4.0*10^8 \pm 2.3*10^8$ b
ASL-500	22	500	Silty clay loam	$5.3*10^8 \pm 2.0*10^8$ c
IFS control	na‡	nd†	Sand	$1.7*10^8 \pm 6.8*10^7$ b
IFS-0	22	5	Sand	$2.7*10^8 \pm 1.6*10^8$ b
IFS-500	22	500	Sand	$3.8*10^8 \pm 1.4*10^8$ b
WL control	na‡	1	Silty clay loam	$2.3*10^8 \pm 1.5*10^8$ b
WL TRT	116 (multiple)	41	Silty clay loam	$5.2*10^8 \pm 1.6*10^8$ c
KK control	na‡	0.8	Sand	$2.1*10^8 \pm 7.3*10^7$ b
KK TRT	154(multiple)	18	Sand	$2.8*10^8 \pm 1.4*10^8$ b

nd†: Concentrations were below the detection limit of the instrument

na‡: No application of biosolids

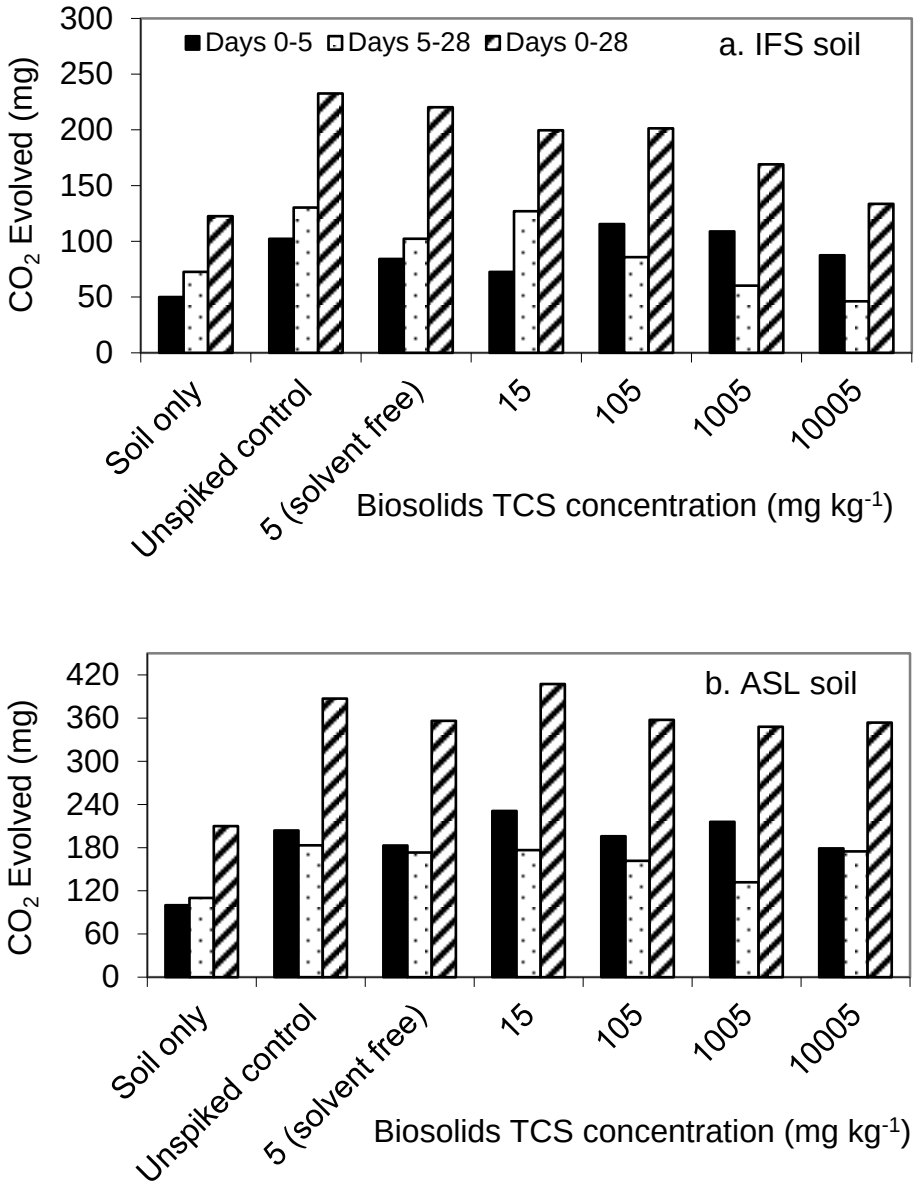


Figure 6-1. Total CO_2 evolution (mg) over various times in the (a) IFS and (b) ASL soils amended with biosolids spiked with a range of TCS concentrations, unreplicated range-finding test.

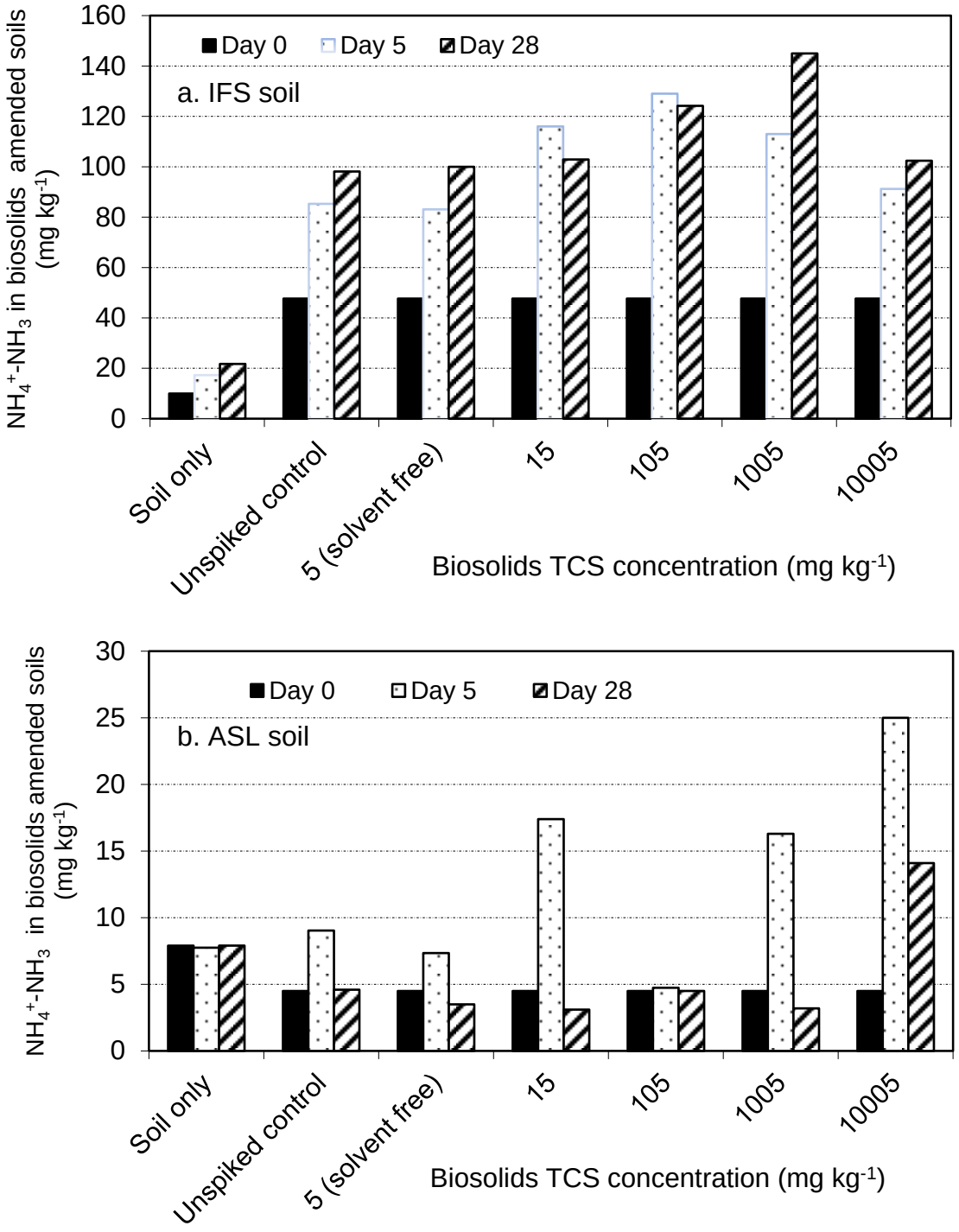


Figure 6-2. NH_4^+-NH_3 concentrations (mg kg^{-1}) over time (Days 0-28) in the (a) IFS and (b) ASL soils amended with biosolids spiked with a range of TCS concentrations, unreplicated range-finding test.

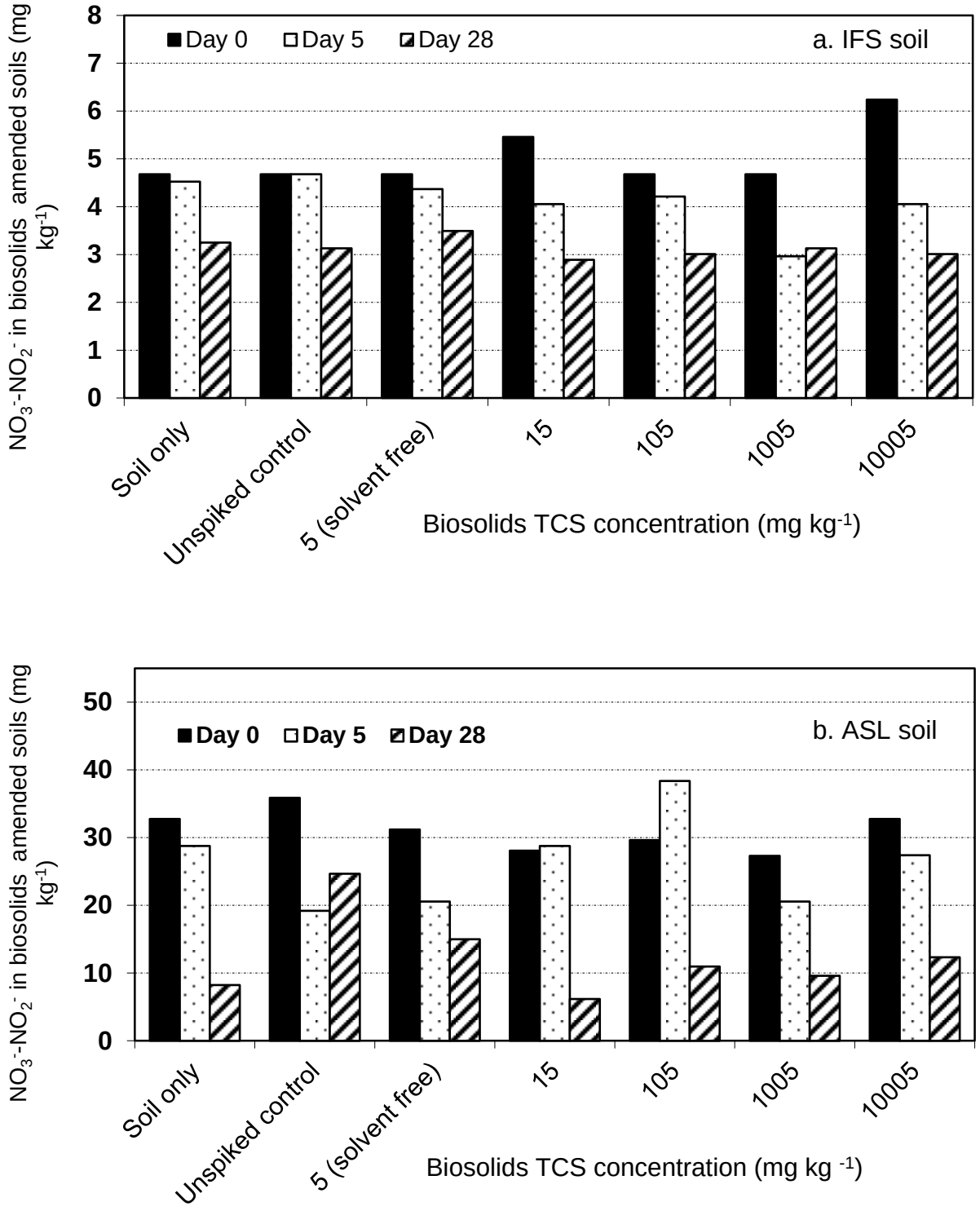


Figure 6-3. $N0_3^-$-$N0_2^-$-N concentrations (mg kg^{-1}) over time (Days 0-28) in the (a) IFS and (b) ASL soils amended with biosolids spiked with a range of TCS concentrations, unreplicated range-finding test.

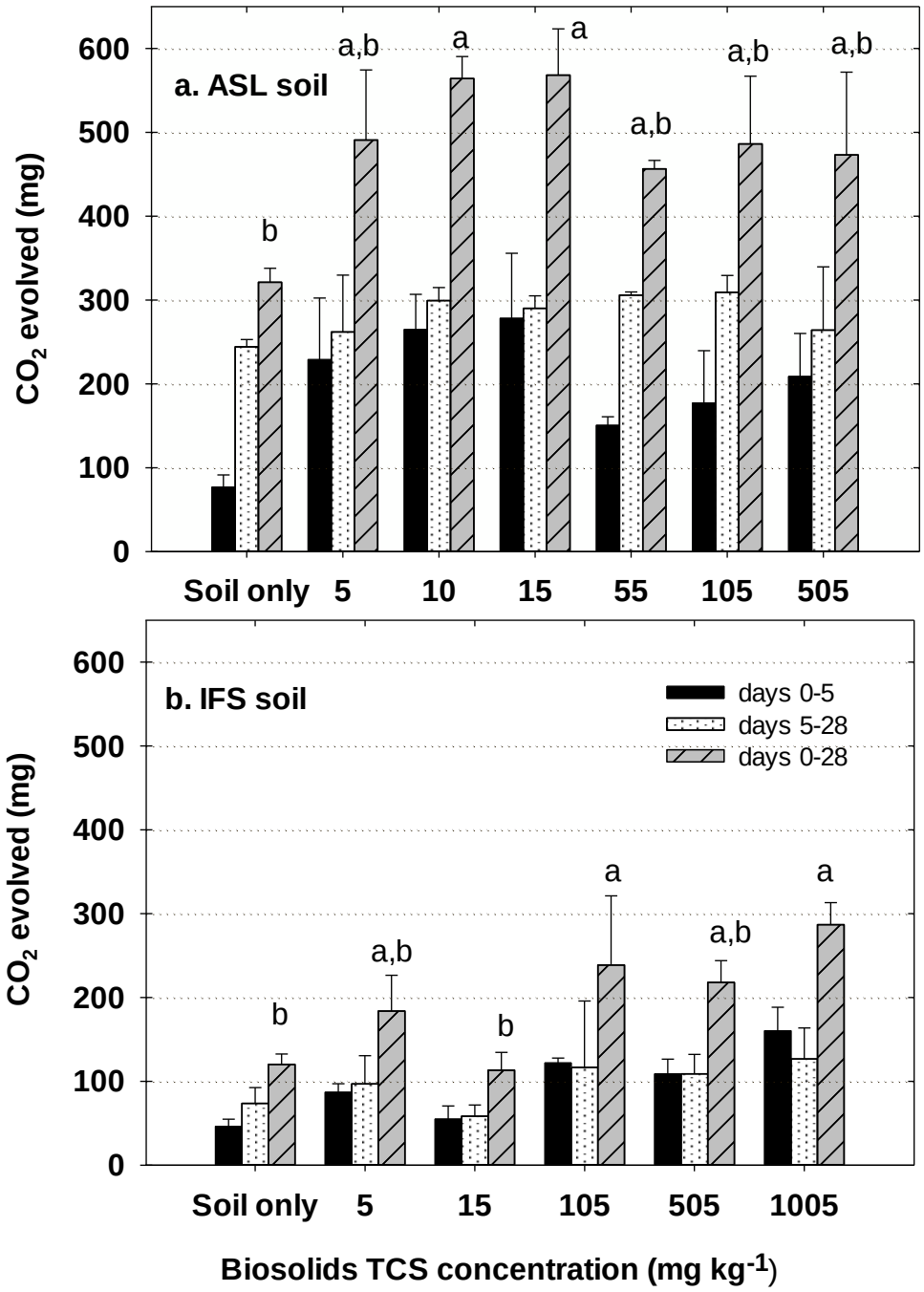


Figure 6-4. Mean total CO_2 (mg) as a function of TCS concentrations and time (Days 0-28) in (a) ASL and (b) IFS soils (like letters indicate no significant difference between treatments), definitive test.

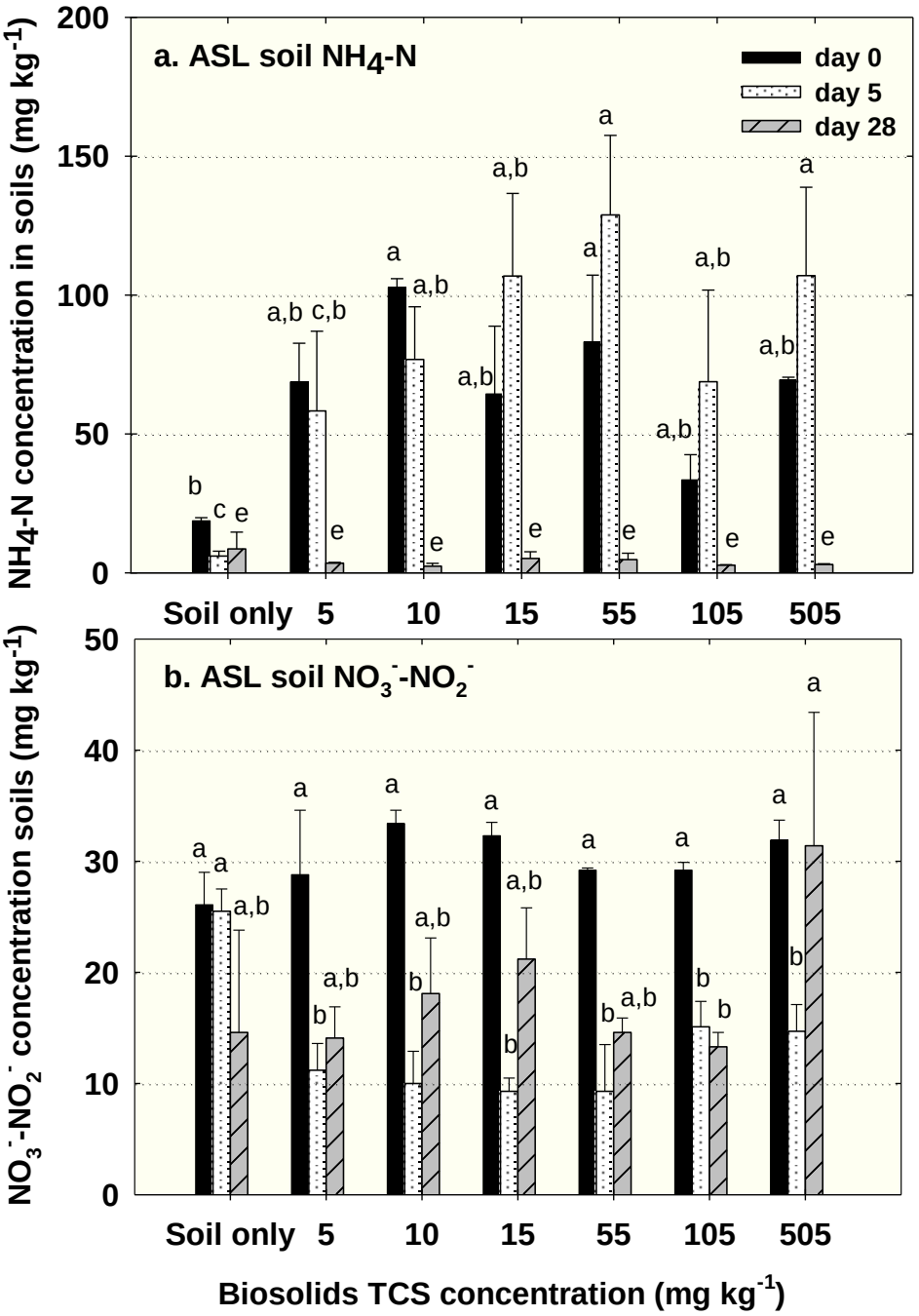


Figure 6-5. Mean (a) NH_4 –N and (b) $N0_3^-$-$N0_2^-$-N concentrations (n=3) as a function of biosolids TCS concentration and time (Days 0-28) in ASL soils (like letters indicate no significant difference between treatments), definitive test.

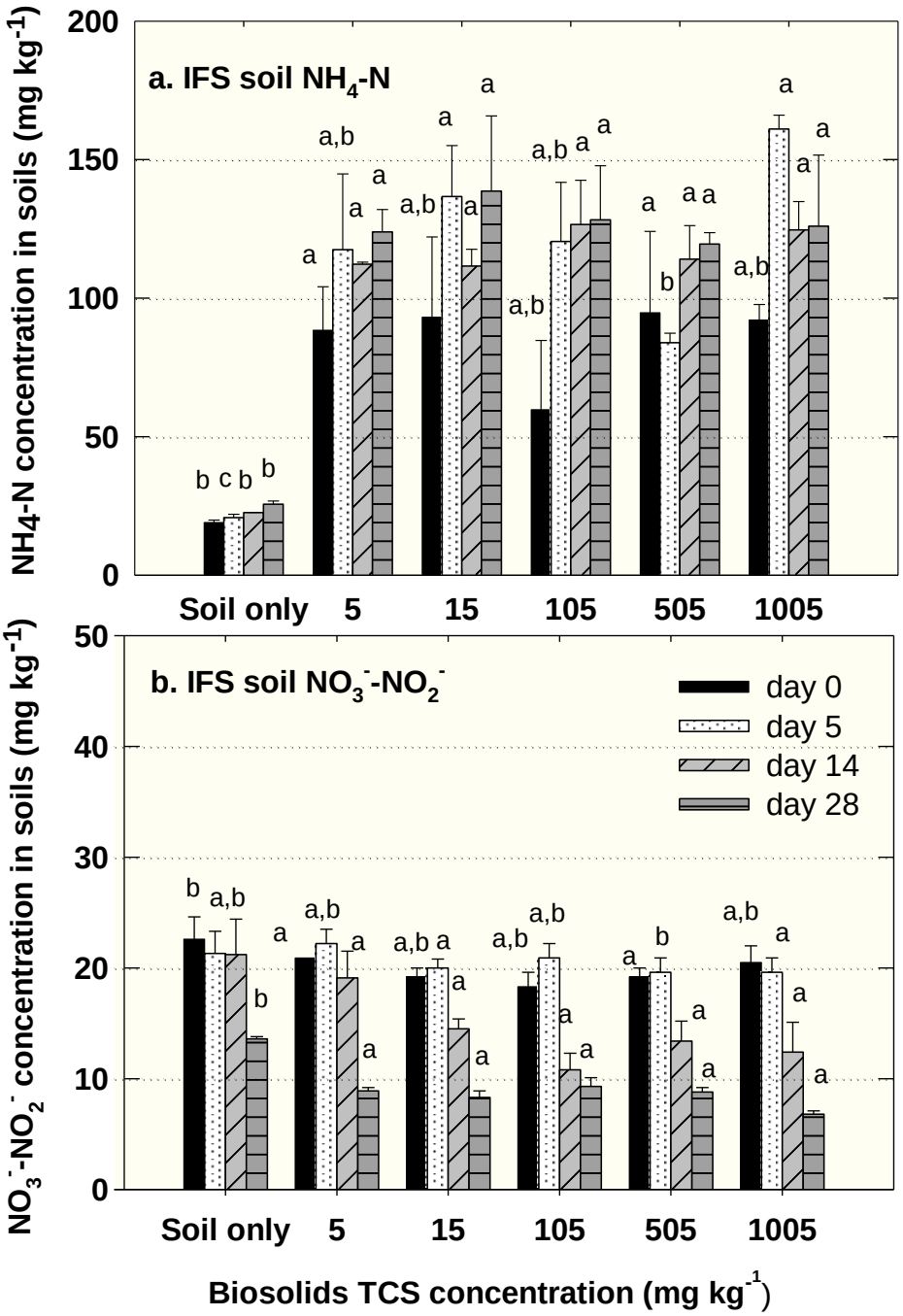


Figure 6-6. Mean (a) NH_4 -N and (b) $N0_3^-$-$N0_2^-$-N concentrations (n=3) as a function of biosolids TCS concentration and time (Days 0-28) in IFS soils (like letters indicate no significant difference between treatments), definitive test.

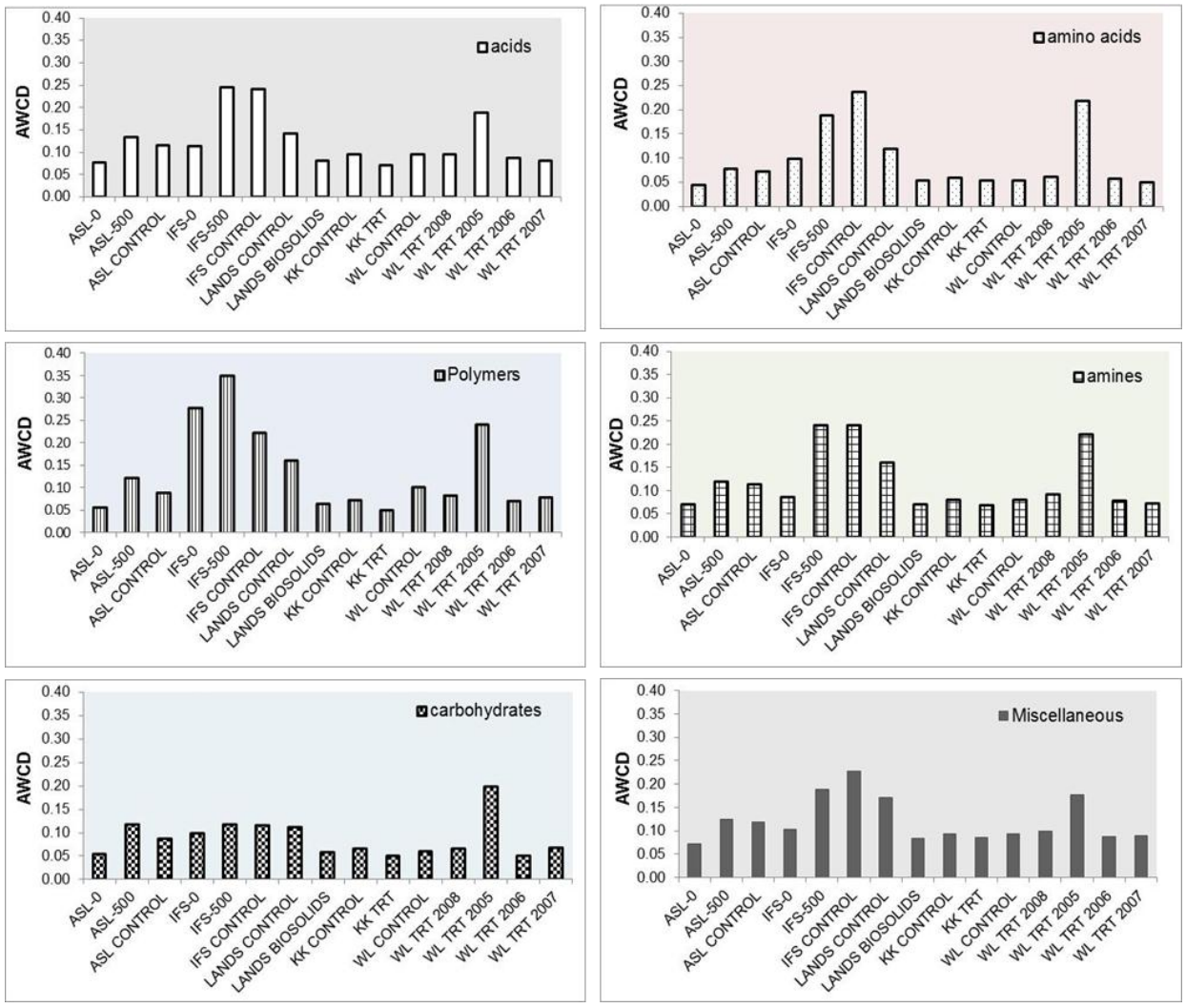


Figure 6-7. Average (n=3) well color development (AWCD) for the substrate types in the various soil samples developed 40 days after the incubation of the biolog plates.

CHAPTER 7
PLANT TOXICITY AND BIOACCUMULATION OF BIOSOLIDS-BORNE TCS

Background

Food chain bioaccumulation begins with chemical uptake by plants. Chemicals can enter plants by partitioning from contaminated soil solutions to the roots, and be translocated through xylem tissue (the xylem transports water from roots to leaves by transpiration). Chemicals may enter the vegetation directly by gas-phase and particle-phase deposition on leaves, enter the stomata and be translocated through phloem tissue (the phloem transports photosynthesis products from leaves to plant parts) (Simonich and Hites, 1995). Chemicals may also directly partition from soil particles to the root epidermis or the cortex and accumulate in the root (Leewen and Vermeire, 2007). The pathway of chemical uptake by plants depends on (a) chemical's properties such as water solubility, lipophilicity, and vapor pressure; (b) environmental conditions like temperature, organic carbon (OC) content of soil, and (c) plant species characteristics like surface area of the leaf and root mass (Simonich and Hites, 1995).

Root uptake from soil solution is a dominant pathway for hydrophilic compounds characterized by high water solubility, low vapor pressure and low log K_{ow} values (<4). Such compounds move from the outer to the inner root, and are translocated via the xylem to various plant parts (Paterson et al., 1994). However, the translocation to plant parts is limited by the TSCF (transpiration stream concentration factor). The TSCF is a function of chemical's K_{ow} and is the ratio of chemical concentration in the xylem sap to the chemical concentration in the solution surrounding the root. Lipophilic organic compounds (log K_{ow} > 4) such as organochlorine pesticides and polycyclic aromatic hydrocarbons do not enter the root xylem, but preferentially partition to the root

epidermis or the soil solids (Paterson et al., 1994; Schroll et al., 1994) and accumulate in the root. Petersen et al. (2003) proposed an alternate mechanism of plant contamination through rainfall induced splashing of soils when biosolids are applied. However, the authors later concluded that the added biosolids did not increase the risk of rain induced splashing and the proposed mechanism is insignificant (Petersen et al., 2003). Thus, plant uptake of a chemical depends on chemical properties (solubility, K_{ow}), plant characterstics (lipid content, leaf orientation, TSCF) and soil properties (organic carbon).

Hulster et al. (1994) found that three *cucurbitaceae* species (zucchini, pumpkin and cucumber) grown in soils accumulated and translocated high concentrations of highly lipophilic PCDD/Fs (Polychlorinated Dibenzo-p-dioxins and dibenzofurans). Uptake by cucumber occurred through particle-phase deposition. The exact mechanism of fruit (zucchini and pumpkin) accumulation was not elucidated, but Hulster et al. (1994) opined that uptake occurred through roots due to increased chemical availability in the presence of unique species-specific root exudates. Several studies (Farkas et al., 2009; Kumar et al., 2005; Boxall et al., 2006; Dolliver et al., 2007) reported plant accumulation and phytotoxic effects of antibiotics in un-amended and manure-amended soils. Triclosan (TCS) is an antimicrobial, and a common component of waste-water. At the wastewater treatment, TCS partitions mainly into biosolids. Biosolids-borne TCS can be land applied, and plants exposed to TCS may exhibit phytotoxicities or accumulate TCS in a manner similar to antibiotics. In a recent presentation, Kumar (2010) presented a ‘Kumar’s rule of 3’ to prioritize pharmaceuticals and personal care products for plant uptake studies. According to the rule, the chemicals that have a molecular

weight less than 450, log K_{ow} less than 3, number of H-bond donors less than three, and number of H-bond acceptors of less than six should be studied in field uptake studies for conducting risk assessment. Triclosan obeyed all the rules except that the log K_{ow} of TCS is greater than three.

Herklotz et al. (2010) studied TCS toxicity in hydroponic system, and suggested adverse effects of TCS on the germination of cabbage (*Brassica rapa var. pekinensis*) and Wisconsin fast plants (*Brassica rapa*) with a lethal concentration (LC) of 0.44 mg TCS L^{-1} water. Stevens et al. (2009) evaluated TCS effects on the germination and bioaccumulation in three wetland plants (*Sesbania herbacea, Eclipta prostrata*, and *Bidens frondosa*). A TCS concentration of 100 mg L^{-1} water reduced the rate of seed germination in two species (*S. herbacea* and *B. frondosa)*. Triclosan also accumulated in the shoots of *S. herbacea*, with a bioconcentration factor (BCF) of <10, and in roots of *B. frondosa* with a bioaccumulation factor (BAF) ranging from 53 to 101. A few unpublished studies (Hoberg, 1992; Schwab and Heim, 1997; cited in Reiss et al., 2009), and a published study (Liu et al., 2009) reported TCS plant toxicity in soil systems not amended with biosolids. Hoberg (1992) suggested adverse effects of TCS on cucumber, with a shoot length No Observed Effect Concentration (NOEC) of 0.096 mg kg^{-1} soil, and a Lowest Observed Effect Concentration (LOEC) of 0.28 mg kg^{-1} soil. Schwab and Heim (1997) evaluated TCS effects on cucumber seedling emergence, shoot length, root and shoot weight and reported a NOEC of 1 mg kg^{-1} soil. Liu et al. (2008) suggested a shoot height inhibition effect concentration (EC_{10}) of 6 mg TCS kg^{-1} soil for cucumber, and a LOEC of 10 mg kg^{-1} soil for rice root length. The studies described above suggested toxicity and bioaccumulation of TCS to plants grown in

hydroponics and in un-amended soil. Simlar to accumulation in un-amended soil, plants grown in biosolids-amended soil may accumulate TCS or experience TCS toxicity. Thus, the consumers eating plants grown in amended soil may be unknowingly ingesting TCS.

A few studies evaluated TCS plant accumulation following land-applied biosolids. Xia et al. (2010) found TCS concentrations <6.5 ng g^{-1} [Limit of quantitation (LOQ) of the instrument] in corn (*Zea Mays*) stover collected from long-term biosolids-amended (various rates) field soils (calcareous mine spoil; pH 7.8) with TCS soil concentrations ranging from 10 to 50 ng g^{-1}. The results suggest minimal TCS accumulation by corn, a monocotyledon (monocot). However, the toxicity and bioaccumulation of TCS might vary with the plant species, and characterstics of soil (Duarte-Davidson and Jones, 1996; Suter, 2007). Wu et al. (2010) evaluated the TCS uptake by a soybean (*Glycine max*), a dicotyledon (dicot), under green house conditions. The biosolids were spiked with TCS and amended (11 Mg ha^{-1}) to soil (sand, pH = 5.1, OC = 16 g kg^{-1}) to achieve a final TCS concentration of 70 ng g^{-1} amended soil. Results suggested TCS contamination of root tissue, and translocation to above-ground biomass. The plants, harvested at the full seed stage (110 d), accumulated TCS in the root (76.8 ± 3.1 ng g^{-1}), stem (136 ± 66 ng g^{-1}), leaf (120 ± 37 ng g^{-1}), and beans (12.6 ± 2.3 ng g^{-1}). The BCF values were measured at first harvesting (60d) and at full seed stage (110d). The BCF from soils to roots were 3 to 6.5 and 1 to 2 in the roots to leaves (Wu et al., 2010). The Wu et al. (2010) study was conducted in a spiked system, where TCS bioavailability and accumulation potential may differ from systems with inherent TCS (biosolids-borne). Further, the study utilized a single crop, and the biosolids had a solids content (19 g L^{-}

^{1}), which is much less than the solids content of biosolids (~300 g kg^{-1}) routinely used for land application in the U.S. Chemical transport potential and bioavailability is likely influenced by the type of biosolids applied to the soils (Edwards et al., 2009), with a greater chemical availability in biosolids with low solids content. In addition, a TCS degradation half-life of 100 d and the appearance of a metabolite [Methyl-TCS (Me-TCS)] (Chapter 4) of greater K_{ow} suggest that both TCS and Me-TCS could exist in soils for extended times following biosolids amendment. The metabolite may also possess phytoaccumulative potential.

The present study investigated the TCS toxicity and accumulation in multiple food crops [lettuce (*Lactuca sativa*), radish (*Raphanus sativus*), cucumber (*Cucumic sativus*), bahia grass (*Paspalum notatum)*], utilizing cake biosolids (commonly used in the U.S.), and a field-equilibrated soil. The soil received a single large application (228 Mg ha^{-1}) of biosolids that was incorporated and mixed with 15 to 20 cm of soil in 2008. The amended soil sampled in 2010 contained an inherent TCS concentration of ~1 mg kg^{-1} soil.

A chemical similar to TCS [i.e triclocarban, (TCC)] was minimally phyto accumulated by bahia grass (Snyder et al., 2011) grown in a biosolids-amended soil. We speculated that, because the partitioning coefficient of TCS (log K_{oc} = 4.26) is even greater than TCC (log K_{oc} = 3.82) (Agyin-Birikorang et al., 2010), TCS should partition more extensively to soil OC and be less phytoavailable. We hypothesize that biosolids-borne TCS has minimal phytotoxicity and phytoaccumulation potential. Our study objective was to evaluate the toxicity and plant uptake of TCS by several crops grown in a soil previously amended with biosolids. Further, the uptake of an expected metabolite

was assessed by analyzing the plants for Me-TCS. Plant toxicity and accumulation was investigated in vegetables grown for edible portions in temperature controlled growth rooms, and bahia grass grown in a greenhouse. In addition, empirical models and equations were used for bioaccumulation estimation and comparison with measured values.

Material and Methods

Soils and Chemicals

Triclosan (CAS No. 101-20-2; >99.9% purity) standard was purchased from United States Pharmacopeia (USP) (Maryland, USA). Internal standard ($^{13}C_{12}$-TCS), pyridine, BSTFA (bis (trimethylsilyl) trifluoroacetamide) +1% TMCS (trimethylchloro silane) were obtained from Sigma-Aldrich (St. Louis, MO). Methyl-TCS (Me-TCS) standard was purchased from Wellington laboratories (Shawnee Mission, KS). Potassium chloride (KCl), methanol (MeOH), acetone of HPLC grade or greater were purchased from Sigma Aldrich (St. Louis, MO), JT Baker (Phillipsburg, NJ) or Fisher Scientific (Atlanta, GA). The biosolids-amended soil was collected in 2010 from a landscaping site in Illinois last amended with biosolids (~228 Mg ha^{-1}) in 2008. Control soil samples were collected from an adjoining site (18 m away, same soil texture) with no known history of receiving land-applied biosolids or sludge. The collected soil samples were air-dried for 1 week and sent to our laboratory in ~18 L buckets. Select physico-chemical properties of the control and the biosolids-amened soils are presented in Table 7-1. Water soluble fertilizer was supplied as Miracle Grow [24-8-16; nitrogen (N)-phosphorus (P)-potassium (K)] obtained from a local store. The vegetable seeds were purchased from Burpee seeds (Warminster, PA). Oasis HLB extraction cartridges

(3 cm^3, 250 mg) for solid phase extraction (SPE) were purchased from Waters (Mississauga, ON, Canada).

Toxicty and Bioaccumulation Study Design

The study included 4 crops × 3 treatments × 4 replicates and a control (four replicates). Biosolids-amended and control soils were air-dried and sieved through a 2 mm sieve. Organic carbon was determined using the standard Walkley-Black method (Walkley and Black, 1934), assuming that 77% of the total OC was oxidized (Nelson and Somers, 1996). Soil ammonium concentration was determined by extracting with 2M KCl (USEPA, 1993b, Method 350.1). Approximately 2 kg (air dried) of control and biosolids-amended soils were weighed in 15 cm (diameter) pots (bulk density of 1.6 g cm^{-3}) lined with a cloth screen to hold the soil. After weighing, the soils were thinly spread in plastic trays to facilitate uniform application of treatments. The treatments included amended soil with inherent TCS (990 ng g^{-1}), and the same soil spiked with 5000 and 10,000 ng TCS g^{-1} soil. Treatment (Trt) spikes were supplemented to the inherent TCS concentration in the amended soil; thus, the final nominal concentrations were 990 (Trt 1), 5990 (Trt 2), and 10,900 ng TCS g^{-1} soil (Trt 3). The TCS concentrations utilized are much greater than normally expected in soils amended with typical biosolids applied at agronomic rates, but were useful to obtain a dose-response relationship and to maximize potential plant accumulation of TCS. The Trt 1 was unique as TCS occurred inherent to the biosolids and is expected to represent a real world (field-equilibrated) scenario. Methanol was utilized as a carrier solvent for spiking in the other treatments, and was allowed to evaporate in a hood as the soils equilibrated with the spikes for 48 h. After spiking and equilibration, the soil was packed into pots and brought to pot holding capacity. In a preliminary study, the pot holding capacity was

determined by saturating a pot with excess water, and allowing free drainage for 24 h. Water retained by the soil column after free drainage stopped was considered the pot holding capacity. Pot holding capacity (water content = 400 g kg^{-1} soil) is greater than the field capacity (water content = 300 g kg^{-1}) due to the textural discontinuity between the soil, the cloth liner, and drainage holes present in the pots.

The crops consisted of gourmet blend lettuce (*Lactuca sativa*), crimson giant radish (*Raphanus sativus*), bahia grass (*Paspalum notatum*), and baby cucumber (*Cucumic sativus*). Crop species represented monocots (bahia grass), dicots (lettuce, radish, cucumber), above-ground (lettuce, cucumber, radish leaves and bahia grass), and below-ground (radish) biomasses. All the crops were planted from seeds. For bahia grass, the recommended seeding rate of 10.9 Mg ha^{-1} was increased to 22 Mg ha^{-1} (equivalent to 2 g seeds per pot) to ensure sufficient and rapid soil surface coverage. Lettuce and radish were planted using an excess seeding rate of ~30 seeds per pot, and cucumber was planted using four seeds per pot. Seeds were covered with wet paper towels and misted every 3 to 4 h until germination. Growth conditions for vegetables included controlled chambers with a 24-h temperature maintained between 20 to 25°C, and a provision of 40-watt florescent bulbs for 15h photoperiod, corresponding to a light intensity of 7000 to 10,000 lux. Bahia grass was grown in a greenhouse, maintained at a suggested temperature range of 26 to 35°C (Newman et al., 2010). Nanopure water was utilized for irrigation when the water content in the pots was ≤80% of the pot holding capacity or when the soil surface appeared dry. On an average, plants received water once daily. Saucers placed beneath the pots collected

any leachate, and the collected water was immediately poured back on to the soils to avoid TCS leaching losses.

The first vegetable thinning occurred at a plant height of at least 2.5 cm, which was 5 d for radish, and 10 d for lettuce and cucumber. No thinning was required for bahia grass. During the first thinning, excess or weak plants were removed. The second thinning (15 d) reduced the number of plants to 6 to 10 in each pot for radish and lettuce, and to two plants for cucumber. A suggested solution for growing pot vegetables consisted of 30g of 20-20-20 (N-P-K) analysis water-soluble fertilizer mixed with 20 L of water (Stephens, 2009). A commercially available soluble fertilizer “Miracle grow” (N-P-K: 24-8-16) was deemed sufficiently close to the recommended dosage, and was prepared by mixing 4 g of solid fertilizer with 4 L of water. Ten days after the germination, control pots were provided with liquid fertilizer at weekly intervals until harvesting. Biosolids-amended soil was deemed to contain sufficient nutrients to permit healthy plant growth as illustrated by the physico-chemical properties of the amended soil (Table 7-1), and were not fertilized.

Bioaccumulation Field Study

We utilized paired soil and plant samples from a site in Fulton County, IL amended at high biosolids application rates for 16 years, resulting in total loads of 1084 to 1180 Mg biosolids ha^{-1}. The last biosolids application occurred in 2000, and the plant samples were collected in 2001, 2002, 2004 and 2006. Plant samples included three field replicates for each crop and for each year. The soil and plant samples were collected, air-dried and stored in a cool and dry place at room temperature until they were sent to us in 2010. The available samples included a monocot (corn leaves) and a dicot

(soybean grains). The field samples addressed the possibility of long-term TCS and Me-TCS plant bioaccumulation in a monoct and a dicot.

Plant Harvesting and Sample Preparation

Harvesting of radish and lettuce plants occurred 40 d after germination. Bahia grass was harvested 60 d after the initial soil preparation, as the grass required reseeding due to poor initial crop cover. Harvested plants were washed with nano-pure water, fresh weights determined, and biomass (leaves and roots) was separated, dried separately at 50°C to constant weight, and the dried tissue ball milled. Soils were collected from each treatment and replicate immediately before and after the study, and dried to constant weight at 50°C.

A sample extraction technique for TCS was modeled after that described by Snyder et al. (2011) with some modifications. The dried soil and plant tissues (0.5-1 g) were loaded into 25 mL glass centrifuge tubes and extracted with 10 mL of MeOH+acetone (50:50, v/v). The extraction was performed on a platform shaker for 18 h, followed by 60 min of sonication in a water bath (Branson 2210, Danbury, CT; temp. 40°C, 60 sonications min^{-1}). Suspensions were centrifuged at 800 x g, and the supernatant transferred to 20 mL glass scintillation vials. The extraction procedure was performed twice and the extracts were combined, dried (under N_2), reconstituted in MeOH and transferred to microcentrifuge tubes. The microcentrifuge tubes were centrifuged at 18,000 × g for 30 min, and supernatant transferred to GC vials, and again dried (under N_2). Dried extracts were then reconstituted in MeOH, followed by cleanup using Oasis HLB SPE cartridges (Chu and Metcalfe, 2007). After the cleanup, the extract was transferred to amber GC vials, an internal standard $^{13}C_{12}$-TCS (50 ng mL^{-1}) was added, and the extracts dried.

Derivatization was performed according to Shareef et al. (2006) with slight modification. Briefly, the dried extracts were reconstituted in a mixture of 4:1 of derivatization agent (BSTFA +1% TMCS) and a solvent (pyridine), vortexed for 10 s, and heated in a dry bath for 1h. The samples were then transferred to fresh GC vials with glass inserts and Teflon lined caps. A set of plant samples from the control soil was spiked with TCS and Me-TCS, and subjected to the same extraction procedure. The average percent recoveries of the spiked TCS and Me-TCS in the plants were >90%.

Instrument Analysis and Quantitation

The samples obtained after the derivatization step were analyzed by splitless injection (5 µL) on a Varian 4000 Gas Chromatograph equipped with a Restek Rxi-5Sil column coupled with a Varian 4000 MS/MS. The GC/MS conditions were modeled after Balmer et al. (2004) with some modifications.The GC column temperature was initially held at 100ºC for 1 min, and then increased to 310ºC at a rate of 10ºC min^{-1} with no final hold time. The carrier gas was helium, the ion trap, manifold and transfer line temperatures were 200, 80 and 270ºC, respectively, and the ionization source was internal/electron ionization. Data acquisition monitored two fragment ions for each compound. The ion masses were m/z of 345/347 for TCS trimethylsilylether, 302/304 for Me-TCS. The internal standard $^{13}C_{12}$ TCS-trimethylsilyl ether mass was monitored at 357/359. The samples ran for 22 min, with an average retention time of 14.8 min for all compounds. For quantification, an 8-point internal calibration curve was generated in the TCS concentration range of 1 to 1000 ng g^{-1}, average R^2 >0.999. The detection limits were determined at a signal to noise (S/N) ratio of >10. The limit of detection (LOD) was 0.28 ng g^{-1} and limit of quantitation (LOQ) was 1 ng g^{-1} for both TCS and Me-TCS in the plant tissues. The LOD and LOQ values were calculated as 3-fold and

10-fold, respectively, the standard deviation in the signal from multiple runs of the lowest calibration standard (Signal/Noise >10) (USEPA, 1984). The details of detection limits and recoveries are provided in the Appendix C.

Results and Discussion

Plant Biomass Yields

Assessment of adverse effects of biosolids-borne TCS on plant growth was by visual examination and by measuring plant biomass yields. Figures 7-1 and 7-2 are representative pictures of lettuce, radish and bahia grass, and show no obvious differences in the health of plants exposed to various soil TCS concentrations. Height and vigor appeared to be superior in all the plants grown in treated soils (biosolids amended) than in the control soil irrespective of the TCS concentration; i.e. TCS spiking did not adversely affect the appearance of any plant species. Our observation was consistent with reports of better growing grass (*Poa pratensis*) in fields of the biosolids-amended soil than the control soils utilized in our study. Differences in field growth were not quantified, but were visually obvious (Kuldip Kumar, personal communication, 2011).

Biosolids addition significantly increased the lettuce yields (fresh and dry wt.) in Trt 1 relative to the control. Lettuce growth in Trt 2 and Trt 3, however, was inhibited and plant fresh weights were significantly smaller than in Trt 1 (Table 7-2). The observed difference appeared at a concentration of 5990 ng TCS g^{-1} amended soil. The lettuce dry weights were not adversely affected at any concentration used.

Fresh weights of bahia grass were significantly greater in all of the TCS treatments than in the controls, suggesting enhancement of plant growth by biosolids or TCS addition (Table 7-2). Fresh weights of radish root in control, Trt 1 and Trt 2 were

not significantly different, but the weight in Trt 3 was significantly smaller than all other treatments (Table 7-2). The data suggest some inhibition of radish root growth at an amended soil concentration of 9150 ng TCS g^{-1}. Thus, using fresh biomass yields as the criteria, an amended soil concentration of 9150 ng TCS g^{-1} (Trt 3) can be regarded as the LOEC for radish root growth. For lettuce, Trt 1 (4570 ng g^{-1}) is regarded as the LOEC, and for bahia grass the NOEC is at least the highest TCS concentration (9150 ng g^{-1}) utilized in the study. In contrast, dry biomass yield data suggest essentially no inhibition effect of spiked TCS. Only the radishes grown in Trt 2 had significantly smaller yields, but the yields were similar to the control, suggesting no overall growth inhibition (Table 7-2). Greater yield as a result of biosolids treatments was the only significant effect. Thus, based on dry biomass yields, NOEC for all the plants is at least 9150 ng g^{-1} (Trt 3).

Most TCS toxicity studies conducted in soils are either un-published or limited to TCS effects on germination rates [Stevens et al. (2009); Hoberg (1992) Schwab and Heim (1997)]. Only Liu et al. (2009) quantified TCS toxicity by growing cucumber and rice plants exposed to a range of TCS concentrations (1-300 mg kg^{-1}). The authors suggested a shoot height inhibition effect concentration of 6 mg TCS kg^{-1} soil for cucumber and a LOEC of 10 mg TCS kg^{-1} soil for rice root length. Comparisons of our study results with those of Liu et al. (2009) are confounded because the latter study involved no biosolids. Nevertheless, our estimated LOEC and NOEC values are within the range reported in the Liu et al. (2009) study. Data suggest that fresh biomass yields of lettuce and radish are adversely affected at TCS concentrations ranging from 5990 to 10990 ng g^{-1} amended soil, but no TCS concentration affected the dry biomass yields.

Cucumber plants utilized in our study behaved different than the other crops. Due to delay in fruiting of the plants, we allowed the cucumber to grow for a longer time. The cucumber variety chosen for the study was cross-pollinated, but the closed growth rooms had no facility for bee pollination. Thus, we hand pollinated the female flowers when they appeared, but were apparently unsuccessful as few fruit formed and plants started to senesce before fruiting. Overall, cucumber growth was poor in all the treatments and is unexplained. However, we do not believe the severe senescence and poor fruit growth was a biosolids or TCS treatment effect because similar problems occurred with plants grown in the control treatment. Due to problems in growing the cucumber plants and poor fruit yield, bioaccumulation data were of limited use. A few fruit were extracted to quantify the bioaccumulation. The TCS and Me-TCS concentrations were below the detection limit (<LOQ of 1 ng g^{-1}) of the instrument in all the treatments, suggesting minimal accumulation based on the limited samples.

Uptake in the Above-Ground Biomass (Lettuce, Radish and Bahia Grass Leaves)

Uptake assessment occured through the calculation of bioaccumulation factors (BAFs) expressed as the ratio of TCS concentration in the plant to the TCS concentration in the soil in which the plant grew. Triclosan accumulated in the above-ground biomass of all the crops, but the extent of accumulation was crop specific, and varied with the amended soil TCS concentration. The greatest TCS concentration (897 ± 117 ng g^{-1}) accumulated in lettuce grown in amended soil with initial TCS concentration of 10145 ± 1042 ng g^{-1} (Trt 3). The average BAF values, irrespective of the TCS treatments, were 0.04 ± 0.04 for lettuce leaves (Table 7-3), and 0.004 ± 0.002 for the radish leaves (Table 7-4). The BAF values in Trt 3 for both lettuce and radish leaves were significantly greater ($p<0.001$) than in Trt 1 and 2. Bahia grass leaves

accumulated no detectable TCS (<LOQ of 1 ng g^{-1}), resulting in an estimated BAF of <0.0001 for all the treatments (Table 7-5). On an average, lettuce leaves experienced 10-fold greater TCS accumulation than the radish leaves and ~40-fold more than bahia grass. Duarte-Davidson and Jones (1996) suggested that physico-chemical properties of organic chemicals and plant species affect the accumulation of polar and non-polar compounds. Schroll and Scheunert (1992) suggested greater bioaccumulation of hexachlorobenzene in dicots (lettuce and carrots) than in monocots (like maize, oats and barley), but did not suggest an explanation. Our study included both dicots (radish, lettuce) and monocots (bahia grass), and TCS accumulation was crop specific. Greater TCS accumulation (0.004-0.43) occured in the dicots (radish and lettuce) than in the monocot (bahia grass) (Tables 7-3 through 7-5). The 10-fold difference in BAF value between the radish and lettuce leaves suggest variable accumulation in plants due to different plant characterstics (e.g., lipid content). Suter (2007) suggested that water and lipid content in plant tissues may affect the contaminant uptake by plants. Bioaccumulation factors ≤ 0.01 are typically considered inconsequential (O'Connor, 1996). Thus, the data suggest inconsequential accumulation in radish (BAF = 0.004) and bahia grass leaves (BAF = <0.001) but some accumulation in lettuce leaves (BAF = 0.04) grown in soil spiked with exceptionally high TCS concentrations. The bioaccumulation potential increased with TCS concentration

Uptake in the Below-Ground Biomass (Radish Root)

Radishes grown in soil spiked with Trt 3 (TCS concentration = 9905 ± 702 ng g^{-1}) accumulated the greatest amount of TCS (9150 ± 1187 ng g^{-1}), corresponding to a BAF value of 0.93 ± 0.14 (Table 7-6). The average BAF in radish root (0.43 ± 0.38), was 10-fold the BAF (0.04 ± 0.04) in lettuce, and 100-fold the BAF (BAF = 0.004) in radish

leaves. Figure B-1 (Appendix B) compares the BAF values in the radish root and lettuce leaves at various TCS concentrations. The data suggest significantly ($p<0.05$) greater TCS accumulation in below-ground biomass (i.e radish roots) than in above-ground biomass (i.e. lettuce leaves). Triclosan plant concentrations and BAFs increased with TCS concentration in amended soil (Trt 1 to 3), but the accumulation in Trt 3 was significantly greater ($p <0.001$) than in the Trt 1 and 2 (Table 7-6).

The above-ground and below-ground plant tissues analyzed for TCS were simultaneously analyzed for Me-TCS, and the Me-TCS concentrations were below the detection limit (1 ng g^{-1}) for all the treatments.

Bioaccumulation Field Study

Concentrations in the soils collected from soybean fields were relatively constant (51-56 ng TCS g^{-1} soil) in various years (Table 7-7), whereas soil concentrations in the corn leaf fields were variable (43-100 ng TCS g^{-1} soil) (Table 7-8). The plant samples also displayed variable TCS accumulation (Table 7-7 and 7-8). The monocot (corn leaf) accumulated less TCS than soybean (dicot) grain. Soybean grain BAF values varied widely across years with a greater accumulation (average BAF = 0.16 ± 0.15) in 2002 than 2001 (average BAF = 0.06 ± 0.09) (Table 7-7). Two of the three soybean samples from 2001 had BAF values <0.01, representing minimal accumulation (Table 7-7). The BAF values for all the corn leaf samples collected in 2006 were <0.01(Table 7-8), representing inconsequential accumulation. Some corn samples collected in 2004 accumulated non-negligible amounts of TCS, with an average BAF value of 0.07 ± 0.05 (Table 7-8), but variability among the samples was large. Methyl-TCS concentrations were non-detectable in all the field grown plants. Collectively, the field samples represented longer-term measures of TCS phytoavailability than the growth chamber

studies. The field results were highly variable, but, were generally consistent with the growth chamber and greenhouse data. Thus, TCS accumulation was slightly greater in the dicot (soybean) than the monocot (corn) plants. The maximum BAF value calculated for the field grown plants was 0.16.

Model-Predicted TCS Concentrations in Plant Tissue

The measured BAFs in various plants can be compared to BAFs predicted by a Biosolids-Amended Soil: Level 4 (BASL4) computer model (Webster and Mackay, 2007). The model parameters were chosen based on the physico-chemical properties of TCS and soils in which the plants were grown. The model predicted different BAFs for above-ground and below-ground biomasses and grass, but failed to distinguish between the radish and lettuce leaves. Further, the model predicts no significant difference among BAFs at various soil TCS concentrations. The BASL4 predicted BAFs (dry wt.) were 23 ± 1.2 for radish root, 6.1 ± 0.32 for lettuce and radish leaf, and 10.8 ± 0.45 for bahia grass tissue (Tables 7-3 through 7-6). Modeled (using BASL4) BAF values over predicts bioaccumulation in all plants and plant parts, and suggest significant TCS bioaccumulation. In contrast, measurement suggest that plant uptake of biosolids-borne TCS is generally minimal and strongly influenced by soil TCS concentrations. The only similarity between the modeled and measured values is the prediction of greater TCS accumulation in below-ground (i.e roots) than the above-ground (i.e leaves) biomass.

Plant accumulation of organic chemicals has been predicted using empirical equations and the accumulation is believed to depend heavily on chemical partitioning coefficient (K_{ow}) with no consideration of soil characterstics or chemical exposure times. Such equations were previously utilized for risk estimation of various chemicals by USEPA. Travis and Arms (1988) derived an equation (equation 7-1) to describe

bioaccumulation of 29 hydrophobic chemicals including pesticides and dioxins in above-ground plant biomass (multiple crop species). The equation was derived for pesticides with a log K_{ow} ranging from 1.75 and 6.15. The log K_{ow} of TCS is 4.8 and thus, TCS bioaccumulation may be expected to follow the prediction.

$$\log Up = -0.578 \log K_{ow} + 1.588 \quad (7\text{-}1)$$

Where Up : Uptake coefficient (equivalent to BAF), and the numerical values are regression parameters; using log K_{ow} of TCS as 4.8,

Calculated uptake coefficient (Up) for TCS was 0.065 for all the above-ground biomass. The estimated BAF value exceeded the measured BAF values for lettuce, radish and bahia grass leaves and underestimated BAF for radish root. Suter (2007) opined that the accumulation of chemicals is less likely to follow the empirical model predictions if the chemical, soil and plant species are different than the ones used to derive the model. A definitive prediction could only be made using the empirical models if the models are validated to a wide range of chemicals, soils and plants. Thus, the models and empirical equations (Equation 7-1) overpredict the phytoaccumulation of TCS, and the estimates should be used with caution.

Mechanism of Bioaccumulation and Comparison with Other Studies

The BAF values obtained in our study suggest some accumulation in the below-ground biomass (roots), and translocation to the above-ground biomass (leaves), but accumulation was greater in the root crop than in above-ground biomass. Mechanistic explanations for the difference are not known. The Henry's constant (H) for TCS at 25°C is small (10^{-9}), so vapor phase movement is likely minimal. Rather, movement in the aqueous phase and/or direct partitioning from soil to lipid rich plant tissue is likely critical

(Trapp and McFarlene, 1995). Trapp and McFarlene (1995) and Wild and Jones (1992) suggested that lipophilic compounds with log K_{ow} >4 (like TCS) have a limited transport across the endodermis membrane through soil solution. Further, Trapp and McFarlene (1995) suggested that non-ionized chemicals entering the plants through root or leaves move in the plant with water flow in the xylem, but TCS movement with water is limited because of the low water solubility.Thus, TCS is expected to move (partition) from soil solids to lipid enriched outer root parts and accumulate there. The abovementioned concept is partially true in our study as we observed a greater accumulation in the radish roots than in the leaves suggesting a direct partitioning to roots, but detection of small levels of TCS in the leaves suggests that at least some translocation occurred from the root to the leaves even when the expectation of movement was minimal.

Wu et al. (2010) evaluated the TCS uptake by soybean (*Glycine max*) from a soil (sand, pH = 5.1, OC = 16 g kg^{-1}) amended with spiked biosolids (11 Mg ha^{-1}) to obtain a final TCS concentration of 70 ng g^{-1} amended soil. Data suggested plant TCS accumulation, and the range of BAFs (1-6.5) was greater than the range of BAFs obtained in our greenhouse and field samples (<0.0001 to 0.43). Difference in biosolids type and application rates likely caused different bioaccumulation in the two studies. The biosolids utilized in the Wu et al. (2010) study contained much less solids content (19 g L^{-1}) than biosolids (300 g kg^{-1}) used in our studies; lower solids content would mean a greater relative TCS aqueous phase concentration, greater bioavailability and greater plant accumulation. In addition, the two studies differed in the biosolids loading rates. Wu et al. (2010) applied a biosolids load of 11 Mg ha^{-1}, compared with loading rate of 228 Mg ha^{-1} used herein, which may have affected TCS bioavailability. Trapp

and McFarlene (1995) suggested that high biosolids application increases the OC content of soils and reduces TCS uptake by roots and translocation via xylem; thus, reducing the extent of plant accumulation.

Previous studies also suggested variable accumulation of antimicrobials and antibiotics (Boxall et al., 2006; Dolliver et al., 2007) in various plant parts. Boxall et al. (2006) investigated the accumulation of antibiotics (concentration = 1 mg kg^{-1} soil) in soil (loamy sand), and found that some of the antibiotics accumulated more in the body of the carrot, whereas some accumulated more in the outer peel of the carrot. Similarly, Dolliver et al. (2007) evaluated the accumulation of antibiotic sulfamethazine in potatoes grown in silt loam soil (pH: 7.0; OC: 27 g kg^{-1}). Sulfamethazine accumulated more in the outer skin of the tuber than the center of the potato. The molecular weight, pK_a and structure of TCS is similar to the antimicrobials (trimethoprim, sulfadiazine) described in Boxall et al. (2006), and was expected to accumulate in a similar manner. Thus, similar to our study, the abovementioned studies suggest variable accumulation in various plant parts.

Degradation in Soils

The percent disappearance of TCS was calculated based on the TCS concentrations measured in soil immediately before and after the greenhouse study (Table 7-8 to 7-10). The data suggest TCS disappearance in all treatments utilized in our study. The soil used to grow lettuce and radish was collected after 40 d, whereas soil sample used to grow bahia grass was collected after 60 d. Previous TCS degradation data (Chapter 4) and values obtained from the literature suggest a primary degradation half-life (time for 50% disappearance) of TCS of ~100 d (across soil types), and formation of a metabolite (Me-TCS). Thus, after 40-60 d of plant growth in the soil,

we expected ~20-25% degradation/disappearance of TCS. Data (Table 7-9), however, suggest a maximum disappearance of 15%. The estimated percent disappearance was variable (shown by large standard errors associated with the data) and did not increase with TCS concentration (Tables 7-8 through 7-10). Further, no metabolite (Me-TCS) was detected in the system suggesting that either the metabolite (Me-TCS) appeared and became non-extractable with time, or the Me-TCS did not form due to reduced bioavailability of TCS. Reduction in TCS bioavailability may occur because high biosolids application (228 Mg ha^{-1}) is expected to favor strong interaction of TCS with OC matrix (Alexander, 1995). Wild and Jones (1991) observed slower degradation of diethylhexyl phthalate (DEHP) in soil amended with high biosolids application (90 Mg ha^{-1}), than in same soil amended at agronomic rates (22 Mg ha^{-1}). These authors opined that the high biosolids dosages either decreased the DEHP bioavailability or created partial anaerobic soil conditions that slowed the degradation. Our study may also possess anaerobic microsites due to high water content (450 g kg^{-1} soil) of the soils. Appearance of Me-TCS and subsequent conversion to non-extractable fraction with time may also occur, similar to previous observations (Chapter 4). Further, Me-TCS was not detected in any of the plant parts, consistent with the absence of detectable Me-TCS in biosolids and soils utilized herein.

Comparison with Real World TCS Concentrations

The spiked TCS concentration range utilized in the present study was 990-10990 ng g^{-1} amended soil. The TCS concentration in Trt 1 (~990 ng g^{-1} amended soil) is equivalent to an agronomic application rate (22 Mg ha^{-1}) of biosolids containing a TCS concentration of 16000 ng g^{-1} (mean concentration from TNSSS, 2009) for at least 6 years assuming no TCS losses. The Trt 1 was unique among other treatments as TCS

occured inherent to the biosolids (no spike additions), and is expected to represent a real world scenario.

Soils with high biosolids applications resulting in high TCS concentrations similar to Trt 1 utilized herein are sometimes used in real world for landscaping purposes. In fact, a biosolids application of 228 Mg ha^{-1} occurred on the landscaping field-equilibrated soil utilized herein. Landscaping soils similar to the one mentioned above may be used for growing grass, but likely not for vegetables. Thus, the vegetables grown in the landscaping soil represents a worst-case estimate. Treatments 2 (5990 ng g^{-1}) and Trt 3 (10990 ng g^{-1}) were included in the study design to obtain a dose-response relationship. Biosolids containing a TCS concentration of 133 mg TCS kg^{-1} (highest biosolids concentration from TNSSS, 2009), applied for 10 years and assuming no TCS loss, results in a TCS concentration of 13300 ng TCS g^{-1} amended soil. Thus, the TCS concentration in Trt 3 was unrealistic. The Trt 1 and 2 are more reasonable, but still represent applications of representative biosolids at agronomic rates for up to 6-30 years, and assume no TCS loss over time. The BAFs were reestimated by excluding Trt 3. The resulting average BAF values were 0.02 for lettuce leaves and 0.26 for radish roots (Table B-1, Appendix B), as compared to the 0.04 and 0.43 with all treatments included.

Further, plant accumulation may differ when TCS is inherent vs spiked into the biosolids. Langdon (2010, personal communication) found different dissipation/ degradation rates of TCS when TCS occurs inherently vs when TCS is spiked into biosolids. Difference in dissipation rates may occur as the spiked TCS is likely to sorb to the outer portion of the biosolids and therefore be more available to microbes in the

presence of O_2 causing faster TCS degradation. Different dissipation rates between the inherent vs spiked systems might have created variable bioavailabilities in our Trt 1 (inherent TCS), Trt 2 and 3 (spiked TCS) causing different accumulation. A relationship between accumulation and chemical occuring in spiked vs inherent form could not be established, as our study was confounded by the different concentrations in the two treatments; however, accumulation data in plants exposed to Trt 1 are likely more accurate.

Thus, while the TCS concentrations utilized in our study were unrealistic for typical biosolids applied at agronomic rates, results may mimic phytoavailability following long-term biosolids applications, or exceptionally high application rates. Results suggest that inherent or spiked biosolids-borne TCS applied at excessively high concentrations and applied for multiple years will not result in significant accumulation of TCS in above-ground plant parts; however some accumulation is possible in root crops like radish.

Table 7-1. Selected physico-chemical properties of the soils and biosolids used in the present study.

Soils	Organic carbon	Mehlich-1 P	NH_4^+-N	pH (1:1)	EC	Texture
	g kg^{-1}	——mg kg^{-1}——			µS cm^{-1}	
Control	30 ± 0.4	3.2 ± 0.2	8.9 ± 0.2	6.0 ± 1.7	512 ± 25	Clay loam
Filed biosolids amended	84 ± 0.3	790 ± 12	27 ± 1.4	7.1 ± 0.0	506 ± 2.3	Silty clay loam

Table 7-2. Yield of plant parts of three plant species represented by fresh weights (g) in the control and biosolids-amended treatments (same letters represent no statistical difference among treatments).

Plant type	Amended- soil TCS concentration (ng g^{-1}) (Treatment)	Fresh weight (g)	Dry weight (g)
Lettuce			
	Control	25.7 ± 0.90 c	6.17 ±1.00 a
	990 (Trt 1)	79.7 ± 6.77 b	13.2 ± 4.94 b
	5990 (Trt 2)	55.6 ± 9.23 a	13.7 ± 4.15 b
	10990 (Trt 3)	55.9 ± 6.28 a	15.2 ± 3.69 b
Bahia grass			
	Control	12.5 ± 1.07 a	0.10 ± 0.02 a
	990 (Trt 1)	42.2 ± 3.70 b	7.82 ± 0.61 b
	5990 (Trt 2)	30.2 ± 7.23 b	4.54 ± 1.26 b
	10990 (Trt 3)	27.7 ± 9.83 b	6.41 ± 4.78 b
Radish			
	Control	22.5 ± 6.31 a	3.70 ± 0.64 a
	990 (Trt 1)	26.9 ± 9.70 a	7.98 ± 0.97 b
	5990 (Trt 2)	17.3 ± 2.60 a	4.55 ± 0.75 a
	10990 (Trt 3)	11.1 ± 2.10 b	7.56 ± 3.16 b

Table 7-3. Measured TCS concentrations (average; n = 3 or 4 and SD) and bioaccumulation factors (BAF) and BASL4 model calculated BAFs in the lettuce leaves grown in a biosolids- amended silty clay loam soil (same letters represent no statistical difference among treatments).

Type of application (Treatment)	Measured biosolids-amended soil concentration	Measured plant tissue concentration	Mean BAF dry wt.	Calculated BAF (BASL4)
	——————ng g^{-1}——————			
Inherent (Trt 1)	1015 ± 94.2	10.7 ± 2.1	0.01 ± 0.00 a	5.92 a
Spiked (Trt 2)	4570 ± 448	119 ±14	0.03 ± 0.00 b	6.47 a
Spiked (Trt 3)	10145 ±1043	897 ±117	0.09 ± 0.01 c	5.93 a
		Average	0.04 ± 0.04	6.10 ± 0.32

Table 7-4. Measured TCS concentrations (average; n = 3 or 4 and SD) and bioaccumulation factors (BAF) and BASL4 model calculated BAFs in the radish leaves grown in a biosolids amended silty clay loam soil (same letters represent no statistical difference among treatments).

Type of application (Treatment)	Measured biosolids-amended soil concentration	Measured plant tissue concentration	Mean BAF dry wt.	Calculated BAF (BASL4)
	——————ng g^{-1}——————			
Inherent (Trt 1)	989 ± 68.4	<1	<0.001 a	5.92 a
Spiked (Trt 2)	4531 ± 439	16.9 ± 2.20	0.004 ± 0.00 a	6.47 a
Spiked (Trt 3)	9905 ± 702	62.8 ± 21.3	0.006 ± 0.00 b	5.93 a
		Average	0.004 ±0.002	6.10 ± 0.32

Table 7-5. Measured TCS concentrations (average; n = 3 or 4 and SD) and bioaccumulation factors (BAF) and BASL4 model calculated BAFs in the Bahia grass grown in a biosolids-amended silty clay loam soil (same letters represent no statistical difference among treatments).

Type of application (Treatment)	Measured biosolids-amended soil concentration	Measured plant tissue concentration	Mean BAF dry wt.	Calculated BAF (BASL4)
	——————ng g^{-1}——————			
Inherent (Trt 1)	977 ± 68.4	<1	<0.0001 a	10.9 a
Spiked (Trt 2)	4492 ± 395	<1	<0.0001 a	11.2 a
Spiked (Trt 3)	9221 ± 832	<1	<0.0001 a	10.3 a
		Average	<0.0001	10.8 ± 0.45

Table 7-6. Measured TCS concentrations (average; n = 3 or 4 and SD) and bioaccumulation factors (BAF) and BASL4 model calculated BAFs in the radish root grown in a biosolids-amended silty clay loam soil (same letters represent no statistical difference among treatments).

Type of application (Treatment)	Measured biosolids-amended soil concentration	Measured plant tissue concentration	Mean BAF dry wt.	Calculated BAF (BASL4)
	——————ng g^{-1}——————			
Inherent (Trt 1)	989 ± 68.4	101 ± 18.7	0.10 ± 0.02 a	22.4 a
Spiked (Trt 2)	4531 ± 439	1244 ± 132	0.27 ± 0.04 a	24.5 a
Spiked (Trt 3)	9905 ± 702	9150 ± 1187	0.93 ± 0.14 b	22.4 a
		Avearge	0.43 ± 0.38	23.1 ± 1.21

Table 7-7. Bioaccumulations factors (BAF) [average (n=3) and standard error (SE)] obtained in grains of soybean grown in field soils.

Soybean grain Year collected	TCS soil concentration (ng g^{-1})	TCS plant concentration (ng g^{-1})	BAF (dry wt.)
2001	51.8	<1†	<0.01
	52.8	<1†	<0.01
	55.0	10.1	0.18
		Average ±SE	0.06 ± 0.09
2002	51.0	<1†	<0.01
	54.9	17.1	0.31
	55.8	10.1	0.18
		Average ±SE	0.16 ± 0.15

Table 7-8. Bioaccumulations factors (BAF) [average (n=3) and standard error (SE)] obtained in leaves of corn grown in field soils.

Corn leaves Year collected	TCS soil concentration (ng g^{-1})	TCS plant concentration (ng g^{-1})	BAF (dry wt.)
2004	55.2	6.62	<0.01
	100	<1†	<0.10
	43.8	5.38	0.12
Average ±SE			0.07 ± 0.05
2006	53.1	<1†	<0.01
	52.0	<1†	<0.01
	55.0	<1†	<0.01
Average ±SE			<0.01

† The BAF in non-detects were calculated by assuming a TCS soil concentration of ½ LOQ (1 ng g^{-1}) of the instrument

Table 7-9. Measured TCS soil concentrations in lettuce treatments (means; n = 3 or 4 and SD) before and after the plant accumulation study and the corresponding % disappearance.

Type of application (Treatment)	Initial TCS soil concentrations	Final TCS soil concentrations	% disappearance
	——————ng g^{-1}——————		
Inherent (Trt 1)	1015 ± 94.2	873 ± 95.2	13 ± 10
Spiked (Trt 2)	4570 ± 448.4	4372 ± 438	3.2 ± 17
Spiked (Trt 3)	10145 ±1043	8826 ± 875	12 ± 5.9

Table 7-10. Measured TCS soil concentrations in radish treatments (means; n = 3 or 4 and SD) before and after the plant accumulation study and the corresponding % disappearance.

Type of application (Treatment)	Initial TCS soil concentrations	Final TCS soil concentrations	% disappearance
	——————ng g^{-1}——————		
Inherent (Trt 1)	989 ± 68.4	874 ± 93	11 ± 10
Spiked (Trt 2)	4531 ± 439	4224 ± 386	7 ± 6
Spiked (Trt 3)	9905 ± 702	8422 ± 472	15 ± 9

Table 7-11. Measured TCS soil concentrations in bahia grass treatments (means; n = 3 or 4 and SD) before and after the plant accumulation study and the corresponding % disappearance.

Type of application (Treatment)	Initial TCS soil concentrations	Final TCS soil concentrations	% disappearance
	——————ng g^{-1}——————		
Inherent (Trt 1)	977 ± 68.4	956 ± 43.4	1.8 ± 8.0
Spiked (Trt 2)	4492 ± 395	4080 ± 330	8.8 ± 8.9
Spiked (Trt 3)	9221 ± 832	8654 ± 613	5.8 ± 8.0

Figure 7-1. Representative photos of lettuce (A and B) and radish (C and D) plants that compare plant growth in the control and treatments.†

†Control: no biosolids, Trt 1(no spike) = TCS spiked concentration of 990 ng g^{-1}
†Trt 2 (5 ppm TCS) = TCS spiked concentration of 5990 ng g^{-1}

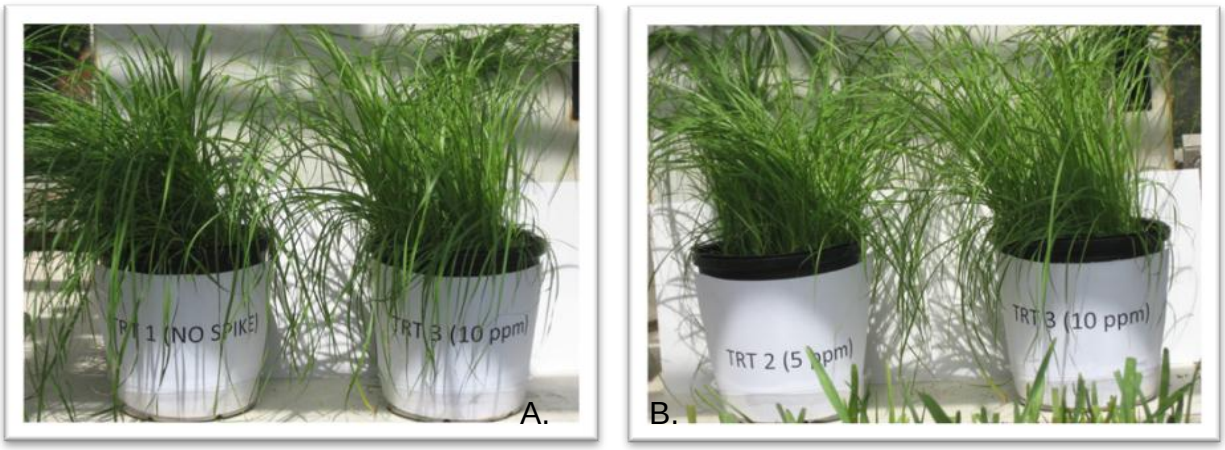


Figure 7-2. Representative photos of bahia grass that compare plant growth in various TCS treatments.†

†Trt 1(no spike) = TCS spiked concentration of 990 ng g^{-1}
†Trt 2 (5 ppm TCS) = TCS spiked concentration of 5990 ng g^{-1}
†Trt 3 (10 ppm) = TCS spiked concentration of 10,990 ng g^{-1}

CHAPTER 8
MOBILITY OF TRICLOSAN (TCS) IN BIOSOLIDS-AMENDED SOILS

Background

Triclosan [5-chloro-2-(2,4-dichlorophenoxy)-phenol] (TCS) is an antimicrobial agent commonly added to a number of consumer products. Plastic and textile manufacturing industries use TCS as an additive. The inherent antibacterial property of TCS makes it an effective agent for use in household articles like sponges and kitchen chopping boards. The incorporation of TCS in a vast array of products results in its discharge to wastewater treatment plants (WWTPs).

The products of WWTPs are liquids (effluents) and solids (sludge). Processed sludge forms biosolids, which may then be land applied. The hydrophobic nature of TCS (log K_{ow} 4.6 to 4.8: Halden and Paull, 2005; Ying et al., 2007) portends significant partitioning of TCS originally in wastewater streams into biosolids. Reported biosolids TCS concentrations in the extensive (78 WWTPs) 2009 Targeted National Sewage Sludge Survey (TNSSS) (USEPA, 2009a) were 0.4 to 133 mg kg^{-1} with an overall mean of 16 ± 65 mg kg^{-1} (including statistical outliers). An average WWTP in U.S. produces 240 kg dry weight of biosolids per million litres of wastewater treated (Kinney et al., 2006). Nationally, WWTPs produce about 7 million Mg of biosolids each year, ~63% of which is land applied (NRC, 2002). Assuming an average biosolids-borne TCS concentration of 16 mg kg^{-1} (mean value from TNSSS), land application represents a potentially significant source (~70 Mg $year^{-1}$) of TCS to the land.

Some worry that the TCS presence in the environment could contribute to the spread of bacterial resistance and threaten human drug therapy (Birosova and Mikulasova, 2009; Pycke et al., 2010; McMurry et al., 1998). McMurry et al. (1998)

recovered TCS-resistant *E.coli* on agar plates with a mutation of *fab* 1 gene. Besides, TCS affects aquatic organisms by blocking enzyme carrying proteins, causing concerns of the possible build-up of bacterial resistance in these organisms (Nghiem and Coleman, 2008). Numerous aquatic TCS toxicity data are summarized in Table 1-1 (Chapter 1). Triclosan can be toxic to fish with chronic toxicity value of 22 mg L^{-1} (Lindstrom et al., 2002). Orvos et al. (2002) reported TCS toxicity to *Daphnia magna* with a 48-h median effective concentration (EC_{50}) of 390 mg L^{-1} and fathead minnow (*Pimephales promelas*) with a 96-h median lethal concentration (LC_{50}) of 260 mg L^{-1}. Therefore, movement of TCS through and over biosolids-amended soils to surface waters could be an environmental concern.

Despite the occurrence of TCS in biosolids, and frequency of biosolids land application, little is known about the mobility of TCS in amended soils. Cha and Cupples (2010) assessed the leaching potential of TCS in biosolids-amended soils using biodegradation and sorption data in a simple leaching model developed for pesticides (Gustafson, 1989). The model involved calculation of leaching potential using groundwater ubiquity scores (GUS); and chemicals with GUS<2.8 were termed non-leachable. This model assumed reversible chemical sorption and first order chemical degradation half-life. The model predicted that TCS in biosolids amended to sandy and loam soils (concentration = 0.05-2 mg TCS kg^{-1} amended soil) would experience insignificant leaching (GUS<0.7). Topp et al. (2008) used simulated rainfall in field studies to estimate the runoff potential of TCS in soil amended with surface applied and incorporated liquid biosolids. Triclosan appeared in runoff water from the surface applied liquid biosolids treatment collected immediately and 266 d after the biosolids

application. The reason for TCS appearance at day 266 was not known but was attributed to the persistence of TCS in bound residues especially during the winter months. Sabourin et al. (2009) conducted a similar TCS leaching study following the application of dewatered biosolids. Less than 1% of the total biosolids TCS mass appeared in the runoff water suggesting minimal mobility.

Lapen et al. (2008) suggested a potential for TCS transport via shallow tile drainage systems to surface waters. Triclosan was detected in tile drains when soils received either liquid or dewatered biosolids (Lapen et al., 2008; Edwards et al., 2009). Greater TCS concentrations (3.68 μg L^{-1}) in tile drains appeared following liquid biosolids application (Lapen et al., 2008) than appearing following dewatered biosolids application (0.24 μg L^{-1}) (Edwards et al., 2009). Xia et al. (2010) reported that 49 to 64% of total TCS mass applied in biosolids treatments was found at a depth of 30 to 120 cm, suggesting significant leaching in soils amended with biosolids for 33 years. The studies described above represent somewhat unique conditions. Lapen et al. (2008) utilized liquid biosolids (11.9 g solids L^{-1} biosolids), the soil in Xia et al. (2010) was a calcareous mine spoil (pH = 7.8) and the biosolids used in Edwards et al. (2009) had high pH (~7.5). Liquid biosolids portends greater amounts of TCS in the aqueous phase as the fluidity of liquid biosolids is similar to water. The high pH in two systems was close to the pK_a of TCS (~8); likely making the dissociated TCS more available for transport within and over the soil surface.

The log K_{oc} values determined in soils, biosolids, and biosolids-amended soils for TCS were large (>4), and reasonably constant for all solid matrices (Agyin-Birikorang et al., 2010). The large values suggest a propensity of TCS to partition onto organic

carbon (OC) associated with biosolids, soil and sediment in the environment. Hysteresis coefficients for TCS determined in soils, biosolids, and biosolids-amended soils matrices were <<1 (0.002 to 0.005 for biosolids, 0.25 to 0.30 for biosolids-amended soils, and 0.41 to 0.42 for un-amended soils) suggesting highly restricted desorption (Agyin-Birikorang et al., 2010). The small hysteresis coefficients, particularly for the biosolids, suggest that reincorporation of TCS to the solution during desorption is practically negligible (Mamy and Barriuso, 2007). Triclocarban (TCC) with soil log K_{oc} (3.82) similar to log K_{oc} of TCS (4.26) experienced minimal leaching (<1%) in a biosolids-amended soil, and formation of TCC bound residues (Snyder, 2009). Triclosan bound residues were also observed in biodegradation study of TCS conducted in biosolids-amended soil (Chapter 4). Based on the expected behavior of TCS, we hypothesized that the mobility of biosolids-borne TCS is strongly retarded in amended soils. Besides TCS, a degradation metabolite of TCS is likely to appear in soils, as the primary degradation half-life of TCS in amended soil is 100 d (Chapter 4). The objective of the present study was to determine the mobility of TCS (and possibly Me-TCS) in biosolids-amended soil. Leachability was assessed in a laboratory column study utilizing a sand soil with a pH<pK_a and a low organic carbon (3 g kg^{-1}) content. The soil was amended with cake biosolids (solids: 320 g kg^{-1}) as the source of spiked (^{14}C) TCS. Sandy soils with low organic carbon and clay contents are more prone to chemical leaching, given the abundance of macropores and low capacity for TCS retention (Yaron et al., 1996). The 30 cm long columns were packed with 20 cm sand, amended with biosolids spiked with ^{14}C-TCS and periodically leached with two irrigation regimes for upto 2 months. Leachates from the columns were collected after each irrigation

event, and at study termination, the soils in the columns were sectioned and extracted to quantify the depth of TCS and Me-TCS movement.

Materials and Methods

Experimental Design

The design consisted of incorporation of TCS spiked biosolids, or soils, into soil columns and application of two irrigation regimes. The irrigation regimes were based on the rainfall received in Gainesville, FL area. The 40-year (1970-2009) average annual rainfall in Gainesville is 132.8 cm (occurring in 126 days). More than half of the rainfall (74.8 cm) occurs in the summer (June – September, occuring in 49 days). In the summer, an average of ~1.53 cm rainfall occurs at a frequency of 2.7 days, yielding a weekly average of 3.97 cm. Irrigation consisted of empirical and stochastic regimes and soil treatments included controls (no biosolids), and biosolids incorporated with the top 2.5 cm of soil. The treatment combinations were:

- Empirical control
- Stochastic control
- Empirical biosolids treatment
- Stochastic biosolids treatment

The irrigation regime consisted of the following:

- Empirical irrigation: Quantity of water applied to the columns was based on the long-term average weekly summer rainfall amounts in Gainesville: ~3.97 cm ≈ 77.9 mL ≈ 0.5 pore volumes/week.
- Stochastic irrigation: Random water applications (quantity and frequency) to mimic actual long-term rainfall patterns, but the total quantity of water applied over the experimental period was the same as the quantity applied in the empirical irrigation regime.

Chloride was utilized as a non-interactive conservative tracer. At each leaching event, irrigation water was mixed with chloride to assess water movement during the experiment.

Soils, Biosolids and Chemicals

As the study was conducted in Gainesville, we utilized soil (sand; OC: 3 g kg^{-1}) collected from the E-horizon of an uncoated Myakka fine sand (sandy, siliceous, hyperthermic Aeric Alaquods). ^{14}C–TCS radiolabeled uniformly on the chlorophenol ring (specific activity - 48 mCi $mmol^{-1}$ and 99% purity) was custom synthesized by Tjaden Biosciences (Burlington, IA). Ecoscint A liquid scintillation cocktail was purchased from National Diagnostics (Atlanta, GA). Potassium chromate, silver nitrate, methanol (MeOH), acetone and other solvents were purchased from Fisher Scientific (Atlanta, GA). An anaerobically digested class A cake biosolids (solids content: 320 g kg^{-1}) was collected from a domestic WWTP in Illinois. The biosolids contained 5 mg kg^{-1} of TCS and non-detectable (<0.7 mg kg^{-1}) Me-TCS (Chapter 2).

Tagging and Application of Biosolids

The biosolids (100 g) were spiked with 0.06 mCi of ^{14}C-TCS (specific activity = 48 mCi $mmol^{-1}$), resulting in a total TCS biosolids concentration of ~8.6 mg TCS kg^{-1}. Although the biosolids TCS concentration was below the mean concentration (16 mg kg^{-1}) found in TNSSS, it was within the range reported in the TNSSS (USEPA, 2009a). The biosolids (10 g dry weight equivalent/tube) were weighed into 50-mL Teflon tubes (total of 10 tubes), and spiked with 5 mL of MeOH containing 6 µCi of ^{14}C-TCS. The ^{14}C-TCS spiked biosolids samples were sealed and equilibrated for 24 h, the MeOH was evaporated for an additional 24 h in a hood. The biosolids samples from various tubes were composited and thoroughly mixed. The columns utilized in the study were 30 cm in length and 5 cm in diameter. Plain sand filled 20 cm of the column and was supported by porous plate. To encourage uniform water distribution, 10 cm of pea gravel was placed at the top and a suction of 0.1 bar was applied to the column. Appropriate

quantities of the spiked thoroughly mixed biosolids (~6 g) were incorporated to a depth of 2.5 cm into the soil at equivalent rate of 22 Mg ha^{-1} (1%, by wt). Control treatments consisted of spraying ^{14}C directly on the top of the soil (no biosolids) to achieve the same TCS concentration as in the biosolids-amended treatments. The suction head of 0.1 bar applied to the columns prevented saturation at the column bottom, and maintained uniform moisture content (10% by weight, roughly field capacity) throughout the column. The schedule of the leaching events and the amount of irrigation applied is described in Table 8-1.

Pore Volume Determination

A preliminary experiment was conducted to determine a representative column pore volume (PV), using the gradual wetting method. Four columns were filled with 600 g of dry soil and weighed. Water was gradually introduced into the columns through the column bottom using Mariotte bottles, until the soil was saturated. A suction head of ~0.1 bar (similar to the experimental conditions) was then applied to the columns until the excess water drained out. The columns were then re-weighed and the difference in weight (~148 g) was assumed to be 1 PV.

Leachate Collection and Analysis

After each leaching event, leachate was collected from each column, and analyzed for tracer (chloride) concentration, and ^{14}C activity. Chloride concentration was determined in an aliquot (5 mL) of leachate using the Argentometric titration procedure (Sheen and Kahler, 1938; Kumar, 2006). The method consisted of titration with silver nitrate using potassium chromate as indicator. Another aliquot (1 mL) of the leachate was mixed with 10 mL of scintillation cocktail to determine ^{14}C activity. Measurement was performed for 5 min on a liquid scintillation counter (LS 6500, Beckman Coulter

Inc., Fullerton, CA, USA) with background correction against blank samples (^{14}C-free cocktail).

Determination of ^{14}C Activity in the Soil

After the study termination, pea gravel on the soil surface was carefully removed and the soil in each column was gently pushed up from the bottom of the columns with a plunger. The soils in biosolids-amended treatment and controls were sectioned into 0.5 cm increments up to 2.5 cm, and at 2.5 cm increments from 5 to 20 cm. A subsample of each soil section was combusted at 900°C in a Harvey Oxidizer Model OX-500 (Tappan, NY), and analyzed for total ^{14}C activity. Additionally, a subsample from the zone of biosolids incorporation (0-2.5 cm) from each column was extracted (twice) with MeOH: acetone (50:50; v/v) solvent as described previously (Chapter 4). The extract was then subjected to RAD-TLC analyses (for ^{14}C-speciation) as described previously (Chapter 4).

Results and Discussion

Leachate Recoveries and Tracer Breakthrough

Leachate recoveries were calculated as the ratio of the volume of irrigation water collected beneath the columns to the volume of irrigation water applied to the columns. Recoveries in the empirical and stochastic irrigation treatments at each leaching event were similar and >90% and approximately constant for the entire study period (62 d) (Figure 8-1). Recoveries in the control and biosolids treatments were also similar. Most of the applied water was recovered with no indication of soil saturation and biosolids application did not retard water movement.

Consistent with the constant leachate recoveries, column masses were nearly constant for the study period, and similar to the masses measured at time zero (data not

presented). Constant column masses suggest that the study conditions remained constant through the study period.

The tracer (chloride) breakthrough curve is represented in Figure 8-2. Breakthrough of Cl did not differ between the control and biosolids-amended treatments and was similar for both irrigation treatments. Similar breakthrough indicates similar water flow despite different frequency of water application. At 1 PV, 50% of Cl appeared in the leachate suggesting uniform convective transport and lack of preferential flow (Mamo et al., 2005). However, the breakthrough curve for the empirical treatments (Figure 8-2a) was more symmetrical than for the stochastic treatments (Figure 8-2b). After leaching for about 4 PV, the relative concentration of Cl was almost zero suggesting that all the Cl was recovered from the columns.

^{14}C in Leachates and Recoveries by Combustion

Leachate collected from columns in all the treatments contained no detectable ^{14}C throughout the study period, suggesting that all the applied ^{14}C-TCS remained in the soil. Addition of up to 4PV of water did not move ^{14}C to the bottom of the columns. Different irrigation regimes and TCS spiked onto biosolids or directly on to the soil surface affect the TCS leaching behavior.

The percent ^{14}C recoveries from the soils sectioned at various depths are presented in Table 8-2. Average recovery of ^{14}C from the top (0-2.5 cm) depth in the empirical treatment was 97.8 ± 0.83%. The soil segment immediately below incorporation (2.5-5 cm) contained 2.08 ± 0.84%, but only 0.01% of ^{14}C was detected at the depth >5 cm. Similarly, for the stochastic treatment, the average recovery was 93.7 ± 2.05% at the 0-2.5 cm depth, 6.17 ± 2.04% at 2.5 to 5 cm depth, only 0.01% of ^{14}C beyond the 5 cm depth. Data for biosolids treatments suggest that the majority of the

^{14}C (93-97%) was contained within the depth of incorporation (0-2.5 cm) irrespective of the irrigation treatments (Table 8-2). Triclosan association with biosolids was likely greater than in the soils, as the partitioning coefficient of TCS is greater in the biosolids (log K_d = 3.76) than in the soils alone (log K_d = 2.25) (Agyin-Birikorang et al., 2010). Appearance of ^{14}C below 2.5 cm likely represents contamination from the top depth during the soil sectioning process. Minimal ^{14}C detected at depths beyond 5 cm suggests that biosolids-borne TCS did not move beyond the depth of biosolids incorporation (0-2.5 cm) except for a little ^{14}C in the 2.5 to 5 cm depth.

Recoveries in the controls were 77 to 82% in the 0 to 1 cm depth and from 17 to 22% in the soils in 1 to 2 cm depth (Table 8-2). As the initial TCS spiking occurred at the surface of the soil column, significant ^{14}C detected at 1 to 2 cm depth suggest some TCS movement. Absence of biosolids in the controls likely allowed some movement with irrigation water, but TCS did not move beyond 2 cm. Triclosan experienced greater sorption in biosolids amended soils than in the un-amended soils (Agyin-Birikorang et al., 2009). Lower sorptive capacity of an un-amended soil suggests a greater movement with soil solution. Cha and Cupples (2010) predicted the leaching of TCS spiked (0.05-2 mg kg^{-1}) in biosolids and amended to sandy and loam soils, using biodegradation and sorption data in a simple leaching model. Predictions for both soils are similar to our results, suggesting insignificant TCS leaching in the presence of biosolids.

In contrast, Lapen et al. (2008) reported leaching of TCS into tile drains (concentration of 3.68 µg L^{-1}) located 80 cm below the soil surface following a liquid biosolids application. Liquid biosolids (11.9 g solids L^{-1} biosolids) have greater (relative) amounts of TCS aqueous phase and could be expected to promote greater TCS

mobility. Triclosan in the aqueous phase may move by gravity-based flow through large pores and worm borrows (preferential flow) (Turpin et al., 2007), and may intersect the shallow tile drains. Further, movement by facilitated flow may occur when TCS associates with dissolved organic carbon (DOC). Edwards et al. (2008) conducted a similar leaching study in soils amended with dried biosolids, and found detetable TCS concentrations (0.24 µg L^{-1}) in tile drains, but the concentrations were much lower than in the Lapen et al. (2008) study. Leaching occuring with dried biosolids application was due to the pH (7.5) of the biosolids that was sufficiently close to the pk_a (~8) to favor greater TCS concentrations in aqueous phase. Similarly, Xia et al. (2010) reported TCS leaching to a depth of 30 to 120 cm in soils annually amended with biosolids for 33 years. Soil was calcareous mine spoil (pH = 7.8) and the high pH likely increased TCS solubility making it more susceptible to movement. A column study was conducted with biosolids-borne TCC leached with 0.5 PV at each leaching event resulting in ~4PV of leachate at the study termination. The amount of water (PV) was similar to our study and a leaching of <1% was observed in a 3.5 month leaching study conducted in 17 cm long columns (Snyder, 2009). The study utilized biosolids with varying OC contents. The authors opined that partitioning coefficient (K_d) influenced the leaching; as a smaller K_d portends a greater readily leachable (aqueous) fraction and a greater mobility. Gibson et al. (2010) measured the leaching of TCS (84-1032 ng L^{-1}) applied with irrigation water to 30 cm long soil columns for 90 days. No TCS was detected in the column leachate; and soil sectioning confirmed minimal leaching beyond the 10 cm soil depth. Greater leaching of TCS in the Gibson et al. (2010) study than our study was likely due to greater TCS concentration in the aqueous phase. The study thus reemphasizes that the

relative TCS movement would be greater when TCS is present in the aqueous form as opposed to TCS occurring bound to biosolids OC.

^{14}C Recoveries by Extraction and Extract Speciation

Extraction was performed on soil samples taken from the zone of biosolids incorporation (0-2.5 cm), as the combustion data (Table 8-2) indicated that the majority of ^{14}C added was present at this depth. The average recoveries of ^{14}C in amended treatments for both irrigation treatments (85-86%) (Table 8-3) were less than the recoveries obtained through combustion (93-97%) (Table 8-2). Extraction recoveries were expected to be smaller than recoveries through combustion, as the combustion procedure measures total (i.e. TCS associated with labile and non-extractable fractions) concentrations. The recoveries in the controls were 96 to 98% in the both irrigation treatments (Table 8-3), similar to combustion procedure recoveries. Data suggest that biosolids application reduces the extractable ^{14}C recoveries.

There are no published reports of the leaching or runoff potential of Me-TCS. Because >95% of ^{14}C was recovered in the 0-2.5 cm depth; these samples were subjected to RAD-TLC analysis to determine ^{14}C speciation. The data (Table 8-4) suggest degradation of TCS and appearance of a metabolite (Me-TCS). Appearance of Me-TCS was similar to our previous observations (Chapter 4). In the biosolids-amended treatments, the average ^{14}C identified as TCS was 51 to 62%, and the average ^{14}C identified as Me-TCS was 37 to 48%, irrespective of the irrigation treatment. The TCS recoveries were generally greater in the empirical than the stochastic irrigation regime. The corresponding Me-TCS recoveries were greater in the stochastic treatments. In the controls, 58 to 62% of ^{14}C was identified as TCS and 38 to 42% as Me-TCS. The TCS and Me-TCS recoveries were variable among the treatments; but the recoveries were

not significantly different suggesting no effect of irrigation or biosolids treatments on TCS speciation/degradation.

In biosolids treatments, >95% of ^{14}C remained in the zone of biosolids incorporation (0-2.5 cm). Extraction and speciation data from that depth suggest that Me-TCS was formed but remained in the top depth segment, suggesting minimal movement in amended soils. In controls, appearance of TCS and Me-TCS beyond the initial surface spiking of TCS suggest that either TCS leached to the lower depth and degraded to Me-TCS or Me-TCS appeared in the top depth and subsequently leached to the lower depth. The first mechanism is more probable as the reported log K_{ow} (5) of Me-TCS is greater than the log K_{ow} (4.8) of TCS and, therefore, Me-TCS movement is likely more retarded than TCS. Thus, Me-TCS mobility/leaching would be equal or smaller than to the mobility of TCS, and rate of TCS infiltration is sufficiently slow to allow degradation.

The speciation data allowed the estimation of time for 50% disappearance of TCS. Data suggest that ~40% of Me-TCS (Table 8-4) was detected after 60 days which suggests a 50% disappearance time of ~75 days. The disappearance time calculated herein is within the range measured in our incubation study (Chapter 4), and other published studies of TCS half-lives (Lozano et al., 2010; Kwon et al., 2010; Wu et al., 2009).

Comparison with CMLS Model

The Chemical Movement in Layered Soils (CMLS) is a model that can be utilized to estimate the chemical movement in response to movement of water as well as chemical degradation. The CMLS has the ability to provide leaching estimates varying with weather and spatial variability of soils and uncertainties in chemical properties.

The model estimates the depth of center of mass of a chemical (non-polar) with time and assumes that a chemical only moves in the liquid phase in response to soil-water environment. Water that is already in the profile is pushed by the inflowing water (piston flow) and water is also lost from the root zone by evapotranspiration and deep percolation. Chemical movement is assumed to be retarded due to reversible sorption on the soil solids. The model makes several simplifying and conservative assumptions, including: complete first order degradation (metabolites not considered), uniform soil and chemical properties within a soil layer, and linear and reversible chemical sorption. Preferential flow is not considered. The model estimates the location of the center of chemical mass rather than chemical concentration profile.

The simplifying assumptions are not true for TCS movement in biosolids-amended soil, as our study (Chapter 4) suggested that TCS degradation followed zero-order kinetics, the formation of a metabolite, and strongly hysteretic desorption (Agyin-Birikorang et al., 2010). Thus, CMLS serves as only a first approximation of biosolids-borne TCS movement. Figure 8-3 present CMLS model simulation of the movement of a model-default chemical [Dimethyl Tetrachloroterephthalate (DCPA)] with K_d (log K_d = 3.7) and half-life (100 days) values similar to TCS. Simulations suggest that a chemical with such characteristics applied to a sand soil (with low organic carbon content), under the rainfall conditions similar to the average annual rainfall in Gainesville requires many years to move completely through a soil column of 20 cm. After 1 year of rainfall, the chemical is predicted to remain in the top 2 cm of the soil profile. Our column study results were similar to the CMLS model prediction as we observed a TCS movement to 2.5 cm in ~2 months (60 days). Our data are also consistent with the recent modeling

results by Cha and Cupples (2010). As the CMLS modeling approach is relatively simple, future work should include simulations utilizing more sophisticated models that consider irereversible and non-equilibrium chemical sorption and the fate and transport of the metabolite as well.

Implications of TCS Movement

Leaching of TCS depends on the equilibrium between TCS in the soil solution and sorbed phase. As expected, the leaching was slower in a biosolids-amended soil than a soil without biosolids, as TCS associates with the OC of the biosolids causing retardation to movement. Similar to TCS, Me-TCS formed in the control and amended soil remained in the top depth (0-2.5 cm). Mobility of contaminants in a soil can be assessed using the relative mobility factor (RMF) (USEPA, 2008a). The RMF is defined as the ratio of leaching distance of test substance (i.e. TCS or Me-TCS) to the leaching distance of the reference substance (i.e. chloride). The chemical is defined as immobile if the RMF is ≤0.15 (USEPA, 2008). Triclosan and Me-TCS moved to a maximum depth of 2.5 cm and the reference chemical moved up to 20 cm depth. The calculated RMF is 0.125, and both TCS and Me-TCS would be regarded as immobile in an un-amended and a biosolids-amended soil in 2 months of leaching period.

Our column leaching study represents a scenario where biosolids-borne TCS was amended to a sand soil of low OC content and subjected to extensive (4PV) leaching. Mobility of TCS depends on the partitioning coefficient (K_d). The K_d in a sandy soil is 5.88× smaller than the K_d in a silty loam soil (Agyin-Birikorang et al., 2010) meaning that a greater fraction of TCS would be available for leaching in a sandy soil as compared to a loam or clay soil. Gibson et al. (2010) observed a variable leaching potential of pharmaceuticals due to difference in K_d values. Thus, the leaching of TCS is expected

to be even less under common field conditions characterized by soils with greater organic carbon contents and vegetative cover.

Previous studies found TCS concentrations (0.24-3.68 μg L^{-1}) in tile drains in soils amended with liquid and dried biosolids (Edwards et al., 2008; Lapen et al., 2008). It may be noted that the tile drain TCS concentrations would be diluted upon interception with the surface water and further reduce TCS concentrations. Implications of TCS concentrations to aquatic organisms are critical. Langdon et al. (2010) quantified the risk of biosolids-borne TCS to aquatic organisms from surface runoff and leaching and suggested that maximum concentration of TCS reported in the leaching and runoff water did not adversely affect the aquatic ecosystem. Mobility data obtained herein and other data available in the literature are utilized in our risk discussion (Chapter 9).

Table 8-1. Amount and schedule of leaching events for the empirical and stochastic irrigation regimes.

Empirical irrigation regimes		Stochastic irrigation regimes	
Day	Amount (mL)	Day	Amount (mL)
5	79	5	50
12	78	11	30
19	78	15	70
26	78	17	75
33	78	26	70
40	78	31	90
47	78	41	80
54	78	46	65
61	78	55	60
68	78	67	90
75	78	73	80
82	78	79	60
		86	65
Total	936	Total	885

Table 8-2. Average percent recoveries ± standard deviation of ^{14}C (by combustion procedure) by depth in the control and biosolids treatment for each irrigation regime.

	^{14}C Recovery (%)	
Depth (cm)	Empirical	Stochastic
	Treatment	Treatment
0-2.5	97.8±0.83	93.7±2.05
2.5-5	2.08±0.84	6.17±2.04
5-20	0.01±0.00	0.01±0.00
	Control	Control
0-1	77.4±3.31	82.5±3.70
1-2	22.4±3.20	17.4±3.70
2-2.5	0.06±0.00	0.01±0.00
2.5-20	0.00±0.00	0.00±0.00

Table 8-3. Average percent extraction recoveries of ^{14}C ± standard deviation in the top depth of the control and biosolids treatment for each irrigation regime.

	^{14}C Recovery (%)	
Depth (cm)	Empirical	Stochastic
	Treatment	Treatment
0-2.5	85.1±10	86.4±10
	Control	Control
0-2.5	96.2±10	97.9±8.1

Table 8-4. Speciation of ^{14}C extracted ± standard deviation in the top depth of control and biosolids treatments for each irrigation regime (same letters represent no significant difference among treatments).

Depth (cm)	TCS (%)	Me-TCS (%)
	Empirical treatment	
0-2.5	62.5 ± 0.7a	37.5 ± 0.7b
	Stochastic treatment	
0-2.5	51.9 ± 5.3a	48.1 ± 5.3b
	Empirical control	
0-2.5	62 ± 2.5a	37.7 ± 2.5b
	Stochastic control	
0-2.5	58 ± 2.6a	42 ± 2.6b

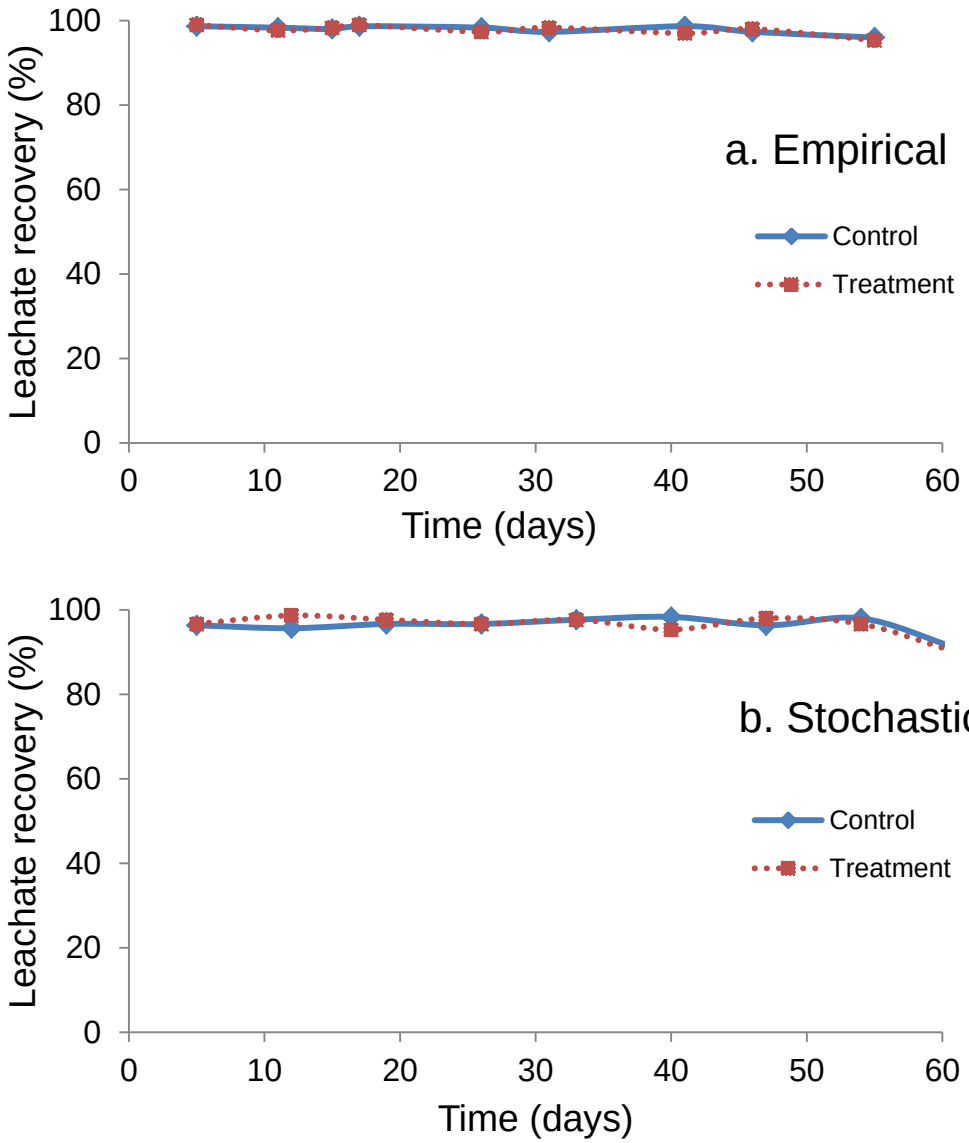


Figure 8-1. Percent recovery of the amount of water that was collected at bottom of the column at various intervals during the study period in the (a) empirical and (b) stochastic irrigation regimes.

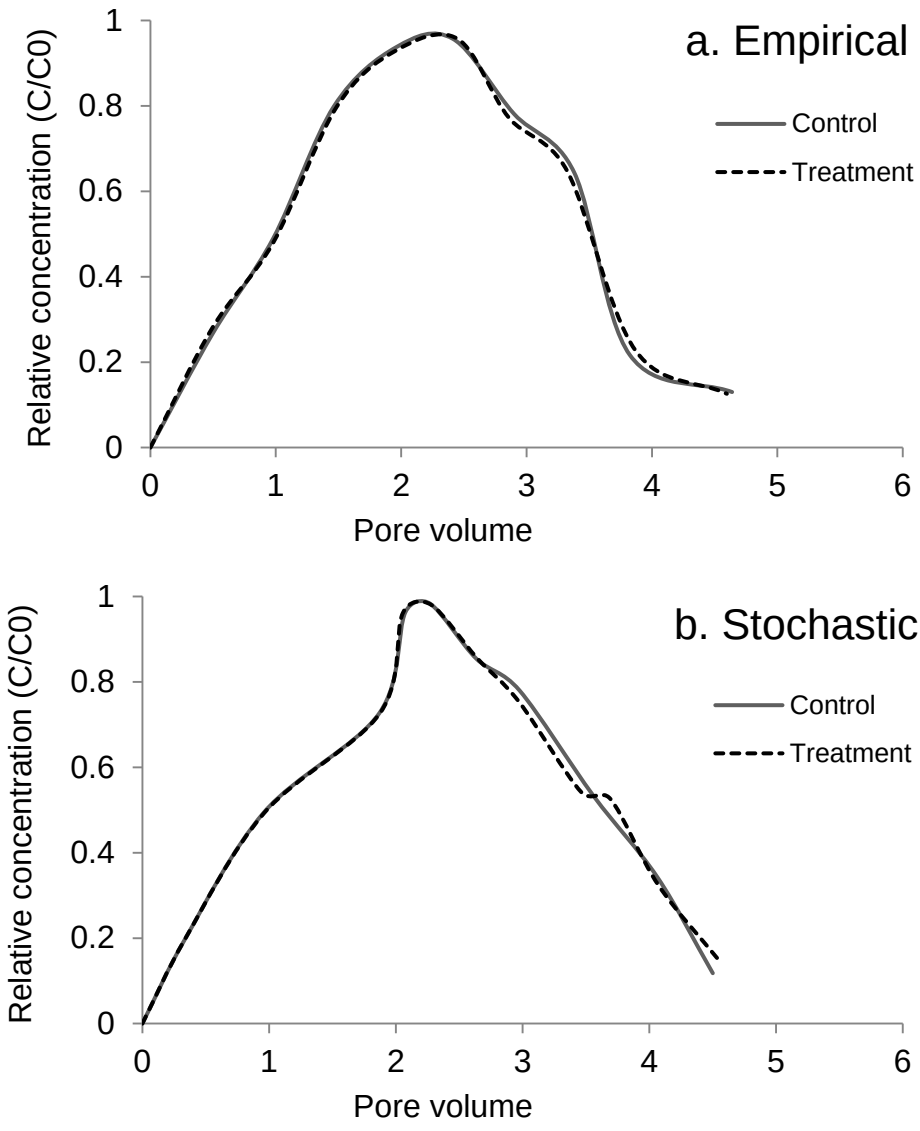


Figure 8-2. Chloride breakthrough curves in control and biosolids-amended soils of the (a) empirical and (b) stochastic irrigation regimes.

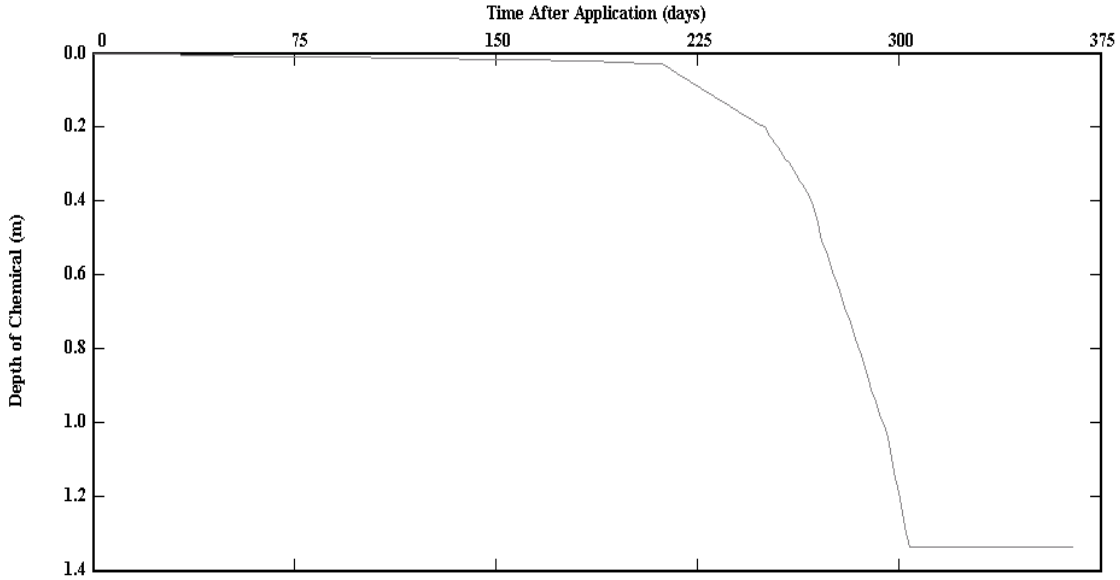


Figure 8-3. Prediction of chemical movement obtained using the CMLS model. The graph represents the depth of DCPA movement (chemical properties similar to TCS) in a sandy soil with time.

CHAPTER 9
RISK ASSESSMENT OF BIOSOLIDS-BORNE TCS

Background

Risk assessment is a process of assigning magnitudes and probabilities to the adverse effects of human activities. The process involves identifying a hazard, and quantifying the relationship between event and its effect (Suter, 2000). Screening level risk assessments are often conducted initially by assuming worst-case scenarios to narrow the scope of subsequent assessments. Specific pathways in which the screening-level assessments are above critical limits are selected for more detailed risk evaluations (Suter, 2007).

Human health risk assessments are sometimes considered as alternatives to ecological assessments. The notion is that protection of humans (typically, the most sensitive receptors) will automatically protect non-human species. However, non-human receptors are possibly exposed to greater chemical concentrations, or are more sensitive than humans. Thus, rather than conducting independent assessments, ecological and human risk (combined) assessments should complement each other (Suter, 2007) and be more protective overall.

Several ecological risk assessments were conducted recently (Reiss et al., 2009; Fuchsman et al., 2010; Langdon et al., 2010) to address the risk of biosolids-borne triclosan (TCS). Fuchsman et al. (2010) estimated the exposure to terrestrial organisms (soil microbes, plants, mammals, birds) using a probabilistic fugacity-based model. Triclosan concentrations in various organisms and their exposures were estimated assuming either equilibrium or steady state conditions. Toxicity values from the literature were utilized to establish effect concentrations and to identify sensitive species

Results suggested no adverse effects of biosolids-borne TCS (except for nitrogen cycling disruption) under even worst-case scenarios (considering the most sensitive toxicity endpoint), and the effects were transient. The study addressed ecological risks utilizing simple fugacity models and a probabilistic approach, but did not consider TCS degradation. Further, corresponding data for validation of the probabilistic model are lacking (MacKay and Barthouse, 2010).

A screening-level assessment (Langdon et al., 2010) quantified the risk of surface runoff and leaching of biosolids-borne TCS to aquatic organisms using the hazard quotient (HQ) approach. Runoff concentrations were predicted using literature values for biosolids TCS concentrations and modeled TCS physico-chemical properties (K_{ow}, K_{oc}, K_d). The assessment identified some risk (HQ>1) to the most sensitive aquatic species (green algae) in a worst-case scenario using the greatest predicted runoff concentrations. Additional assessment that protected 95% of the study population resulted in lower risk, but HQ values remained >1. Langdon et al. (2010) opined that hydrophobic chemicals like TCS are expected to have low aqueous phase concentrations due to higher partitioning coefficients than used in their study and to hysteretic sorption-desorption behavior. Consistently, Agyin-Birikorang et al. (2010) reported strong hysteretic desorption behavior of TCS, incomplete desorption, and lower aqueous phase TCS concentrations than expected.

Reiss et al. (2009) followed the European Union System for the Evaluation of Substances (EUSES) model and supplemented his approach with United States regulatory guidance. The margin of safety (MOS) concept was utilized to assess the risk of biosolids-borne TCS (5 and 15 mg TCS kg^{-1} biosolids). Soil TCS concentrations were

estimated from agronomic biosolids applications (5-10 Mg ha^{-1}) to assess risks to earthworms, soil organisms and plants. Estimated TCS concentrations in earthworm tissues were utilized to assess risk to earthworm and fish-eating birds and mammals. The authors suggested no significant risk (MOS>>100) to the species from the TCS concentrations typically found in soils, even after applying the biosolids annually for 10 years.

Most of the data (half-lives, K_d, microbial and plant toxicity, earthworm accumulation) utilized in the Reiss et al. (2009) and Fushman et al. (2010) assessments were either derived from models or were measurements extracted from unpublished sources. Reiss et al. (2009) considered a relatively short half-life (35d) for TCS in amended soils and Fushman et al. (2010) did not consider TCS degradation. Thus, a characterization of biosolids-borne TCS behavior, and a risk assessment based on measured data is expected to be more accurate. Our approach for the preliminary risk estimation was similar to Reiss et al. (2009). We began by identifying the relevant exposure pathways from the list of pathways used in the Part 503 Biosolids Rule (USEPA, 1994). We then chose the most relevant measured parameters for the assessment (half-life, LC_{50}, NOAEL, K_{oc} etc.), and calculated hazard indices (HI) for each pathway. Hazard indices are the ratio of exposure concentration of a species to the corresponding species endpoint. Pathways in which HI values exceeded one were identified and subsequently subjected to detailed evaluation. Our risk estimation utilized measured data generated herein to build on the previous assessments, and extended the risk evaluation to humans. Thus, our results provide a more comprehensive evaluation of risk of biosolids-borne TCS than previously published evaluations.

Pathways of Exposure

The biosolids-borne TCS risk assessment included 14 exposure pathways utilized in the risk assessment supporting the Part 503 Biosolids Rule (Table 9-1). Similar to the recent triclocarban (TCC) risk assessment (Snyder, 2009), additional pathways were identified to incorporate terrestrial animal exposure to contaminated fish (pathway 15), and aquatic organism exposure to contaminated surface water (pathway 16). The receptor for each pathway was a highly exposed individual (HEI), the individual from a population that has the greatest realistic exposure. The HEI differs from a most exposed individual (MEI) sometimes used in risk assessment, who has unrealistic exposures and does not actually exist (Epstein, 2003).

Exclusion of Exposure Pathways

Risk to Humans

Previous assessments examined the human risk to TCS exposure through the use of personal care products containing TCS (NICNAS, 2009) or through drinking water contaminated with TCS (USEPA, 2008b). NICNAS (2009) utilized the margins of exposure (MOE) concept; the MOE is similar to the HI approach of USEPA for characterizing occupational and domestic risk of chemicals to humans. The MOE is a measure of the likelihood that a particular adverse health effect occurs in an organism after an exposure as compared to an unexposed organism. The greater the MOE, the smaller is the potential of an adverse effect or risk. If MOE >100, an adverse affect is not expected. The exposure pathways in the assessment included inhalation and dermal exposure through TCS containing household products (NICNAS, 2009). Results suggested that even repeat dosages over long-periods of time (~2 year) posed no risk to humans, as all the MOEs were >100. Risks to children (assumed to be the most

sensitive population) through exposure to TCS in toy paints and breast milk were also calculated. The MOEs for all the exposure pathways for children were >100 and there was minimal expected risk from inhalation and dermal exposure to TCS from the use of personal care products (NICNAS, 2009).

The USEPA conducted a screening assessment of TCS human risk through consumption of TCS-containing drinking water as a part of the Reregistration Eligibility Decision (RED) for TCS (USEPA, 2008b). The assessment utilized TCS concentrations of 56 ng L^{-1} in drinking water found in southern California (Loraine and Pettigrove, 2006). Assuming a water intake rate of 2 L, the calculated TCS intake rate is 1.4 ng $kg^{-1}d^{-1}$ for a 70 kg adult. The USEPA suggested a TCS reference dose (RfD) of 300,000 ng kg^{-1} d^{-1} (McMahon, 1998), which was same for the acute and chronic dietary end-points. The TCS intake rate (1.4 ng $kg^{-1}d^{-1}$) was many fold smaller than the RfD (300,000 ng $kg^{-1}d^{-1}$) and the exposure concentration never reached the RfD. Hazard was deemed negligible even after a 100 year exposure. Further, TCS degrades in aquatic environments (Canosa et al., 2005), so the actual exposure concentration would be even smaller. As the screening assessment of TCS from drinking water did not suggest a risk, a quantitative risk estimation was not conducted by USEPA. More recently, Benotti et al. (2009) reported TCS concentrations in source water and finished drinking water derived from streams impacted by wastewater effluent. The maximum (6.4 ng L^{-1}) and mean (3 ng L^{-1}) TCS concentrations were nearly 10 fold smaller than the California concentrations used for the USEPA risk estimation above. Thus, estimated risks using these concentrations would be even smaller. Realistic TCS concentrations in drinking water will not represent a risk to humans even over long-term (100 year) exposure.

Risk to Aquatic Organisms

Several aquatic risk assessments for TCS have been conducted (Lyndall et al., 2010; Langdon et al., 2010; Brausch et al., 2010). Abundant TCS toxicity data are available for aquatic organisms like fishes, amphibians, algae and vascular plants (Tables 9-2 and 9-3), and include values from both acute and chronic toxicity studies. Algae and invertebrates are generally more sensitive to TCS exposure than fish and vascular plants. Brausch et al. (2010) attributed algal sensitivity to the disruption of lipid biosynthesis through fab 1 (fatty acid synthesis). McMurry et al. (1998) and Lu and Archer (2005) identified TCS toxicity to FASII (enoyl acyl carrier protein reductase) pathways. Lyrge et al. (2003) and Franz et al. (2008) identified membrane destabilization and Newton et al. (2005) identified uncoupling of phosphorylation.

Langdon et al. (2010) focused on the risk assessment of pharmaceuticals and personal care products entering the aquatic environment through land applied biosolids using the HQ approach. Land application allows TCS transfer to soils and a potential for TCS migration to surface waters through leaching or runoff. Estimated runoff water TCS concentration predicted using an equilibrium partitioning (reversible desorption) approach was 4.5 µg L^{-1}. Predicted environmental concentrations were compared with the aquatic toxicity end-points to determine possible adverse effects. Conservative assumptions included using the greatest runoff TCS concentrations, and toxicity end-points for the most sensitive aquatic species. A preliminary assessment, utilizing conservative parameters, suggested some hazard of TCS (HQ>1) and warranted detailed assessment. Consistently, a preliminary assessment by Braush and Rand (2010) also suggested some risk of TCS to aquatic species (HQ>1), but the authors acknowledged that the estimated risk was for a worst-case scenario. Detailed

investigation by Langdon et al. (2010) included more realistic aquatic toxicity and runoff concentrations to protect 95% of the aquatic species. The HQ was still >1 implying that a runoff TCS concentration (0.59 µg L^{-1}), predicted to protect 95% of the population, posed some threat to the most sensitive species. However, the runoff TCS concentration (0.59 µg L^{-1}) used exceeded the range of TCS concentrations (0.025-0.10 µg L^{-1}) previously reported in runoff water and tile drainage from field application of cake biosolids (Topp et al., 2008; Sabourin et al., 2009; Edwards et al., 2009). Tile drain TCS concentrations of 3.68 g μL^{-1} were measured following liquid biosolids application, but may represent unique conditions. Application of liquid biosolids portends greater TCS aqueous phase concentrations as the fluidity of liquid biosolids is similar to water. Langdon et al. (2010) utilized an estimated log K_{oc} of 3.97 that was smaller than the measured value of 4.26 (Agyin-Birikorang et al., 2010), and hysteretic adsorption was not considered (Agyin-Birikorang et al., 2010). Additionally, Langdon et al. (2010) ignored degradation or dilution of TCS despite previous evidence of significant degradation of TCS in biosolids-amended soil over time (Lozano et al., 2010; Kwon et al., 2010; Wu et al., 2009). Thus, the risk predicted by Langdon et al. (2010) study was conservative and the actual risk to the aquatic organisms is likely less.

Lyndall et al. (2010) evaluated TCS risk to aquatic (algae, bacteria, and invertebrates), sediment-dwelling organisms, and aquatic-feeding wildlife from WWTP effluent entering the surface waters. Triclosan concentrations in surface water were either obtained from literature or predicted from a fugacity based model assuming steady state conditions. Wild life exposures were calculated using standard wildlife dietary exposure equations (USEPA, 1993c). Toxicty to aquatic organisms was

evaluated based on a species sensitivity distribution (SSD) approach, which Capedevielle et al. (2008) used to assess TCS risk in freshwater environments. The SSD consisted of a toxicity distribution based on chronic toxicity data for several species, and is regarded as more realistic than NOEC toxicity values based on the most sensitive species. The estimated 95th percentile TCS concentrations in surface waters, sediments, and biota tissues were well below the 5th percentile of the respective species sensitivity distributions, suggesting no adverse effect of TCS exposure. Triclosan risk assessments in aquatic species (Langdon et al., 2010, Lyndall et al., 2010) consistently conclude that TCS risk to aquatic organisms is unlikely. Thus, we did not perform an independent assessment on aquatic species in our study.

The risk estimations conducted for humans (USEPA, 2008b; NICNAS, 2009) and aquatic organisms (Langdon et al., 2010; Lyndall et al., 2010) suggested limited TCS risk. Thus, the aquatic and human exposure pathways initially included for our risk assessment can be excluded. The pathways were excluded when detailed studies were either previously conducted (aquatic organisms) or there was minimal predicted risk through particular pathways (human). The six pathways that were excluded include-

- Biosolids→soil→airborne dust→human
- Biosolids→soil→air→human
- Biosolids→soil→groundwater→human
- Biosolids→soil→surface water→human
- Biosolids →soil →surface water →animal
- Biosolids→soil→surface water→aquatic organism

The remaining pathways considered in the risk evaluation are presented in Table 9-4. Further, the ingestion exposure of a non-gardener human is considered similar to the home gardener and represents the worst-case scenario for the exposure. Pathways 1 and 2 (Table 9-1) were grouped together and considered in pathway 3 (Table 9-4).

Reference Dose Calculation

The USEPA (1993c) defines the human reference dose (RfD) as an estimate of "a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime". The RfD is typically expressed in units of milligrams per kilogram of bodyweight per day (mg kg^{-1} bw d^{-1}). Reference doses can be calculated from non-human endpoints as follows:

$$RfD = \frac{NOAEL}{UF * MF}$$

Where:

UF: uncertainty factors

MF: modifying factor based on professional judgement (default = 1)

NOAEL: no observed adverse effect level

Uncertainty factors for obtaining human RfD for non-cancerous chemicals are calculated according to the following criteria (USEPA, 1993). Uncertainty factors are applied when extrapolating toxicity data from:

- Animal study data to humans (10_a)
- Prolonged exposure data to average healthy humans (10_b)
- Use of a less-than-chronic study exposure (3_s to 10_s)
- Deriving an RfD from a LOAEL rather than a NOAEL (10_l)

Modifying factors accounting for scientific uncertainties typically range from 3_{mf} to 10_{mf}. As discussed earlier, acute toxicity of TCS to humans via oral and dermal routes is low. Triclosan also did not cause in-vivo genotoxicity, carcinogenicity, reproductive or developmental toxicity in rodents. Various animal toxicity studies are available (Table 9-5) that suggest rat and mouse sensitivity to TCS. The majority of the studies were either derived from unpublished literature or lack sufficient evidence of the credibility. A 13

week repeat TCS toxicity study conducted in mice suggested a NOAEL of 25 mg kg^{-1} d^{-1} (NICNAS, 2009), and the mice was considered more sensitive to TCS exposure. However, the adverse effects were peroxisome proliferator type, considered irrelevant for human risk assessments. So, this end-point (mouse study) was not appropriate for calculating the RfD for humans.

A chronic study (2 years) tested the sensitivity of rat to mild clinical chemistry and hematology changes (hypertrophy and hepatocyte vacuolization) in male cells (NICNAS, 2009). An ingestion NOAEL of 40 mg kg^{-1} bw d^{-1} was identified (Table 9-5) in the study, and was deemed appropriate for our risk calculations. The RfD for humans was calculated using uncertainty factors for extrapolation of animal to human study (10_a) and prolonged exposure data to average healthy human (10_b) as follows:

RfD = (40 mg kg^{-1} bw d^{-1}) / ($10_a \times 10_b$) = 0.40 mg kg^{-1} bw d^{-1}

Similarly, an RfD value for terrestrial animals was calculated from the same rat study utilizing uncertainty factors specific for ecosystem risk assessment. An uncertainty factor of 5 is applied if the test species and endpoint species of interest are in the same class but different order (5_a). Differences in species sensitivity, laboratory-field extrapolation, and intraspecific variability can each be accounted for with uncertainty factors of two each (2_b, 2_c, 2_d, respectively) (Suter, 2007). The RfD for all terrestrial animals was calculated as follows:

RfD = (40 mg kg^{-1} bw d^{-1}) / ($5_a \times 2_a \times 2_b \times 2_c$) = 1 mg kg^{-1} bw d^{-1}

Predators like American woodcock, herring gull, and short tailed shrew feed on earthworms, and may consume TCS that has accumulated in the worm bodies. Reiss et al. (2009) suggested an acute LD_{50} of 862 mg kg^{-1} d^{-1} for avian species. The RfD for

predators is estimated by applying appropriate uncertainty factors. An uncertainty factor of 10 is applied if the toxicity end-point is derived from less than chronic studies (10_s), if endpoint is based on LOAEL (or LD_{50}) rather than NOAEL (10_l), and a modifying factor of 3 to account for scientific uncertainties (3_{mf}).

$$RfD = (862 \text{ mg kg}^{-1} \text{ bw d}^{-1}) / (10_s \times 10_l \times 3_{mf}) = 2.87 \text{ mg kg}^{-1} \text{ bw d}^{-1}$$

Parameters for Risk Estimation

Environmental Fate

Although our study (Chapter 4 and 8) and other published studies (Lozano et al., 2010; Wu et al., 2009; Xu et al., 2009) suggested TCS degradation in biosolids-amended soils, we assumed no loss of TCS through degradation (worst case scenario) for the screening level risk estimation.

Effect on Soil Dwelling Organisms

Toxicity to earthworms

The acute toxicity to earthworms was assessed according to OPPTS guidelines (USEPA, 1996b). Mortality was assessed in a 28 d exposure of earthworms to TCS spiked in biosolids, and amended to sand (IFS), silty clay loam (ASL) and an artificial soil (Chapter 5). An unreplicated range-finding test suggested TCS effects on the earthworm mortality exposed to TCS concentration (≤5 mg kg^{-1} biosolids) only in the sand. A replicated definitive test conducted on the sand showed no effects of TCS on earthworm mortality, and no abnormal symptoms were observed in the live earthworms up to a TCS concentration of 105 mg kg^{-1} biosolids (Chapter 5). The estimated 28d LC_{50} value was >105 mg kg^{-1} biosolids or >1 mg kg^{-1} amended sand soil. The estimated 28d LC_{50} for ASL and artificial soil was >10,000 mg kg^{-1} biosolids or >100 mg kg^{-1} amended

soils. For screening level risk assessment, the most sensitive toxicity end-point of 1 mg kg^{-1} amended soil was considered.

Bioaccumulation in earthworms

The measured earthworm BAF values were 6.5 ± 0.84 in the IFS soil and 12 ± 3.08 in the ASL soil. The BASL4 model underestimated the bioaccumulation potential of TCS, with calculated BAF values of 5 ± 0.0 (IFS soil) and 2.2 ± 0.0(ASL soil). The earthworms collected from field soil (similar in texture to ASL soil) had an average BAF of 4.3 ± 0.7 (Chapter 5). A conservative estimate of 10 as the earthworm BAF was utilized to calculate the predicted environmental concentrations in earthworms (Table 9-6) grown in soils amended with biosolids-borne TCS.

Toxicity and Bioaccmulutaion in Plant Biomass

The average BAF values in a greenhouse study were 0.43 ± 0.38 in radish root, 0.04 ± 0.04 in lettuce, 0.004 ± 0.002 in radish leaves, and the BAF value in bahia grass was <0.0001. The greatest BAF value (0.93) was obtained in the highest TCS treatment (10 mg kg^{-1} amended soil) utilized in our study. The BAF values were also measured in the field collected samples. The average BAF in field grown soybean grain were 0.06 to 0.16 (Chapter 7). The BASL4 model greatly overestimated the bioaccumulation potential of TCS in plant tissues. The calculated average BAF values were 6 to 23. A worst case conservative estimate of 0.93 was used for the screening level risk estimation.

Avian and Mammalian Toxicity

The risk posed to birds that feed on earthworms was examined using predicted earthworm concentrations. In a manner similar to Reiss et al. (2009), we selected two avian species that consume high quantities of earthworms, the American woodcock and

the herring gull. The risk posed to mammalian species was also examined in a species with high earthworm consumption, the short-tailed shrew. As no other reliable data sources were available, the TCS toxicity data (Table 9-6) for the avian and mammalian species were obtained from Reiss et al. (2009) and appropriate uncertainty factors were applied to calculate RfD that applies to all predators.

Screening-Level Assessment

Exposure Concentration Calculation

According to the USEPA Part 503 risk assessment for the biosolids rule (USEPA, 1994), an HI calculation is performed to determine if a particular pollutant/pathway is a potential problem. The HI is the ratio of the predicted environmental concentration (PEC) of a chemical to the established terrestrial or human health criteria (values obtained from toxicity studies). The terrestrial toxicity values are NOAEL, LOAEL, LC_{50} or LD_{50}, and the human toxicity values are represented by RfDs. A chemical/pathway with an HI of >1 is considered problematic and requires a tier-2 assessment of hazard rankings (USEPA, 1995). Reiss et al. (2009) conducted a terrestrial risk assessment utilizing the European Union's rules, and concept of margin of safety. Snyder (2009) used the USEPA HI approach and conducted an integrated human and environmental risk assessment of biosolids-borne triclocarban (TCC). We utilized the equations described in Reiss et al. (2009) and Snyder (2009) for our initial risk estimation.

Triclosan exposures were calculated by multiplying concentrations in relevant environmental media by the corresponding animal or human intake rates in that media. Exposure concentrations were calculated using measured or predicted data. Screening level exposures assumed two biosolids application scenarios: a one time application rate of 50 Mg ha^{-1} ("worst case" scenario), and an application of 5 Mg ha^{-1} for 100 years

("100-year" scenario). The biosolids application scenarios were similar to the Part 503 Biosolids Rule risk assessment (NRC, 2002). Typical one time biosolids application rates are 5-10 Mg ha^{-1} as opposed to 50 Mg ha^{-1} considered here, and land-application at a single location is unlikely to occur for 100 consecutive years. The TCS concentration used was the 95th percentile TCS concentration (62 mg kg^{-1}) from TNSSS (USEPA, 2009a), using the 95th percentile contaminant concentration was specified in the Part 503 risk assessment. All other parameters and equations required for the risk estimation are described in Table 9-6.

Screening-Level Hazard Index Calculation

Hazard indices for each exposure pathway were calculated as the ratio of exposure concentration to the RfD for the corresponding species endpoint (Table 9-7). The screening level assessment indicated an HI value of well below one for the majority of the exposure pathways, under both biosolids application scenarios (worst case and 100-year). The resulting HI values suggest that the HEIs would experience minimal risk from exposure to biosolids-borne TCS over their life time even when exposed to the 95th percentile TCS concentration. The critical pathways (pathways 1, 8 and 9) are the only ones in which the HI value was greater than one, and those were selected for the second tier assessment.

Tier 2 Assessment

Consideration of TCS Degradation

For the tier-2 assessment, we considered TCS degradation to recalculate the HI for the pathways that were critical (HI>1) in the screening-level risk assessment. The time required for 50% disappearance of biosolids-borne TCS estimated from the ^{14}C-TCS biodegradation study was 77 d for the ASL soil (silty clay loam) and >126 d for the

IFS (sand) soil (Chapter 4). The leaching study (Chapter 8) estimated time taken for 50% TCS disappearance in a sandy soil as 75 d. According to the EPA's persistence and bioaccumulation criteria (http://www.Pbt profiler.n et/criteria.asp), a chemical is termed persistent if the half-life in soil is greater than 60 d. Thus, biosolids-borne TCS is considered persistent in both soils (USEPA, 1999). For simplicity, we assumed a half-life of 100 d for risk estimation (Table 9-6). Further, our short-term (18 week) laboratory incubation study suggested zero order kinetics for TCS disappearance, but other published studies assumed a first order half-life. Thus, for simplification and conservatism, we also assumed a first order rate equation, as the zero-order kinetics result in non-detectable TCS after a year.

Assuming an average half-life of 100 d, the average TCS concentrations in soils (CS) over time were calculated as follows:

$$CS_{(time=t)} = \frac{CS_{(time=0)} * [1 - \exp(-kt)]}{kt} \qquad (9\text{-}2)$$

Where: At t=0, $CS_{(time=0)} = \frac{CB * BAR}{DT_{soil} * Rho_{soil}}$ (Table 9-6)

k = first order decay rate constant ($0.693/t_{1/2}$) = 0.693/100 = 0.007 d^{-1}

t = time after biosolids application

Parameters required for the calculation are described in Table 9-6. A comparison of TCS concentrations estimated using a half-life of 100d, with concentrations assuming no TCS loss (no degradation) is described in Figure 9-1. The results suggest that, assuming degradation, ~10% of TCS remains in the soil after 1 year, and the TCS

concentration did not change (reached steady state) even when the biosolids containing TCS are applied for 100 years.

Pathway 1: Biosolids→soil→plant (direct phytotoxicity)

Plants grown in amended soils (pathway 1) may experience some risk of TCS following biosolids application. Biosolids with 95th percentile TCS concentration applied at worst case application rates (50 Mg ha^{-1}) did not present a risk (HI = 0.159); however, longer term (100-year) scenario presented a risk (HI = 1.59). The HI in the 100-year scenario was 10-fold greater than the risk associated with biosolids applied through the single application (Table 9-7). If the biosolids TCS concentration is adjusted to the mean concentration from the TNSSS (16 mg TCS kg^{-1}), the HI values for the 100-year scenario reduces to 0.38, which is well below the critical value of one. Alternatively, considering a TCS degradation half-life of 100 d, the majority of TCS would disappear in 1 year (Figure 9-1), and the HI for both the worst case and 100-year scenario is less than one, even when considering the 95th percentile TCS concentration.

Pathway 8: Biosolids→soil→soil organism

An HI of one was exceeded for the acute toxicity to *Eisenia Foetida* (earthworms) for the 100-year scenario. The HI values were calculated using a conservative toxicity end point obtained from our study in sand soil. The end-points in the silty clay loam and the artificial soils were 10-fold greater, and the HI reduces to 0.16 for the 100-year scenario in these soils. If we adjust the HI using the more realistic average TCS concentration from TNSSS (16 mg kg^{-1}), the adjusted HI reduces to 0.04 for the worst-case and 0.41 for the 100-year scenarios. Further, considering TCS degradation, very little TCS would be detected at the end of 1 year (Figure 9-1) or 100 years and the adjusted HI would be much less than one.

Pathway 9: Biosolids→soil→soil organism→predator

For the worst case scenario, the HI was less than one for herring gull, but greater than one for the American woodcock and short- tailed shrew. The 100-year scenario resulted in HI of 33 for the American woodcock, 14 for the short-tailed shrew and 3.3 for herring gull (Table 9-7). The different HI values for the predators reflected variable earthworm diets. The most sensitive species was the American woodcock, whose earthworm diet was assumed to be 77%. Considering a TCS degradation half-life of 100d, the majority of TCS would disappear in 1 year and the HI for both the worst case and 100-year scenario would be less than one, even using the 95th percentile biosolids TCS concentration.

Thus, the adjusted HI values for plants, earthworms and predators were all less than one when TCS degradation was considered. However, the HI value of greater than one for various organisms (estimated in the screening risk assessment) under 100-year scenario suggests that biosolids-borne TCS pollutant limits are needed to guide sustainable use of long-term biosolids land application practice.

Sources of Uncertainty in Our Risk Estimation

- Absence of reliable data on TCS toxicity to predators.
- Using the estimated toxicity values for earthworms and plants. The highest concentration values selected in our studies did not cause any adverse effects, and the toxicity end-points were estimated to be greater than the highest concentration tested in each study. So, the actual toxicity to organisms may be much lower (i.e greater toxic end-points).
- Exclusion of toxicity and accumulation of major metabolite of TCS i.e. Me-TCS in the risk assessment, as the fate and transport data for Me-TCS is essentially unknown.

Calculation of Preliminary Biosolids-Borne TCS Pollutant Limits

The USEPA Part 503 Biosolids Rule (USEPA, 1995) includes pollutant limits to ensure the sustainable land application of biosolids. The various pollutant limits include: Cumulative pollutant loading rates (CPLRs), Annual pollutant loading rates, Ceiling concentration limits, and Pollutant concentration limits. The pollutant limits were calculated herein based on the most sensitive pathway that had an HI of greater than one (following the USEPA's risk estimation)

Cumulative pollutant loading rates (CPLRs)

A CPLR is the maximum pollutant load (kg ha^{-1}) that can be applied to a soil through biosolids application. When the pollutant load reaches CPLR at a site, biosolids application does not have to cease, but only biosolids that meets the pollutant concentration limits can be applied thereafter. The CPLRs for TCS were obtained from the risk assessment results and represent the maximum cumulative TCS load in amended soil that remains protective of human and ecological health. The CPLR can be calculated by setting the HI for a relevant exposure pathway to one and back calculating the TCS concentration in the amended soil.

Our screening level assessment suggested that American woodcock under the "100-year" scenario is most sensitive of all the species (greatest HI) and thus the CPLR should be protective of the bird. The CPLR is the concentration in soils calculated by setting the HI equation (pathway 9) equal to one as follows:

$$HI = 1 = (FI * WD * CS * BAF_{worm} / BW) / RfD$$

Where:

FI = food ingestion rate (g d^{-1} d.w.)

WD = Worm diet (%)

CS = Soil concentration (mg TCS amended soil^{-1})

BAF_{worm} = Bioaccumulation factor in the worm

BW = Body wt. (g)

RfD = Reference dose

The calculated CPLR for the American woodcock is 5.8 kg ha^{-1} (equivalent to 2.9 mg TCS kg^{-1} amended soil). The CPLR is thus the amount of TCS that can be land applied without expectation of adverse effects. The calculated CPLR translates to one time application of biosolids containing TNSSS 95th percentile TCS concentration (62 mg kg^{-1} TCS kg^{-1}) at a rate of 93 Mg ha^{-1}.

Annual pollutant loading rate (APLR)

The APLR is the maximum amount of pollutant that can be annually applied from biosolids or given away in a bag or container for land application (USEPA, 1995). Estimation is based on a conservative estimate of a 20-year of site life of lawns and home gardens. The APLR for TCS is calculated by dividing the CPLR by 20, assuming 20 years of land application and no TCS loss. The resulting APLR (0.29 kg TCS ha^{-1} y^{-1}) is protective of HEI present in the amended soils.

Ceiling concentration limit

Ceiling concentration limits are the maximum pollutant concentrations allowable in land applied biosolids. The limits were set to prevent the use of low quality biosolids containing extremely high pollutant concentrations. A ceiling concentration limit is the less stringent of the 99th percentile TCS concentration in biosolids and pollutant limit calculated from risk assessment. The 99th percentile TCS concentration from the TNSSS (USEPA, 2009a) was 197 mg kg^{-1} biosolids. Alternatively, the ceiling concentration was calculated from the CPLR value obtained in the amended soils. The

resulting ceiling concentration was 127 mg TCS kg^{-1} biosolids for the worst-case scenario and 12.7 mg TCS kg^{-1} biosolids for the 100-year scenario.

Pollutant concentration limit

Pollutant concentration limits are the most stringent concentration limits for pollutant land applied in biosolids. Biosolids that meet the pollutant concentration limits have few regulatory requirements for land application. The pollutant concentration limit assumes a total biosolids load of 1000 Mg over 100 years or less, and is calculated by dividing the CPLR by 100 years of application at 10 Mg ha^{-1} $year^{-1}$. The resulting risk based TCS concentration limit based on the most sensitive organism (predator, pathway 9) for is 6.3 mg TCS kg^{-1} biosolids. The concentration is much lower than the representative TCS concentration (10-20 mg kg^{-1}) in the biosolids across U.S. (Chapter 2), and mean concentration (16 mg kg^{-1}) reported in the TNSSS (USEPA, 2009a), but similar to the TCS concentration of CHCC biosolids (5 mg kg^{-1}) utilized in our study.

We accept our overall hypothesis of minimal risk of biosolids-borne TCS to human and environmental health. Thus, biosolids containing a 95^{th} percentile TCS concentration (62 mg kg^{-1}) applied at agronomic rates (5 Mg ha^{-1}) for multiple years (up to 100 years) did not adversely affect the majority of the organisms (according to tier-2 assessment) included in the exposure pathways described herein.

Table 9-1. Human and ecological exposure pathways for land-applied biosolids (US EPA, 1995).

Pathway	Description of HEI
1. Biosolids→soil→plant→human	Human (except for home gardener) lifetime ingestion of plants grown in biosolids-amended soil
2. Biosolids→soil→plant→human	Human (home gardener) lifetime ingestion of plants grown in biosolids-amended soil
3. Biosolids→soil→human	Human (child) ingesting biosolids
4. Biosolids→soil→plant→animal→human	Human lifetime ingestion of animal products (animals raised on forage all of which is grown on biosolids-amended soil)
5. Biosolids→soil→animal→human	Human lifetime ingestion of animal products (animals ingest biosolids directly)
6. Biosolids→soil→plant→animal	Animal lifetime ingestion of plants grown on biosolids-amended soil
7. Biosolids→soil→animal	Animal lifetime ingestion of biosolids
8. Biosolids→soil→plant	Plant toxicity due to uptake of biosolids-borne TCS when grown in biosolids-amended soils
9. Biosolids→soil→soil organism	Soil organism (e.g earthworms, microbes) ingesting biosolids/soil mixture
10. Biosolids→soil→soil organism→predator	Predator of soil organisms that exist in biosolids-amended soils
11. Biosolids→soil→airborne dust→human	Adult human lifetime inhalation of particles (dust)

Table 9-1. Continued

Pathway	Description of HEI
12. Biosolids→soil→surface water→human	Human lifetime drinking surface water and ingesting fish containing TCS from biosolids
13. Biosolids→soil→air→human	Adult human lifetime inhalation of volatilized TCS from biosolids-amended soil
14. Biosolids→soil→groundwater→human	Human lifetime drinking well water containing TCS from biosolids that leached from soil to ground water
15. Biosolids→soil→surface water→animal	Animal lifetime drinking surface water and ingesting fish containing TCS from biosolids
16. Biosolids→soil→surface water→aquatic organism	Aquatic organism exposed to runoff water containing TCS from biosolids

Table 9-2. Acute aquatic toxicity endpoints of TCS from published studies.

Species	Trophic group	End point	Result (LC_{50},mg L^{-1})	Source
D.magna	Invert.	48 h	0.39	Orvos et al. (2002)
Ceriodaphnia dubia	Invert.	24, 48 h (pH= 7)	0.2	Orvos et al. (2002)
Pimephales promelas	Fish	24,48, 72, 96 h	0.36, 0.27, 0.27, 0.26	Orvos et al. (2002)
Lepomis macrochirus	Fish	24, 48, 96 h	0.44, 0.41, 0.37	Orvos et al. (2002)
Oryzias latipes	Fish	96 h	0.602 (larvae) 0.399 (embryos)	Ishibashi et al. (2004)
Xenopus laevis	Amphibian	96 h	0.259	Palenske et al. (2010)
Acris blanchardii	Amphibian	96 h	0.367	Palenske et al. (2010)
Bufo woodhousii	Amphibian	96 h	0.152	Palenske et al. (2010)
Rana sphenocephala	Amphibian	96 h	0.562	Palenske et al. (2010)
Pseudokirch-niriella subcapitata	Algae	72 h growth	0.53 µg L^{-1}	Yang et al. (2008)

Table 9-3. Chronic aquatic toxicity endpoints of TCS from published studies.

Species	Trophic group	End point	Result (LC_{50},mg L^{-1})	Source
D. magna	Invert.	21d survival, reproduction	200 (NOEC) 200 (LOEC)	Kopperman et al. (1974)
C. dubia	Invert.	7 d survival, reproduction	50 6	Kopperman et al. (1974)
C. dubia	Invert.	7 d survival, reproduction	IC 25=170	Carlson and Caple (1977)
Chironomus riparius	Invert.	28 d survival, emergence	440	Schultz and Riggin (1985)
Chironomus tentans	Invert.	10 d survival, growth	LC25= 100	Schultz and Riggin (1985)
Hyalella Azteca	Fish	10 d survival, growth	LC25=60	Schultz and Riggin (1985)
O. mykiss	Fish	96 d Hatching, survival	71 3	Kopperman et al.(1974)
O. latipes	Fish	14 d hatchability	IC25=290	Carlson and Caple (1977)
Gambusia affinis	Fish	35 d sperm count	101.3	Orvos et al. (2002)
Danio rerio	Amphibian	9 d hatchability	IC25=160	Carlson and Caple (1977)
Xenopus laevis	Amphibian	21 d metamorphosis	No effect	Ishibashi et al.(2004)
Rana catesneiana	Amphibian	18 d development	300	Palenske et al.(2010)
Rana pipiens	Amphibian	24 d survival, growth	230 2.3	Yang et al.(2008)

Table 9-3. Continued.

Species	Trophic group	End point	Result (LC_{50},mg L^{-1})	Source
S. capricornutum	Algae	96 h growth	EC50 =4.46 EC25=2.44	Kopperman et al. (1974)
S. subspicatus	Algae	96 h biomass, growth	EC50=1.2 0.5	Kopperman et al. (1974)
S. costatum	Algae	96 h growth	EC50>66	Kopperman et al. (1974)
flos-aquae	Algae	96 h biomass	EC50=0.97	Kopperman et al. (1974)
P. subcapitata	Algae	72 h growth	EC25=3.4	Ura et al. (2002)
N. pelliculosa	Algae	96 h growth	EC50=19.1	Kopperman et al.(1974)
Natural algal assemblage	Algae	96 h biomass	0.12	Marchini et al.(1992)
Closterium ehrenbergii	Algae	96 h growth	NOEC=250	Canton et al. (1985)
Dunaliella tetriolecta	Algae	96 h growth	NOEC=250	Calamari et al. (1982)
L. gibba	Plant	7 d growth	EC50>62.5	Kopperman et al. (1974)
S. herbacea	Plant	28 d seed germination, morphology	100 100	Abernathy et al.(1986)
E. prostrata	Plant	28 d seed germination, morphology	No effect 1000	Abernathy et al.(1986)
B.frondosa	Plant	28 d seed germination, morphology	100 10	Abernathy et al.(1986)

Table 9-4. Redefined human and ecological exposure pathways for land-applied biosolids.

Pathway	Description of HEI
1.Biosolids→soil→plant	Plant toxicity due to uptake of biosolids-borne TCS when grown in biosolids-amended soils
2. Biosolids→soil→human	Human and child ingesting biosolids
3. Biosolids→soil→plant→human	Human (home gardener) lifetime ingestion of plants grown in biosolids-amended soil
4. Biosolids→soil→plant→animal	Animal lifetime ingestion of plants grown on biosolids-amended soil
5. Biosolids→soil→plant→animal→human	Human lifetime ingestion of animal products (animals raised on forage grown on biosolids-amended soil)
6. Biosolids→soil→animal	Animal lifetime ingestion of biosolids
7. Biosolids→soil→animal→human	Human lifetime ingestion of animal products (animals ingest biosolids directly)
8. Biosolids→soil→soil organism	Soil organism ingesting biosolids/soil mixture
9. Biosolids→soil→soil organism→predator	Predator of soil organisms that have been exposed to biosolids-amended soils

Table 9-5. Data utilized for the calculation of RfD for humans and animals.

Study type	Species	Endpoint	Exposure	Result (mg kg^{-1} day^{-1})	References
Subchronic toxicity	Rat	NOAEL	Dietary feeding exposure for 90 d	100 50	SCF (2000) Ciba (2000)
	Hamster		Dietary feeding for 13 weeks	75	Ciba (2000)
Chronic/carcinogenicity	Rat	NOAEL	Dietary feeding for 2 years	52	Borzelleca (1992)
	Hamster	NOAEL	Dietary feeding for 90-95 weeks	75	Ciba (2000)
Reproductive or developmental	Rat	NOAEL	Exposure by gastric intubations and effects up to 2 generations	150 (reproductive performance) 50 (offspring) 25 (maternal development)	Borzelleca (1992) Ciba (2000) Borzelleca (1992)
	Mouse	NOAEL			
Repeat toxicity	Mouse	NOAEL	13 week Changes in liver weight	25	NICNAS(2009)
Repeat toxicity	Rat	NOAEL	Oral feeding (2 years) Clinical chemistry and liver changes	40 (male) 56 (female)	NICNAS (2009)

Table 9-6. Various parameters used for conducting the preliminary risk estimation.

Abbreviation	Parameter definition	Value	Assumptions/Explanation	Pathway	Reference
BAF	Chemical concentration in an organism divided by the concentration in an environmental medium, when the concentrations are near steady state, and multiple uptake routes contribute (Suter, 2007)	BAF_{worm} = 10 (worm, d.w.)	Conservative BAF values obtained from our study	9	Chapter 5
		BAF_p = 0.93 (plant, d. w.)	Conservative BAF value assuming a worst case	3, 4, 5	Chapter 7
BAR	Biosolids application rate	50 Mg ha^{-1} (d.w.)	One-time application rate	1, 2, 3, 4, 5, 6, 7, 8, 9	NRC (2002)
		5 Mg ha^{-1} y^{-1} (d.w.) x 100 y	Application rate; applied annually for 100 years		
BW	Body weight (live weight)	Adult: 70 kg Child: 16 kg	Mean	2, 4, 5, 6, 7, 9	USEPA (1997)
		Cow: 590 kg			USEPA (1993c)
		American woodcock: 0.181 kg			
		Short-tailed shrew: 0.016 kg			USEPA (1993c)
		Herring gull: 1.09 kg			USEPA (1993c)

Table 9-6. Continued.

Abbreviation	Parameter definition	Value	Assumptions/Explanation	Pathway	Reference
CA	TCS concentration in consumed animal either eating plant or soil	Concentrations vary with the animal species	CS* FS* FI/BW	4, 5, 6, 7	Snyder (2009)
		CA_m (animal meat)	CA* FF		
CB	TCS concentration in biosolids	62 mg kg^{-1}	95th percentile concentration in biosolids in TNSSS	1, 2, 3, 4, 5, 6, 7, 8, 9,	USEPA, (2009a)
CS	Predicted concentration in soils	Worst case:1589 µg kg^{-1} 100 year: 15897 µg kg^{-1}	$CS = \frac{CB * BAR}{DT_{soil} * Rho_{soil}}$	1, 2, 3, 4 5, 6, 7, 8, 9	
DT	Depth	15 cm	Depth of biosolids incorporation	1, 2, 3, 4 5, 6, 7, 8, 9	
DS or Rho	Density	1300 kg m^{-3}	Bulk density of soils		Brady and Weil (2002)

Table 9-6. Continued.

Abbreviation	Parameter definition	Value	Assumptions/ Explanation	Pathway	Reference
CW	TCS concentration in earthworms	Worst case: 15897 µgkg^{-1} 100-year: 158974 µg kg^{-1}	PEC $_{soil}$ * BAF$_{worm}$ Earthworms acquire chemical via ingestion of the soil	8, 9	Chapter 5
FF	Fat fraction in meat	Beef: 0.10 Pork: 0.090 Poultry: 0.060	Mean	5, 7	USEPA, 1997
FI	Food ingestion rate	Cow: 9100 g d^{-1} (d.w.) American woodcock: 139 g d^{-1} (d.w.) Short tailed shrew: 13 g d^{-1} Herring gull : 213 g d^{-1}		4, 5, 6, 7, 9	Nagy (1987) in Suter (2007) In Reiss et al. (2009)
FS	Soil fraction of diet	Cow: 0.025	Value used in Part 503 biosolids risk assessment	6	USEPA (1995)
FVC	Combined fruit and vegetable consumption	7.7 g kg^{-1} d^{-1} (w.w.)	Mean	3	USEPA (1997)

Table 9-6. Continued.

Abbreviation	Parameter definition	Value	Assumptions/Explanation	Pathway	Reference
HFS	Hectare-furrow-slice mass	2.2×10^6 kg	Soil bulk density = 1.3 g cm^{-3}	1, 2, 3, 4, 5, 6, 7, 8, 9,	Brady and Weil, 2002
MC	Meat consumption	Adult			
		Beef: 90 g d^{-1} Pork: 27 g d^{-1} Poultry: 67 g d^{-1}	Mean		USEPA, 1997
				5, 7	
		Child			
		Beef: 1.8 g kg^{-1} d^{-1} Pork: 0.84 g kg^{-1} d^{-1} Poultry: 1.5 g kg^{-1} d^{-1}	Mean		USEPA, 1997
LD_{50}	Lethal dose of TCS that kills 50% of the organisms	Avian toxicity 862 mg kg^{-1} d^{-1} Short tailed shrew: NOAEL: 75 mg kg^{-1} d^{-1}	The organisms feed on earthworms as their primary diet	9	In Reiss et al. (2009)
PC	Concentration in the plant tissue	Worst case: 1478 µg kg^{-1} 100 year: 14784 µg kg^{-1}	CS * BAF_p	3, 4, 5	Chapter 7
PEC	$PEC_{predators}$	Predator TCS concentrations vary with the species	Calculated for two biosolids application scenarios	9	Chapter 5

Table 9-6. Continued.

Abbreviation	Parameter definition	Value	Assumptions/Explanation	Pathway	Reference
RfD	Reference dose: daily dose of chemical that appears to be without appreciable risk during an entire life time.	Human: 0.40 mg kg^{-1} bw d^{-1} Animal: 1 mg kg^{-1} bw d^{-1} Predators: 2.87 mg kg^{-1} bw d^{-1}	Calculated using toxicity end-points obtained from literature	1, 2, 3, 4, 5, 6, 7, 8, 9	Chapter 9
SI	Soil ingestion	Adult: 0.05 g d^{-1} Child: 0.2 g d^{-1} Pica child: 10 g d^{-1}	Mean	2	USEPA (1997)
WD	Percent of worm diet	Hearing gull : 7.7 American woodcock: 78 Short tailed shrew: 31.4		9	Reiss et al. (2009)

Table 9-7. Equations used to calculate screening level hazard indices (HI) (considering no TCS degradation).

Pathway	Hazard Index Equations	Worst case Hazard Index	100-year Hazard Index	Comments/ Assumptions
1. Biosolids→soil→plant	CS / RfD *CF	0.159	1.59	Direct TCS effects on plant growth
2. Biosolids→soil→human	(CS * SI / BW) / RfD *CF	Adult: $2.6*10^{-6}$ Child: $4.5*10^{-5}$ Pica child: $2.2*10^{-3}$	Adult: $2.6*10^{-5}$ Child: $4.5*10^{-4}$ Pica child: $2.2*10^{-2}$	
3. Biosolids→soil→plant→ human	(PC * FVC) / RfD * CF	Adult: 0.026	Adult: 0.26	All produce consumed grown in biosolids-amended soil
4. Biosolids→soil→plant→ animal	(PC*FI/BW)/RfD*CF	Cow: 0.023	Cow: 0.23	100% of diet consists of plants growing biosolids-amended soil
5. Biosolids→soil→plant→ animal→human	(CA_m * MC / BW) / RfD * CF	Beef: $6.7*10^{-6}$ Pork: $1.8*10^{-6}$ Poultry: $2.9*10^{-6}$	Beef: $6.7*10^{-5}$ Pork: $1.8*10^{-5}$ Poultry: $2.9*10^{-5}$	Individual HI values calculated for each meat product consumed 100% of diet consists of plants grown in biosolids-amended soil

Table 9-7. Continued.

Pathway	Hazard Index Equations	Worst case	100 years	Comments/ Assumptions
6. Biosolids→soil→animal	(CS * FS * FI / BW) / RfD * CF	Cow: $6.1*10^{-4}$	Cow: $6.1*10^{-3}$	
7. Biosolids→soil→animal→ human	(CA_m * MC / BW) / RfD * CF	Beef: $1.8*10^{-7}$ Pork: $4.8*10^{-8}$ Poultry: $8*10^{-8}$	Beef: $1.8*10^{-6}$ Pork: $4.8*10^{-7}$ Poultry: $8*10^{-7}$	HI values calculated assuming consumption of each meat product
8. Biosolids→soil→soil organism	SC / RfD * CF	Eisenia foetida: 0.16	Eisenia foetida: 1.59	Based on the most sensitive LC_{50} Lifetime spent in biosolids-amended soil
9. Biosolids→soil→soil organism→predator	(FI* WD *CS* BAF_{worm}/BW) / (RfD * CF)	Herring gull: 0.08 American woodcock: 3.3 Short-tailed shrew: 1.4	Herring gull: 0.83 American woodcock: 33 Short-tailed shrew: 14	Earthwom diet by the birds is variable among species

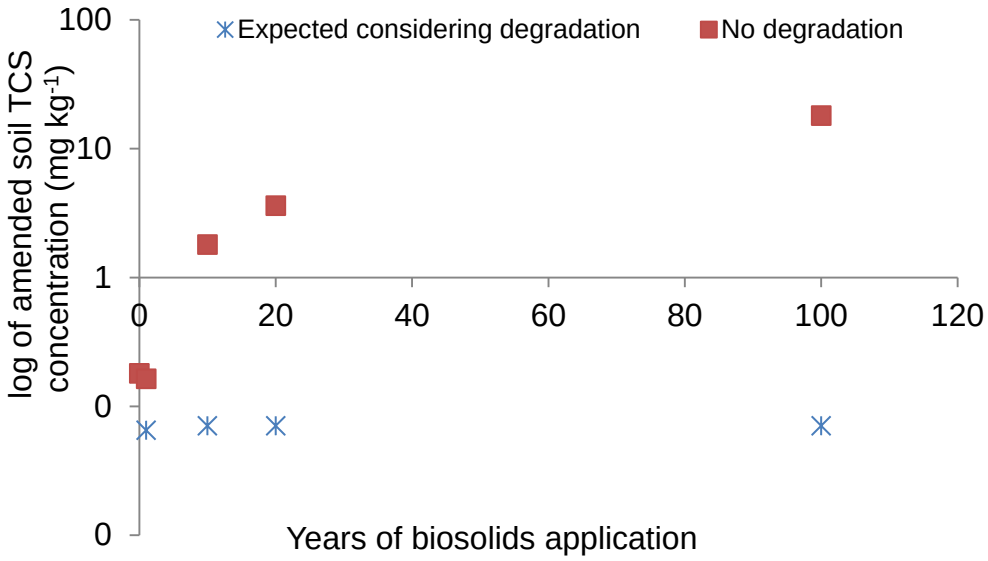


Figure 9-1. Log of predicted TCS concentrations (mg kg^{-1}) assuming no TCS loss, and expected TCS concentrations (considering degradation) varying with time (years) of annual biosolids applications.

SUMMARY AND CONCLUSIONS

Summary of Intermediate Objective Results

Objective 1: Quantify TCS Concentration in Biosolids

The measured TCS concentrations in 15 biosolids were 0.40 to 40 mg kg^{-1} with an average of 18 ± 12 mg kg^{-1} and a median concentration of 21 mg kg^{-1}. The average and median concentrations were consistent with the mean value (16 ± 65 mg kg^{-1}) reported in TNSSS (USEPA, 2009a) and majority of recently published studies. Biosolids utilized in our study were anaerobically digested, but differences in TCS concentrations occured likely due to differences in digestion periods (time), inputs, or dewatering methods (air-dried vs cake). Triclosan concentrations may also differ between WWTPs serving residential and industrial communities. A typical representative range of biosolids TCS concentration appears to be ~10 to 20 mg kg^{-1}.

Objective 2: Determine/Verify Basic Physico-Chemical Properties of TCS

Water solubility of a chemical influences a chemical's transport, bioavailability and degradation rate in soils. Measured TCS water solubilities were 9 mg L^{-1} (pH = 6.14), 27 mg L^{-1} (pH = pK_a = 8.14) and 800 mg L^{-1} (pH = 10.14). The overall 90-fold increase in solubility when pH exceeded the pK_a resulted from the dissociation of the neutral acid to anionic species. Increased solubility at higher pH portends greater concentration in the aqueous phase during sewage treatment processes utilizing lime stabilization and in high pH soils. High pH levels are not common in most sewage treatment systems or in most soils, except in some sodic soils. Solubility of TCS measured at pH 6.14 (i.e., 9 mg L^{-1}) appears reasonable for predicting the fate and transport of TCS in many soils. Partitioning coefficient (K_{ow}) values determined by using models and published measurements were in close agreement, and the value (log K_{ow} = 4.8) changed

negligibly when pH exceeded the pK_a. A log K_{ow} of 4.8 was considered representative at pH values typically found in most sewage treatment systems and in amended soils.

Mobility of TCS in biosolids-amended soil determines the potential for soil and groundwater contamination. The low water solubility (9 mg L^{-1}) and relatively high log K_{ow} (4.8) of TCS suggests extensive retention in (or on) biosolids and limited transport in soil systems. The mean (n=7) inherent log K_d values for biosolids was 4.15 ± 0.03 and log K_{oc} was 4.68 ± 0.07. The mean (n=18) spiked log K_d value for biosolids was 3.76 ± 0.04 and log K_{oc} value was 4.30 ± 0.03. Based on the limited number of biosolids (n=7) analyzed for inherent K_d and K_{oc} , the differences between inherent and spiked coefficients were not statistically significant, but the inherent log K_d and K_{oc} values tend to be greater than the spiked values. The log K_d values determined for biosolids, soils and biosolids-amended soils were variable. Following normalization to organic carbon, the coefficients (K_{oc}) determined in all matrices were not significantly different and averaged 4.26 ± 0.31 (Agyin-Birikorang et al., 2010). Thus, a specific or narrow range of TCS partitioning coefficients (K_{oc}) can serve as a first approximation to describe the behavior of TCS in soils or other matrices. The high log K_{oc} suggests preferential partitioning of TCS into organic material and thus, the mobility of biosolids-borne TCS is expected to be low, with minimal risk of leaching.

Objective 3: Determine the Degradation (Persistence) of Biosolids-Borne TCS

Biodegradation of TCS was studied in biosolids-amended sand (IFS) and silty clay loam (ASL) soils. Mineralization of ^{14}C-TCS to $^{14}CO_2$ was minimal (<0.5%) in both soils through 18 week in both biotic and inhibited biotic treatments. Biosolids-amended soil TCS concentrations (up to 0.40 mg kg^{-1}) did not adversely affect CO_2 evolution at any time. Methyl-TCS was a major metabolite of TCS in the biotic treatment in both

soils. Absence of Me-TCS in inhibited treatment suggested that Me-TCS is formed by a biomethylation reaction. Biosolids-borne TCS is persistent with a time for 50% disappearance of 77 to >126 d depending on soil texture. Triclosan formed bound (non-extractable) residues of expected limited lability with time. Degradation data were inconsistent with the TCS lability expectations according to our operationally-defined extraction scheme. Triclosan degraded faster in silty clay loam soil (than the sand soil), but the silty clay loam has a smaller labile pool. So, the operationally-defined scheme imperfectly distinguishes the labile and bound pools, and/or some of the compound in bound fraction may also be bioavailable/biodegradable. Further investigation is needed of the lability-inferred extraction schemes and the relationship to other measures of lability (degradation and bioaccumulation data). Triclosan degradation is typically slower in biosolids-amended soils (laboratory study) than in un-amended soils, but faster than in field soils. We accept our hypothesis that biosolids-borne TCS is persistent in the environment and forms bound residues; however, the fate of metabolite (Me-TCS) is not known.

Objective 4: Determine the Impacts of Biosolids-Borne TCS to Soil Organisms

Earthworm survival in the silty clay loam (ASL) soil and the artificial soil was not adversely affected at TCS concentration ≤10,005 mg kg^{-1} biosolids (equivalent to a maximum amended soil concentration of 100 mg kg^{-1}), suggesting an LC_{50} of ~10,005 mg kg^{-1} biosolids. The estimated LC_{50} value in the IFS soil is ~105 mg TCS kg^{-1} biosolids. The application of biosolids at 22 Mg ha^{-1} followed by incorporation at 15 cm depth (~100 fold dilution of the TCS concentration), results in LC_{50} of ~1 mg TCS kg^{-1} soil in the IFS soil and 100 mg TCS kg^{-1} soil in the ASL and artificial soils.

Earthworms grown in the silty clay loam soil (ASL) accumulated more TCS than in the sand (IFS) soil. The average BAF values in the two soils, irrespective of the spiked TCS concentration, were 6.5 ± 0.84 for the IFS soil and 12 ± 3.08 for the ASL soil. The average values were significantly different ($p<0.05$) from each other and likely reflected variable physico-chemical soil properties. The soils differed in native soil organic carbon (OC) contents (11 g kg^{-1} for IFS soil and ~34 g kg^{-1} for ASL soil), suggesting more accumulation in soil with greater OC content. The average TCS concentration measured in the earthworms collected in a field equilibrated soil was 4.3 ± 1.9 mg kg^{-1}, corresponding to a BAF value of 4.35 ± 0.7. Greater BAF values occurred where TCS was spiked into biosolids (laboratory conditions) as opposed to inherent TCS in field soils. The spiked log K_d values are less than the inherent log K_d values suggesting more availability in a spiked system. Biosolids-borne TCS accumulated in, but was not toxic to, earthworms. We accept the hypothesis that biosolids-borme TCS is not toxic to earthworms but acknowledge that earthworms may accumulate TCS. Earthworm accumulation of TCS varied significantly between laboratory and field conditions, but the accumulation did not vary with TCS concentration in biosolids. Earthworm TCS bioaccumulation would be similar irrespective of the TCS concentration in soils where the worm is grown.

Objective 5: Determine the Toxicity of Biosolids-Borne TCS on Microbial Reactions

The TCS concentration of 505 mg kg^{-1} biosolids (5.05 mg kg^{-1} soils) was regarded as NOAEL for the microbial community toxicity test in both soils. Biolog plate analyses suggested that micro-organism exposed to TCS do not differ in substrate utilization and, thus, community structure effects were not anticipated. Biosolids-borne TCS has no

adverse effect on soil micro-organisms, using microbially-mediated processes, community structure, and bacterial counts as indicators, and our hypothesis of no adverse TCS effects was accepted. Assuming a representative biosolids TCS concentration of 16 mg kg^{-1}, biosolids applied at 22 Mg ha^{-1} $year^{-1}$ would have to be applied for at least 30 years (assuming no loss of compound and no decrease in bioavailability) to expect possible adverse effects on the processes of ammonification, nitrification, or microbial respiration.

Objective 6 and 7: Quantify the Phytoavailability and Leaching Potential of Biosolids-Borne TCS

Plant toxicity and bioaccumulation tests were performed using three plant species including radish, lettuce, and bahia grass. Using fresh biomass yields as the criteria, an amended soil concentration of 9150 ng g^{-1} was regarded as the LOEC for radish root growth. For lettuce, 4570 ng g^{-1} was the LOEC, and for bahia grass the NOEC is at least 9150 ng g^{-1}. Based on dry biomass yields, NOEC for all the plants is at least 9150 ng g^{-1}. Food chain bioaccumulation begins with chemical uptake by plants. Plant bioaccumulation tests suggested minimal accumulation in radish (BAF = 0.004) and bahia grass leaves (BAF = <0.001), but some accumulation in lettuce leaves (BAF = 0.04) and more in radish roots (BAF = 0.43). Dicots (radish, lettuce) accumulated more TCS than the monots (bahia grass). The BAF values obtained in our study suggest some accumulation in the below-ground biomass (roots), and translocation to the above-ground biomass (leaves), but accumulation was greater in the root than in the leaves. Thus, a diet of tuber (root) plants is likely to pose greater risk of TCS to animals and humans than a diet of leafy plants (lettuce).

As expected from the measured K_d and K_{oc} values, the mobility of TCS was minimal in leached soil columns amended with ^{14}C-TCS spiked biosolids. The leaching was less in a biosolids-amended soil than a soil without biosolids, as TCS association with the OC of the biosolids retards movement. In amended soil, both TCS and Me-TCS remained within the depth of biosolids incorporation (0-2.5 cm), but in controls, surface applied TCS (and Me-TCS) moved to a depth of 2.5 cm. Thus, a relatively long time will be required for both chemicals to move through a soil profile and reach groundwater. Estimated time for 50% TCS degradation in the leaching study was 76 d, consistent with 50% disappearance time in the incubation study (77 to >126d). We accept our hypothesis that biosolids-borne TCS has minimal mobility and phytoavailability.

Ultimate Objective: Risk Assessment of Biosolids-Borne TCS

A screening level risk assessment suggested minimal risk to human, terrestrial or aquatic populations (HI<1) in most of pathways, except in pathways 1, 8 and 9 (HI>1). A tier-2 assessment considering TCS loss through degradation reduced the HI value for all critical pathways to less than one, suggesting minimal risk to even sensitive organisms like American woodcock, herring gull, short tailed shrew, earthworms and plants. We accept our overall hypothesis of minimal risk of biosolids-borne TCS to human and environmental health. Biosolids containing a 95th percentile TCS concentration (62 mg kg^{-1}) applied at agronomic rates (5 Mg ha^{-1}) for multiple years (up to 100 years) did not adversely affect the majority of the organisms (according to tier-2 assessment) included in the exposure pathways described herein.

American woodcock was identified as the most sensitive species to TCS exposure and, thus, pathway 9 was utilized for the calculation of preliminary pollutant limits. The estimated CPLR was 5.8 kg TCS ha^{-1}. The resulting pollutant concentration limit was 6.3

mg TCS kg^{-1} biosolids, which portends that if biosolids contain a TCS concentration <6.3 mg kg^{-1}, biosolids land application may not have restrictions regarding TCS risk. However, our pollutant limits are preliminary estimates not intended to make changes to the biosolids land application regulations. Rather, we calculated the limits to guide future TCS work. We utilized the measured data (collected from our study) wherever possible for risk estimation, but the assessment for some organisms (e.g. predators) was limited by the lack of reliable data sources. Snyder (2009) identified the predator pathway (pathway 9) as the most critical pathway in the TCC risk assessment, and same pathway was critical for TCS. Our estimates are preliminary, as the toxicity end point in the most critical exposure pathway (pathway 9) was derived from unpublished sources and the risk was assessed based on our best scientific judgement (use of uncertainty factors). The critical exposure pathways (HI>1) identified herein needs re-assessment based on reliable toxicity endpoints. Additionally, the TCS pollutant limits were established assuming no loss of TCS through degradation, but TCS disappears with a relatively short half-life (100d) and should be considered when estimating the pollutant limits. As Me-TCS was deemed as a major metabolite, risk assessment of biosolids-borne TCS should consider the contribution of Me-TCS.

Future Studies

Appearance of metabolite (Me-TCS) raises some concern, as limited information is available about Me-TCS. Leaching behavior of Me-TCS addressed in our leaching study was similar to TCS. However, basic physicochemical properties of Me-TCS (solubility, K_{ow}, K_d), biodegradation, toxicity, plant accumulation are essentially unknown. A recent study detected Me-TCS in soils amended with biosolids-borne TCS (Lozano, personal communication, 2011), and future studies should address the fate

and transport of Me-TCS in biosolids-amended soils. Further, the behavior of bound fraction of TCS is still not certain. Our short-term (18 week) incubation study suggested formation of bound resiues but long-term studies are needed to explore possible changes in bioavailability of TCS bound fraction with time. Data gaps also remain regarding the potential development of antibiotic resistance and endocrine disruption effects of TCS. Conflicting data reports the ability of TCS to cause endocrine disruption (Veldhoen et al., 2006; Fort et al., 2010) in aquatic environments. Veldhoen et al. (2006) suggested endocrine disruption effects at TCS concentrations (0.15-1.4 µg L^{-1}), as opposed to no adverse effects observed in Fort et al. (2010) study. Improved risk assessments also require more reliable toxicity end points for terrestrial species.

Other research might include TCS leachability studies to explore the effect of soils, biosolids, application methods and ground cover on the TCS movement. Aquatic risk assessments only consider TCS concentrations in the aqueous phase but given the high partitioning coefficients of TCS, it is expected to sorb to dissolved organic carbon (DOC) and reduce the TCS availability to aquatic organisms. Characterization of bioavailability of DOC-associated TCS could improve the risk assessments of runoff and tile drianage waters entering the surface waters and affecting aquatic environments.

APPENDIX A
EXPLANATION OF THE SEQUENTIAL EXTRACTION SCHEME

Labile is defined as the fraction of the chemical that is readily transformed by micro-organisms or readily available to plants (https://www.soils.org /publications/soils-glossary#). Mild extraction methods are commonly used to mimic the chemical uptake by soil organisms and plants and, thereby, estimate the labile fraction of the chemical. Common extraction agents used to characterize lability of compounds similar to TCS (chlorophenols) are a mixture of water and MeOH (Yu et al., 2005; Hu et al., 2005). Water extractions alone do not always correlate well with bioavailability (Hickman and Reid, 2005). In particular, the bioavailability of hydrophobic chemicals can be underestimated by water extractions because low water solubility limits the amount of chemical extracted (Semple et al., 2003). Combining the amount of chemical extracted by water and MeOH is one approach to circumvent the problem with chemicals characterized by low water solubility. The humic fractions with expected low lability can be successfully extracted by a strong base like NaOH (Shirshova et al., 2006). The residual activity left in the dried soil after the sequential extractions is expected to represent the bound or non-labile fraction. However, chemicals considered bound are sometimes sorbed to the OM, Fe and Al oxides by either weak electrostatic forces or covalent bonds. The TCS sorbed by weak electrostatic forces is expected to be extracted by a mixture of MeOH+acetone (50:50 v/v), as the two solvents differ in polarity and should extract the loosely sorbed TCS. Indeed, the solvent mixture is utilized to determine total TCS and triclocarban concentrations in biosolids (Snyder et al., 2010; Heidler et al., 2006) and is expected to be a more rigorous extractant than MeOH alone. The ^{14}C bound by covalent bonds was considered bound to the soil. The

sequential extraction scheme is proposed to extract labile (water, MeOH) and the non-labile TCS in the soils. The non-labile fractions will include: humic-associated, (NaOH) loosely sorbed (MeOH+acetone), and bound (combustible) fractions of TCS.

APPENDIX B
SUPPLEMENATAL DATA FOR CHAPTER 7

Table B-1. Average bioaccumulation factors (BAF) in the radish and lettuce leaves after excluding the highest treatment (Trt 3).

Plant	Average BAF including all treatments	BAF excluding Trt 3
Radish roots	0.43	0.26
Lettuce leaves	0.04	0.02

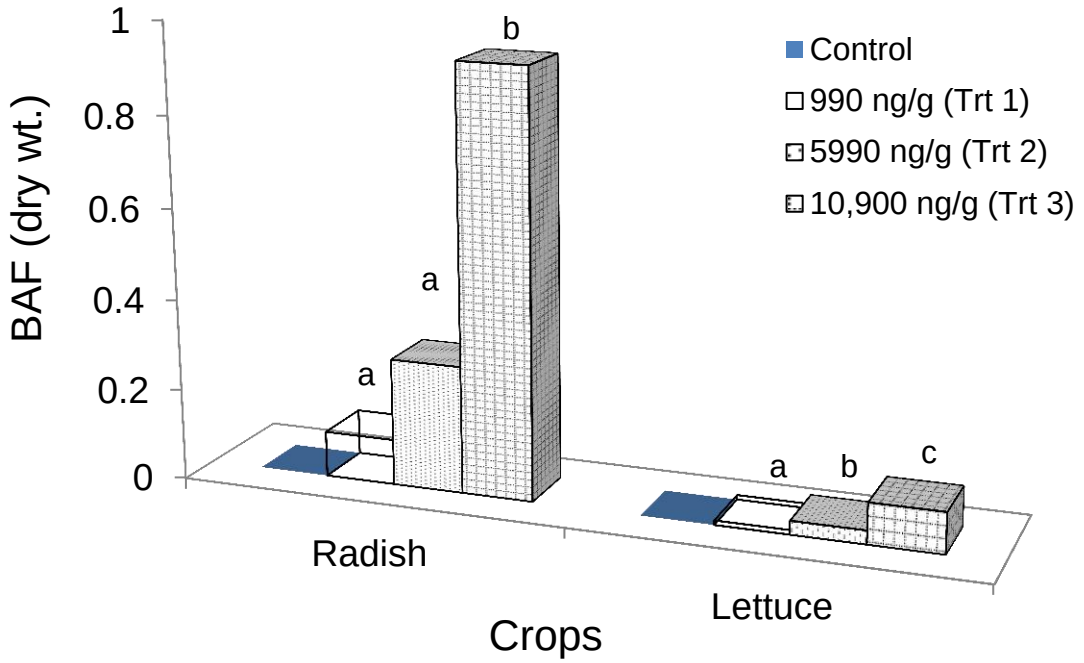


Figure B-1. Comparison of bioaccumulation factors in radish root (below-ground) and lettuce leaves (above-ground) grown in a biosolids-amended soil with a range of TCS concentrations (same letters represent no statistical difference among treatments).

APPENDIX C
LIMITS OF DETECTION, QUANTITATION AND RECOVERIES

Table C-1. Limits of detection, quantitation and percent recoveries for TCS and Me-TCS in various matrices.

Matrix	Chemical	% recovery ± S. E	LOD (ng g^{-1})	LOQ (ng g^{-1})	Instrument
Biosolids	TCS	64 ± 5.0	0.40	1.3	LC/MS
Soil	TCS	95 ± 7.6	0.28	1.0	GC/MS
	Me-TCS	90 ± 8.6			
Earthworm	TCS	93 ± 5.0	0.22	0.7	GC/MS
	Me-TCS	91 ± 4.5			
Plants	TCS	90 ± 11	0.28	1.0	GC/MS
	Me-TCS	89 ± 6.9			

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BIOGRAPHICAL SKETCH

Manmeet Waria was born in the beautiful state of Punjab (India), and is the youngest of the 3 three sisters with a younger brother. She received a Bachelor of Science (Hons.) in agriculture from Punjab Agricultural University in India and moved to U.S. in 2005. She obtained a Master of Science in environmental soil science from University of Nebraska, Lincoln in 2007. In the spring of 2008, she moved to sunny Florida and joined Ph.D. in the Soil and Water Science Department and graduated in spring 2011. She is a married woman and along with her Veterinarian husband, she dreams to travel the world.