Method Development for Contaminant Uptake Pathways Using Triclosan and Macroinvertebrates

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ABSTRACT

Triclosan (TCS) is an antibacterial chemical added to personal care products. Because large quantities of triclosan are produced and subsequently introduced into environmental systems, it is important to understand how it behaves in these systems, including its movement through food webs. Analytical methods were modified to extract triclosan and methyl-triclosan (MTCS) from water and biota samples. A bioaccumulation and biomagnification study was performed, using *Chironomus riparius* and Aeshnids (Odonata: Aeshnidae). *C. riparius* was exposed to a controlled amount of triclosan for 20 days and sampled periodically. Aeshnids, in separate aquaria, were fed triclosan-exposed *C. riparius* concurrently. They were sampled at the completion of the 23 days. Results indicate a decrease in concentration of triclosan in water, no detectable concentrations in biota, having the sand as the major sorption pool. Future work will focus on the improvement of the analytical methods and the transfer of these chemicals into higher trophic levels.
OVERVIEW
In order to study the behavior of TCS and MTCS in environmental, aquatic systems, methods were developed for its analysis in water and biota samples. The objectives of this thesis were (1) to refine methods for detecting organochlorine pollutants in water, biota, and sediment, (2) to refine a method for the bioaccumulation of organochlorines pollutants using model organisms, and (3) to develop a method to track the movement of the organochlorine pollutants up trophic levels in a laboratory setting.

INTRODUCTION
Humans have been affecting the environment through all of recorded history. Some effects can have minimal impact, and nature can maintain its traditional functions. Others can have drastic impact, affecting the balance of the ecosystem. Despite better living through chemistry, when synthetic compounds are released into the environment, the magnitude of their effects is shrouded by natural processes. Moreover, some of these processes are compounding, resulting in field data that might not explicitly show how much one process influences the results. Before an attempt can be made to distinguish these processes, a general understanding of their fate must explained.

Contaminant Fate
Focusing on organochlorines pollutants, Figure 1 depicts a simplified snapshot of the fate of a chemical into an aquatic system. The chemical has been introduced into the water, usually from point-source pollution (1). The chemical will reach equilibrium with the abiotic elements. The chemical may stay dissolved in the water (2), it may evaporate into the atmosphere (3), and it may sorb to the sediment (4). The chemical will also reach equilibrium with the biotic elements. The chemical will be absorbed by organisms through several pathways, such as diffusion or consumption of matter full of the chemical. When the concentration in the organism is higher than the environment (5), this is defined as bioaccumulation. Moreover, when the concentration in the first trophic level organism is lower than the concentration in the second trophic level organism (5), this is defined as biomagnification.

Figure 1: The fate of organic pollutants in aquatic ecosystems
Bioaccumulation, Bioconcentration, Biomagnification

Kravitz et al. (2000) defined bioaccumulation as the uptake of chemicals into the tissue of organisms through any route: respiration, ingestion, or direct contact with contaminated water, sediment, and pore water in the sediment. To quantify an appropriate cumulative factor, the ratio of the concentration in biota to the concentration in the environment (water) is defined as the bioaccumulation factor (BAF). Curiously, Kravitz et al. (2000) define bioconcentration as the accumulation of a contaminant by water alone, and define the bioconcentration factor (BCF) identically as BAF. Kravitz et al. (2000) categorize biomagnification as the result of both bioaccumulation and bioconcentration, amplifying the concentration up the trophic levels. However, Kravitz et al. (2000) quantify the biomagnification factor (BMF) as the ratio of the concentration in biota by contaminated food alone to the concentration in the environment (water). These terms are describing how the chemical will interact with the biotic elements of the system, but obtaining samples where the contamination is only from one source than the other is difficult. It can can occur such is the case of the bald eagle and DDT where the only source of DDT came from the food it consumed (reference). Situations like this work best when the organism does not inhabit the phase that has the most contamination. The only manner to determine this for aquatic organisms is to perform a controlled experiment.

Objectives

The objectives of this thesis were (1) to refine methods for detecting organochlorine pollutants in water, biota, and sediment, (2) to refine a method for the bioaccumulation of organochlorines pollutants using model organisms, and (3) to develop a method to track the movement of the organochlorine pollutants up trophic levels in a laboratory setting.

LITERATURE BACKGROUND

To assess which organochlorine contaminants and organisms to study, scientific literature was collected to determine which would be suitable. The following sections describe the model chemicals and model organisms.

Triclosan and Methyl-triclosan

Use in Commercial Products

TCS was first introduced into the healthcare industry in 1972 as an antimicrobial component of surgical scrub, and since its debut, it has become one of the most prominent biocide used in personal care products (PCPs) in the world (Jones et al. 2000). To highlight its prominence, Schweizer analyzed 700 samples from 1992 to 1999 and found that most of the antibacterial products found on the consumer market had TCS as a FDA-regulated active ingredient (Schweizer 2001). This suggests how quickly a medical-grade product can enter into the consumer market within 20 years. This marks TCS as a great organochlorine to study given its ubiquitous distribution. Another reason is its bioaccumulative nature.

Triclosan in Humans

TCS can enter the human body and build up over time. Ingestion and dermal absorption are the likeliest methods of introduction. A pilot study in Sweden raised concern over the concentrations in the human body. It looked into randomly selected breast milk, a human fluid having a higher concentration of organic compounds (Adolfsson-Erici et al. 2002). From five randomly selected milk samples from the Mother’s Milk Center in Stockholm, they found an interesting mix of results. Two of the samples had concentrations of TCS less than 20 μg*kg⁻¹, but one sample had 300 μg*kg⁻¹.

While the authors did not provide insight into possible explanations for these numbers, it would seem reasonable that TCS was entering into the body in some fashion. Since TCS has
been known to be used in toothpaste, it is possible that some TCS was absorbed into the body through this way. How long it persists in human fatty tissues is still unknown. It is possible that TCS might be metabolized after some time has passed, but this might only occur if it is in a place of high metabolic activity. Furthermore, there is a likelihood that once TCS establishes itself on fatty tissue that is unlikely to be consumed, it can persist a whole lifetime.

TCS can have considerable concentrations in places where adipose lies in high quantity. It is likely if TCS were found in any other body tissue, it would travel until it were either excreted or settled in a fat cell. As awakening as this study was, this showed the scientific community the need for further surveys of the concentration of TCS in human bodies.

Dayan (2007) evaluated 62 samples from the Mother’s Milk Banks in California and Texas for the presence of TCS in the human body. Of the 62 samples, 51 samples had concentrations ranging from 100 to 2100 ug*kg⁻¹ lipid. As high as these concentrations are, Dayan concluded that there is a 6500-fold safety margin between the concentrations humans are exposed to and the concentrations needed for having an adverse effect on human health. Dann and Hontela (2011) suggest that while this may hold true, the continuous exposure to TCS and its high probability of bioaccumulation in human systems may cause adverse build up, requiring more research into the effects that it may have on infants.

Apart from its evidence in human tissues, there are also some known health effects TCS can have on a person if there are high enough concentrations. TCS has been shown to cause skin irritation and induce allergies in children (Campbell and Zirwas, 2006). The legitimacy of its concern can come into question. For humans, the likelihood that concentrations of TCS can cause adverse effects the body is unlikely. Moreover, Campbell and Zirwas tested TCS at near 99% purity in solid form. The amount of concentration one can be exposed at any given time in PCPs is around 0.05% in a dissolved state. While that study certainly raises questions as to the safety of the chemical, there are certainly high tolerance thresholds that might warrant a milder reaction to the safety of human health. What might require more attention is the fate of TCS is after it has been used.

Because it is used in a number of PCPs, high concentrations of it are entering wastewater treatment systems. Depending on system, TCS removal can be highly efficient, having 100% removal success, to none at all (Dann and Hontela 2010). While regulations do exist in the quality of cleanliness of sewage water, TCS, like many other pollutants, are not regulated.

Triclosan in Wastewater Treatment Plants

The Swedish pilot study found varying concentrations of TCS in effluent discharge from three different wastewater treatment plants (WWTPs), which had differing intensities of cleaning steps (Adolfsson-Erici et al. 2002). According to their findings of the fish bile samples, there was a difference between the concentrations of TCS. In the fish exposed to effluent that had only a chemical precipitation aerobic treatment, the concentration was 47 mg*kg⁻¹ fresh weight, while the fish bile of fishes from other WWTPs, which had both an aerobic and anaerobic treatment process, had concentrations <0.08 mg*kg⁻¹ fresh weight. There is clear indication that different treatments may be better at removing TCS than others. However, when looking into this more deeply, one might begin to question how bioaccumulative TCS is. Furthermore, one might postulate as to how much of an influence biomagnification might have on the concentrations described by Adolfsson-Erici et al (2002). Nevertheless, because of its biocidal nature, its breakdown happens significantly more so in aerobic treatments than in anaerobic ones (McAvoy et al. 2002).

A possible explanation might be found looking to the monitoring study Kantiani et al.
performed in 2008, looking into the river systems of Spain. Having taken various water samples from WWTPs, they compared the use of conventional activated sludge (CAS) and two pilot scale membrane bioreactors (MBR) on the same effluent in one of the WWTP. What was noticed when all things kept constant, such as type of water, time of residence, and volume, CAS had an average removal success of 40% whereas MBR had a removal rate of 70%.

While clearly one treatment was more efficient than the other, other WWTPs that used CAS had a total removal rate higher than 80%. As they suggest, the concentration of TCS is mainly attributed to being within the particulate matter, thus being displaced into sewage sludge, allowing for the CAS to remove about 40-50% of the remaining TCS contamination. As it stands, the success of removing TCS from effluent is largely due to its lipophilic properties, adhering to the influent organic matter. This adherence that suggests TCS will sorb onto sediment in aquatic systems, meaning the model organism should live in the benthos.

From Triclosan to Methyl-triclosan

Apart from the removal of TCS by eliminating the activated sludge, other pathways disintegrate TCS into various metabolites and derivatives. One of particular interest is MTCS. MTCS is a result of microbial resistance; while TCS is toxic to many microorganisms, one defense is to methylate TCS to cease its toxicity (Boehmer et al. 2004). The flora in the activated sludge create this metabolite. Because it is no longer has the hydroxyl group, MTCS is much more lipophilic than TCS; moreover, it has become resistant to biodegradation and photolysis (Lindström et al. 2002), unlike the original TCS. While its persistence is greater, its impact on the environment can be considered mute (Bester 2005). However, if found in biota, it may be great indicator of how far wastewater contaminants can affect aquatic systems. TCS could also affect the terrestrial ecosystem.

Triclosan in Biosolids

As that is allowed to settle and be digested, some TCS is degraded and the rest lies dormant in the removed sludge that is eventually converted into biosolids. This, however, can lead to other complications as the practical use of biosolids increases.

While TCS can primarily be categorized as an aquatic contaminant, there is a potential for it to be introduced into terrestrial systems. Numerous studies have found considerably high concentrations of TCS in CAS and the consequent biosolids (Dann and Hontela 2011). While the concentrations of TCS vary significantly, levels in activated sludge can be as low as 500 to as high as 15,600 ug*kg⁻¹ (McAvoy et al. 2002) while biosolids can have concentration of 10,500 to 30,000 ug*kg⁻¹ (Kinney et al. 2008). Interestingly, biosolids may have a higher final concentration of TCS. This can raise much concern when one looks into the agricultural uses biosolids have.

For example, the Milwaukee Metropolitan Sewage District has been producing a fertilizing product Milorganite® for the past 85 years. Dr. George Snyder from the University of Florida, Gainesville, FL conducted a study to see the uptake potential TCS can have with vegetables grown in TCS-containing fertilizer (Snyder 2013). The fertilizer used had a TCS concentration of 2 mg*kg⁻¹. Using such common vegetables as lettuce, tomato, corn, and carrots, Snyder found that corn had the highest concentration of TCS, 29.7 ug*kg⁻¹. The other vegetables had concentrations ranging from 0.8 to 8.2 ug*kg⁻¹. Because of their considerable small concentrations, Snyder (2013) concluded that there is little worry for negative effects on a person consuming produce grown from TCS-containing biosolids, since, using corn as an example, the amount of corn necessary to cause concern is about 1349 pounds of the produce consumed daily, which is an unrealistic possibility for any human. With so much mention of TCS’s behavior,
some necessary information of the compound are needed.

**Physicochemical Properties**

Having the chemical name 5-chloro-2-(2,4-dichlorophenoxy)phenol or 2,4,4′-trichloro-2′-hydroxydiphenyl ether, TCS is registered with the Chemical Abstract Service Registry Number (CASRN) 3380-34-5. It has the chemical formula of C₁₂H₇Cl₃O₂ with a molecular weight of 289.55. TCS is lipophilic in nature, having an octanol-water coefficient at 4.76, in terms of \( \log K_{ow} \). Despite this, TCS is slightly soluble in water, having a maximum concentration of 10 mg*l⁻¹ at 20 °C. Other properties such as photolysis and hydrolysis vary according to pH and dissolved organic material in the water.

MTCS has the chemical name 5-(chloro-2-(2,4 dichlorophenoxy)anisole, with its CASRN as 4640-01-1. It has the chemical formula of C₁₃H₉Cl₃O₂ with a molecular weight of 303.57. While it is synthesized by microbes in activated sludge, it can be purchased commercially as well. It is even more lipophilic than TCS, having the \( \log K_{ow} \) of 5.2. There is very little toxicology of MTCS; however, TCS has been studied extensively for its effects on the biotic elements of the environment.

**Ecotoxicity of Triclosan**

TCS, because of its antimicrobial properties and slight water solubility, has the potential to have the most dramatic effect to the microecology of an aquatic ecosystem. Because of its usually small concentration in the environment, the damage may be undetectable to those not seeking it. In the WWTP, the main cleaning process is a biological chemical cleaning, so the concern for the microorganisms in CAS is highly relevant. Interestingly, Orvos et al. (2002) found that most of them were not severely affected by TCS at levels under its aqueous solubility.

The group which showed severe toxicity was algal in nature. Specifically, *Scenedesmus subspicatus* showed the highest sensitivity to TCS. After a 96-hour study, the EC₅₀ biomass (median effective concentration) was 1.4 ug*l⁻¹ and the NOEC (no-observed-effect concentration) was 0.69 ug*l⁻¹. Marine phytoplankton are not exempt from this toxicity either. DeLorenzo and Fleming (2008) found that for the microorganism *Dunaliella tertiolecta*, a 96-hour experiment showed that its EC₅₀ population density was 3.5 ug*l⁻¹ (DeLorenzo and Fleming 2008). While these were in laboratory setting with static water, these results suggest that aqueous concentration of TCS should relatively stay at low concentrations for them to not affect essential organisms at the productive level of the food web. There is not enough information as to how TCS might affect an ecosystem as it goes up the trophic levels.

Surveys and pilot studies have found TCS concentrations in fish bile (Adolfsson-Erici et al. 2002). There have also been other studies done with aquatic snails (Coogan et al. 2008). Nevertheless, these do not address the behavior of TCS as it progresses through different trophic levels. They do present how TCS is related to bioaccumulation (Orvos et al. 2002), but these do not allow for there to be a clear distinction between bioaccumulation and biomagnification. In addition, some of the model organisms do not have niches were TCS is found in the highest concentrations. This is a drawback of laboratory studies, but if the correct organisms are chosen, a better proxy can be established.
Biota
In the field of ecotoxicology, the biological effects of a toxin is assessed by taxon; however, it does not readily address the behavior of the chemical according to the likeliest location it would be and how it would behave in an ecosystem. For instance, *Daphnia magna* is a common aquatic arthropod used for toxicology. This can be very useful since it provides a standard method of comparison for ecotoxicologists and agencies, but sometimes the chemicals of interest are not found in the location where these organisms live. While *D. magna* could provide method of easy comparison, it might fail in its accuracy of representing the ecosystem when applied to a real-world system. TCS and MTCS are lipophilic compounds that will adhere to the organic carbon in a system, i.e. the sediment. Therefore, model organisms in the benthos are required to track TCS’s movement in a ecosystem’s food web.

**Bloodworms**

Chironomids (Diptera: Chironomidae) are abundant freshwater insects found globally. They are known for their common name of non-biting midge flies. Because of their benthic lifestyle, they can represent a grand amount of insect biomass. A study done on a stream in Indiana reported that the annual chironomid secondary production was about 80% of the total insect secondary production, which is the first trophic level of consumption (Berg & Hellenthal, 1992). They are found near water bodies since the majority of their life-cycle is spent in their aquatic states. Their life-cycle can be divided into four major stages: the egg stage, the larval stage, the pupal stage, and the terrestrial imago stage. The number of larval instars vary from species to species. One species has four larval instars and can have its entire life-cycle occur within three to four weeks. This species is *Chironomus riparius*.

The larvae of *C. riparius* are benthic macroinvertebrates, living in sediment on the beds of rivers and lakes. They indiscriminately eat fine particulate organic matter (microdetritus), silt, periphyton, and other fine particles in the water (Rasmussen, 1984), classifying them as deposit feeders or collectors. This guild constitutes just under 50% of the fauna found in first to third order streams (Vannote et al. 1980). Because of their benthic lifestyle, they can represent a grand amount of insect biomass. A study done on a stream in Indiana reported that the annual chironomid secondary production was about 80% of the total insect secondary production, functionally serving as the first trophic level that is a producer (Berg & Hellenthal, 1992). The larvae are tolerant to varying water qualities, including dissolved oxygen levels (Learner and Edwards 1966), salinity (Bervoets et al. 1996), pH (Havas and Hutchinson, 1982), and sediment grain size (Ingresoll and Nelson 1993). They have also been numerous in polluted waters (Lindegaard 1995, Potsma 1995). This hardy nature makes them suitable subjects to evaluate the behavior of chemicals in the environment. It is also considered an opportunistic species because of its presence in environments

![Figure 4: C. riparius larvae, bloodworm](image)
that might otherwise be ill-established or heavily polluted. Because of its high tolerance to highly eutrophic environment, which are low-oxygenated as well, *C. riparius* is an ideal organism to study the uptake of sorbed contaminants (Hooper *et al.*, 2003).

In the case of TCS, it is only mildly water-soluble, and using a model organism to observe its effects on the environment that would otherwise not be found in the same location is erroneous. Sediment-dwelling organisms are the best to be considered since TCS is lipophilic and would consequently be found in organic matter, which is the case of most eutrophic aquatic sediments. Chironomids, non-biting midge might be the best group of organisms to consider. They have an aquatic-feeding larval stage, and a non-feeding, alate stage. When under laboratory conditions, they have a short life cycle, and the feeding larvae consume sediment-deposited detritus. Because of its high tolerance to highly eutrophic environment, which are low-oxygenated as well, *C. riparius* is an ideal organism to study the uptake of TCS (Hooper *et al.*, 2003).

**Dragonflies**

Another organism is necessary to show the movement of the chemical through direct consumption. Given this is not a common work in ecotoxicology, other likely predators that are atypical were sought. To keep it within the same trophic level, benthic macroinvertebrates, dragonfly naiads were a reasonable choice. They also provided a range of scientific appeal (Córdoba-Aguilar 2008), particular as a bioindicator of aquatic health (Caro and O’Doherty 1999, Corbet 1999, Hornung and Rice 2003).

Dragonfly naiads can spend one to six years in their aquatic state before emerging into an alate adult. In both adult and immature forms, dragonflies are known to be predators. They are capable of consuming other macroinvertebrates and other naiads. As adults, they are able to consume large quantities of flying insects near a water source (Whitfield & Purcell 2013). Moreover, dragonflies present themselves as a hearty meal for the next trophic level. They are commonly consumed by fish and amphibians, and where their are no higher trophic levels, intratrophic predation and intrataxa predation occur. As adults they are consumed by other dragonflies, birds, and some amphibians. In fishless water bodies, dragonfly naiads are the apex predator in their macroinvertebrate ecosystem. These interactions allow for interesting occurrences of shared prey competition and the partitioning of resources (Wissinger & McGrady 1993).

While odonates have not been extensively analyzed as model organisms, some scientists have found their critical value in both aquatic and terrestrial systems for the behavior of chemicals in the environment. For example, Buckland-Nicks *et al.* (2014) were curious to see the bioaccumulation of methylmercury (MeHg) in dragonflies as
naiads, their exuviae, and as adults. The examined two populations of odonates (Odonata: Anisoptera) in two different lakes in Nova Scotia, Canada. They found 232 ± 112 ng·g⁻¹ dry wt in naiads, 236 ± 50 ng·g⁻¹ dry wt in emerging adults, and 231 ± 74 ng·g⁻¹ dry wt in mature adults. These findings suggest that the concentration in odonates is not significantly different from different life stages. In terms of trophic level transfer, both predators of the odonates in aquatic and terrestrial systems have the potential to bioaccumulate MeHg.

Heitzman (2013) also showed how odonates can be useful indicators of other organic pollutants. He studied polycyclic aromatic hydrocarbons (PAH) that were a result of urban runoff into wetlands. He collected samples of both water and odonates, and while the data were variable, the concentrations of PAHs were considerably higher in the biota. This suggested that bioaccumulation was occurring as opposed to passive chemical exposure. Odonates are useful organisms that can be utilized for showing biomagnification occurring in a laboratory setting.

Hypotheses

With the organochlorine pollutants and organisms chosen, the hypotheses are (1) TCS and MTCS will bioaccumulate to biota in a steady-state system to a predicted concentration (2) TCS and MTCS will be transferred to the next trophic level at detectable concentrations.

METHODOLOGY

A mathematical model was developed to help predict the movement of triclosan and methyl-triclosan in a microcosm. This model is a synthesis of previous work done on the partitioning of organic pollutants using physicochemical properties, but there are a several limitations to this model. The equations assumes equilibrium is reached in the system; other variables such as temperature and vapor pressures are not present as influential factors; and other processes that may change the total amount of contaminants in the system (biodegradation, biotransformation, hydrolysis, photolysis) are not present. However, the benefit of using this model was to determine certain experimental parameters such as the time of experiment and total amount of pollutant.

Mathematical Model Development

There are many ways to predict how a chemical will behave in the environment. One manner is to predict its movement through a mathematical model. These models are determined experimentally, distilled into an equation. There was not one simply equation to predict how the movement of triclosan and methyl-triclosan in an aquatic system. In a very simple model, the chemical can partition into different phases in the environment; in this model, they are water (C_w), sediment (C_s), and biota (C_B). This can be written where the total amount of the pollutant (C_T) is the sum of it in the water, sediment, and biota (1). Air is not considered because triclosan and methyl-triclosan do not vaporize at room temperature.

\[ C_T = C_W + C_S + C_B \]  

(1)

However, concentrations are what are reported. Water concentrations (W_c) are mass of contaminant per water volume (V) (2a); sediment concentrations (S_c) are mass of contaminant per total mass of sediment (S_M) (2b); biota concentrations (B_c) are mass of contaminant per wet mass of tissue (B_M) (2c). Some experimentally determined equations relate these concentrations to each other.

\[ W_c = \frac{C_W}{V} \]  

(2a)

\[ S_c = \frac{C_s}{S_M} \]  

(2b)
Bioconcentration factor (BCF) is defined as the ratio of the concentration in an organism to concentration of the water when the exposure has only been through water (Kravitz et al 2000) (3a). Biota-sediment accumulation factor (BSAF) is the ratio of the concentration in the biota divided by the fraction of lipids in the organism (f_L) to the concentration in the sediment divided fraction of organic carbon in the sediment (f_oc) (Ankley et al. 1992) (3b). The soil-water partition coefficient (K_d) is the ratio of the concentration in the sediment to the concentration in water (Karickhoff et al. 1979) (3c).

\[ B_C = \frac{C_B}{B_M} \]  
\[ BCF = \frac{B_C}{W_C} \]  
\[ BSAF = \frac{B_C/f_L}{S_C/f_{oc}} \]  
\[ K_d = \frac{S_C}{W_C} \]  

Karickhoff et al. (1979) experimentally determined that K_d related to the octanol-water partition coefficient (K_ow) through the fraction of organic carbon (f_oc) found in the sediment (4a). Many have found a correlation between log BCF and log K_ow (Samiullah 1990), but Chiou (1985) determined that the BCF determined by f_L than total mass was approximately equal to the triolein-water partition coefficient (K_tw) for slightly water-soluble organic compounds. Jabusch and Swackhamer (2005) found that the log K_ow and log K_tw for their organochlorine pollutants to be approximately equal. Given K_tw for triclosan is not available, but K_ow is considered a proxy for organic partition, the BCF could then be estimated to be directly proportion between the K_ow and f_L(4b).

\[ K_d = 6.3 \times 10^{-4} f_{oc} K_{ow} \]  
\[ BCF = f_L K_{ow} \]  

With these definitions, the BCF and K_d can be defined and expanded to be 5a and 5b respectively.

\[ \frac{B_C}{W_C} = f_L K_{ow} \rightarrow \frac{C_B}{B_M} / \frac{C_w}{V} = f_L K_{ow} \]  
\[ \frac{S_C}{W_C} = 6.3 \times 10^{-4} f_{oc} K_{ow} \rightarrow \frac{C_S}{S_M} / \frac{C_w}{V} = 6.3 \times 10^{-4} f_{oc} K_{ow} \]  

These equations can be rearranged to isolate C_B (6a) and C_S (6b).

\[ C_B = \frac{f_L K_{ow} B_M C_w}{V} \]  
\[ C_S = \frac{6.3 \times 10^{-4} f_{oc} K_{ow} S_M C_w}{V} \]  

With C_B and C_S defined, they can be reinserted into equation 1 (7a) to isolate for C_w (7b).
\[ \begin{align*}
C_T &= C_W + \frac{6.3 \times 10^{-4} f_{oc} K_{ow} S_M C_w}{V} + \frac{f_L K_{ow} B_M C_w}{V} \\
C_W &= \frac{C_T V}{6.3 \times 10^{-4} f_{oc} K_{ow} S_M + f_L K_{ow} B_M + V}
\end{align*} \]  \tag{7a} \tag{7b}

Finally, related values can be condensed to present \( C_W \) (8a), \( C_S \) (8b), and \( C_B \) (8c). These equations rest on the knowledge of \( V, f_L, f_{oc}, K_{ow}, M_S, \) and \( M_B \). With these estimates, \( W_C, S_C, \) and \( B_C \) can be calculated, and the contaminant amount per given sample size can be estimated. Furthermore, the amount of contaminant loss can be estimated experimentally, allowing for a range of where to expect the concentration of contaminant.

\[ C_W = \frac{C_T V}{K_d S_M + BCFB_M + V} \] \tag{8a}

\[ C_S = \frac{K_d S_M C_w}{V} \] \tag{8b}

\[ C_B = \frac{BCFB_M C_w}{V} \] \tag{8c}

**Uptake and Storage**

The rigor of the mathematical model was tested using a method from OCED (2004). This protocol was chosen because equilibrium had to be reached to test the model. It was modified for observing the chemicals partitions with the organisms as well as their movement to another trophic level (Gallego-Gallegos et al. 2013, Marinkovic et al. 2012, Servia et al. 2006). In order to test hypothesis 1, a time series was created to show the movement of the chemical in the aquatic system over time. The following setup facilitates observing this movement.

**Bioaccumulation**

There were six 710 ml glass jars (3.8 cm radius, 17.3 cm height). Within each jar, there was \( \sim 170 \) g of silica play sand (1), reaching the approximate height of 3 cm in the jar. The sand was sieved to have a particle diameter of 250 \( \mu \)m to 500 \( \mu \)m. The sand was rinsed with water three times to reduce the total suspended solids. The jars had 250 ml of tap water (2), reaching about 7-8 cm up the jar, creating a ratio of 1:4 with the sediment level. The jars had continuous aeration at the rate of 1-3 bubbles per second through a glass Pasteur pipette (4) 2-3 cm above the sediment level. They had a photoperiod of 16 h light 8 h dark. The jars are covered with aluminium foil (5) to slow evaporation.

Each jar had 5 *C. riparius* specimen (3) from their first instar (approximately 2-3 days old from hatching). They were provided by Environmental Consulting & Testing, Inc. in Superior, WI. The larvae were in the jars 24 hours prior to the water being spiked with 20 uL of 1000 mg/L of TCS/MTCS. Aeration recommenced after the spike to mix the compounds. The blanks were spiked with 20 uL of pure hexane.
The specimens were fed 0.25 mg of Tetramin© per larvae per day within the first 10 days. After the 10 days, their food intake was increased to 0.5 mg of Tetramin© per larvae per day. After the first 8 days, the first test jar of the bioaccumulation portion was removed for sample preparation. Using a metal spatula, the sand was stirred to find the specimens and carefully removed. The specimens were placed into a separate mason jar with 250 ml of water for 24 hours to allow depuration. The water in the experimental jar was removed as sufficiently as possible. It was decanted into a 250 mL amber jar. Placing a piece of aluminium foil approximately 1350 cm² (45x30 cm, 18x12 in) into a metal pan, the foil is folded to create a malleable pan. The sand was periodically moved into this foil pan, spread out using the metal spatula. If any specimens are found, they were placed into their appropriate depuration jars. Once all the sand has been moved onto the foil, the experimental jar was rinsed with small amounts of water to transfer as many grains as they can. The total number of specimens recovered was recorded. The amber jar was placed into a refrigerator. The foil was placed into a vacuum hood to allow the water to evaporate from 24 to 48 hours. Once all the water is evaporated, the foil was folded up and was placed into a refrigerator. This same process was repeated for specimens on days 15, 18, 22, and 23. On the 23rd day, two specimens had emerged. They did not escape given the jars were covered with foil. The exuviae of the specimen were grouped with the other instar in that sample. Using an aspirator, the midge flies were caught in a plastic tube. Using an acetone-moistened paper towel, the adults were stunned within 1-2 minutes. They were picked up and carefully transferred into an amber GC vial. The vial was labeled and placed into the freezer with the rest of the samples.

**Biomagnification**

In order to see the movement of the chemicals into a higher trophic level, the parameters had to stay the same as the setup above, while a time series was not attempted in this set up, this part of the experiment tested hypothesis 2. Six additional experimental jars with the same parameters were established concurrently. Six other jars had 250 ml of deionized water with a constant, gentle aeration at the rate of 1-3 bubbles per second through a glass Pasteur pipette 2-3 cm above the bottom of the tank. One dragonfly naiad (Odonata: Aeshnidae) was placed into each jar. The dragonfly naiads were provided by Carolina Biological Supply Company in Burlington, NC.

Instead of the *C. riparius* specimens being frozen, they were fed to the naiads, one per naiad. The intention was to have 100% recovery of the *C. riparius* specimens, but uneven recovery caused for some naiads to be fed at different times. Table 1 shows the dates when the naiads were fed.
The extraction and analysis of triclosan and methyl-triclosan is a modified method from Rubinfeld *et al.* (2010). The water extraction, biota extraction, and sediment extraction begin differently, but they follow the same pattern once they are in the cleanup and concentration/derivatization stages.

**Water Extraction**

The sample water was thawed in room temperature. The water was measured in a 250 mL graduated cylinder to 100 mL and weighed; weight and volume were recorded. The water was decanted into a 250 mL separatory funnel. Two drops of 7 M hydrochloric acid and 5 mL of dichloromethane (DCM) were added to the sample; the separatory funnel was vigorously shaken for 2 minutes. The water/DCM mixture was allowed to sit for 15 minutes to allow separation of the liquids; the DCM was drained into a 40 mL vial; this process was repeated twice. In the final drain, the foam created in the water/DCM interface was allowed to go into the vial; DCM was exchanged for hexane using a nitrogen evaporator and concentrated to ~5 mL. The vial was wrapped in parafilm and placed into a refrigerator if the next step did not follow immediately.

**Sediment Extraction**

The sample sediment was thawed and weighed out to ~5 g; the exact mass was recorded. The sediment was placed into a 50 mL beaker; 40 mL of volumetric 1:1 hexane/aceton (H/A) was added to the beaker; two to three scoops of anhydrous sodium sulfate was added to the beaker. Using a sonicator probe positioned 1-2 cm above the sediment line, it pulsed for 6 minutes in 15 second intervals; the beaker was allowed to sit for 15 minutes; the liquid contents were decanted into a 125 mL Erlenmeyer flask through filter paper with anhydrous sodium sulfate. This process was repeated twice. In the final drain, the sediment was allowed into the filter paper, and the glassware that could have had any contact with H/A was rinsed with more H/A. The flask was taken to the nitrogen evaporator to exchange the solvent to only hexane; the liquid was decanted into a 40 mL vial concentrated to ~5 mL. The vial wrapped in parafilm and placed into a refrigerator if the cleanup stage did not follow immediately.

**Biota Extraction**

The sample biota was thawed and weighed out; the exact mass was recorded. The biota was placed into a mortar and pestle; one to two scoops of anhydrous sodium sulfate was added. Small quantities of liquid nitrogen were decanted into the mortar, and the biota was ground to dust. This process was repeated twice or thrice as needed. The biota mixture was transferred into a 50 mL beaker; the mortar and pestle were rinsed with 5 mL H/A into the 50 mL beaker. 35 mL H/A was added to the beaker, and the sample then followed the sediment extraction.

### Table 1: The feeding cycle of the dragonflies. Red marks the naiad had died by that feeding cycle.

<table>
<thead>
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<th>3</th>
<th>4</th>
<th>5</th>
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<td>11/20/2015</td>
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</table>
Cleanup

The water extract has two to three scoops of anhydrous sodium sulfate added to it just prior to this step. A vacuum manifold has a 15 mL centrifuge tube inside of it; a silica SPE cartridge placed onto one of the manifold holds was rinsed with with 5 mL of hexane using the negative pressure of the vacuum. The rinse waste was dripped into a waste beaker; the sample extract was rinsed with 10 mL of hexane inside the cartridge; the cartridge was placed on top of the 15 mL centrifuge tube, and 10 mL DCM was poured into the cartridge. The DCM dripped into the 15 mL centrifuge tube; once all the DCM was dripped into the centrifuge tube, the tube was moved into the nitrogen evaporator to solvent exchange into hexane, maintaining ~10 mL of liquid once solvent exchange was complete.

Concentration and Derivatization

The filtered extract was concentrated from ~10 mL to 200 uL using the nitrogen evaporator. During this concentration, 1 mL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) from Sigma-Aldrich in Milwaukee, WI was added to the 15 mL centrifuge tube. Once the extract was concentrated to 200 uL, the sample was transferred into a gas-chromatography (GC) vial for analysis using a gas chromatograph-mass spectrometer (GC/MS). Prior to analysis, 20 uL of internal standard [name of internal standard here] was added to the GC vial.

Data Analysis

Gas Chromatography-Mass Spectrometry

The analyses of the samples were done by using Agilent Technologies 7890B/5977A GC/MSD instrument. It worked in two parts: the gas chromatograph volatilized a small liquid sample; the gaseous sample traveled through a capillary column carried by helium. The different phases separated heavier compounds from lighter ones. It resulted with different compounds separating in the capillary column with a specific retention time. At the end of the column, the molecules passed through a transfer line into the mass spectrometer. The molecules entered a quadrupole in which electron ionization occurred, splitting the molecules in a characteristic manner to which the detector can assign a mass to charge ratio (m/z), creating a fingerprint of the molecules present. To get an accurate reading, samples were run with a full-range m/z scan and a selected-ion monitoring (SIM) scan.

Calibration Curves

Using Agilent’s MassHunter WorkStation - Quantitative Analysis (Mass Quant) program, two calibration curves of different magnitudes (1 mg/L to 10 mg/L and 100 mg/L to 2000 /L) were created. The calibration curves corresponded to standards of derivatized triclosan and methyl-triclosan and had three points on each curve. Theses curves were used to determine the concentrations of derivatized triclosan and methyl-triclosan in each sample. The standards and samples were run three times.

Statistical Analyses

Linear regressions were established using the biota, water, and sediment samples from the bioaccumulation set up. Linear regressions of the water and sediment of the biomagnification set up were created to infer the relationship each microcosm had with the biota. Relevant concentrations and ratios were calculated from the experimental data and a one tail t-test was done to compare experimental results to the predicted results.
RESULTS

Analytical Efficiency
Spike recovery experiments were also done to see the efficiency of extracting TCS/MTCS, and the data found suggest a high yield loss for TCS and MTCS in the water phase, whereas there was a reasonable yield loss for the biota phase (Table 2). Spike recoveries were not conducted on the sand due to logistical constraints. Given the percent recoveries of the original method had better yield recoveries, it may be necessary to return to that original method.

Percent Recoveries

<table>
<thead>
<tr>
<th></th>
<th>Original Method</th>
<th>Modified Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water (n=2)</td>
<td>Water (n=3)</td>
</tr>
<tr>
<td>TCS</td>
<td>43%±5%</td>
<td>4%±1%</td>
</tr>
<tr>
<td>MTCS</td>
<td>56%±6%</td>
<td>5%±1%</td>
</tr>
<tr>
<td></td>
<td>Biota (n=3)</td>
<td>Biota (n=3)</td>
</tr>
<tr>
<td>TCS</td>
<td>62%±10%</td>
<td>50%±6%</td>
</tr>
<tr>
<td>MTCS</td>
<td>51%±12%</td>
<td>53%±4%</td>
</tr>
</tbody>
</table>

Table 2: Results from the spike-recovery experiments in water and biota.

Bioaccumulation Results
No detectable amounts of TCS/MTCS were found in any of the bioaccumulation biota samples. TCS/MTCS concentrations were found decreasing in the bioaccumulation water samples. (Graph 1). Linear regressions showed that the concentration of TCS could be described as: the concentration of TCS = -0.234*(time in days) + 5.1802, having an R² value of 0.8272. MTCS could be described as: the concentration of MTCS = -0.1597*(time in days) + 3.4511, having an R² value of 0.7806. Under full scan, dichlorophenol (DCP) was found in the water sample (Figure 8). Without a proper calibration curve for DCP, the response factors were measured as a semi-quantitative proxy. The ANCOVA test comparing the response factor slopes was done for all three contaminants. With the results (p-value>0.05), there was not a significant difference between the chemicals.

Having found no traces of TCS/MTCS in biota, the other likely pool of sorption were the sand samples. Samples found significant amounts TCS/MTCS in the sand. The relationship could be described as the concentration of TCS = -0.1617*(time in days) + 45.657 with an R² of 0.0352. For MTCS, it can be described as the concentration of MTCS = 0.3595*(time in days) + 75.517 with an R² of 0.1242. DCP was also found in the sample and showed similar behavior. Finally, the predicted results were compared to the laboratory results (Table 3). The statistical results suggest that the experiment did reach equilibrium in water by the end of the experiment, but because the model assumes equilibrium experimental data did not show a significant difference between the predicted results and the obtained results. Conversely, the biota samples did not match the predicted results; therefore they were significantly different values.
Graph 1: The concentrations of TCS and MTCS in the water phase over time

Semi-Quantitative Analysis of DCP, TCS, MTCS (Water)
Graph 2: The response of the GC/MS of DCP, TCS, and MTCS in the water phase over time.

Graph 3: The concentrations of TCS and MTCS in the sand phase over time.
Biomagnification Results

The data also suggests that no detectable amounts of TCS/MTCS transferred to dragonfly naiads; however, dragonfly naiad three shows some TCS/MTCS to have transferred up the food chain (Figure 9). To verify this finding, a full scan was ran on that sample and the chromatogram shows high noise level in that sample. Given that it cannot be said for certain if the spectra found are TCS/MTCS, the results for that sample are reasonably dismissable (Figure 8, 9).
DISCUSSION

Interpretations of Experimental Data and Evaluation of Method

The data collected yielded interesting results. On one hand, the system reached equilibrium within the 23-days of the experiment; however, the lack of uptake of TCS/MTCS in biota suggests various unaccounted processes affected the system. Several fates could have occurred to TCS/MTCS to suggest this loss. The first, and the most probable, is the compound partitioned into the sand. While this was attempted to be accounted for in the mathematical model, the data suggests that the contaminants disappeared elsewhere. While some photoreactions may explain the loss of TCS, the fact that MTCS followed the same path but at a much higher rate can redirect the thinking to being connected to hydrophobic tendencies. This concurs with the log $K_{ow}$ of TCS/MTCS, 4.9 and 5.2 respectively. To verify that the contaminants reached equilibrium with the rest of the system, it may be necessary to extract from the sediment samples collected. This could explain why no detectable amount of triclosan was found in biota tissue.

A methodological reasoning for the lack of contaminant is the depuration period. The depuration period was done to ensure a proper indication of the contaminant in the tissue and not in the gut content of the specimen. Since the water was free of any TCS/MTCS, it is possible that some of the contaminants partitioned into the depuration water during those 24-hours.

Another possible explanation for the lack of contaminants is the lack of enough biotic mass to amass the pollutants. With the relatively low mass (<0.1 g), the amount that could have partitioned into the biota could have been lost in the extraction method. The failure to accumulate in the bloodworms would also explain the biomagnification data.

The data showed no detected amounts of TCS/MTCS in the majority of the dragonfly samples. Because of the inconsistency of feeding, if any TCS/MTCS was present, the one with the highest amount of pollutant would have been dragonfly one. However, the data showed detected levels of the quantifying ions of TCS/MTCS in dragonfly three. It is not known with certainty why the MassHunter program determined these concentrations in the sample, but when running a full scan of the sample, the data showed a different magnitude of noise compared to the other dragonfly samples. When looking at the peaks of the data, the one’s identified as TCS/MTCS showed signals of possible chlorine atoms in their mass spectra; however, the noise was to great to ascertain what could have cause such numbers. Given the lack of confidence and
that there was only one sample that showed this behavior, it is highly probable that it is a false-positive. Before evaluating the mathematical model, a possible connection to other other studies and the photoreactive nature of TCS should be explored as another source of loss of TCS.

While the photodegradation of TCS has been studied by numerous scientists, Sánchez Prado (2007) best mapped the photodegradation routes of TCS with various components relating to pH, and the presence of chlorine species. The most likely of compound that TCS could have become is 2,8-dichlorodibenzodioxin (DCDD). Latch *et al.* (2003) found that 1-12% of TCS could be converted to DCDD, most likely being the dissociated species given their solutions were buffered at pH ≥8.0 and TCS has a pKa of 7.9. Sánchez Prado (2007) expanded this to being independent of sample pH and having isomers of DCDD or dichlorohydroxydibenzofuran (DHBF). The best support can be found with Aranami & Readman (2007) who showed that both freshwater and saltwater irradiated with artificial lighting showed DCDD after 3 days of exposure. If DHBF was also created, the sample may be silylated because of the derivatizing agent.

Apart from dioxins and furans, TCS can also degrade into chlorophenols. These compounds, 2,6-dichlorophenol and 2,4,6-trichlorophenol, require the presence of chlorine radicals or chloramines to form, even though the original experiment that presented this reaction used concentrations that were not environmentally relevant (Kanetoshi *et al.* 1987). Sánchez Prado (2007) demonstrated that even at low concentrations, TCS conversion yield could reach as much as <10%, being significantly stabler than the parent compound and potentially more toxic. These toxins would also be silylated.

Chloroform could also have formed with chlorine radicals or chloramines, Rule *et al.* (2005) showed that within minutes of combining TCS-imbued dish soap and chlorinated water, 15 μg/L of chloroform was produced. It may possible, however, that since the systems were aerated, this compound, if formed, was likely evaporated into the air and could potentially not be found in the samples. Moreover, since the extraction method focuses on the organic phases and the aqueous phases disposed, this may have been lost in the process.

Evidently there were processes that were not considered for the uptake method. Since tap water was used, though aerated, the presence of chlorine species could have existed and chlorophenols and chloroform could have been formed. DCDD and silylated DHBF could have also formed independent of that, the likelihood it could be found in the water samples are small, given DCDD has a log $K_{ow}$ 5.75 (Burkhard & Kuehl 1986). DHBF, having similar chemical properties and aspects might also have a high log $K_{ow}$, also the presence of the hydroxyl group could make it slightly water soluble.

There are many regions in which the analytical portion could be improved. The low percent recovery of TCS/MTCS suggest a significant loss of contaminant. The location as to where the loss occurred is unspecified. Experimental research data indicated a potential increase in extraction of TCS/MTCS with the modification done on the original method, however, the percent recoveries done on the whole method indicated a loss of sample. It may be necessary to return to the original method for better understanding of method modification.

**Evaluation of Mathematical Model**

There are benefits for composing mathematical models and there are drawbacks. Some of the most evident benefits are in relating chemical properties to chemical behavior. This runs under several assumptions that could benefit the theorist but not the realist. In the particular case of the mathematical model derived, there were quite a few assumptions that occurred that could explain why the model failed to provide a more accurate picture of the results obtained.
The most evident was assuming BCF is proportionally related to the fL and Kow. Attempts have been made to establish this relationship. Chiou (1985) perhaps came closest to finding the answer. The study found that for a variety of slightly water-soluble compounds (such as TCS), the triolein-water partition coefficient (Ktw) was approximately equal to the BCF based on fish lipid weight. This meant that biotic lipids were intrinsically related to the compounds partitioning into biota tissue based. However, Log Ktw is not a value readily reported with environmental contaminants as much as the Kow. Könemann & Van Leeuwen (1980) found that the log BCF and log Ktw has a very strong correlation in guppies, and when the log Kow was related to log BCF a similarly strong correlation was found, suggesting that Kow and Ktw were approximately the same. A probable error in the model might have been to assume the values themselves were the same, whereas their log form were. Using Könemann & Van Leeuwen (1980) equation of relating log BCF to log Kow might have yielded a better result. However, because this problem was not tackling high trophic levels such as fish, the rationale, though supported experimentally, it is not directly related to fatty tissues found in macroinvertebrates. A more accurate direction would be to conduct an experiment where several species of a particular taxon (or trophic level) were exposed to a contaminant, calculating the correlation between the taxon’s fL to BCF and Kow,. This experimentally derived formula would relate these properties, and it could substitute the BCF estimate in the current mathematical model.

Another aspect of improvement in the model is related to data fed to the equation. An assumption of the experiment was that the sediment did not contain any organic carbon. Given the results, it may be possible that such an assumption was flawed. By changing the input of foc, the contaminant concentration estimate shifted to it being more likely to be in the sediment. It may be necessary to conduct an experiment to determine the fraction of organic carbon. Various methods exist; for example, the chromic acid titration method (Walkens & Black 1934) is a relatively simple that requires no extensive analytical instruments while yielding acceptable results. The sand should be tested for the experimentally determined foc to determine if there was any organic carbon that could explain the TCS/MTCS decline in water.

**FUTURE DIRECTION**

**Points of Success**

There are many way to measure the success of a project. In this thesis neither objective was met given the data found; however, what the data suggested a step forward into the research of organic pollutants. As a group of compounds, organic pollutants share the intrinsic quality of having a hydrocarbon backbone; the diversity of the group lies in the functional groups and the arrangement of them. In this particular case, it was noted that silylation of planar compounds definitely increases the sensitivity a GC/MS has with compounds like this. The increase of sensitivity of 218% from normal TCS to silylated TCS, surpassing even the sensitivity of MTCS on the GC/MS.

It was also noted that while performing various modifications to parts of a method can seem promising, without multiple iterations of the wholly improvement method, the data can be misleading. Moreover, it is necessary to have multiple samples as opposed to only having small numbers of them (n<4). It was also noted that while experimentally derived equations are great at attempting to predict the fate of a chemical in the environment, without having a cohesive model that aims at multiple phases, coefficients can only indicate so much of a chemical’s behavior. It would also be interesting to know how to efficiently rear specific macroinvertebrate taxon. Failure to do so can result in severe effects on an experiment and lead to minimal mass for the intended experiment. Moreover, the selection of the chemical to know how it could react
in a system, i.e. knowing photolytic and hydrolytic properties, can definitely impact the data and suggest certain aspects that may not be found.

**New Directions**

With the experience gained, this research, like almost any other, can lead to new routes and new inquiries, tackling related, but different problems encountered in the last aspect, to returning to the drawing board.

**Short Term Directions**

Within the immediate future there are several aspects of this project that could resolved. The first, and most obvious one, is to analyze the sand samples. The possibility that TCS/MTCS partitioned into this phase could explain the failure for bioaccumulation to have occurred in experiment. Moreover, this provides with better insight into selecting the appropriate sediment for such microcosm studies. Related, the Wilkens-Blacks method (1934) could be used to determine the exact number of f_{oc} present in the sand. Failure to have known this could help explain why the mathematical model improper prediction of what would occur at equilibrium.

When these samples are analyzed, both the water and sediment samples can then be searched for the possible photodegradants of TCS (DCDD, chlorophenols, chloroform) and their silylated forms. Problematically, there are no standard for these compounds in this research, so if found, they would be qualitative at best.

**Long Term Directions**

With immediate improvements at the foresight, long term goals could be given groundwork with this research. The unsuccessful nature of increasing the percent recoveries indicates a return to modify methods to increase extraction of organic chemicals. Several options are plausible. Increasing acidity and introducing brine are two options that could enhance the extraction of the chemical in water samples. Freeze-drying the biota tissue instead of using liquid nitrogen could reduce the risk of sample loss through the pouring and the grinding of the tissue. Continuing to determine how effective derivatization is to the compounds is of prime concern as well. Changing the packing material to cellulose could benefit the extraction.

On the other hand, it may be that this method cannot be improved because it was not originally intended for TCS/MTCS extraction. It may be necessary to look at other methods of extraction and derivatization to obtain more accurate readings of TCS/MTCS. Methods such as Rice & Mitra (2007) or Morales *et al.* (2005) show promising results with high recoveries for TCS and other related chemicals. In addition to changing the extraction method, the instrument could be changed as well. A gas-chromatograph and tandem mass-spectrometer could increase the sensitivity and the detection limit, allowing for more environmentally relevant concentrations to be analyzed. Liquid chromatography-mass spectrometry would be another excellent instrument to improve the methods.

**ACKNOWLEDGMENTS**

I would like to thank Dr. Dan Choffnes, Dr. Janice Pellino, and Dr. David Brownholland for their insight in modifying methods, and we would also like to thank Carthage College, the Environmental Science Program, the Society for Environmental Toxicology and Chemistry, and the Summer Undergraduate Research Experience (SURE) for funding and research facilities. Finally, I would like to thank Dr. Tracy Gartner for her insight in science writing, and most of all, I would like to thank Dr. Sarah Rubinfeld for being a mentor and fellow researcher, making every moment an opportunity to improve.
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Hornung, J. P. Odonata and wetland quality in southern Alberta, Canada: a preliminary study.


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