CONCENTRATIONS OF TRICLOSAN IN THE CITY OF DENTON WASTEWATER TREATMENT PLANT, PECAN CREEK, AND THE INFLUENT AND EFFLUENT OF AN EXPERIMENTAL CONSTRUCTED WETLAND

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The Pecan Creek Waste Reclamation Plant in Denton, Texas, an activated sludge WWTP, was sampled monthly for ten months to determine seasonal and site variation in concentrations of triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol), an antibacterial additive. SNK separation after the highly significant ANOVA on ranked data were: summer = fall > winter = spring and influent > downstream = effluent = wetland inflow > wetland outflow (α=0.05). After the plant converted to ultraviolet disinfection, measurements were made before and after the UV basin to determine if significant amounts of triclosan were converted to dioxin. Percent loss at each of the treatment steps was determined. Concentrations of triclosan in the downstream site were below the published NOEC for the most sensitive species.

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CHAPTER 1

INTRODUCTION

Triclosan (5-chloro-2-[2,4-dichloro-phenoxy]-phenol) manufactured in Europe by Ciba Specialty Chemicals of Basel, Switzerland is marketed for a range of antimicrobial functions in North America and Europe (Reiss et al., 2002). Triclosan has been in use in consumer hygiene products as well as within medical settings for 35 years (Ciba Specialty Chemicals, 2004). Many antibacterial soaps, deodorants, face washes, skin creams, plastics and toothpastes contain triclosan (Orvos et al., 2002). Most products labeled 'antibacterial' contain triclosan. Currently, it is difficult to find hand soaps and dishwashing liquid in major grocery stores that do not contain triclosan. In these consumer products, the amount of triclosan (w/w) typically ranges from 0.1 to 0.3%. Originally, it was thought that triclosan exhibited a "broad-spectrum bacteriostatic activity against gram-negative and gram-positive bacteria, molds, and yeasts" (McAvoy et al., 2002) affecting membrane structure and function nonspecifically. However, studies have now shown that triclosan "acts as a site-directed, picomolar inhibitor of enoyl-[acyl carrier protein] reductase by mimicking its natural substrate" (Schweizer, 2001), which effectively inhibits bacterial lipid biosynthesis (Adolfsson-Erici et al., 2002). Most of the consumer products that contain triclosan are disposed of down the drain. In fact, they comprise about 96% of the total uses of triclosan (Reiss et al., 2002). The fate of triclosan in a wastewater treatment plant was measured in a field study. It was found that 79% biologically degraded, 15% sorbed to sludge and 6% discharged into the receiving surface water system (Singer et al., 2002). The discharge of triclosan from wastewater effluent poses "a potential for triclosan exposure to aquatic organisms" in the receiving system (Orvos et al., 2002). Though some environmental

bodies may be used as sources for drinking water, it is unlikely that a public health concern exists because triclosan is not toxic to humans at low levels. There is a possibility that triclosan concentrations in a water body may be toxic to the biota. The no observable effects concentration (NOEC) for *Scenedesmus subspicatus* was 0.5 μg/L and 1.2 μg/L for the lowest observable effects concentration (LOEC) for both biomass and growth rate endpoints, which was the most sensitive species tested for this particular pollutant (Orvos et al., 2002). In four wastewater treatment plants in Ohio, USA and four wastewater treatment plants in the United Kingdom, final effluent triclosan concentrations ranged from 0.24 to 2.7 μg/L (Reiss et al., 2002). Concentrations ranged from 0.4 to 22.1 μg/L in the effluent in another study (Aguera et al., 2003). In "Pharmaceuticals, Hormones and Other Organic Wastewater Contaminants in US Streams 1999-2000: A National Reconnaissance" (Kolpin et al., 2002); triclosan was one of the most frequently detected compounds. In 85 samples (detection limit of 0.05 μg/L), it was detected with a frequency of 57.6%, a maximum concentration of 2.3 μg/L and a median level of 0.14 μg/L (Kolpin et al., 2002). The station in Texas that was analyzed for and in which triclosan was detected was the Trinity River below Dallas TX01 08057410 (32˚42'26" latitude and 96˚44'08" longitude), which had a triclosan concentration of 0.30 μg/L (Barnes et al., 2002). There is reason to be concerned when the dilution of wastewater in a river is low because concentrations of triclosan may exceed the algae NOEC for a segment downstream from the discharge point (Reiss et al., 2002). Other concerns about triclosan include the possibility of it bioconcentrating. The bioconcentration factor (BCF) in fish was estimated to be 2.7-90 L/kg (Wezel and Jager, 2002). In Lake Greifensee in Switzerland, direct phototransformation accounts for 80% of the observed total elimination of triclosan (Tixier et al., 2002). It is unclear what triclosan phototransforms into from that study, but it is important to note that triclosan can

undergo direct photolysis in UV light to yield 2,8-dichlorodibenzo-*p*-dioxin (2,8-DCDD) in both buffered and natural (Mississippi River) water with yields ranging from 1-12% (Latch et al., 2003).

This project examined the amounts of triclosan entering the City of Denton wastewater treatment plant, in the effluent, in Pecan Creek approximately 1000 feet downstream from the discharge point, and both the influent and effluent of a constructed wetland. In a risk assessment paper written about triclosan from wastewater treatment plant discharge into receiving systems, it was assumed "that no triclosan is present in the river before the point of wastewater treatment plant discharge" (Reiss et al., 2002). In this paper, it was assumed that systems either had high flow, high dilution and multiple wastewater treatment plants or systems had low flow, low dilution and one wastewater treatment plant (Reiss et al., 2002). The Pecan Creek system studied here is the unlikely case as far as their assumptions go. Pecan Creek is a low flow river. Often the river is 100% effluent from the Denton wastewater treatment plant. For this reason, the Denton wastewater treatment plant is very interesting as far as triclosan risk is concerned. The Trinity River system, of which Pecan Creek is part, is a system that has multiple wastewater treatment plants with low dilution. If triclosan is going to be a risk, the Trinity River system is most likely where that risk will manifest. The Denton wastewater treatment plant is also interesting in that it has an experimental constructed wetland, which could indicate whether the treatment type could mediate the risk or completely remove the triclosan before it can reach the receiving system. For this reason, measurements were made of the amount of triclosan entering the constructed wetland and the amount of triclosan leaving the wetland.

CHAPTER 2

OBJECTIVES OR HYPOTHESES

The purpose of this study was to determine triclosan concentrations in the Denton wastewater treatment plant in the influent, effluent and inflow and outflow of an experimental constructed wetland. Measurements were also made 1000ft downstream from the discharge point. The triclosan concentrations were compared among sites. Seasonal variability was also studied, as well as the variability in the eight-hour flow composite samples. The main analysis done was a 3-factor analysis of variance (ANOVA). Supporting analyses that were done include normality, homogeneity of variances and potential outlier tests. The following additional analyses were also done: 2-factor ANOVA for dates and sites and supporting analyses, ultraviolet treatment effects and supporting analyses, and percent loss of treatment steps. For all tests the alpha level was set to 0.05.

Season, Site, Composite Analysis via 3-Factor ANOVA

The null hypotheses tested by the 3-factor ANOVA for the seasons, sites and composites were: No significant difference between sites exists. No significant difference between composite samples exists. No significant difference between seasons exists. No significant interaction exists between sites and composite samples. No significant interaction exists between sites and seasons. No significant interaction exists between seasons and composite samples. No significant interaction exists among sites, seasons and composite samples.

Corresponding Normality Tests via Shapiro-Wilk

The ANOVA analysis has the assumption that all the levels are normal. Prior to the ANOVA, the null hypotheses tested for normality were: No significant difference exists between the observed frequency distribution of influent samples and expected normal distribution. No significant difference exists between a normal distribution and the distribution of triclosan concentrations in the effluent samples. No significant difference exists between a normal distribution and the distribution of triclosan concentrations in the downstream samples. No significant difference exists between a normal distribution and the distribution of triclosan concentrations in the wetland outflow samples. No significant difference exists between a normal distribution and the distribution of triclosan concentrations in the wetland inflow samples.

No significant difference exists between a normal distribution and the distribution of triclosan concentrations in the spring. No significant difference exists between a normal distribution and the distribution of triclosan concentrations in the summer. No significant difference exists between a normal distribution and the distribution of triclosan concentrations in the fall. No significant difference exists between a normal distribution and the distribution of triclosan concentrations in the winter.

No significant difference exists between a normal distribution and the distribution of triclosan concentrations in the 1-8 composite sample. No significant difference exists between a normal distribution and the distribution of triclosan concentrations in the 9-16 composite sample. No significant difference exists between a normal distribution and the distribution of triclosan concentrations in the 17-24 composite sample.

Potential Outlier Tests via Grubb's

High triclosan concentrations in each of the sites were potential outliers, which were tested for by Grubb's outlier test. The null hypotheses for that analysis were: The largest triclosan concentration is not a significant outlier in each of the separated sites. The largest triclosan concentration is not a significant outlier in each of the seasons. The largest triclosan concentration is not a significant outlier in each of the three composite groups.

Corresponding Homogeneity of Variances via Hartley's Fmax

In addition, the data were tested for equality of variance before analysis. The null hypotheses were: The site variances are not significantly different than homoscedastic. The season variances are not significantly different than homoscedastic. The composite variances are not significantly different than homoscedastic.

Date and Site Analysis via 2-Factor ANOVA

Another ANOVA was run because the composite data were not significantly different. The null hypotheses for the 2-factor ANOVA were: No significant difference between sites exists. No significant difference between monthly sampling dates exists. No significant interaction exists between sites and monthly sampling dates.

Corresponding Normality Tests via Shapiro-Wilk

The null hypotheses for the normality tests, which were necessary to meet the assumptions of the ANOVA were as follows: No significant difference exists between a normal distribution and the distribution of triclosan concentrations in each of the sites, separately. No significant difference exists between a normal distribution and the distribution of triclosan concentrations in each of the months sampled, separately.

Corresponding Homogeneity of Variances via Hartley's Fmax

Homogeneity of variances is one of the assumptions of the ANOVA. The null hypotheses were: The site variances are not significantly different than homoscedastic. The monthly sampling date variances are not significantly different than homoscedastic.

Ultraviolet Treatment Effects on Effluent via 2-Factor ANOVA

The influence of ultraviolet treatment on triclosan conversion was tested following the same steps used for previous analyses. No significant difference between effluent before and after ultraviolet triclosan concentrations exists. No significant difference between monthly sampling dates after ultraviolet treatment initiated exists. No significant interaction exists between sites and monthly sampling dates.

Corresponding Normality Tests via Shapiro-Wilk

The null hypotheses tested for the normality of the levels in order to meet the assumptions of the ANOVA were as follows: No significant difference exists between a normal distribution and the distribution of triclosan concentrations in each of the sites, separately. No significant difference exists between a normal distribution and the distribution of triclosan concentrations in each of the months sampled, separately.

Corresponding Homogeneity of Variances via Hartley's Fmax

These were the null hypotheses for the homogeneity of variances test in order to meet the assumptions of the ANOVA. The site variances are not significantly different than homoscedastic. The monthly sampling date variances are not significantly different than homoscedastic

Percent Loss of Treatment Steps

The hypotheses tested for triclosan fate during treatment, in the wetland and in Pecan Creek were: There does not exist a percent loss from influent to effluent. There does not exist a percent loss from effluent to 1000ft downstream from the discharge point. There does not exist a percent loss from the effluent to the wetland inflow. There does not exist a percent loss from the wetland inflow to the wetland outflow. There does not exist a percent loss from the effluent before the ultraviolet treatment to the effluent directly after the ultraviolet treatment.

CHAPTER 3

LITERATURE REVIEW

Though triclosan has been in manufactured products for the past 35 years (Ciba Specialty Chemicals, 2004), only recently has it gained attention from environmental researchers. For this reason, there is not as much literature available about 'environmental' triclosan, as there is for other chemical pollutants. However, researchers are continuously expanding knowledge about triclosan and publishing important information.

There were quite a few methods that could have been used had the equipment been available or inexpensive. One method (Kolpin et al., 2002) involved a continuous liquid – liquid extraction with dichloromethane and subsequent identification via gas chromatography – mass spectrometry (GC-MS). The method made use of a microdroplet dispersing frit. This frit improved extraction efficiency by recycling distilled solvent through it. Brown, Zaugg and Barber (1999) detail the exact method in "Wastewater Analysis by GC-MS." The detection limit for triclosan was 0.05 μg/L.

In "GC-MS, Determination of Triclosans in Water, Sediment and Fish Samples via Methylation Diazomethane" by Okumura and Nishikawa (1996), the detection limit for triclosan in water was 0.037 ng/mL or 0.037 μg/L. The researchers also determined detection limits for its three chlorinated derivatives. Triclosan was spiked in surface water, such as river or lake water, at the following three concentrations (0.05, 0.10 and 0.15 ng/mL). The relative standard deviations of the extracted concentrations ranged from 3.0 to 12.8%.

In "Measurement of Triclosan in Wastewater Treatment Systems" by McAvoy et al. (2002) the analytes were isolated using C18 solid phase extraction. The extract was then

derivatized to form trimethylsilylethers. The researchers spiked wastewater samples in the laboratory. The recoveries ranged from 79 to 88% in the influent and 36 to 87% for the final effluent. Influent triclosan concentrations (before spiking) ranged from 3.8 to 16.6 μg/L. Final effluent concentrations ranged from 0.24 to 2.7 μg/L. Each of the wastewater treatment plants in this study was only monitored over a 24-hour period once. Thus, reported triclosan values for each plant are only based on one measurement. Raw influent and final effluent for the activated sludge treatment plants were 5.21 μg/L and 0.24 μg/L for the Columbus plant and 10.70 μg/L and 0.41 μg/L for the Loveland plant, respectively. Therefore, an average (2 wastewater treatment plants in Ohio, USA) of 96% of triclosan was removed by activated sludge treatment. Raw influent and final effluent for the trickling filter treatment plants were 3.83 μg/L and 1.61 μg/L for the Glendale plant, 16.6 μg/L and 2.10 μg/L for the West Union plant in August 1997, and 15.4 μg/L and 2.70 μg/L for the West Union plant in October 1997. Only 75.5% of triclosan (on average, from 3 wastewater treatment plants in Ohio, USA) was removed by trickling filter wastewater treatment plants. The detection limit for this method was 0.01 μg/L.

In "Developmental Evaluation of a Potential Non-Steroidal Estrogen: Triclosan" by Foran, Bennett and Benson (2000), triclosan was examined for potential endocrine disruption due to the similarity of its chemical structure to non-steroidal estrogens. This was accomplished by testing the development of Japanese medaka. The 48-hour lethal concentration 50% (LC50) for medaka fry was 352 ± 68 μg/L. It was found that triclosan is not potently estrogenic. "However, changes in fin length and non-significant trends in sex ratio suggest triclosan is potentially weakly androgenic" (Foran, Bennett and Benson, 2000).

In "Pharmaceuticals and Personal Care Products (PPCPs) in Surface and Treated Waters of Louisiana, USA and Ontario, Canada" by Boyd, Reemtsma, Grimm, and Mitra (2003),

triclosan was found in Louisiana sewage treatment plant effluent at 0.010 μg/L to 0.021 μg/L. This method uses solid-phase extraction and derivatization. The method detection limit for triclosan was 0.2 ng/L. Triclosan was not detected at the drinking water treatment plants sampled or in the two surface waters tested. Three 1-L ultra-pure laboratory water samples were spiked with 1mL of 5.06 mg/L triclosan to determine the percent recovery, which was 60.1% and the relative standard deviation was 22.8%.

In "Triclosan, a Commonly Used Bactericide Found in Human Milk and in the Aquatic Environment in Sweden" (Adolfsson-Erici et al., 2002), five human milk samples were randomly selected from the Mothers' Milk Center located in Stockholm, Sweden. Three of them were found to contain measurable amounts of triclosan. The concentrations found in the milk samples were 60, 130 and 300 μg/kg lipid weight. Concentrations of triclosan were also found in the bile of fish that were exposed to municipal wastewater in both caged and tank experiments. Wild fish living in streams that had effluent discharges from three wastewater treatment plants also contained triclosan in their bile. One of the wastewater treatment plants examined did not use anaerobic digestion and received predominantly domestic wastewater, whereas the other two were modern plants that utilized both anaerobic and aerobic digestion. The caged rainbow trout exposed to treated wastewater had bile concentrations ranging from 0.71 mg/kg (upstream) and 17 mg/kg fresh weight to 120 mg/kg fresh weight (downstream). The wild living fish had concentrations ranging from 0.24 mg/kg to 4.4 mg/kg fresh weight (Adolfsson-Erici et al., 2002).

In "Effects of Three Pharmaceutical and Personal Care Products on Natural Freshwater Algal Assemblages" by Wilson et al. (2003), three representative pharmaceutical and personal care products (PPCPs), including triclosan, were tested separately to determine what effects, if any, they had on natural algal communities using dilution bioassays performed in the laboratory.

Sampling was done upstream and downstream of the wastewater treatment plant in Olathe, KS. Algal biomass yields were not significantly affected when exposed to a triclosan concentration of 0.12 μg/L, nor were algal community growth rates significantly altered during the exponential phase of growth. However, algal biomass for the chroococcalian cyanobacteria and for *Chlamydomonas*, a green alga, were significantly affected relative to the upstream site in November of 2001. Marked shifts were found in the community structure of both attached and suspended algae with the addition of triclosan at 0.12 μg/L downstream and upstream of the wastewater treatment plant ($p<0.05$). A second experiment was done with concentrations of triclosan at three treatment levels: 0.012 μg/L, 0.12 μg/L and 1.2 μg/L. When these three treatment levels were compared in the bioassays, increased triclosan concentrations resulted in decreased final algal genus richness. The structure and function of algal communities may be impaired by triclosan entering the receiving stream ecosystem via wastewater treatment plant effluent. Shifts in the nutrient processing capacity may result, as well as structural changes in the natural food web of these streams (Wilson et al., 2003).

Measurements of triclosan were made in September of 2000 at two wastewater treatment plants in the River Aire Basin in the UK using the method described in McAvoy et al. (2002) for determination of triclosan (Sabaliunas et al., 2003). As mentioned earlier, this method has a detection limit of 0.01 μg/L for triclosan in wastewater. At the trickling filter wastewater treatment plant in Meltham, concentrations of triclosan were measured to be 7.5 μg/L in the influent, 5.9 μg/L in the primary effluent, and 0.34 μg/L in the final effluent. This leads to 21.3% primary removal and 95.5% total removal. Triclosan concentrations were also measured at an activated sludge wastewater treatment plant in Crofton: 21.9 μg/L in the influent, 13.35 μg/L in the primary effluent, and 1.1 μg/L in the final effluent. Primary removal was 39% and total

removal was 95%. A triclosan die-away rate study was done in Mag Creek, which has effluent discharge from the Meltham wastewater treatment plant, which had a flow of 0.048 m^3 s^{-1} during the study. The flow-based dilution factor during the study was 2.65. Triclosan was measured 50 m upstream from the Meltham wastewater treatment plant effluent discharge point to be 19 ± 1.4 ng/L (mean ± SE, n = 3). Triclosan was also measured in the water at various sites downstream from the discharge point (mean ± SE, n = 3) in Mag Brook: 20m downstream (travel time 1min) the concentration was 80 ± 15 ng/L, 750 m downstream (travel time 55 min) the concentration was 53 ± 3.2 ng/L, 1500 m downstream (travel time 165 min) the concentration was 43 ± 5.6 ng/L, and 3500 m downstream (travel time 310 min) the concentration was 44 ± 4.2 ng/L. This leads to a die away rate of 0.21 to 0.33 h^{-1} and a half life ($t_{½}$) of 2.1 to 3.3 h calculated from the 20 m site to the 1500 m site due to complications involving the 3500 m site. A fluorescent dye tracer was used to determine travel times of the water plugs in order to directly measure in-stream removal of triclosan. Bioavailable concentrations of triclosan were also measured using semipermeable membrane devices (SPMDs) ranging from 93 ng/3 units at the upstream site to 489 ng/3 units 20m downstream to 205 ng/3 units 1500m downstream from the discharge point in Mag Creek. Concentrations of triclosan were also modeled in the River Aire Basin (Sabaliunas et al., 2003).

In Cibolo Creek, which is a moderate sized stream in South Central Texas with a discharge of approximately 0.1 m^3/s, a dilution factor less than 3, and an often-dry riverbed upstream of the treatment plant (Morrall et al., 2004). To put the triclosan concentrations in perspective, the total suspended solids (TSS) and the five-day biological oxygen demand (BOD_5) were measured to be 16.2 ppm and 6.6 ppm, respectively, at the 200 m downstream site. The die away rate of triclosan from wastewater treatment plant effluent was calculated to be 0.06 h^{-1}.

This die away rate corresponds to a half-life of 11.3 h. What these numbers mean is that over an 8 km river reach downstream from the effluent discharge point the amount of triclosan was reduced by 76%. Approximately 19% of this loss is attributed to sorption and settling as determined by mathematical modeling. The concentration of triclosan measured in the effluent was 0.785 μg/L. Triclosan was measured at a concentration of 0.431 μg/L 200 m downstream from the outfall. Triclosan was measured in the wastewater treatment plant effluent, as well as both upstream and downstream from the wastewater treatment plant using the method described in McAvoy et al. (2002). As previously noted, this method has a detection limit of 0.01 μg/L. "When removing sorption and settling, the remaining amount of triclosan had an estimated first-order loss rate of 0.25 h^{-1}. This loss rate was presumably due to other processes such as biodegradation and photolysis. These data show that loss of parent triclosan from the water column is rapid. Additional data are needed to fully document loss mechanisms" (Morrall et al., 2004).

In "Fate and Effects of Triclosan in Activated Sludge," Federle, Kaiser, and Nuck (2002) found that greater than 98.5% of the incoming triclosan at a wastewater treatment plant was removed when triclosan was incrementally increased from 40 μg/L to 2000 μg/L. The amount of triclosan from the influent that was sorbed to the wasted solids was between 1.5% and 4.5%. Greater than 94% of the triclosan was removed through primary biodegradation. The amount of triclosan that was either incorporated into biomass or mineralized to carbon dioxide was between 81% and 92%. This study demonstrates that triclosan undergoes extensive mineralization in activated sludge by microorganisms.

The aquatic toxicity of triclosan was studied in regards to activated-sludge microorganisms, algae, an aquatic plant, invertebrates and fish (Orvos et al., 2002). The 3-hour

sludge median oxygen consumption inhibitory triclosan concentration was determined to be 20 mg/L, while the median glucose utilization inhibitory triclosan concentration was determined to be 239 mg/L for wastewater microorganisms. The no observable effects concentration (NOEC) and lowest observable effects concentration (LOEC) for *Scenedesmus subspicatus* were 0.5 μg/L and 1.2 μg/L, respectively, for both biomass and growth rate endpoints. The effect concentration 50% (EC50) for *Scenedesmus subspicatus* was 0.7 μg/L for the biomass endpoint and 2.8 μg/L for the growth rate endpoint. The EC50 for *Selenastrum capricornutum* (recently renamed *Raphidocelis subcapitata*) was 4.46 μg/L. The EC50 for *Anabaena flos-aquae* was determined to be 0.97 μg/L and it was 19.1 μg/L for *Navicula pelliculosa*. The EC50 for *Skeletonema costatum* was greater than 66.0 μg/L and for *Lemna gibba* it was greater than 62.5 μg/L. The 48-h EC50 for *Daphnia magna* in triclosan was 390 μg/L (95% confidence interval: 330 - 460μg/L). The NOEC for *Daphnia magna* was 200 μg/L based on survival. Based on reproduction of *Daphnia magna*, the NOEC and LOEC were 40 μg/L and 200 μg/L, respectively. The 96-h LC50 triclosan concentration for fathead minnows (*Pimephales promelas*) was 260 μg/L, while it was 370 μg/L for bluegill sunfish (*Lepomis macrochirus*). "Sublethal effects observed during the study included loss of equilibrium, fish jaw locked open, erratic swimming, spinal curvature, and quiescence" to rainbow trout (*Oncorhynchus mykiss*) (Orvos et al., 2002). The average triclosan accumulation factor in *Danio rerio* (zebrafish) was 4,157 in an environment of 3 μg/L triclosan and only 2,532 in an environment of 30 μg/L triclosan over the five-week test period. From these results, algae were considered the most susceptible organisms to triclosan toxicity (Orvos et al., 2002).

CHAPTER 4

STUDY AREA

The study area for this project was the Pecan Creek Waste Reclamation Plant in Denton, Texas, which is a conventional activated sludge wastewater treatment plant, also known as the Denton wastewater treatment plant. At the beginning of this study, the plant was permitted for 15 MGD average daily flow. During this project, the plant underwent a number of modifications, including changeover from chlorination/dechlorination to ultraviolet disinfection treatment as part of its expansion. After expansion, plant capacity increased to 21 MGD average daily flow (City of Denton, 2004a). Denton, Corinth, Argyle and Krum are served by this wastewater treatment plant (North Central Texas Council of Governments, 2003). After discharging into Pecan Creek, water flows into Lake Lewisville, which contains Denton's drinking water intake structure (City of Denton, 2004b).

The treatment train at the Denton wastewater treatment plant is as follows. First, raw sewage enters the plant through the bar screen structure, which takes out objects with a diameter larger than 1 inch. Then, heavier sands and gravels are removed by the grit removal/flow splitting structure. Flow "is measured through a venturi flow tube downstream of the lift station" (City of Denton, 2004b). Next, the wastewater passes through primary treatment, which involves sedimentation of solids and removal of floating material. The primary clarifiers provide this treatment and lower the biological load by removing 25% to 40% of the biochemical oxygen demand (BOD). Primary clarifiers may remove up to 60% of the total suspended solids in the waste stream. The solids are removed for primary and secondary digestion by microorganisms to stabilize the solids. This stabilized material is applied to land adjacent to the plant. After

clarification, the water then passes into the aeration basins. Return activated sludge organisms are added to this waste and for four to six hours it will stay in the aeration basins. Air is continually added to these basins in order for microorganisms to aerobically digest organic material still present in the waste stream. This treatment removes even more of the BOD and large amounts of ammonia. "The aeration basins are considered the 'heart' of an activated sludge process" (City of Denton, 2004b). After aerobic digestion, water enters into the final clarifiers. These units, much like the primary clarifiers, collect gravity settleable solids, otherwise known as activated sludge. These solids are then recovered and used in the aeration basins, which links the whole activated sludge process together. In a final clarifier, notched weirs allow the treated wastewater to flow through. From all seven of the collection troughs in the final clarifiers, treated effluent flows to sand filters that remove any solids left in the water.

After sand filtration, effluent is disinfected in the chlorine contact tanks. Twenty minutes of contact is all it takes to kill disease-causing microorganisms. After chlorination, the chlorine must be removed before the water is discharged into the environment. Sulfur dioxide is added to the chlorinated effluent in the dechlorination basin, after which the effluent is discharged into Pecan Creek. This creek is dominated by the discharge so that there is low dilution and stream flow is sometimes 100% effluent (City of Denton, 2004b).

Once the expansion of the plant was complete, the chlorination/dechlorination disinfection was replaced by ultraviolet disinfection (North Central Texas Council of Governments, 2003). Ultraviolet disinfection works because the ultraviolet light penetrates the cell walls of microorganisms and inhibits their ability to reproduce. An electrical discharge through mercury vapor generates the ultraviolet radiation (Environmental Protection Agency,

1999). UV disinfection does not change the chemical and physical properties of the treated water, as opposed to chlorination disinfection (Trinity River Authority of Texas).

The Pecan Creek Water Reclamation Facility is unique in that it has an experimental constructed wetland that if expanded to accommodate the effluent flow could possibly mediate the risk of triclosan to the environment by significantly reducing the amount of triclosan released into the receiving system. The approximately 46 by 46-square meter wetland was constructed in the fall of 1992 with a potential maximum volume of 570,000 L. It has four lanes separated by three earthen berms and a layer of clay isolates the wetland from groundwater. Depths range from a few centimeters at the inflow to 0.6 m at the wetland outflow. It receives a portion of the treated effluent before it is discharged into pecan creek; this wetland inflow is adjustable (Lazorchak et al., 2001).

CHAPTER 5

METHODS

Method Development

There are quite a few different methods employed to determine triclosan concentrations in wastewater and in surface waters. Each of these was evaluated for applicability to this project. Since funding was not available, a relatively inexpensive method was necessary, preferably using equipment that the lab already had. There were many methods (for example: Okumura and Nishikawa, 1996; McAvoy et al., 2002; Ternes et al., 1998) that involved derivatizing the triclosan for easier detection. Some researchers noticed problems with peak tailing on the gas chromatography – mass spectrometry (GC-MS) instrument that were rectified by the derivatization. After triclosan was obtained, it was run on TRAC Lab's GC-MS. It was determined that the tailing was not bad enough to make life more complicated by adding the derivatization step. Also, there were concerns about incomplete derivatization. These concerns were in addition to the fact that the more complex a procedure, the more chances for error, sample losses and contamination. Due to concerns about the tailing, however, the injector sleeve was changed right before the samples were run, along with determining that the instrument was performing efficiently and near optimally, otherwise the tailing caused severe quantification problems. Also because of quantification problems, multiple standard curves were made throughout the project because the method was so sensitive to instrument changes. GC-MS methodology was chosen because of familiarity with GC-MS techniques and the fact that diazinon is detected and quantified reasonably well using GC-MS methodology. Access to liquid chromatography – mass spectrometry (LC-MS) instrument was not available and only one

method using high performance liquid chromatography (HPLC) was found, whereas there were a plethora of methods using GC-MS. As an experiment, the diazinon methodology using methods 614 (Environmental Protection Agency, 1993) and 3510 (Environmental Protection Agency, 1996) was modified by using methylene chloride as the extraction solvent to more effectively extract the triclosan. This modified methodology was used to extract a blank and blank spike along with an effluent and effluent spike to determine recovery of triclosan. The samples were spiked with a triclosan concentration of 1ppb. The blank and effluent had concentrations of triclosan undetectable with this methodology. The percent recovery of triclosan, assuming a concentration of zero for the blank and effluent samples, was 124% for the effluent and 114% for the blank. The spike was recovered with minimal tailing. The exact temperature program for triclosan was determined by Dr. B. Venables (personal communication, February 2003). After these initial analyses, concerns that pH may have a significant effect on the triclosan extraction efficiency were evaluated. The initial experiment had been done at a reduced pH of about 2 for both the blank and effluent spikes, because it was assumed that lower pH would help extraction efficiency. For this reason, a raw neutral pH influent and a base/acid extraction were done. The base/acid extraction involved raising an influent sample's pH to around 11 and then extracting the sample into a clean amber glass bottle. The pH of the sample was then lowered to 2 and the extraction repeated. This extract was collected in a separate clean amber glass bottle. Both of these samples were run in the GC-MS after concentrating. The theory was that the base extraction would clean up the chromatogram and possibly reduce quantification problems. More triclosan should be extracted at the acidic pH and none at all at the basic pH. The basic extract (14.46 μg/L) had more than twice as much triclosan in it as did the acidic extraction (5.58 μg/L), but it also had a very messy chromatogram. The acidic extract had a much cleaner

chromatogram, but did not have as much triclosan in it as the neutral raw influent (27.54 μg/L), more than likely due to unavoidable extraction losses. Based on these results it was determined that a neutral extraction was most efficient for these matrices.

Many methods to analyze triclosan involve using solid phase extraction; for example: Ternes et al, 1998; Singer et al., 2002; Lindstrom et al., 2002 and Aguera et al., 2003. In order to use solid phase extraction (SPE) methods, the sample must be filtered first. This is necessary especially since the raw influent was so dirty. For this reason, a sample was filtered before extraction to determine if significant losses of triclosan resulted. An influent sample filtered through an AE glass fiber filter was analyzed at the same time as the neutral influent extraction discussed earlier. The filtered influent had a lot less recovered triclosan (2.2 μg/L) than the unfiltered one (27.54 μg/L). From these experiments it was concluded that it would be best to do a neutral extraction without filtration. Hence, solid phase extraction methodologies were not an option and dichloromethane liquid-liquid extraction was determined to be the most appropriate methodology for this study.

Materials and Methods

Sampling Collection, Preservation and Handling

Grab samples and composite samples were collected in amber glass containers by the City of Denton, specifically Chris Havis and coworkers. All samples were kept at 4°C from the time of collection until extraction. All samples were extracted within seven days of collection and were completely analyzed within forty days of extraction.

Grab samples were collected in 4-L amber glass containers from the following sites: influent and effluent of the wastewater treatment plant for quality control analyses. Eight-hour flow composite samples were collected from the influent and effluent of the wastewater

treatment plant along with influent and effluent of the constructed wetland and approximately 1000 ft downstream from the effluent discharge point for a 24-hour period once a month. For each site, there were 1-8 hour, 9-16 hour and 17-24 hour flow composite samples collected in 1-L amber glass containers. This study went from April 2003 to January 2004 with monthly sampling.

Glassware Washing

All glassware was washed with generic laboratory soap and water. Then, the glassware was rinsed three times with tap water and three times with deionized (DI) water, followed by an acetone rinse and drying. Some glassware, like the graduated cylinders, were rinsed with 20% HCl prior to rinsing with tap and DI water.

Separatory Funnel Extraction and Concentration

The methods used were modified EPA method 3510 (1996) for the separatory extraction and EPA method 614 (1993) for the GC-MS analysis. The extraction method was modified such that rather than doing an acid/base extraction, a neutral extraction was done. For all samples but the influents, a large glass graduated cylinder was used to measure 1000 mL of sample, which was then poured into a glass 2000 mL separatory funnel. Stock triphenyl phosphate, TPP, (1000 ppm) from Absolute Standards was diluted with methylene chloride to 10 ppm. With a 1-10 μL pipette or a 10 μL glass syringe, 10 μL of the surrogate compound, TPP (10 ppm), was added to yield a final concentration of 1 ppb surrogate standard spike in the extract. Using a small glass graduated cylinder, 60 mL of methylene chloride was added to the separatory funnel. The separatory funnel was shaken well and vented to release excess pressure. When there was no longer any excess pressure, the sample was extracted by shaking the funnel for 2 minutes. The organic and water phases were allowed to separate for a minimum of 10 minutes. If the emulsion

interface between layers was more than one-third the volume of the solvent layer then mechanical techniques were used to separate the two layers. First, stirring the emulsion with a glass rod was attempted. If that did not reduce the emulsion, then the emulsion and organic layer were transferred to two plastic or glass centrifuge tubes. These were then centrifuged between 1500 and 2000 rpm for 10 minutes. A glass transfer pipette was used to return the aqueous layer to the separatory funnel. Then, the remainder of the centrifuged sample was poured over anhydrous sodium sulfate in order to absorb the water, followed by a methylene chloride rinse. The organic layer was collected in a labeled small amber glass bottle. Then, the extraction was repeated using a second 60 mL volume of methylene chloride. The extract was repeated a third time with all extracts combined in the labeled small amber glass bottle. Attaching a 10 mL concentrator tube to a 500 mL evaporative flask assembled a Kuderna-Danish (K-D) concentrator. The glassware joints were secured with plastic joint clips. Several clean boiling chips were added and then the combined extract was poured into the evaporative flask. A three-ball Snyder column was attached to the evaporative flask. Each Snyder column was pre-wet by adding about 1mL of methylene chloride to the top. The K-D apparatus was placed in a hot water bath (80°C) so that the concentrator tube was immersed in hot water below the joint and the entire lower rounded surface of the flask was bathed with hot vapor. At the proper rate of distillation, the balls of the column actively chattered. When the apparent volume of liquid reached 1 mL, the K-D apparatus was removed from the water bath and allowed to drain and cool. The Snyder column was removed. The evaporative flask was rinsed with methylene chloride into the condenser tube. A glass Pasteur pipette was used to transfer the condensed extract to a clean Teflon-sealed screw-cap vial, which was appropriately labeled. The condenser tube was rinsed with methylene chloride and this was also added to the vial. This procedure was

repeated for each sample collected. Each vial was labeled separately. Each extract was condensed to 100 μL using a stream of nitrogen and a warm water bath.

The procedure was identical for the influent samples. However, only 100 mL from each sample was extracted 3 times with only 6 mL of methylene chloride using a 250 mL separatory funnel. Stock TPP (1000 ppm) from Absolute Standards was diluted with methylene chloride to 100 ppm, which was then added (10 μL) to yield a final concentration of 1 ppb in the extract and the final extract was condensed to 1000 μL (1 mL).

Quality control analyses were also done. For each sampling event, a blank deionized water sample was extracted along with a blank spike. An effluent grab sample was extracted along with an effluent grab spike and duplicate spike. To each spike sample, 10 μL of 10 ppm stock triclosan from Absolute Standards was added for a final extract concentration of 0.1 ppb. All quality control samples were done using 1000 mL samples condensed to a final extract of 100 μL.

GC-MS Analysis

All samples were analyzed using a Hewlett Packard (HP) 5970 MS with an HP 5890 Series II GC. Using a 1-10 μL micropipette or glass syringe, 5 μL of 1000 ppm stock tokuthion from Absolute Standards was added to each 1mL sample and 5 μL of 100 ppm tokuthion (stock diluted with methylene chloride) was added to each 100 μL sample. Tokuthion was the internal standard. The extract was shaken well. Then, 2 μL of extract was injected into the GC-MS for analysis. A standard curve was made using the following concentrations of triclosan (target analyte) and TPP (surrogate): 10 μg/mL, 5 μg/mL, 2.5 μg/mL, 1.25 μg/mL, 0.625 μg/mL, 0.312 μg/mL, 0.156 μg/mL and 0.078 μg/mL; with tokuthion (internal standard) at a constant concentration of 5 μg/mL. The lowest standard on the standard curve was divided by 10 because

of the 10-fold concentration factor in all samples but the influent. This value (0.0078 μg/L) was established as the practical quantification limit (PQL). Each day before any samples were run, a continuing calibration standard (usually either 0.625 μg/mL or 1 μg/mL) was run to assess how well the GC-MS was performing. The following GC-MS method was saved on the computer as "Triclosa." The injection temperature was 260 °C (static). The oven started at 40 °C and held at that temperature for 1 minute. Next, at a rate of 70 degrees per minute, the oven ramped up to 140 °C and held for 5 minutes. Then, at a rate of 10 degrees per minute, the oven ramped up to a final temperature of 300 °C and held for 17 minutes. The detector did not start scanning until 14 minutes. Then, it scanned every 0.100 seconds for 20 minutes with subscans of 0.400 AMU wide and 5 samples used. The GC-MS capillary column (DB5.625) had an internal diameter of 0.25 mm and a length of 30 m. The carrier gas was helium with an inlet pressure of 10 psi.

CHAPTER 6

RESULTS

Data

Refer to Table 7 in the Appendix for the raw data for the April through January sampling events. Unfortunately, the wetland sometimes did not flow due to pump troubles; in those instances data are not available (NA). During the first month of data collection, it was assumed that the wetland would not have measurable triclosan concentrations. For this reason, only wetland composite 1-8 was extracted and analyzed. Because concentrations of triclosan were not detectable (ND), the other two composites were not extracted (NA) and are also assumed to have triclosan concentrations that are undetectable with this methodology. For samples that have undetectable triclosan concentrations, it was assumed that the concentration was 0 μg/L. After the April 2003 sampling event, the flow into the wetland was increased to more than 1% of the wastewater treatment plant effluent. After the September 2003 sampling event, the ultraviolet disinfection system was implemented instead of the previous chlorination and dechlorination steps. For the November 2003 sampling event, only a grab sample was available for the wetland inflow and outflow sites. This grab sample concentration is written in the 1-8 composite column in the table, but was not used in the statistical analyses. Also, an extra site was added 15 ft west of the ultraviolet basin for direct comparison of triclosan concentrations before and after the ultraviolet step. In December 2003, the wetland outflow sample site moved 100 ft from prior sampling site. Refer to Table 8 in the Appendix for quality control analyses.

Statistics

All of the statistical analyses were performed using Base SAS® software, Version 8.2 (SAS Institute Inc., http://www.sas.com).

Due to the complexity of the data collected: ten months of sampling at five main sites: influent, effluent, downstream, wetland inflow and wetland outflow with three eight-hour time composites at each site, there were quite a few possible analyses that could have been done. The sampling site concentration data were analyzed using a model III, 3-way analysis of variance (ANOVA). This type of ANOVA works best with replicates. Since samples were only taken once per month and the composites taken at each sampling site are not true replicates because they differ in time, the data were split into seasons. The sampling months were allocated using the official version of seasons, as written on calendars. The following are the definitions of seasons that were used: spring (March 21 - June 21), summer (June 21 - Sept 23), fall (Sept 23 - Dec 21), and winter (Dec 21 - March 21) (Campbell and Stewart, 1999). With this method, there was only one month in the winter season. For this reason, the data from the December sampling were moved from fall to the winter season. Prior to performing the 3-way ANOVA, the normality and homoscedasticity of the individual levels (season, site, composite) was determined, in order to determine whether a parametric ANOVA or an ANOVA on ranked data was appropriate. The concentrations in several samples were below the detection limit. There are a few ways to deal with these points: they can be assumed to be zero, assumed to be at the detection limit, or assumed that they are at some fraction of the detection limit (usually half). For the following statistics, it was assumed that they were zero. Since only the wetlands have points below the method detection limit, the rest of the sites were unaffected. First, normality tests were run on all of the data separated by level, including the influent. Table 1 summarizes the results.

All normality analyses are done using the Shapiro-Wilk normality test. Concentrations are in μg/L.

Table 1: Descriptive statistics for each level by factor for the raw, untransformed data with undetectable triclosan concentrations set to 0.0μg/L. The data are split up by the three bold factors (site, season and composite) along with being separated by level. Composite 1-8 (A) is the first eight-hour flow composite sample (10am-6pm for all samples but the influent, influent is from 12am-8am), composite 9-16 (B) is the second eight-hour flow composite and composite 17-24 (C) is the third eight-hour flow composite. Sample size (n), mean, sample standard deviation (SD), variance (s^2), Shapiro-Wilks normality test probability (normal prob.) and the five number summary are listed. Probabilities below the alpha limit (α=0.05) are not normal and the appropriate descriptive statistics to completely describe the distribution of the level would be the five number summary and the sample size. Otherwise the appropriate descriptive statistics are the mean, standard deviation and sample size.

Level					Normal	Five Number Summary				
Site	n	Mean	SD	s^2	Prob.	Min	Q1	Q2	Q3	Max
Downstream	30	0.123	0.0794	0.0063	0.0066	0.030	0.060	0.1065	0.159	0.318
Effluent	30	0.122	0.0782	0.0061	0.0165	0.028	0.072	0.0975	0.177	0.363
Influent	30	7.94	6.463	41.77	<0.0001	2.70	4.30	5.95	7.90	26.80
Wetland Inflow	20	0.110	0.1094	0.0120	0.0095	0.000	0.024	0.0825	0.152	0.409
Wetland Outflow	20	0.042	0.0452	0.0020	0.0029	0.000	0.0105	0.0265	0.0645	0.165
Season										
Fall	24	1.549	2.6978	7.2780	<0.0001	0.000	0.0865	0.119	2.131	8.000
Spring	30	1.771	5.0074	25.0736	<0.0001	0.000	0.0300	0.0535	0.192	25.86
Summer	46	2.722	5.8363	34.0623	<0.0001	0.021	0.103	0.216	4.20	26.80
Winter	30	1.101	2.258	5.0969	<0.0001	0.016	0.029	0.090	0.133	7.90
Composite										
1-8 (A)	42	2.435	5.8927	34.7243	<0.0001	0.000	0.0600	0.1195	0.286	26.80
9-16 (B)	44	1.669	3.7841	14.3197	<0.0001	0.000	0.0485	0.1120	0.3255	21.00
17-24 (C)	44	1.654	3.6483	13.3098	<0.0001	0.000	0.0440	0.1205	0.3635	20.00

While running the normality tests, the data were examined to determine if there were statistically significant outliers. There were potential outliers for all of the levels and these results are summarized in Table 2. Grubb's test for outliers was used, even though one of the assumptions of this test is that the data are normal. However, it is still the most appropriate outlier test for these type data.

Table 2: Triclosan concentrations for each level were examined for potential outliers using Grubb's test. A probability less than α=0.05 is considered significant. The data were split up by the three bold factors (site, season and composite) along with being separated by level. Composite 1-8 (A) is the first eight-hour flow composite sample (10am-6pm for all samples but the influent, influent is from 12am-8am), composite 9-16 (B) is the second eight-hour flow composite and composite 17-24 (C) is the third eight-hour flow composite.

Site	Potential Outliers?	Outlier points (μg/L)	Grubb's stat	Prob.
Downstream	Yes	0.318	2.46	p>0.10
Effluent	Yes	0.363	3.08	0.025>p>0.01
Influent	Yes	25.86	2.77	0.05>p>0.025
Wetland Inflow	Yes	0.409	2.73	0.025>p>0.01
Wetland Outflow	Yes	0.165	2.72	0.025>p>0.01
Season				
Fall	Yes	8.0 and 7.5	2.39 and 2.21	p>0.10
Spring	Yes	25.86	4.81	p<0.005
Summer	Yes	26.8	4.12	p<0.005
Winter	Yes	7.9	3.01	0.025>p>0.01
Composite				
1-8 (A)	Yes	25.86	3.98	p<0.005
9-16 (B)	Yes	21.0	5.11	p<<0.005
17-24 (C)	Yes	20.0	5.03	p<<0.005

There were quite a few outliers in this dataset. It was decided not to delete all three high points that occurred in the month of June in the influent, because a reason to do so was not found besides the fact that they are statistically significant outliers. They could be real points because statistically unlikely events sometimes do happen. The downstream outlier was deleted, even though it is not a significant outlier because it is approximately three times higher than the other two composites for the downstream site in the month of August. When it was run on the gas chromatography – mass spectrometry (GC-MS) instrument, along with having a high triclosan concentration, it also had a high surrogate standard (triphenyl phosphate or TPP) concentration (7.87 μg/mL when it should have been 1 μg/mL), which led to the belief that something unusual went on with that sample. The TPP concentration could have been adjusted by a factor to make it comparable to the other two composites and then the triclosan concentration adjusted with the

same factor, but this approach has questionable validity, which led to the decision to delete the point. The effluent outlier was deleted because it is a statistically significant outlier and it is not representative of the composites for the month of September. The influent outlier at 25.86 μg/L was deleted because it is a statistically significant outlier and because it is a magnitude higher than the other two composites for the month of April. The TPP concentration was reasonable for this sample, but the sample also differed from the other two composites in that instead of extracting a total volume of 100 mL, the total volume extracted was 1000 mL. The reason for this was it was done in the first month of actual sampling and there was uncertainty about how much influent needed to be extracted to get a good result. Perhaps this large extraction volume led to the high result. Also, this result was not on the standard curve, which only went up to 10 μg/L, so there was not a lot of confidence in this number. The wetland inflow outlier was deleted because it was a statistically significant outlier and it was almost twice the other composite for the month of August. When this sample was run on the GC-MS, it had a high TPP concentration (28.33 μg/mL when it should have been 1 μg/mL), which led to belief that something went wrong with that sample. As mentioned previously, the TPP concentration could have been adjusted by a factor to make it comparable to the other composite and then the triclosan concentration adjusted with the same factor, but the validity of this approach was uncertain, which led to the decision to delete the point. The wetland outflow outlier was deleted because it was a statistically significant outlier and because it was not representative of the composites for the month of December.

Before deleting the outliers, the samples with undetectable triclosan concentrations were set to the practical quantification limit (PQL), rather than zero. The PQL is 0.0078 μg/L. Normality analyses were then rerun. Table 3 summarizes the levels.

Table 3: Descriptive statistics for each level by factor for the data with undetectable triclosan concentrations set to the PQL of 0.0078μg/L. The data were split up by the three bold factors (site, season and composite) along with being separated by level. Composite 1-8 (A) was the first eight-hour flow composite sample (10am-6pm for all samples but the influent, influent is from 12am-8am), composite 9-16 (B) was the second eight-hour flow composite and composite 17-24 (C) was the third eight-hour flow composite. Sample size (n), mean, sample standard deviation (SD), variance (s^2), Shapiro-Wilks normality test probability (normal prob.) and the five number summary are listed. Probabilities below the alpha limit (α=0.05) are not normal and the appropriate descriptive statistics to completely describe the distribution of the level would be the five number summary and the sample size. Otherwise the appropriate descriptive statistics are the mean, standard deviation and sample size.

Level					Normal	Five Number Summary				
Site	n	Mean	SD	s^2	Prob.	Min	Q1	Q2	Q3	Max
Downstream	30	0.123	0.0794	0.0063	0.0066	0.030	0.060	0.1065	0.159	0.318
Effluent	30	0.122	0.0782	0.0061	0.0165	0.028	0.072	0.0975	0.177	0.363
Influent	30	7.94	6.463	41.77	<0.0001	2.70	4.30	5.95	7.90	26.80
Wetland Inflow	20	0.111	0.1082	0.0117	0.0057	0.0078	0.024	0.0825	0.152	0.409
Wetland Outflow	20	0.044	0.0434	0.0019	0.0008	0.0078	0.0144	0.0265	0.0645	0.165
Season										
Fall	24	1.550	2.6976	7.2770	<0.0001	0.0078	0.0865	0.119	2.131	8.000
Spring	30	1.772	5.0067	25.0670	<0.0001	0.0078	0.0300	0.0535	0.192	25.86
Summer	46	2.722	5.8363	34.0623	<0.0001	0.021	0.103	0.216	4.20	26.80
Winter	30	1.101	2.258	5.0969	<0.0001	0.016	0.029	0.090	0.133	7.90
Composite										
1-8 (A)	42	2.436	5.8925	34.7215	<0.0001	0.0078	0.0600	0.1195	0.286	26.80
9-16 (B)	44	1.670	3.7839	14.3179	<0.0001	0.0078	0.0485	0.1120	0.3255	21.00
17-24 (C)	44	1.655	3.6481	13.3086	<0.0001	0.0078	0.0440	0.1205	0.3635	20.00

There were not many significant changes in the results observed by changing the undetectable concentrations to the PQL rather than having them as 0.000 μg/L. Therefore, the concentrations were set to 0.000 μg/L for the remaining analyses.

The outliers were deleted from the sites only and the normality tests were rerun.

Table 4: Descriptive statistics for each level by factor for the raw data with the site outliers deleted. The data are split up by the three bold factors (site, season and composite) along with being separated by level. Composite 1-8 (A) is the first eight-hour flow composite sample (10am-6pm for all samples but the influent, influent is from 12am-8am), composite 9-16 (B) is the second eight-hour flow composite and composite 17-24 (C) is the third eight-hour flow composite. Sample size (n), mean, sample standard deviation (SD), variance (s^2), Shapiro-Wilks normality test probability (normal prob.) and the five number summary are listed. Probabilities below the alpha limit (α=0.05) are not normal and the appropriate descriptive statistics to completely describe the distribution of the level would be the five number summary and the sample size. Otherwise the appropriate descriptive statistics are the mean, standard deviation and sample size.

Level					Normal	Five Number Summary				
Site	n	Mean	SD	s^2	Prob.	Min	Q1	Q2	Q3	Max
Downstream	29	0.116	0.0715	0.0051	0.0113	0.030	0.060	0.106	0.127	0.286
Effluent	29	0.113	0.0646	0.0042	0.1089	0.028	0.072	0.096	0.162	0.254
Influent	29	7.32	5.604	31.400	<0.0001	2.7	4.3	5.7	7.5	26.8
Wetland Inflow	19	0.094	0.0860	0.0074	0.0412	0.000	0.020	0.078	0.131	0.288
Wetland Outflow	19	0.036	0.0358	0.0013	0.0127	0.000	0.000	0.025	0.064	0.133
Season										
Fall	24	1.549	2.6978	7.2780	<0.0001	0.000	0.0865	0.119	2.131	8.0
Spring	29	0.940	2.1283	4.5296	<0.0001	0.000	0.030	0.052	0.173	8.3
Summer	43	2.886	6.0058	36.070	<0.0001	0.021	0.101	0.194	4.30	26.8
Winter	29	1.133	2.2905	5.2466	<0.0001	0.016	0.029	0.090	0.131	7.9
Composite										
1-8 (A)	40	1.906	4.6922	22.0164	<0.0001	0.000	0.056	0.1165	0.270	26.8
9-16 (B)	43	1.700	3.8235	14.6191	<0.0001	0.000	0.042	0.107	0.288	21.0
17-24 (C)	42	1.716	3.7248	13.8739	<0.0001	0.000	0.041	0.1125	0.260	20.0

Obviously, parametric ANOVA was not an option due to the fact that most of the levels violate the normality assumption. Several transformations (log transform, square root transform, natural log transform, arcsin transform, arcsin squareroot transform) were attempted and none of them resulted in normality. For this reason, the variates were ranked and a parametric ANOVA was run on ranked data. Because a parametric ANOVA on ranked data was used, the assumptions regarding normality and homogeneity of variances had to be met for the ranked data. Hence, the variates were ranked and normality tests were analyzed.

Table 5: Descriptive statistics for each level by factor for the ranked data. The data were split up by the three bold factors (site, season and composite) along with being separated by level. Composite 1-8 (A) was the first eight-hour flow composite sample (10am-6pm for all samples but the influent, influent is from 12am-8am), composite 9-16 (B) was the second eight-hour flow composite and composite 17-24 (C) was the third eight-hour flow composite. Sample size (n), mean, sample standard deviation (SD), variance (s^2), Shapiro-Wilks normality test probability (normal prob.) and the five number summary are listed. Probabilities below the alpha limit (α=0.05) were not normal and the appropriate descriptive statistics to completely describe the distribution of the level would be the five number summary and the sample size. Otherwise the appropriate descriptive statistics are the mean, standard deviation and sample size.

Level					Normal	Five Number Summary				
Site	n	Mean	SD	s^2	Prob.	Min	Q1	Q2	Q3	Max
Downstream	29	58.0	23.47	550.79	0.1347	20.0	37.5	61.0	73.0	95.0
Effluent	29	57.4	23.65	559.16	0.0893	17.0	41.0	57.0	79.0	92.0
Influent	29	111.0	8.51	72.43	0.2737	97.0	104.5	111.0	118.0	125.0
Wetland Inflow	19	45.8	31.96	1021.70	0.1047	4.5	10.0	44.5	74.0	96.0
Wetland Outflow	19	23.2	19.74	389.51	0.0113	4.5	4.5	15.0	39.0	76.0
Season										
Fall	24	69.6	29.84	890.38	0.3706	4.5	50.0	66.0	90.5	120.0
Spring	29	44.5	37.64	1416.73	0.0013	4.5	50.0	33.0	80.0	121.0
Summer	43	79.1	31.46	990.04	0.0507	11.5	59.0	86.0	104.5	125.0
Winter	29	52.1	35.57	1265.29	0.0120	9.0	19.0	53.0	74.0	119.0
Composite										
1-8 (A)	40	64.0	36.32	1319.08	0.1225	4.5	35.25	64.5	93.5	125.0
9-16 (B)	43	62.6	36.84	1357.16	0.0510	4.5	31.0	62.0	96.0	124.0
17-24 (C)	42	62.4	36.35	1321.58	0.0512	4.5	30.0	63.5	93.0	123.0

The bulk of the levels were normal (alpha=0.05) using Shapiro-Wilks normality test. The wetland outflow, spring, and winter distributions were significantly different than a normal distribution. Only the spring distribution was significantly different than a normal distribution using an alpha of 0.01. ANOVA is robust, but if significance is not obtained, it may be because of the breaking of the normality assumption. Homoscedasticity was determined using Hartley's Fmax test for each level separately (α=0.05). The results of this test are summarized in Table 6.

Table 6: Homoscedasticity was tested via Hartley's Fmax test for each factor. Probabilities larger than the alpha level of 0.05 for a factor are homoscedastic.

Factor	Largest s^2	Smallest s^2	v	Fmax	Probability
Site	1021.70	72.43	18	14.11	<<0.01
Season	1416.73	890.38	23	1.59	>0.05
Composite	1357.16	1319.08	39	1.03	>0.05

Sites were not homoscedastic, but the season and composite were. ANOVA is robust and the heteroscedasticity of the site should not ruin the validity of the analysis. If significance was not obtained in the final analyses, this may be due to the breaking of the homogeneity of variances assumption necessary for running a parametric ANOVA.

The following were the summary statements for the ANOVA. Season triclosan concentrations were highly significantly different ($p=0.0008$), as were the sites ($p<0.0001$); however, composites ($p=0.9934$), the interaction between season and site ($p=0.0817$), the interaction between season and composite ($p=0.9471$), the interaction between site and composite ($p=0.9969$) and the interaction among season, site and composite ($p=0.9999$) were not significant (3-way, model III, with replication parametric ANOVA on ranked data). Seasons were separated into two statistically distinct groups: summer = fall > winter = spring (SNK, $\alpha=0.05$). Sites were separated into three statistically distinct groups: influent > downstream = effluent = wetland inflow > wetland outflow (SNK, $\alpha=0.05$). The site separation is what one would expect. Low sample size or the breaking of the assumptions of the parametric ANOVA may have hindered separation into statistically significant groups for the season data.

The results were essentially the same, though the probabilities change, when a 3-way, model III, with replication parametric ANOVA on ranked data was done on all of the data except for the influent. This analysis was done because the influent data were so much different from the other groups, that it may significantly affect the season and site groups such that the ANOVA results would be compromised. This apparently is not the case.

Remarks on the power of tests are generally important to fully understand the results of statistical analyses. A way to determine the power of a parametric ANOVA on ranked data retrospectively was not found. In order to have an approximation of the power of this test, the

minimum significant difference was determined from a parametric ANOVA. The assumptions for the parametric ANOVA are heavily violated, which is why a parametric ANOVA on ranked data was done. For this reason, the parametric ANOVA on ranked data should be more powerful than the parametric ANOVA, but at least this will give an estimate of the power of the test. The mean of all triclosan concentrations was 1.77 μg/L, regardless of season, site or composite. The minimum significant difference the Tukey's test following parametric ANOVA could detect was 2.28 μg/L for the season data, which was not significant ($p=0.5961$, model III, 3-way with replication parametric ANOVA). The least significant difference that could be detected following parametric ANOVA for the season data was 1.72 μg/L (LSD proc in SAS, alpha of 0.05). This test split the seasons into two overlapping groups from greatest to smallest: summer fall winter spring. The means of the seasons range from 2.88 μg/L in summer to 0.94 μg/L in spring. The minimum significant difference the Tukey's test following parametric ANOVA could detect was 2.70 μg/L for the site data, which was significant ($p<0.0001$, model III, 3-way with replication parametric ANOVA). The least significant difference that could be detected following parametric ANOVA for the site data was 1.92 μg/L (LSD proc in SAS, alpha of 0.05). This test split the sites into two distinct groups: influent > downstream = effluent = wetland inflow = wetland outflow. The means of the sites range from 7.32 μg/L at the influent to 0.036 μg/L at the wetland outflow. The minimum significant difference the Tukey's test following parametric ANOVA could detect was 1.75 μg/L for the composite data, which was not significant ($p=0.9799$, model III, 3-way with replication parametric ANOVA). The least significant difference that could be detected following parametric ANOVA for the composite data was 1.46 μg/L (LSD proc in SAS, alpha of 0.05). This test did not differentiate the composites, so that they were all in the same group: A (1-8) = C (17-24) = B (9-16). The means

of the composites range from 1.91 μg/L for the 1-8 time composite to 1.70 μg/L for the 9-16 time composite.

Due to the fact that the composites were not found to be significantly different in the ANOVA, a two-way parametric ANOVA on ranked data with date and site as the two levels was also done. The results of that test are as follows. First normality was tested on the ranked data to determine if it met that assumption. Seven of the ten months were normally distributed and four of the five sites were normally distributed (α=0.05, Shapiro-Wilks normality test). Therefore, the bulk of the levels are normally distributed. ANOVA is robust and should be able to handle the deviations made from meeting the normality assumption. Next, homogeneity of variances was tested to see if the data met that assumption. The monthly dates were homoscedastic ($p>0.05$, Fmax = 4.07, a=10, v=8, Hartley's Fmax test). The sites were heteroscedastic ($p<<0.01$, Fmax=14.11, a=5, v=18, Hartley's Fmax test). Once again ANOVA is robust and should be able to handle the breaches of assumptions made. The following are the summary statements for the ANOVA. Triclosan concentrations separated by month were highly significantly different ($p<0.0001$), as were the sites ($p<0.0001$), and the interaction between month and site ($p<0.0001$) was significant (2-way, model III, with replication parametric ANOVA on ranked data). Monthly sampling dates were separated into seven overlapping groups:

7/9 6/27 11/26 8/13 12/11 9/10 10/16 5/23 1/7 4/11 (SNK, α=0.05). Sites were separated into four statistically distinct groups: influent > downstream = effluent > wetland inflow > wetland outflow (SNK, α=0.05). However, due to the significant interaction between month and site, it is risky to remark on the factors separately.

Power determinations were made for this test similar to the determinations made for the parametric ANOVA on ranked data, which used the three levels of season, site and composite. A

parametric ANOVA was done using the two levels of monthly dates and sites, even though the assumptions of normality are heavily violated, in order to determine the minimum significant difference and the least significant difference. As before, the parametric ANOVA on ranked data should be more powerful than the parametric ANOVA, but at least this will give an estimate of the power of the test. The mean of all triclosan concentrations was 1.77 μg/L, regardless of season, site or composite. The minimum significant difference the Tukey's test following parametric ANOVA could detect was 1.00 μg/L for the monthly data, which was significant ($p<0.0001$, model III, 2-way with replication parametric ANOVA). The least significant difference that could be detected following parametric ANOVA for the monthly data was 0.615 μg/L (LSD proc in SAS, alpha of 0.05). This test split the monthly dates into five overlapping groups: 6/27 7/9 8/13 12/11 10/16 11/26 5/23 9/10 1/7 4/11. The means of the months range from 7.62 μg/L at 6/27/03 to 0.484 μg/L at 4/11/03. The minimum significant difference the Tukey's test following parametric ANOVA could detect was 0.608 μg/L for the site data, which was significant ($p<0.0001$, model III, 2-way with replication parametric ANOVA). The least significant difference that could be detected following parametric ANOVA for the site data was 0.434 μg/L (LSD proc in SAS, alpha of 0.05). This test split the sites into two distinct groups: influent > downstream = effluent = wetland inflow = wetland outflow. The means of the sites range from 7.32 μg/L at the influent to 0.036 μg/L at the wetland outflow.

It was of interest to compare the triclosan concentrations in the effluent before the ultraviolet basin to the effluent after the ultraviolet basin because researchers have reported high conversion of triclosan to dioxin in ultraviolet light. The concentrations in the samples for the three sampling dates for only the effluent before and after the ultraviolet basin all met the normality assumption ($\alpha = 0.05$, Shapiro-Wilks normality test on raw data). Both sites were also

normal ($\alpha = 0.05$, Shapiro-Wilks normality test on raw data). The normality assumption of a parametric ANOVA was met. The three sampling dates were heteroscedastic because the highest variance was 0.0005664 and the lowest was 0.0000252 both with an n of 6, so that the Fmax was 22.4 ($p<0.01$, Hartley's Fmax test). The two sites were homoscedastic ($p>0.05$, Fmax = 1.24, a=2, v=8, Hartley's Fmax test). ANOVA is robust and should be able to handle the fact that the dates were heteroscedastic. The following are the summary statements from the ANOVA. Mean triclosan concentrations separated by sampling date were highly significantly different ($p<0.0001$); however, the two site means were not significantly different ($p=0.3190$) and the interaction between month and site ($p=0.8108$) was not significant (2-way, model III, with replication parametric ANOVA). Monthly sampling date means were separated into three statistically distinct groups: 0.159 μg/L (11/26/03) > 0.096 μg/L (12/11/03) > 0.034 μg/L (1/7/04) (SNK, $\alpha=0.05$).

Power determinations were made for this test directly from the parametric ANOVA, since the assumptions were mostly met. The mean of all triclosan concentrations for the effluent data regardless of season, site or composite was 0.096 μg/L. The minimum significant difference the Tukey's test following parametric ANOVA could detect was 0.027 μg/L for the monthly data. The least significant difference that could be detected following parametric ANOVA for the monthly data was 0.022 μg/L (LSD proc in SAS, alpha of 0.05). The minimum significant difference the Tukey's test following parametric ANOVA could detect was 0.018 μg/L for the two sites. The least significant difference that could be detected following parametric ANOVA for the two sites was 0.018 μg/L (LSD proc in SAS, alpha of 0.05). The mean of the effluent before the ultraviolet basin was 0.100 μg/L and the mean of the effluent after the ultraviolet basin was 0.092 μg/L.

It was also of interest to determine the percent loss at each step of the wastewater treatment process from influent to effluent to downstream, from influent to effluent to wetland inflow to wetland outflow to downstream, and from influent to effluent before ultraviolet treatment to effluent to downstream. The volume of water that the wetland outflow contributes to the downstream site was minimal when compared to the volume of water that the effluent discharge contributes. The effluent before ultraviolet treatment site only exists after the ultraviolet system was put in place in November, so only the November through January sites were used in this analysis. Only the months that have wetland flow triclosan concentrations were used in the influent to effluent to wetland inflow to wetland outflow to downstream analysis. All months were used in the influent to effluent to downstream analysis. Because the bulk of the sites were not normal, it was more appropriate to determine the median percent loss rather than mean percent loss. The median is a resistant statistic and is not changed by outliers, whereas the mean is a nonresistant statistic and would be affected by the high values, which were present. The median, after deletion of the significant outliers, was used. The percent reduction was calculated as the amount from the first step minus the amount from the second step with that quantity divided by the amount from the first step and multiplied by one hundred to convert to percent. For example, the median influent triclosan concentration was 5.7 μg/L and the median effluent triclosan concentration was 0.096 μg/L. Then, 5.7-0.096 is 5.604 μg/L, divided by 5.7 and multiplied by 100% is 98.3%. Hence, the median percent loss of triclosan from influent to effluent was 98.3%. Therefore, the bulk of the triclosan coming into the wastewater treatment plant was being removed before being discharged. There was not a median percent loss from effluent to 1000 ft downstream from the discharge point because the median from the downstream site (0.106 μg/L) was higher, but not statistically significantly different than the

effluent site median of 0.096 μg/L. Instead, there was a 10.4% increase in the concentration. The downstream samples were always much dirtier than the effluent samples. The downstream sites often had algae, plants and dirt in the sample, whereas the effluent samples rarely had that problem. Triclosan has a high K_{ow} or octanol / water partition coefficient (Log K_{ow} (L/kg) = 4.76), along with having a high K_{oc} or organic carbon partition coefficient (Log K_{oc} (L/kg) = 4.26), which means that significant amounts of triclosan may adhere to the sediment or plant and algae material (Wezel and Jager, 2002). Because of this triclosan may have been extracted using the methodology employed. This may have led to the increased triclosan concentration in the downstream samples compared to the effluent samples. There was high variability in the data, which also may have contributed to the apparent increase in triclosan. The median percent losses for the following medians are only for the months in which there were wetland data available. The median influent was 5.55 μg/L, the effluent was 0.0865 μg/L, the downstream was 0.088 μg/L, the wetland inflow was 0.078 μg/L and the wetland outflow was 0.025 μg/L. This led to a percent reduction of triclosan from influent to effluent of 98.4%, from effluent to wetland inflow of 9.8%, and from wetland inflow to wetland outflow of 67.9%. As before, the downstream concentration was higher than the effluent concentration as well as being much higher than the wetland outflow concentration. There was a 1.7% increase from the effluent concentration to the downstream concentration. Reasons for this apparent increase have been discussed previously. The following medians are only for the months after the ultraviolet system was installed and the effluent right before the ultraviolet system was sampled. The median influent was 4.3 μg/L, effluent before ultraviolet basin was 0.081 μg/L, effluent is 0.095 μg/L, and the downstream was 0.088 μg/L. This led to a median percent reduction of triclosan from influent to effluent before ultraviolet basin of 98.1%. The median effluent concentration before

the ultraviolet basin was lower than the effluent concentration after the ultraviolet basin. This does not make a lot of sense, but could be caused by the high variability in the data. The concentrations at the two sites were not statistically significantly different. The effluent triclosan concentration increased 17.3% from before the ultraviolet basin to after. The percent reduction of triclosan from effluent at the discharge point to 1000 ft downstream from the discharge point was 7.4%.

CHAPTER 7

DISCUSSION

In the following paragraphs, the results found in this study at the Pecan Creek wastewater treatment plant in Denton, TX, are compared to results from similar studies found in the literature.

Detection Limits

The practical quantification limit (PQL) for the methodology used in this study was 0.0078 μg/L. This was lower than the detection limit from Brown, Zaugg and Barber (1999) of 0.05 μg/L for triclosan in wastewater. It was also lower than the detection limit of 0.037 μg/L (in surface water) from Okumura and Nishikawa (1996). The PQL from this study was lower than the detection limit of 0.01 μg/L in wastewater from McAvoy et al. (2002). Two studies (Morrall et al., 2004 and Sabaliunas et al., 2003) also used the methodology from McAvoy et al. (2002). The detection limit, 0.2 ng/L, from Boyd, Reemtsma, Grimm, and Mitra (2003) was much lower than the PQL in this study. The method used in this study had a PQL lower than all but one of the methods from the articles reviewed. The PQL in this study was well below concentrations found to be toxic to algae, the most sensitive group, according to literature (Orvos et al., 2002).

Influent Concentrations

The median influent concentration (used because the influent data are not normal, Shapiro-Wilks, α=0.05) found at the Pecan Creek activated sludge wastewater treatment facility in this study across all seasons was 5.95 μg/L, though influent concentrations ranged from 2.7 μg/L to 26.8 μg/L. Influent concentrations of triclosan from two activated sludge wastewater

treatment plants in Ohio, USA, were 5.21 μg/L and 10.70 μg/L. Note that these concentrations are from one sampling event each; they are not averages nor medians. The median influent concentration found in this study was very similar to the concentration found in the Columbus, OH wastewater treatment plant and the concentration found at the Loveland, OH wastewater treatment plant was well within the range of concentrations found in the Denton, TX wastewater treatment plant. Influent concentrations from two trickling filter wastewater treatment plants in Ohio, USA, were 3.83 μg/L, 16.6 μg/L and 15.4 μg/L. The two high numbers are from one plant, which was sampled twice, once in August and then again in October of 1997. Though the Denton wastewater treatment plant is not a trickling filter plant, influent concentrations are not affected by treatment process. The median influent concentration from this study was within the range found in the Ohio trickling filter wastewater treatment plants (McAvoy et al., 2002). The influent concentration from an activated sludge wastewater treatment plant in the UK was 21.9 μg/L. This influent concentration is more than three times the median concentration found at the Pecan Creek wastewater treatment plant, but during one month of sampling in June 2003, the concentrations ranged from 20.0 to 26.8 μg/L, which bounds the UK concentration (Sabaliunas et al., 2003).

Effluent Concentrations

The mean triclosan concentration (used because the effluent data were normal, Shapiro-Wilk, α=0.05) in the effluent was 0.113 μg/L, though the concentrations ranged from 0.028 μg/L to 0.254 μg/L. Effluent concentrations of triclosan from two activated sludge wastewater treatment plants in Ohio, USA, were 0.24 μg/L and 0.41 μg/L. Note that these concentrations are from one sampling event each; they are not averages nor medians. The mean effluent triclosan concentration from the Denton wastewater treatment plant was about half of the lowest of the

two activated sludge wastewater treatment plants in Ohio. However, the maximum triclosan concentration found was in between these two wastewater treatment plants. The triclosan effluent concentrations from two trickling filter wastewater treatment plants in Ohio, USA, were 1.61 μg/L, 2.10 μg/L and 2.7 μg/L. The two high numbers are from one plant, which was sampled twice, once in August and then again in October of 1997. The concentrations in the trickling filter wastewater treatment plants were about an order of magnitude higher than the concentrations found in the Denton wastewater treatment plant effluent. This is more than likely due to the difference between triclosan removal in activated sludge treatments and trickling filter treatments (McAvoy et al., 2002). The triclosan effluent concentrations found in this study were about an order of magnitude higher than the concentrations found in a Louisiana conventional secondary wastewater treatment plant (0.010 μg/L and 0.021 μg/L) (Boyd et al., 2003). The final effluent concentration from an activated sludge wastewater treatment plant in the UK was 1.1 μg/L, which is about a magnitude higher than the mean effluent concentration for the Denton wastewater treatment plant (Sabaliunas et al., 2003).

Triclosan Removal

The percent removal of triclosan from influent to effluent in the Pecan Creek activated sludge treatment plant during this study was compared to literature values for both activated sludge and trickling filter wastewater treatment plants. The median percent removal of triclosan from influent to effluent in this study was 98.3%. This is higher than the average 96% removal of triclosan in two activated sludge treatment plants in Ohio, USA as described in McAvoy et al. (2002). The total removal of triclosan from influent to final effluent at an activated sludge wastewater treatment plant in the UK was 95% (Sabaliunas et al., 2003), which is lower than the percent removal found in this study. The median percent removal of triclosan from influent to

effluent of greater than 98.5% is very similar to what was found in this study (Federle, Kaiser and Nuck, 2002). However, all of these values are fairly similar in that they are all above 95%.

Receiving Water

The median triclosan concentration (used because the downstream data were not normal, Shapiro-Wilks, α=0.05) in Pecan Creek 1000 ft (304.8 m) downstream from the effluent discharge point was 0.106 μg/L and ranged from 0.030 μg/L to 0.286 μg/L. The downstream site concentrations were not significantly different than the effluent concentrations (mean = 0.113 μg/L) in this study and travel time to the downstream site is not known. The percent loss from mean effluent concentration to median triclosan concentration 1000 ft downstream was 6.2%. The final effluent triclosan concentration was 0.34 μg/L at the Meltham, UK wastewater treatment plant; 20 m downstream in Mag Creek, UK the triclosan concentration was 0.080 ± 0.015 μg/L and 750 m downstream the concentration was 0.053 ± 0.0032 μg/L. Therefore, compared to the effluent, 20 m downstream the percent reduction of triclosan was 76.5% and 750 m downstream the percent reduction was 84.4%, not corrected for the stream flow dilution factor of 2.65 (Sabaliunas et al., 2003). The attenuation of triclosan in Mag Creek just 20 m downstream was quite a bit greater than what was found in Pecan Creek. The die away rate of triclosan was also examined in another study. In Cibolo Creek, TX, 200 m downstream from the outfall, triclosan was 0.431μg/L, while in the effluent it was 0.785 μg/L. At 2000 m downstream from the outfall, the triclosan concentration was 0.223 μg/L. This means that the percent reduction from effluent to 200 m downstream was 45.1% and from effluent to 2000 m downstream it was 71.6%, not including any dilution factor corrections (< 3). This stream, like Pecan Creek, has low dilution (Morrall et al., 2004). The attenuation 200 m downstream from the discharge point in Cibolo Creek is much higher than what was found in this study, though lower

than what was found in the previous study (Sabaliunas et al., 2003). Concentrations found in the downstream site in this study seem to be quite different than those found in similar studies. This could be due to the lack of dilution in Pecan Creek.

Triclosan concentrations in the receiving system were compared to published toxicity values. As previously noted, the median concentration of triclosan in the receiving system, 1000 ft downstream from the effluent discharge point, was 0.106 μg/L. This was lower than the no observable effects concentration (NOEC) for *Scenedesmus subspicatus*, which has a NOEC of 0.5 μg/L for both biomass and growth rate endpoints. The lowest observable effects concentration (LOEC) for *Scenedesmus subspicatus* (1.2 μg/L) is about an order of magnitude higher than the median concentration found at the downstream site. The maximum triclosan concentration found at the downstream site was 0.286 μg/L, which is approximately half of the NOEC for *Scenedesmus subspicatus*. Algae are the most sensitive species for triclosan in this study (Orvos et al., 2002). However, the median concentration of triclosan in the receiving system (0.106 μg/L) was near the concentration found to cause marked shifts in community structure of both attached and suspended algae (0.12 μg/L) and this concentration is less than half the maximum triclosan concentration found at the downstream site (0.286 μg/L) (Wilson et al., 2003).

The study done here is particularly interesting in that most of the studies in the available literature do not have monthly or even repeated measurements of triclosan concentrations at wastewater treatment plants. It is important to note the seasonal variation that was present in the data from this study, and is likely to also be present at other wastewater treatment plants. From the literature reviewed, this is the only study that repeatedly measured triclosan concentrations in a wastewater treatment plant and downstream for more than a two or three month period.

Analytical Percent Recovery

Percent recoveries of effluent spikes from this study were compared to the available literature. The percent recoveries of a 0.1 ppb triclosan spike in the effluent ranged from 48% to 190.5% in this study. Percent recoveries of two samples were 36 and 87% for final effluents spiked with 0.5 μg/L (final concentration) (McAvoy et al., 2002).

Percent recoveries of blank spikes from this study were compared to literature values. The percent recoveries of a 0.1 ppb triclosan spike in deionized water ranged from 82% to 134%. These values are higher than the percent recovery of triclosan (60.1%) in Boyd et al. (2003).

Each individual lab determines acceptance limits. TRAC laboratories, where samples were extracted and analyzed in this study, has acceptance limits for diazinon, which has a methodology similar to that used for triclosan in this study. These limits are calculated from historical data for a 1 ppb diazinon spike. From April to May of 2003, which was the time period when the triclosan study began, the acceptance limits for the blank spike recovery were 70-130%, matrix spike recovery limits were 70-130% and the duplicate relative percent deviation limit was 25%. From June through November of 2003, the blank spike recovery acceptance limits were 69-126%, the matrix spike recovery acceptance limits were 53-134% and the duplicate relative percent deviation acceptance limit was 42%. From December of 2003 through March of 2004, the blank spike recovery acceptance limits were 49-95%, the matrix spike recovery acceptance limits were 49-95% and the duplicate relative percent deviation acceptance limit was 42%. For April of 2004, the blank spike recovery acceptance limits were 55-128%, the matrix spike recovery acceptance limits were 36-118% and the duplicate relative percent deviation acceptance limit was 19% (Steve Junot, personal communication, May 2004). There were not defined acceptance limits for the triclosan data. However, it was interesting to see how

the triclosan spike recoveries compare to the acceptance limits for diazinon to have some basis for comparison. The percent recoveries of a 0.1 ppb triclosan spike in the blank ranged from 82% to 134%. The percent recoveries of a 0.1 ppb triclosan spike in the effluent (matrix spike) ranged from 48% to 190.5%. The relative percent deviations of the effluent spike and duplicate spike ranged from 1.68% to 40.5%. The lowest blank spike percent recovery was above all of the lower acceptance limits. The highest blank spike percent recovery of 134% was above all of the upper acceptance limits, but it is not exceptionally far off from them. The lowest effluent spike percent recovery was below all of the lower acceptance limits for the diazinon matrix spike, but it is fairly close to a few of those lower limits. The upper bound of the effluent spike percent recovery for triclosan was above the entire set of upper acceptance limits for the diazinon analysis. However, triclosan and diazinon are different analytes and therefore, respond differently in the effluent matrix. Also, the diazinon was spiked at 1 ppb, while the triclosan was spiked at 0.1 ppb. The Environmental Protection Agency, at this time, does not regulate triclosan concentrations in wastewater. If they did, then there would be an approved methodology complete with expected acceptance limits. The upper bound relative percent deviation of the triclosan effluent spikes and duplicate spikes was lower than the duplicate relative percent deviation of the diazinon analysis during most of the months of this study. These results suggest that the triclosan methodology from this study was sound and that the data are acceptable.

CHAPTER 8

FUTURE WORK

There are many other interesting research projects that could be done using the Pecan Creek system as well as the Trinity River system in regards to triclosan. As shown by this research project, 1000 ft downstream from the discharge point in Pecan Creek, the triclosan concentration was not significantly attenuated. It would be important to find out how far downstream the triclosan concentration persisted and how far downstream the triclosan concentration diminished to below the detection limit, or effectively zero. In addition, a die-away rate study could also be done in Pecan Creek to determine the time it takes for half the concentration of triclosan to be removed by these conditions. It would be interesting to redo this study using a different method or sampling regime: bimonthly or continuing it for several years. These studies could further define the seasonal variation, and perhaps a study could be done to find the causes of it, especially since Denton has such a large transient student population from the University of North Texas and Texas Woman's University. A large, well-funded research project could look at many sites above and below wastewater treatment plants along the Trinity River system. This would be especially fascinating in regards to an ecological risk assessment because there exists the possibility that multiple wastewater treatment plants with low dilution could compound the triclosan concentration so that it reaches above the algae no observable effects concentration (NOEC). It would also be appealing to sample Pecan Creek to see if triclosan resistant bacteria were present. In general, more studies need to be done with repeated measurements of the influent, effluent and downstream concentrations of triclosan because of the seasonal variability, as found in this study.

CHAPTER 9

CONCLUSIONS

This study was designed to determine the concentrations of triclosan entering (influent) the Pecan Creek wastewater treatment plant in Denton, TX, as well as the amount discharged (effluent) from the plant into Pecan Creek. Determinations of triclosan concentrations were also made 1000ft downstream from the discharge point in Pecan Creek, as well as at the inflow and outflow of the experimental constructed wetland. The concentrations from these sites were compared using analysis of variance (ANOVA). Attenuation of triclosan from the effluent to the discharge point was calculated, as well as the percent removal of triclosan from the wetland inflow to the outflow and percent loss from each step in the wastewater treatment chain. Seasonal variation of the data was also examined using ANOVA. The results from this study were compared to results from similar studies in the literature. Influent and effluent concentrations from this study are fairly similar to those found in the literature. However, triclosan concentrations at the downstream site were quite different from those in the literature. This could be due to the lack of dilution in Pecan Creek, but further studies should be done to test this. Concentrations of triclosan in the downstream site were below the published no observable effects concentration (NOEC) for the most sensitive species. Concentrations in experimental constructed wetlands were not found in the literature. However, from the results of this study, if the experimental constructed wetland was designed to accommodate all of the effluent flow, then it could significantly reduce the amount of triclosan discharged into the receiving system and significantly mediate associated risks to the biota from triclosan exposure.

Seasonal variation of triclosan concentrations in wastewater treatment plants and downstream was not explored in the literature.

APPENDIX

Table 7: Raw data from April 2003 to January 2004. Influent, effluent, downstream, wetland inflow, wetland outflow and effluent prior to the ultraviolet treatment are included. All concentrations are in μg/L. The 8-hour flow composites are denoted as 1-8 (first 8-hour flow composite, 12am-8am for the influent and 10am-6pm for all other sites), 9-16 (second 8-hour flow composite) and 17-24 (third 8-hour flow composite). NA=not available and ND=not detected.

Sampling	Influent			Effluent			Downstream			Wetland Inflow			Wetland Outflow			Effluent, PreUV		
Date	1-8	9-16	17-24	1-8	9-16	17-24	1-8	9-16	17-24	1-8	9-16	17-24	1-8	9-16	17-24	1-8	9-16	17-24
4/10-4/11/03	25.86	2.70	3.80	0.081	0.031	0.033	0.060	0.030	0.041	ND	NA	NA	ND	NA	NA			
5/22-5/23/03	5.70	8.30	5.40	0.052	0.072	0.047	0.038	0.074	0.055	0.173	0.267	0.192	0.000	0.077	0.036			
6/26-6/27/03	26.80	21.00	20	0.124	0.078	0.090	0.229	0.107	0.122									
7/8-7/9/03	5.00	6.50	8.4	0.119	0.190	0.177	0.159	0.194	0.260									
8/12-8/13/03	6.30	5.40	4.9	0.226	0.216	0.183	0.103	0.101	0.318	NA	0.288	0.409	NA	0.042	0.028			
9/9-9/10/03	6.80	4.20	4.3	0.254	0.363	0.185	0.286	0.216	0.222	0.087	0.078	0.060	0.034	0.021	0.025			
10/15-10/16/03	7.00	8.00	7.5	0.083	0.096	0.119	0.121	0.119	0.127	0.090	0.055	0.055	0.064	ND	0.065			
11/25-11/26/03	4.50	4.10	4.3	0.159	0.162	0.132	0.114	0.117	0.106	0.133			0.029			0.168	0.177	0.155
12/10-12/11/03	7.90	6.20	6.8	0.090	0.095	0.099	0.090	0.088	0.088	0.120	0.131	0.122	0.165	0.133	0.090	0.081	0.140	0.071
1/6-1/7/04	3.20	3.50	3.8	0.029	0.028	0.034	0.033	0.032	0.032	0.020	0.028	0.016	0.023	0.021	0.024	0.034	0.041	0.038

Table 8: Quality control data from April 2003 to January 2004. Triclosan was added to the spikes to yield a final spike concentration of 0.1 ppb. The effluent spike recovery was calculated using the effluent spike and duplicate spike mean.

Sampling	Blank	Blank spk	Blank spk	Effluent grab	Eff spk	Eff dspk	Eff spk	Eff spk	Eff spk
Date	µg/L	µg/L	%Recovery	µg/L	µg/L	µg/L	%Recovery	%Deviation	Mean
4/10-4/11/03	ND	0.088	88.00%	0.159	0.247	0.257	93%	4.00%	0.252
5/22-5/23/03	ND	0.172	101.80%	0.077	0.213	0.224	88.80%	5.00%	0.219
6/26-6/27/03	ND	0.128	128%	0.071	0.295	0.226	159.50%	26%	0.2605
7/8-7/9/03	ND	0.123	123%	0.159	0.301	0.454	142.00%	40.5%	0.3775
8/12-8/13/03	ND	0.082	82%	0.166	0.214	0.181	48%	16.70%	0.1975
9/9-9/10/03	ND	0.134	134%	0.179	0.400	0.339	190.50%	16.50%	0.3695
10/15-10/16/03	ND	0.097	97%	0.079	0.151	0.177	85%	15.80%	0.164
11/25-11/26/03	ND	0.099	99%	0.171	0.296	0.301	127.5%	1.68%	0.2985
12/10-12/11/03	ND	0.098	98%	0.084	0.188	0.165	92.5%	13.03%	0.1765
1/6-1/7/04	ND	0.094	94%	0.047	0.104	0.072	57.00%	29.60%	0.0845

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