TRICLOSAN CONCENTRATIONS IN WESTERN KENTUCKY WATERSHED

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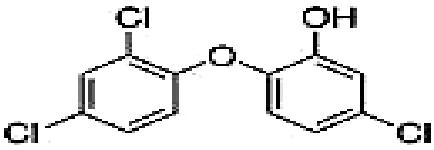
Abstract

Triclosan (2,4,4'-trichloro-2'-hydroxyphenyl ether) is considered as one of the emerging new pollutants in the environment. In this study, triclosan contamination levels were measured in water samples collected from Murray Wastewater Treatment Plant (WWTP), Bee Creek, Clarks River and Kentucky Lake. Enzyme-linked immunosorbent assay (ELISA) method was used to determine triclosan concentrations in the samples. The results revealed that detectable concentrations of triclosan were found in all samples analyzed. The concentrations of triclosan exhibited the following trend: Influent > Effluent > Downstream Bee Creek > Upstream Bee Creek ≥ Clarks River > Kentucky Lake (HBS site). Removal efficiency calculations revealed that about 40% of triclosan enter the receiving waters (Bee Creek). Clarks River and Kentucky Lake water samples contained relatively lower levels of triclosan than WWTP samples.

Introduction

Triclosan (2,4,4'-trichloro-2'-hydroxyphenyl ether) is a common antibacterial ingredients in household and personal care products such as soaps, dental care products, first aids, cosmetics, and many other various products[1-3]. Wide spread use of these products resulted in environmental contamination by triclosan. Residues of triclosan were reported in wastewater treatment plant samples, river water, and lake water samples. Although triclosan has been found to be toxic to plants and aquatic organisms (algae, plankton, fish), there exists no study dealing with contamination levels of triclosan in western Kentucky watershed. Triclosan (2,4,4'-trichloro-2'-hydroxyphenyl ether) is a very stable antibacterial compound and is not easily biodegradable[1,2]. It has a molecular weight of 289.5, a p*K*a of 8.14, is relatively non-volatile with a vapor pressure of 4x 10^{-6} mmHg at 20 ˚C and is poorly soluble in water with a solubility of 10 mg /l at 20˚C [1,3]. Triclosan has been reported to be a thyroid hormone disruptor, can inhibit lipid biosynthesis, and can affect the livers of various organisms[3-8]. It has been reported that triclosan exposure can cause contact dermatitis, photo allergic contact dermatitis (PACD), and there are reported cases of immunotoxic and nuerotoxic reactions to triclosan[2]. However, there exists no study on triclosan in watersheds in western Kentucky.



In order to make clear the magnitude of contamination by triclosan in our regional waters, we selected Murray Waste Water Treatment Plant (MWWTP), Bee Creek (where water from MWWTP is emptied) samples including upstream and downstream from MWWTP, Clarks River and Kentucky Lake (non-point sources) samples. Map showing the sampling locations is given in Figure 1. Understanding the triclosan contamination levels in regional waters is important in order to prevent further contamination and protect the living resources of this region.

Materials and Methods

Water samples were collected, filtered and passed through SPE cartridges and eluted with methanol as described in Kantiani et al. (2008)[9]. Four sampling events were occurred during January, 2009 through March 2009 (January 2, January 11, February 13 and February 21, 2009) at the selected sampling sites (Figure 1). Clean glass test tubes were used for standards, control, and samples. 250 uL of the appropriate standard, control, or sample were added. 500 uL of Triclosan Antibody Coupled Paramagnetic Particles were mixed thoroughly and added to each tube and mixed for 2 seconds without foaming. The samples

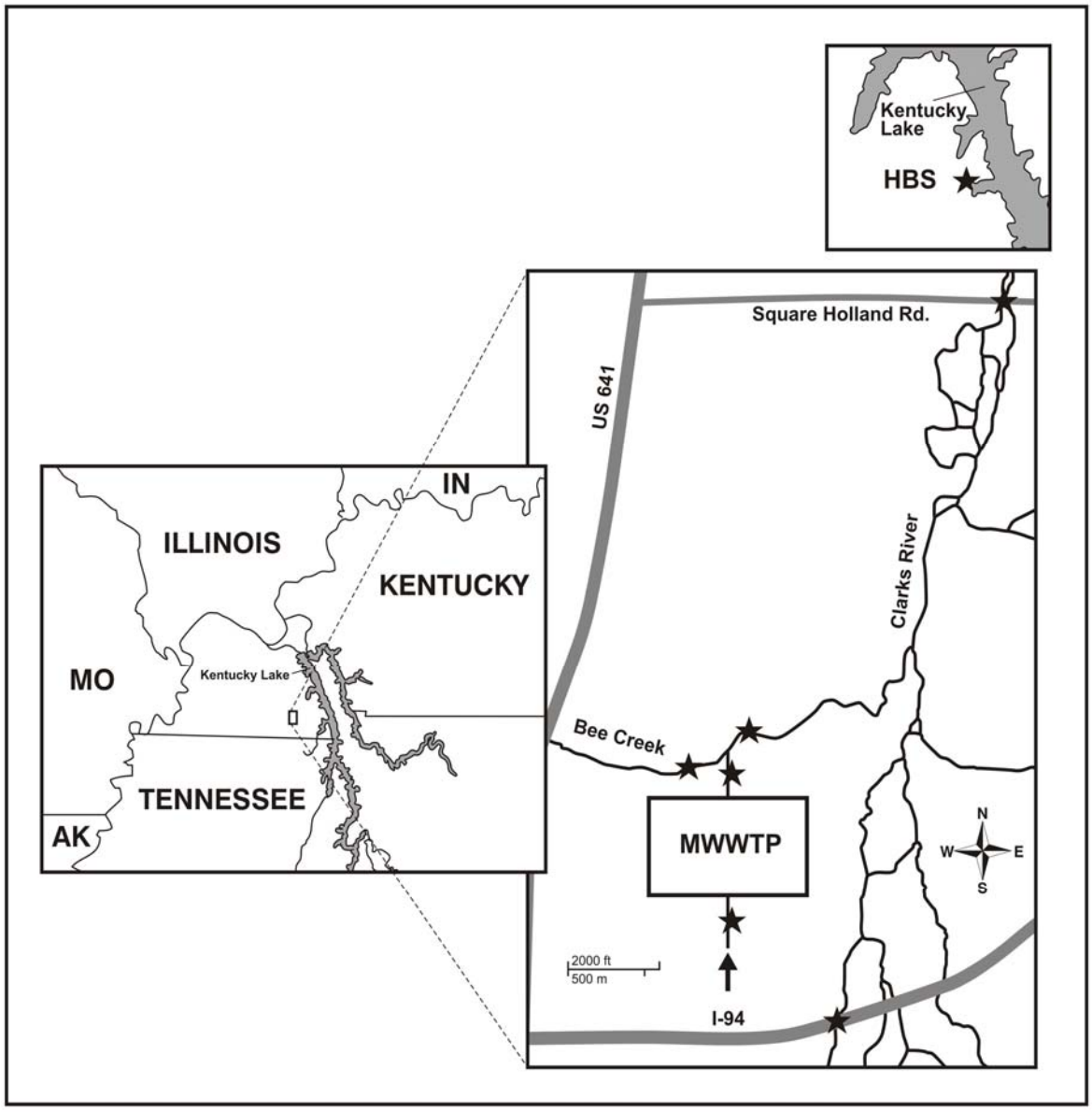


Figure 1. Map showing sampling locations (*) in Murray Wastewater Treatment Plant, Bee Creek, Clarks River and Kentucky Lake, USA.

were incubated for 30 minutes at room temperature. 250 uL of Triclosan Enzyme Conjugate were added to each tube and mixed for 2 seconds. The samples were incubated for 30 minutes at room temperature, and then placed in the Magnetic Separation Rack for two minutes. The tubes were decanted and gently blotted in a consistent manner. 1 mL of washing solution was added to each tube and vortexed for 1-2 seconds. The tubes remained in the magnetic separation unit for two minutes. All tubes were decanted and gently blotted in a consistent manner. The tubes were then washed an additional time. The tubes were then removed from the separator and 500 uL of color solution was added to each tube. Each tube was vortexed for 1 to 2 seconds minimizing foaming. The samples were incubated for 20 minutes at room temperature. 500 uL of stopping solution was added to each tube. 1 mL of washing solution was added to a clean test tube to be used as a blank. The samples were read at 450 nm in a UV-Vis spectrophotometer within 15 minutes after adding the stopping solution.

Results and Discussion

Concentrations (ng/L) of triclosan in MWWTP influent, effluent, Bee Creek upstream, Bee Creek downstream, influent composite and effluent composite samples were shown in Table 1.

Table 1. Triclosan concentrations (ng/L) in Murray Wastewater Treatment Plant influent, effluent, downstream Bee Creek, Upstream Bee Creek, Influent composite and effluent composite samples.

Survey Number	Influent (ng/L)	Effluent (ng/L)	Downstream (ng/L)	Upstream (ng/L)	Influent Composite (ng/L)	Effluent Composite (ng/L)
1	3.2	1.3	1.29	0.92	N/A	N/A
2	2.9	1.2	1.29	0.97	1.18	1.41
3	2.8	1.3	1.18	0.51	N/A	N/A
4	3.0	1.3	1.20	0.72	3.18	1.22

Among the various samples analyzed, MWWTP influent contained highest amount (2.8-3.2 ng/L) than effluents (1.3 ng/L). Upstream Bee Creek contained the lowest concentration of triclosan. Effluent and downstream of Bee Creek had similar range of concentrations indicating that WWTP effluent contributed triclosan to the Bee Creek. Triclosan concentrations in 24-hr composite samples showed similar concentration range (Table 1).

Table 2. Triclosan (ng/L) concentrations in Clarks River and Kentucky Lake water samples.

Survey Number	Clarks River Site 1(ng/L)	Clarks River Site II(ng/L)	Kentucky Lake (HBS) (ng/L)
1	0.73	0.64	0.80
2	0.53	0.49	0.47
3	0.37	0.72	0.25
4	0.59	0.55	0.058

Table 2 shows triclosan concentrations in natural waters such as Clarks River and Kentucky Lake (HBS: Hancock Biological Station site). The natural waters contained approximately one order magnitude less concentration than WWTP samples. Clarks River site 1 and 2 did not show large difference in triclosan concentrations. Clarks River site 1 (near I-94 bridge) is located about 2 km upstream of Bee Creek, whereas Clarks River site 2 (Squire Holland Road bridge) is located at about 1 km downstream Bee Creek. The triclosan concentrations in Clarks River sites 1 and 2 did not show large difference in concentrations indicating that triclosan from downstream Bee Creek (from WWTP) is diluted well or degraded before it reached Clarks River. Kentucky Lake at HBS site had the lowest concentration (0.058 ng/L) of triclosan during survey #4. Kentucky Lake sampling site is about 22 km from Clarks River/Murray WWTP and not linked to Clarks River or WWTP. Triclosan levels in Kentucky Lake waters may be attributed to the non-point source.

Results from other WWTP, lakes, rivers, and streams nationwide is shown in Table 3. Compared to the bigger WWTP, the West Kentucky watershed contained relatively lower amount of triclosan present in the water samples. For example, the Arlington County Water Pollution Control Center which serves a population of 194,000 showed effluent triclosan concentration of 72 ng/L [10], while Murray WWTP effluent showed 1.2-1.3 ng/L. Murray WWTP serves maximum population of 30,000. Relatively low concentration of triclosan in MWWTP may be due to lower population that contributed triclosan to the wastewater (Table 3).

Table 3. Comparison of triclosan concentrations in wastewater treatment plant samples, lakes, river and stream from different regions in the USA.

Samples	Facility/ Body of water	Sample Location	Triclosan Concentration (ng/L)	Reference
Wastewater Treatment Plant (WWTP)	Murray WWTP- Effluent	Murray , KY, USA	1.2- 1.3	Present Study
	Murray WWTP-Influent	Murray , KY, USA	2.8-3.2	Present Study
	Arlington County Water Pollution Control Plant-Effluent	Arlington, VA, USA	72	10
	Arlington County Water Pollution Control Plant-Influent	Arlington, VA, USA	3000	10
	City of Alexandria Sanitation Authority-Effluent	Alexandria, VA, USA	47	10
	City of Alexandria Sanitation Authority-Influent	Alexandria, VA, USA	3300	10
	Norman M Cole Water Pollution Control Plant-Effluent	Fairfax County, VA, USA	28	10
	Norman M Cole Water Pollution Control Plant-Influent	Fairfax County, VA, USA	3600	10
	City of Denton, Texas WWTP	Denton, TX, USA	120	11
	WWTP- Effluent	Greater Baltimore Area, MD, USA	35±20	12
	WWTP- Influent	Greater Baltimore Area, MD, USA	6100±1600	12
	WWTP- Influent	Savannah, GA	6178	13
	WWTP- Effluent	Savannah, GA	836	13
Point Sources (upstream/downstream from WWTP)	Bee Creek- Upstream	Murray , KY, USA	0.51-0.97	Present Study
	Bee Creek- Downstream	Murray , KY, USA	1.18-1.29	Present Study
	Clarks River- I-94	Murray , KY, USA	0.53-0.73	Present Study
	Clarks River- Site II	Murray , KY, USA	0.49-0.64	Present Study
	Detroit River	Detroit, MI, USA	11−98	14
	Pecan Creek	Denton, TX, USA	60-80	10
Non-point sources (lakes)	Kentucky Lake (Hancock Biological Station)	KY, USA	0.058-0.80	Present Study
	Lake Greifensee	Detroit, MI, USA	50	14

The results revealed that detectable levels of triclosan are present in water samples from the MWWTP, Clarks River and Kentucky Lake sites. The concentrations of triclosan exhibited the following trend: Influent > Effluent > Downstream Bee Creek > Upstream Bee Creek ≥ Clarks River > Kentucky Lake (HBS site). Removal efficiency of triclosan in MWWTP was calculated using concentrations in influent and effluent samples. About 40% of triclosan is not removed or not degraded during the wastewater treatment process and enter the receiving waters (Bee Creek). Clarks River and Kentucky Lake water samples contained relatively lower levels of triclosan than Murray Wastewater Treatment Plant samples. Triclosan levels in Clarks River and Kentucky Lake water may be attributable to non-point source.

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