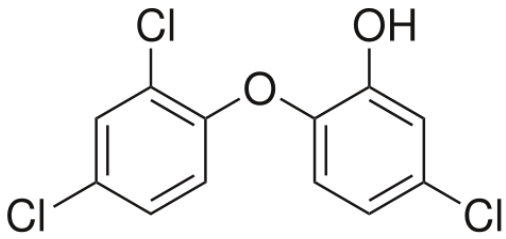
TRICLOSAN CONCENTRATIONS IN SURFACE WATERS: MONITORING OF BEE CREEK, CLARKS RIVER, AND RED DUCK CREEK, KENTUCKY, USA

Benningfield D*[1], Arnett, B[2], Olszowy, K[2], Kemp M[2], Loganathan B G[1]

[1]Department of Chemsitry and Watershed Studies Institute, Murray State University, Murray, KY 4207, USA;
[2]Industrial and Engineering Technology, Murray State University, Murray, KY 42071, USA

Introduction

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is a potent antibacterial and antifungal ingredient in household (fabrics and plastics) and personal care products such as soaps, dental care products, cosmetics etc[1-3]. Triclosan (Figure 1) residues have been reported in wastewater treatment plant samples, river water, and lake water samples[4-8]. Although triclosan has been found to be toxic to plants and aquatic organisms (algae, plankton, and fish) and is a known endocrine disruptor, there exists no study dealing with contamination levels of triclosan in the western Kentucky watershed (Figure 2). Previous studies have shown that triclosan is found throughout food web and it can be photolyzed to 2,8-dichlorodibenzodioxin or methylated to a more bioaccumulative ether form in natural waters[9-14].



In order to elucidate the levels of contamination by triclosan in the western Kentucky watershed, sites from Murray Wastewater Treatment Plant (MWWTP), Bee Creek (where water from MWWTP is emptied), Clarks River, Kentucky Lake (non-point sources), and Red Duck Creek (located in Mayfield, KY) were selected for sampling. Understanding the triclosan contamination levels in regional waters is important in order to prevent further contamination and protect the living resources of this region.

Fig.1. Triclosan **(**5-chloro-2-(2,4-dichlorophenoxy)phenol, Mwt.289.5, p*Ka*: 8.14)

Materials and methods

Surface water samples were collected from selected locations in Murray and Mayfield (Figure 2). Water samples were filtered and passed through Oasys HLB SPE cartridges (Waters Corp. MA, USA.) 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 in Murray, Kentucky and two sampling events occurred at selected sites from Red Duck Creek, Mayfield, Kentucky. Red Duck Creek samples were collected during October, 2010 through December, 2010 (October 30, 2010 and December 4, 2010). Triclosan ELISA (Magnetic particle) Kit (Abraxis, IL, USA) was used to perform triclosan concentrations in the samples. 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 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. Triclosan concentrations in the samples were calculated based on five point calibration curve. Standard methods were used to measure other parameters

incluidng flow rate, dissolved oxygen (DO), total dissolved solids (TDS) and pH. Total coliform and *Escherichia coli* counts were determined using IDEXX Colilert® method[15].

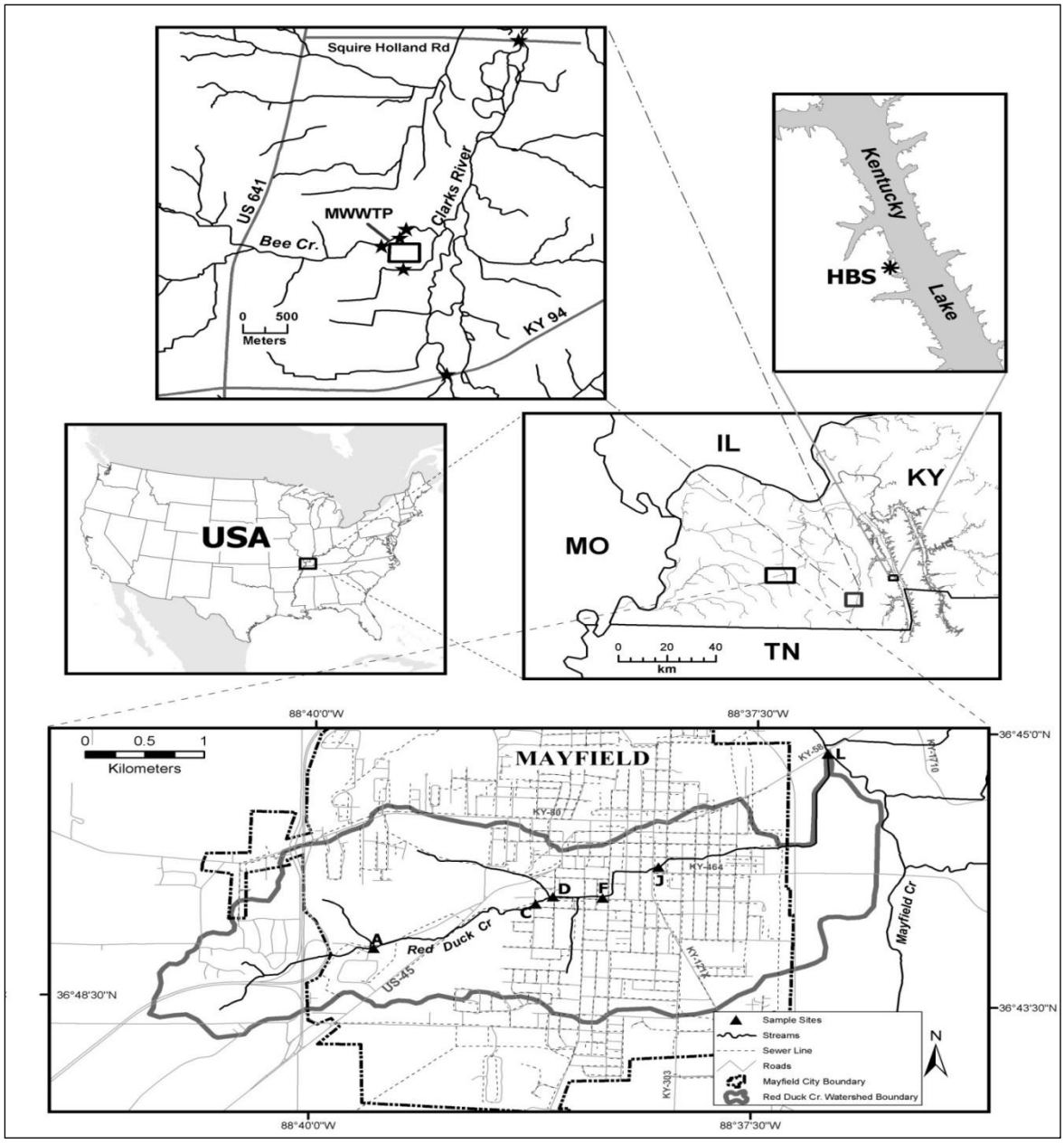


Fig. 2. Map showing sampling locations (*) in Murray Wastewater Treatment Plant (MWWTP), Bee Creek, Clarks River and Kentucky Lake, USA. Sampling sites A through L (bottom part) indicates sampling locations in Red Duck Creek, Mayfield, KY, USA.

Results and discussion

Table 1 and 2 show triclosan concentrations in surface water samples from various locations in Murray, Kentucky. Among the samples analyzed, Murray wastewater treatment plant influent contained the highest

concentrations ranged from 2.9 to 3.2 ng/L and the surface water collected from Kentucky Lake at Hancock Biological station contained the lowest concentrations (0.06 to 0.8 ng/L). Murray wastewater treatment plant effluent contained relatively lower concentrations than the influent, indicating the wastewater treatment process removed about 50% of triclosan entering wastewater treatment plant. Water samples from Bee Creek site at the downstream of wastewater treatment plant is more contaminated with triclosan than upstream indicating input of triclosan from wastewater treatment plant into the Bee creek (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. (N/A: Sample not available for analysis)

Survey Number	Influent (ng/L)	Effluent (ng/L)	Downstream Bee Creek (ng/L)	Upstream Bee Creek (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

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

Survey Number	Clarks River Site I (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 3. Flow, flow rate (cfs), pH, and dissolved oxygen (DO) measurements in Red Duck Creek water samples.

SITE	FLOW/POND		FLOW RATE (cfs)		pH		DO (ppm)	
	Survey I	Survey II	Survey I	Survey II	Survey I	Survey II	Survey I	Survey II
L	FLOW	FLOW	3.7	3.1	5.9	6.2	11.5	5.5
J	FLOW	FLOW	3.0	3.8	6.9	7.0	11.7	12.9
F	POND	POND	0	0	6.7	6.8	12	8.1
D	POND	POND	0	0	6.6	6.6	12	12.6
C	POND	POND	0	0	6.7	6.7	12.2	12.5
A	POND	POND	0	0	6.5	6.5	12	13

Table 3 and 4 show various parameters measured at six sites at Red Duck Creek, Mayfield, Kentucky. During the survey period, water flow was measurable only at sites L and J. Other sites were ponded (Table 3). Triclosan concentrations in Red Duck Creek water samples were several orders of magnitude higher (µ/L) than Bee Creek, Clarks River and Kentucky Lake (Table 1,2 and 4).

Table 4. Total dissolved solids (TDS), total coliform (T-coli), *Escherichia col*i (E.coli), and triclosan (µg/L) measurements in Red Duck Creek water samples, Mayfield, Kentucky.

SITE	TDS		T-Coli (CFU/100mL)		E. Coli (CFU/100mL)		Triclosan (ppb)	
	Survey I	Survey II	Survey I	Survey II	Survey I	Survey II	Survey I	Survey II
L	96	122	935	9610	537	790	45.16	22.2
J	90	118	4360	3130	62	180	23.14	12.71
F	94	139	10112	7560	1789	930	25.21	20.88
D	150	145	10112	2010	2851	130	10.66	5.67
C	212	162	10112	2040	1334	1110	23.8	12.71
A	113	134	3044	6890	1670	360	29.15	N/A

Total coliform and *E.coli* counts (colony forming units/100 mL) were higher in ponded conditions than water collected during flow conditions (Table 4). Triclosan concentrations did not show much variation in the six sites surveyed. Considering triclosan concentrations and bacterial counts in surface water samples (during flow conditions) from sites L and J during survey I and II, it appears to have some relationship with triclosan concentrations and the bacterial count. Since triclosan is an antibacterial agent, higher concentrations of triclosan might have negatively affected the bacteria growth. Further studies with more number of samples are required to elucidate the relationship between triclosan concentrations and the bacterial counts.

Acknowledgements

The authors are thankful to Mr. Jason Henderson, Murray Wastewater Treatment Plant (WWTP) for his help during sampling at the WWTP. We also would like to thank Ms. Maggie Morgan and Miss. Brianna Cassidy, for their help in the field sampling and laboratory analysis. This research was partly supported by the Four River Basin Team (Maggie Morgan), Mayfield, Kentucky. The authors are grateful to the Jones/Ross Research Center, Department of Chemistry, Murray State University for providing necessary equipment and supplies for this study.

References:

1. Pannu MW, O'Connor GA, Toor GS. (2012) *Environ. Toxicol. Chem.*31:646-653
2. Lin H, You Hu Y, Zhang X-U, Guo Y-P, Chen G-R. (2011) *Environ. Toxicol. Chem.*30: 2416-22
3. Nabeshima Y, Hasegawa J, Matsuda M, Kawano M, Wakimoto T, Morita M. (2007) *Organohalogen Compounds.* 69:1503-06
4. Reiss R, Mackay N, Habig C, Griffin J. (2002) *Environ. Toxicol. Chem.* 21:2483-92
5. Waltman E, Venables B, Waller W. (2005) *Environ. Toxicol. Chem.*. 25: 367-72
6. Bester K. (2004) *Arch. Environ. Contam. Toxicol.* 49: 9-15
7. Heidler J, Halden R. (2006) *Chemosphere.* 66: 362-69
8. Sanchez-Prado L, Llompart M, Lores M, Fernandez-Alvarez M, Garcia-Jares, Cela R. (2006) *Anal Bioanal Chem.* 384: 1548
9. Kantiani L, Farre M, Asperger D, et al. (2008) *J. Hydrol.* 361: 1-9
10. Thomas P, Foster G. (2005) *Environ. Toxicol. Chem.* 2005; 24: 25-30
11. Coogan M, Edziyie R, La Point T, Venables B. (2007) *Chemoshpere.* 67: 1911-18
12. Halden R, Paull D. (2005) *Environ. Sci. Technol.* 39: 1420-26
13. Senthilkumar K., Peck A, Palefsky W, Sajwan K.S. (2008) *Oganohalogen Compounds.* 70: 233-36
14. Singer H, Müller S, Tixier C, Pillonel L. (2002) *Environ. Sci. Technol.*. 36: 4998-5004
15. IDEXX Colilert® Test Method for the simultaneous detection of total coliforms and *E. coli* in water. 6/2003