

Development of a Sensitive Magnetic Particle Immunoassay (ELISA) and its Application to the Measurement of Triclosan in Water, Wastewater and Soil Samples



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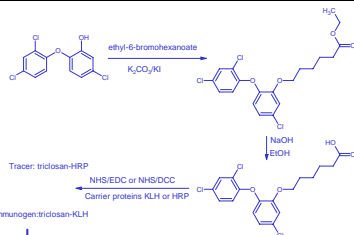
OBJECTIVES

- Development of a magnetic particle enzyme immunoassay for triclosan and methyl-triclosan as a cost-effective, portable, and high throughput analytical method.
- Utilize the triclosan magnetic particle ELISA to measure triclosan and triclosan-methyl levels in water samples obtained from river water, wastewater treatment plants and soil samples.

INTRODUCTION

- Triclosan is a broad spectrum antibacterial compound that is incorporated into numerous household products.
- Structurally, triclosan is related to environmental contaminants such as polybrominated biphenyl ethers (PBDEs).
- Triclosan has been measured in water and human milk samples and bioaccumulates in fish plasma.
- Triclosan breakdown products produced in river water by the action of sunlight include dioxins.
- Methyl triclosan, presumably formed from bacterial metabolism of triclosan in sewage plants, has also been reported in environmental samples.

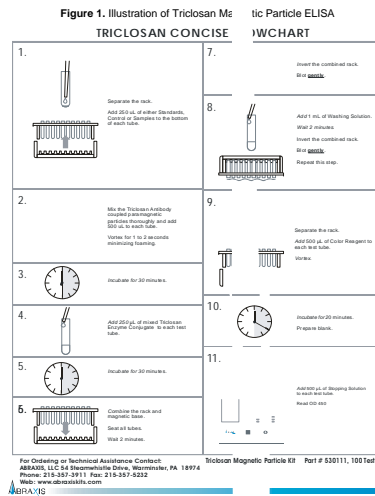
METHODS



- Water Sample Collection:** a) Water samples were collected from different purification stages at the water and wastewater treatment plants from three different cities in the Red River Basin, MN. b) various creek and lake water samples around the city of Denton, TX.
- Water Sample Pretreatment:** Wastewater samples were centrifuged at 2000g for 10 minutes and methanol added to the supernatant to a final concentration of 25%. Water samples were collected and diluted with methanol to 25% methanol.

- Soil Extraction:** Weigh 10 gm of soil. 100 μ L of a 10 μ g/mL solution of Triclosan by the addition of 20 mL of methanol and 5 minutes. Allow the sample to settle, centrifuge. Dilute sample at least 1:500 in 25% methanol/water. Analyze as sample in the ELISA.

The anti-Triclosan coupled magnetic particles were prepared by NHS/EDAC activation, according to the procedure supplied by the manufacturer (Seradyn). The unbound NHS/EDAC was removed by magnetic separation and washed two times with ethane sulfonic acid (MES) buffer (pH 6.0). The reaction was then quenched with glycine buffer and the covalently coupled particles were washed and diluted with a Tris-saline/BSA pre-conjugate was prepared by coupling the Triclosan NHS/DCC coupling procedures. Triclosan (Sigma) was used as standards in the ELISA.



RESULTS AND DISCUSSION

- The ELISA is very sensitive. It can detect methyl-triclosan at 15 ppt (Figure 2) in water samples and showed a relative low variance.
- The ELISA gives good recoveries of triclosan samples and showed a relative low variance.
- The antibody has some (low) cross-reactivity against the more bulky polybrominated biphenyl ethers (Table 2).
- Drinking water tested was below the limit of detection (20 ppt) of the ELISA.

Standard Curve

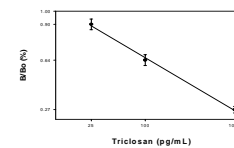


Figure 2. Mean of 30 runs, error bars represent 2 SD around the mean

Assay Performance

Recovery			
Four (4) groundwater samples, were spiked with various levels of Triclosan and then assayed using the Abraxis Triclosan Assay.			
The following results were obtained:			
Amount of Triclosan Added (ppt)	Mean (ppt)	S.D. (ppt)	Recovery (%)
62.5	52.2	4.8	83.4
125	109.1	10.8	87.3
250	252.5	9.1	101.0
500	955.8	28.4	113.1
Average			96.2

Precision			
The following results were obtained:			
Control	1	2	3
Replicates	5	5	5
Days	5	5	5
n	25	25	25
Mean (ppt)	40	124	619
% CV (within assay)	7.5	5.9	4.7
% CV (between assay)	11.4	10.4	6.2

Table 1. Recovery and Precision of Triclosan from spiked water

Specificity		
The cross-reactivity of the Abraxis Triclosan Assay for various Triclosan analogues and other compounds can be expressed as the least detectable dose (LDD) which is estimated as 50% B/B ₀ , or as the dose required for 50% absorbance inhibition (50% B/B ₀).		
Compound (ppt)	LDD (ppt)	50% B/B ₀
Triclosan	20	250
Methyl-triclosan	15	80
BDE-29	34	810
BDE-47	20	390
BDE-49	5,200	17,800
BDE-99	2,150	15,000
4 OH-BDE-49	130	7,800
5 OH-BDE-47	150	5,600
6 OH-BDE-47	890	10,200
2,4,5-Tribromophenyl	>100,000	>100,000
2,3,7,8-TCDF	54,000	>100,000
PCB	940	40,000
L-Threonine (T4)	340	700,000

Table 2. Cross-Reactivity (Specificity) of the Triclosan ELISA

Soil Type	Recovery (%)	
	1:500 Dilution	1:1000 Dilution
Organic	131	118
Top Soil	101	130
Clay	113	134

Table 3. Triclosan Recoveries From Soil Samples

- In general, the concentration of triclosan and methyl-triclosan decreased following the flow of the wastewater treatment plant process (Figure 3).
- Recoveries of soil samples ranged from 101-131% (Table 3)

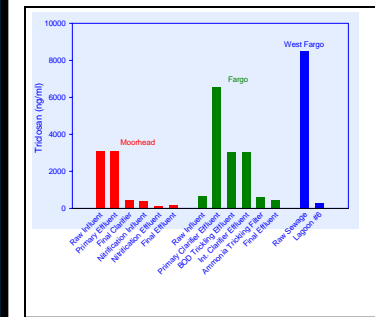


Figure 3. Triclosan and methyl-triclosan levels from wastewater treatment plants in 3 cities of North Dakota.

ELISA vs. Instrumental Analysis

Sample	ELISA (ppt)	GC/MS/SIM (ppt)	
		Triclosan	Methyl-Triclosan
City of Denton Outflow	93.1	86.4	23.0
Pecan Creek Upstream of WWTP	45.3	9.1	<1
Pecan Creek downstream of WWTP	54.0	56.7	18.2
Lake Lewisville*	<LOD	<LOD	<LOD

*Reservoir receiving Pecan Creek discharge

CONCLUSIONS

- This work describes a magnetic particle based ELISA system for the analysis of Triclosan and Methyl-triclosan and its performance characteristics in water.
- The ELISA exhibits within and between assay precision of less than 12% and accuracy between 83-113% which provides for consistent monitoring of environmental samples.
- The magnetic system ELISA is rapid (results within 90 minutes), and very sensitive (Triclosan 20 ppt, methyl triclosan 15 ppt). Water samples do not require sample extraction or pre-concentration and minimizes the use of organic solvents.
- Applications of the Triclosan ELISA to water, wastewater samples from wastewater treatment plants, and from soil samples demonstrates the utility of the ELISA for timely on-site analysis which provides for a cost-efficient monitoring method.

Acknowledgments

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