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Measurement of Triclosan in Water Using a Magnetic Particle Enzyme Immunoassay

WEILIN L. SHELVER,*,† LISA M. KAMP,§ JENNIFER L. CHURCH,§ AND FERNANDO M. RUBIO§

Biosciences Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 1605 Albrecht Boulevard, Fargo, North Dakota 58105, and Abraxis, LLC, 54 Steamwhistle Drive, Warminster, Pennsylvania 18974

A sensitive magnetic particle-based immunoassay to determine triclosan [5-chloro-2-(2,4-dichlorophenoxy)phenol] in drinking water and wastewater was developed. Rabbit antiserum was produced by immunizing the rabbit with 6-[5-chloro-2-(2,4-dichlorophenoxy)phenoxy]hexanoic acid−keyhole limpet hemocyanin. Horseradish peroxidase was conjugated with 4-[3-bromo-4-(2,4-dibromophenoxy)-phenoxy]butyric acid via *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). The triclosan antibody was coupled to magnetic particles via the NHS/EDC reaction. The antibodies were able to recognize some structurally related polybrominated biphenyl ethers but did not recognize various common pollutants that were less similar to the hapten. The ELISA could detect triclosan in standard solution (25% methanol/H_2O v/v) at 20 ppt and its metabolite, methyl-triclosan, at 15 ppt. Water samples from different treatment stages were prepared to contain 25% methanol and analyzed directly without any sample extraction or preconcentration. The results showed that recoveries were >80% and the % CV was <10%, demonstrating the assay was both accurate and precise. Application of the triclosan ELISA to water treatment plants showed that tap water at various purification stages had low concentrations of triclosan (<20 ppt) and required an increased sample size for appropriate detection and measurement. Application of ELISA to the wastewater treatment plants (WWTP) demonstrated high concentrations of triclosan (in general, >3000 ppt in water entering the WWTP) with the levels decreasing as the water proceeded through the processing plant (<500 ppt at outflow sewage). The ELISA measurement was shown to be equivalent to the more specific GC-MS analysis on a number of wastewater treatment samples with a high degree of correlation, with the exception of a few samples with very high triclosan concentrations (>5000 ppt). Measurement of methyl-triclosan (in WWTP) using GC-MS demonstrated the levels of this compound to be low. In summary, a rapid, sensitive, accurate, and precise magnetic particle-based immunoassay has been developed for triclosan analysis, which can serve as a cost-effective monitoring tool for various water samples.

KEYWORDS: ELISA; immunoassay; residue; water monitoring; triclosan

INTRODUCTION

Triclosan [5-chloro-2-(2,4-dichlorophenoxy)phenol] is a broad-spectrum antibacterial often incorporated in personal care products such as soaps, deodorants, toothpastes, and household products such as clothing and trash bags to decrease bacterial contamination. The widespread use of triclosan has raised concerns about the environmental impact of triclosan residues as well as food safety problems caused by its presence in foods. Triclosan has been demonstrated to occur in river water samples in both North America and Europe and is likely widely distributed wherever triclosan-containing products are used. Presumably because of its widespread usage, Koplin found that triclosan is among the most commonly found chemicals in U.S. waterways (*1*). Similar studies in Sweden (*2*) also demonstrated significant contamination of waterways. Although significant amounts are removed in sewage plants, considerable quantities remain in the sewage effluent, initiating widespread environmental contamination. Triclosan undergoes bioconversion to methyl-triclosan (a more lipophilic compound), which has been demonstrated to bioaccumulate in fish (*3*, *4*). In addition, triclosan has been found in human urine samples with a mean of 127 ng/mL from 30 persons with no known industrial exposure (*5*) and was found in significant amounts in three of five samples of mothers' milk (*2*), clearly demonstrating its

* Corresponding author [telephone (701) 239-1425; fax (701) 239-1430; e-mail shelverw@fargo.ars.usda.gov].
† U.S. Department of Agriculture.
§ Abraxis, LLC.

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presence in humans. The action of sunlight in river water has been reported to convert triclosan into dioxin derivatives (*6*) and raises the possibility of pharmacological dangers not envisioned when the compound was originally utilized.

Sophisticated instrumental methods based on various GC-MS or LC-MS technologies are currently utilized to measure triclosan in environmental and food samples (*7*−*9*). However, these methods require sophisticated operating environments and are not amenable to the high-throughput analysis needed for environmental or food safety monitoring work. Immunochemical methods, because of their simplicity, ruggedness, and parallel sample analysis, are eminently suitable as high-throughput methods and retain the high sensitivity and good specificity needed for this type of work. Enzyme immunoassays utilizing magnetic particle technology have been shown to be particularly well suited for the analysis of many environmental contaminants, producing assays that are field portable, user-friendly, and cost-effective (*10*−*12*). Here, we report the development of a triclosan magnetic particle immunoassay. Using this assay, we determined triclosan concentrations in water and wastewater treatment plants in three different cities in the Red River basin. The ELISA was validated by comparison of a number of wastewater samples with GC-MS analysis.

MATERIALS AND METHODS

Materials. *N*-Hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) and other chemicals except as noted below were purchased from Sigma-Aldrich (St. Louis, MO). Supermagnetic particles (1 μm) were obtained from Seradyn (Indianapolis, IN). Triclosan reference standard was obtained from U.S. Pharmacopeia (Rockville, MD). Methyl-triclosan as well as [^{13}C]triclosan and [^{13}C]-methyl-triclosan were obtained from Wellington Laboratories Inc. (Ontario, Canada). The analytical standards for polybrominated diphenyl ethers (PBDEs), bromobiphenyls, triiodothyronine (T3), and thyroxine (T4) were obtained either from Chem Service (West Chester, PA) or AccuStandard (New Haven, CT). Hapten 4-[3-bromo-4-(2,4-dibromophenoxy)phenoxy]butyric acid was a gift from Dr. Qing Li, University of Hawaii; the synthesis method was published previously (*13*). *N*-(*tert*-Butyldimethylsilyl)trifluoroacetimide was obtained from Regis Technologies, Inc. (Morton Grove, IL).

Hapten Synthesis, Tracer and Magnetic Particle Conjugations. To a solution of triclosan (145 mg, 0.5 mmol) in 30 mL of acetone was added ethyl 6-bromohexanoate (123 mg, 0.55 mmol), anhydrous potassium carbonate (83 mg, 0.6 mmol), and potassium iodide (8 mg, 0.05mmol), and the mixture was refluxed for 48 h. After the mixture had been filtered and the acetone evaporated, a yellow oil was obtained (272 mg). Examination of the positive mode electrospray mass spectrum gave a peak of *m*/*z* 431 corresponding to the product, ethyl 6-[5-chloro-2-(2,4-dichlorophenoxy)phenoxy]hexanoate (MW = 430). The residue was dissolved in 6.7 mL of ethanol and 3.3 mL of 1 N sodium hydroxide and refluxed overnight, and the solution was acidified with 2.5 N HCl to pH <2. The product was obtained by extraction with ethyl acetate (10 mL × 5) and removal of the ethyl acetate. The 6-[5-chloro-2-(2,4-dichlorophenoxy)phenoxy]hexanoic acid (triclosan−hexanoic acid) was purified by applying the oil to a preparative silica plate, developing the plate with hexane/dichloromethane (3:1), and extracting with acetone. After evaporation of the solvent, a total of 146 mg of oil was recovered (73% yield, with a positive mode electrospray mass spectra signal at *m*/*z* 403 corresponding to the acid, MW = 402). The triclosan−hexanoic acid (12 mg, 0.03 mmol) was activated by dissolving it in 300 μL of DMF and adding 0.08 mmol of NHS (9 mg) and 0.08 mmol (16 mg) of *N*,*N*′-dicyclohexylcarbodiimide and mixed at room temperature overnight. The precipitated urea was removed, and the supernatant was added dropwise to 20 mg of keyhole limpet hemocyanin (KLH) (in 5 mL of 100 mM borax buffer, pH 9) and reacted at 4 °C overnight. The unconjugated hapten was removed by dialysis against phosphate-buffered saline. The KLH−triclosan−hexanoic acid conjugate was stored at −80 °C until used.

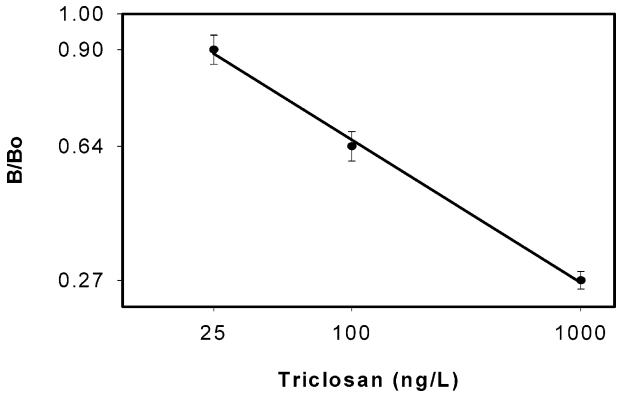


Figure 1. Average of seven calibration curves obtained over a period of 45 days for the ELISA determination of triclosan.

Similarly, 4-[3-bromo-4-(2,4 dibromophenoxy)phenoxy]butyric acid (*13*) was coupled with horseradish peroxidase by a procedure analogous to the production of antigen with the replacement of *N*,*N*′-dicyclohexylcarbodiimide with EDAC. Rabbit anti-triclosan antibodies coupled to the magnetic beads was also accomplished using NHS/EDAC activation in 50 mM 2-(*N*-morpholino)ethanesulfonic acid buffer, pH 5.

Animal Immunization. Four female New Zealand white rabbits were immunized with 200 μg of the KLH−triclosan−hexanoic acid conjugate on days 1, 22, 43, 64, 85, 113, 140, 162, 183, 204, and 225. After the second boost (day 43), blood was obtained from rabbit ears and checked for antibody titer. The seventh boost serum from rabbit 131 was utilized for immunoassay development because of its high sensitivity toward triclosan in comparison to serum from the other rabbits.

Enzyme Immunoassay (ELISA) for Water Samples. Glass vials were used to collect the samples, and methanol was added to a concentration of 25% (v/v) prior to analysis. Sample (250 μL) and suspended antibody-coupled paramagnetic particles (500 μL; 1:30000) were mixed in a glass test tube and incubated at room temperature for 30 min. The HRP−4-[3-bromo-4-(2,4-dibromophenoxy)phenoxy]butyrate (250 μL) was added and incubated for 30 min at room temperature. A magnetic field was applied holding the paramagnetic particles and bound reagents in the tube, and the unbound agents were removed, after which the particles were washed with water. The chromagen, 3,3′,5,5′-tetramethylbenzidine (TMB), and hydrogen peroxide (500 μL) were added to the particles, and the mixture was incubated for 20 min at room temperature. After the reaction had been stopped by the addition of 500 μL of 2% sulfuric acid solution, the absorbance was read at 450 nm within 15 min (Photometric Analyzer, Abraxis, Warminster, PA). The concentration was determined utilizing a regression line constructed from log B/B_0 versus log concentration of standards containing 0, 25, 100, and 1000 ng/L triclosan. **Figure 1** shows the average of seven calibration curves taken over a 45 day period with standard error bars.

Sample Collections. Water samples were collected from three different cities within 15 miles of each other in the Red River basin. In general, four to five samples were obtained at different purification stages at both the water and the wastewater/sewage treatment plants in Fargo and West Fargo in North Dakota and Moorhead in Minnesota. The sewage water processing for Fargo is based on a trickling system, that for Moorhead on an activated sludge system, and that for West Fargo on a lagoon system. The water was centrifuged at 2000*g* for 10 min and the supernatant collected; methanol was added to a final concentration of 25%, and the sample was stored at 4 °C until analysis. For particulate free analysis, the water was first filtered through a 1 μM glass fiber filter, and sufficient MeOH to make a final concentration of 25% was added for immunoassay. For GC-MS analysis the sample was extracted with methylene chloride as described below. For samples collected from drinking water treatment plants, no sample pretreatment was needed for ELISA measurement.

Sample Cleanup for GC-MS Analysis. Wastewater samples (2.5−25 mL, depending on triclosan concentrations) were filtered, and 1 M

citrate−phosphate buffer, pH 3 (2% v/v of the sample), was added along with the internal standard [$^{13}C_{13}$]methyl-triclosan and [$^{13}C_{12}$]-triclosan (100 μL of 4 ng/mL in nonane). The water was extracted once with 10 mL of methylene chloride and once with 5 mL of methylene chloride. Methylene chloride extracts were combined, 100 mg of sodium sulfate was added, and the solution was concentrated by evaporation with a stream of nitrogen using 20 μL of nonane as keeper. The derivatization agent *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetimide (MTBSTFA) (20 μL) was added and reacted at room temperature for 1 h, after which 2 μL of the solution was injected into the GC-MS for analysis.

GC-MS Analysis. A Hewlett-Packard 5890 gas chromatograph (GC) with an AutoSpec mass spectrometer (MS) (Waters Corp., Milford, MA) was used for GC-MS analysis. The GC utilized helium as a carrier gas. The temperature program consisted of an initial hold at 100 °C for 1 min then ramped at 10 °C/min until 310 °C was reached; total GC analysis time was 22 min. The GC column was a 30 m DB5 ms column (Agilent Technologies, Inc., Palo Alto, CA) with 0.25 mm i.d. and a 0.25 μm film thickness. A deactivated precolumn of 1 m × 0.53 mm i.d. was employed. The injector program was cool on column with oven tracking injection temperature. The GC-MS interface and ion source were set at 275 °C. The mass spectrometer was run in a positive ion electron impact mode with the ionization voltage set at 40 eV, resolution at 7500, and trap current at 500 μA. The MTBSTFA derivatized triclosan fragment [M − 57(C_4H_9)]$^+$ and $^{13}C_{12}$-labeled internal standard (retention time = 15:34 min) were monitored with selected ion reaction (SIR) with mass to charge (*m*/*z*) of 344.97/346.96 and 357.01/359.00 and methyl-triclosan was monitored with *m*/*z* of 301.97/303.96 (m/m + 2) parent and 314.01/316.00 $^{13}C_{13}$-labeled internal standard (retention time = 13:44 min). An isotope dilution method using calibration curves of 0.1, 0.5, 1, 5, 10, 50, and 100 ng/mL with 10 ng/mL of ^{13}C-labeled internal standards was used, and the unknown concentration was computed from the standard curve. A least-squares equation was used for the calibration with typical correlation $r^2 > 0.9996$. The level of detection limit was calculated using Opus (version B3.7/1X) Quan (version 3.6) software program (Waters Corp.) and was 0.2−4.0 pg on column for triclosan and 0.4−8.4 pg) on column for methyl-triclosan depending on stages of water processing, the operating parameters of the instrument, particularly the column, and other factors.

RESULTS AND DISCUSSION

Antigen and Antibody Generation. The hapten synthesis and the conjugation of carrier proteins were performed without difficulty. The target compound was confirmed by detection of the correct molecular ion using LC-MS. The success of the carrier protein conjugation was confirmed by SDS-PAGE, in which the protein−hapten conjugate has a higher molecular weight in comparison to the non-hapten-conjugated protein. As the number of immunizations increased, triclosan, PBDE-28, and PBDE-47 showed a progressive decrease in 50% inhibition concentration (IC_{50}) (**Figure 2**), indicating an increase in sensitivity for these compounds, but the IC_{50} of methyl-triclosan showed little change. Because the decreases in the IC_{50} from the fourth bleed to the ninth bleed for triclosan (64%), PBDE-28 (58%), and PBDE-47 (61%) were similar, the latter two compounds showed very little change in cross-reactivity. Because the IC_{50} of triclosan decreases, whereas the IC_{50} of methyl-triclosan remains essentially constant, the cross-reactivity of methyl-triclosan decreased from 307 to 129% from the fourth bleed to the ninth bleed.

Precision and Recovery. Four different types of water samples (creek, pond, well, and municipal from the Warminster, PA, area) were spiked with different levels of triclosan and, after the addition of methanol to a concentration of 25% (v/v), were assayed using the ELISA procedure described above (**Table 1**). In the ELISA, for all spiked levels from 62.5 to 500 ng/L the recoveries were between 83 and 113% with coefficients

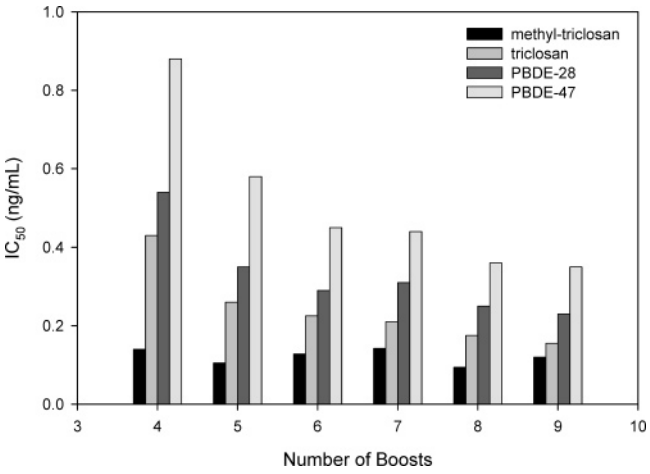


Figure 2. Change in IC_{50} of various substrates with an increased number of booster immunizations.

Table 1. Recovery and Precision of Triclosan and/or Methyl-triclosan from Spiked Water [(ELISA) Municipal Water, Well Water, Pond Water, and Creek Water; (GC-MS) Tap Water]

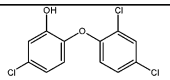
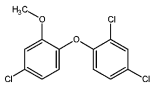
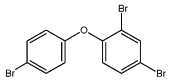
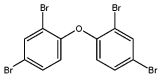
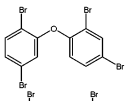
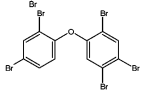
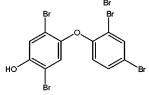
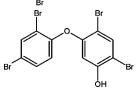
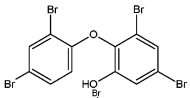
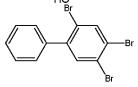
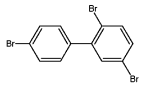
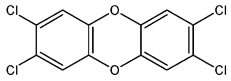
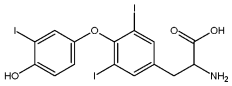
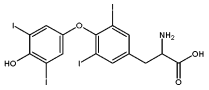
spike (ng/L)	*n*	found	% recovery	% CV
		ELISA (Triclosan)		
62.5	4	52.2 ± 4.8	83	9.2
125	4	109.1 ± 10.8	87	9.9
250	4	252.2 ± 9.1	101	3.6
500	4	565.6 ± 28.4	113	5.0
		GC-MS (Methyl-triclosan)		
100	3	96.4 ± 0.7	96	0.7
		GC-MS (Triclosan)		
100	3	131.4 ± 1.7	131	1.3

of variance (CVs) <10%, indicating the assay is both accurate and precise. In addition, the ELISA showed good within-day and between-day variation; with five replicates and assays in five different days, the within-day assay was <8 % CV for all three spiked levels (recovery means of 40, 124, and 519 ppt), and the between-day assay was <12% CV (data not shown). **Table 1** also showed GC-MS recovery and precision for tap water spiked at 100 ng/L for methyl-triclosan and triclosan. The GC-MS showed good precision, and between-day analyses were typically <5% CV (data not shown). The recovery of spiked triclosan is >125%, indicating a trace level of triclosan exists in tap water. This is not the case for methyl-triclosan, however, which has recovery close to the spiked level.

Sensitivity and Specificity for ELISA. The sensitivity can be expressed as the limit of detection (LOD) using either 90% B/B_0 or the mean of 20 replicate blanks plus 3 standard deviations. The LODs determined by the 20 replicate blank sample method from deionized water, tap water, lake water, and river water were 13.7, 12.9, 14.3, and 13.7 ppt, respectively (data not shown). An analysis of variance (ANOVA) demonstrated no statistically significant differences related to the type of water. The mean LOD using 90% B/B_0 of the standard curves for these sets of samples was 12.0 ppt, demonstrating either method was acceptable.

The cross-reactivity is computed from 100 times the ratio of the midpoint of the competition curve (50% B/B_0) of standard, in this case triclosan, to the corresponding value of the compound. The smaller the value of either the limit of detection or midpoint, the better the antibody binds to the compound. When a compound's LOD is far larger than that of triclosan, the compound will show little or no interference with the assay

Table 2. Sensitivity and Cross-Reactivity of Triclosan Antibody

Compound	Structure	90% B/B_0 (ng/mL)	50% B/B_0 (ng/mL)
Triclosan		0.020	0.25
Methyl-Triclosan		0.015	0.08
PBDE-28		0.034	0.61
PBDE-47		0.020	0.39
PBDE-49		5.2	17.8
PBDE-99		2.15	15.0
4'-OH-BDE-49		0.13	7.8
5-OH-BDE-47		0.15	5.6
6-OH-BDE-47		0.66	10.2
2,4,5-tribromobiphenyl		> 100	>100
2,4',5-tribromobiphenyl		54	9,100
2,3,7,8-TCDD		> 100	>100
T3		0.94	40
T4		340	700

based on this antibody. The high sensitivity to methyl-triclosan is expected because the hapten resembles methyl-triclosan more closely than triclosan.

The antibody recognized PBDE-28 and PBDE-47 containing bromine in the positions corresponding to chlorine (or the hydroxyl) in triclosan, with only a slight decrease in sensitivity (**Table 2**). We have previously demonstrated that analytes containing bromine in a position corresponding to an ether linkage of the hapten are well recognized by the antibody (*11*), so PDBE-47's low 50% B/B_0 is expected. Although bromine is larger than chlorine, the antibody recognition is only slightly impaired with 50% B/B_0 still well below 1 ppb. However, the addition of an extra bromine in a position corresponding to a hydrogen in triclosan dramatically decreased recognition as seen in the BDE-49 and BDE-99, where the 50% B/B_0 is >15 ppb. The hydroxyl-substituted BDEs show slightly better recognition, indicating the hydroxyl group may be slightly better accommodated than bromine, with these compounds showing a 50% B/B_0 of between 5 and 10 ppb depending on the placement of the hydroxyl group. Other compounds including some brominated biphenyls, 2,3,7,8-tetrechlorodibenzo-*p*-dioxin (TCDD), T3, and T4 are essentially not recognized by the antibody.

Triclosan Levels (Nanograms per Liter) from Water Treatment Plants. The tap water samples and water at various purification stages for drinking water were below or close to the limit of detection (20 ng/L) of triclosan using ELISA. The cities draw their water from different sources; Moorhead obtains water from the Red River and wells, West Fargo relies totally upon wells, and Fargo uses water from the Red River. Despite the variety of water sources, triclosan was present in quite low concentrations, demonstrating little contamination in any of the sources. This might be expected because this locality is not downstream or near any large urban population center. A concentration step would be necessary to precisely measure triclosan levels in drinking water using the ELISA, but this approach would negate the rapid and convenient ELISA procedure and be of little practical value.

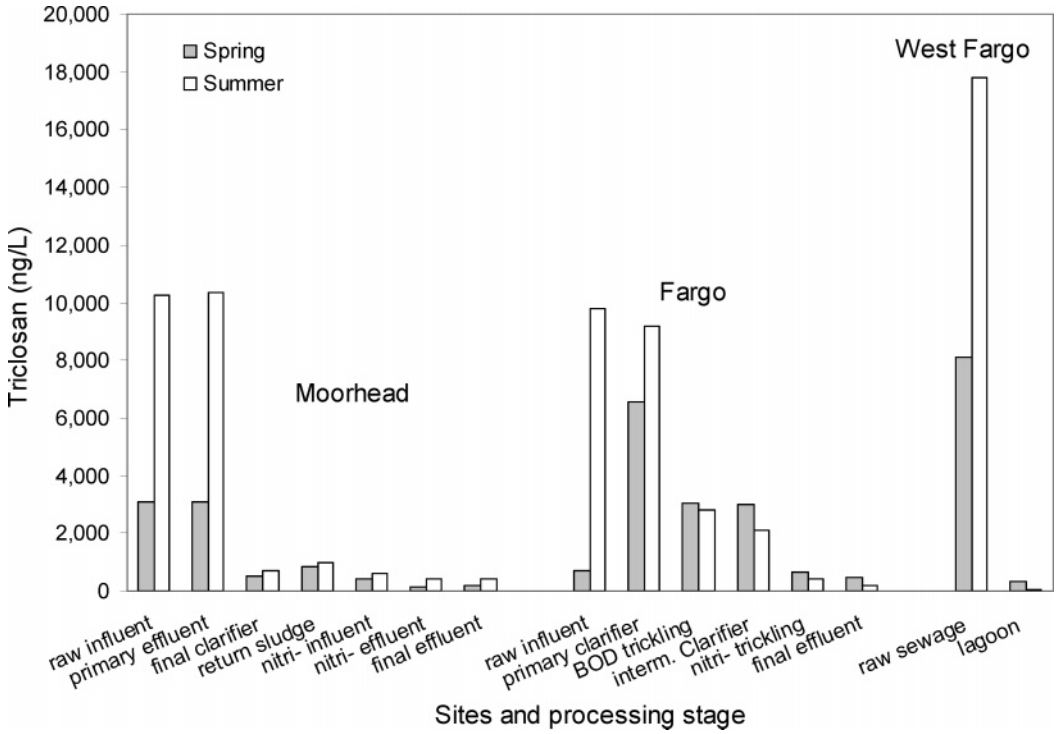


Figure 3. Triclosan levels in different purification stages originating from three different collection sites (Moorhead, activated sludge; Fargo, trickling; West Fargo, lagoons) in spring (gray bars) and summer (white bars) 2006, using ELISA measurement.

Table 3. Levels of Triclosan (Nanograms per Liter) between Filtered and Nonfiltered Samples from ELISA Measurements[a]

Fargo				Moorhead			
description	unfiltered	filtered	% total	description	unfiltered	filtered	% total
raw influent	9770	2145	22	raw influent	10300	6057	59
primary clarifier effluent	9163	3373	37	primary effluent	10300	7491	73
BOD trickling filter effluent	2797	990	35	return sludge	973	438	45
intermediate clarifier effluent	2123	1234	58	final clarifier	701	570	81
North ammonia trickling filter	410	271	66	nitrification influent	617	498	81
final effluent	131	205	156	nitrification effluent	401	381	95
				final effluent	410	371	90
West Fargo							
raw sewage	17800	13869	78				
lagoon 6	67	55	82				

[a] Samples were collected in the summer of 2006.

Triclosan Levels (Nanograms per Liter) from Wastewater Treatment Plants. Figure 3 shows the levels of triclosan from three different wastewater treatment plants utilizing three different water purification systems; trickling (Fargo), activated sludge (Moorhead), and lagoons (West Fargo), for two sampling periods. As can be seen in the figure, all of the processes remove substantial amounts of triclosan, but none of the three processes can completely remove triclosan. There are still detectable levels of triclosan in waters discharged back to the environment. The raw influent ranged from 3000 to 14000 ng/L, whereas the effluent ranged from 161 to 462 ng/L. These results are somewhat higher than those reported in the literature for raw influent, 500−1300 ng/L (Switzerland; *14*) and 1100−1300 ng/L (Germany; *15*), but similar to those reported for effluent, from 42−213 ng/L (Switzerland; *16*) to 70−650 ng/L (Switzerland; *14*) or 240−410 ng/L (Ohio; *17*). The summer sample showed much higher levels in the raw sewage and first processing step, but levels were reduced in later processing steps, indicating the capacity to reduce triclosan was not compromised by the increased input. The two sets of data demonstrated the ELISA performed well, giving consistent results.

The level of triclosan in raw influent is difficult to measure. Much of the triclosan is absorbed on the sediment that is normally removed by filtration or centrifugation prior to analysis. A comparison of the results of samples with and without filtering (**Table 3**) clearly demonstrates that up to 80% of the triclosan may be in the particulates. At most of the processing stages, filtering greatly reduced the amount of triclosan in the samples, although this effect decreased throughout the processing sequence, at the end of which filtering the samples showed no effect. The removal of triclosan was similar to other reported data, although our data show somewhat better removal from the plant utilizing trickling than other studies have reported for this process. As others have observed, the activated sludge system showed the highest ratio of removal of the systems. Because the ratio depends on the measurement of triclosan in raw influx, sample processing (i.e., removal of particulate with bound triclosan), can markedly influence these ratios and could be misleading. Ultimately, the amount of triclosan in the effluent discharged into the environment is of greater concern, because this produces environmental contamination.

Triclosan versus Methyl-triclosan in Wastewater Samples. As can be seen from **Table 4**, the levels of methyl-triclosan are low in the wastewater during all purification stages, ranging from 2 to 30 ng/L, which is in general agreement with the values

Table 4. Comparison of Triclosan and Methyl-triclosan Levels (Nanograms per Liter) in Wastewater Measured by GC-MS and ELISA[a]

	GC-MS		ELISA[b]
description	triclosan	methyl-triclosan	total
Fargo			
raw influent	2556	2.7	2145
primary clarifier effluent	3097	7.0	3373
BOD trickling filter effluent	734	13.5	990
intermediate clarifier effluent	727	27.1	1234
North ammonia trickling filter	168	21.7	271
final effluent	215	15.3	205
Moorhead			
raw influent	2212	3.0	6057
primary effluent	2752	4.1	7491
return activated sludge	358	5.3	438
final clarifier	297	5.6	570
nitrification influent	308	6.2	498
nitrification effluent	235	7.7	387
final effluent	192	13.0	371
West Fargo			
raw sewage	4119	5.8	13869
lagoon 6	70.9	nd[c]	55.4

[a] Samples were obtained in the summer of 2006. [b] Mean of duplicate measurements. [c] Not detected.

reported by others (*14*). Furthermore, unlike triclosan, the methyl derivative does not markedly decrease as processing progresses, thereby increasing as a percent of triclosan, although this exceeds 10% for only one sample.

Comparison of MS and ELISA Analysis. With the exception of three points at high triclosan concentrations (>5000 ng/L; ELISA) the correlation between the ELISA results and the GC-MS results is quite good, with a high r^2 (0.92) and a regression coefficient near 1.00, indicating correspondence between the methods. This confirms that methyl-triclosan does not make an appreciable contribution to the triclosan water levels determined by the ELISA technique. The discrepancies of the three points could be due to fine particulates, coloring, or high dilution factors for ELISA, and all three samples are in earlier wastewater treatment stages.

In conclusion, this ELISA gave reasonably accurate and precise results from water samples. The cross-reactivity with methyl-triclosan or with certain substituted PBDEs did not seem to present a problem in the application of the analysis. Comparison of GC-MS with ELISA demonstrates that the immunoassay would be extremely useful in detecting triclosan in water samples in mass screening or routine monitoring of water processing.

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