Side-by-Side Comparison of Three Sampling Methods for Aerosolized Endotoxin in a Wastewater Treatment Facility

Dale J. Stephenson, Ph.D., C.I.H.
Dean R. Lillquist, Ph.D.
Frank D. DeRosso, M.S.P.H.
Daniel D. Greene, M.S.P.H.
George White, Ph.D.

Abstract

Research studies have established the occurrence of adverse health effects in individuals exposed to organic dusts and water aerosols laden with endotoxin. To determine what exposure levels cause these health effects, it is necessary to quantify airborne endotoxin. Several scientific studies have demonstrated that the quantification of detectable endotoxin is affected by differences in sampling media, analytical method, and aerosol composition. The study reported here performed side-by-side endotoxin sampling using a liquid impinger, a glass fiber filter, and a polycarbonate filter in a wastewater treatment plant. Results show levels of detected endotoxin appear to be highest with the impinger. Coefficients of variation calculated for each sampling method show the glass fiber filter having the least variability when sampling was conducted at the highest endotoxin levels. Lastly, a Spearman rank order correlation test identified an apparent correlation between endotoxin levels obtained with the impinger and the glass fiber filter.

Introduction

Endotoxin is a structural component found in the outer cell wall of Gram-negative bacteria. Various human cells have been found to respond to endotoxin. It appears, however, that the most important physiologic reactions are those that occur in cells of the immune system (Ulmer, 1997). Some of the biologic responses associated with cellular reactions to endotoxin include fever induction, macrophage activation, B-cell mitogenicity, restrictive lung function changes, and reduction in alveolocapillary gas transfer (Cotran, Kumar, & Collins, 1999; Rylander, 1997).

Previous research studies have documented human exposure to endotoxin-containing dusts and aerosols. Several occupational studies have associated the presence of adverse health effects among agricultural workers exposed to organic dusts laden with endotoxin (Clark, Rylander, & Larsson, 1983; Kennedy et al., 1987; Rylander, Haglind, & Lundholm, 1985; Smid et al., 1994). Other studies have suggested that endotoxin-contaminated water aerosols, such as those generated during sewage treatment or atmospheric humidification, may be the source of upper respiratory and gastrointestinal disorders observed in an exposed population (Khuder, Arthur, Bisesi, & Schaub, 1988; Laitinen et al., 1994; Rylander, 1999; Milton, 1996; Teeuw, Vandenbroucke-Grauls, & Verhoef, 1994).

Accurate quantification of airborne endotoxin is necessary for correlation of endotoxin levels with observed health effects. In addition, a standardized endotoxin sampling method is needed to appropriately establish acceptable versus unacceptable exposure levels. The results of past research have shown this to be a difficult task. Several studies have demonstrated that the amount of endotoxin detected when different sampling methods are employed is highly variable and can be affected by factors such as sampling media (filter types), analytical methods, and aerosol composition (Douwes et al., 1995; Gordon, Galanes, & Brosseau, 1992; Thorne et al., 1997). Further, Milton and co-authors and Thorne and co-authors have suggested that endotoxin binds with varying degrees to different filter materials, enhancing the variability in endotoxin recovery during analytical extraction (Milton, Gere, Feldman, & Greaves, 1990; Thorne et al., 1997). Given these findings, this study was undertaken to provide data that will help in establishing a standardized sampling and analytical method that can be used to accurately estimate human exposure to aerosolized endotoxin.

The purpose of this study is to compare three different sampling methods for the collection of aerosolized endotoxin. Different collection media are employed in each method and consist of a liquid impinger, a glass fiber (GF) filter, and a polycarbonate (PC) filter. The GF and PC filter sampling methods were selected as comparative media since they have been shown to give consistent

TABLE 1

Endotoxin Results for Each Sampling Method

Sampling Method	Run 1				Run 2			
	Range (EUs/m3)	Mean (EUs/m3)	SD	CV	Range (EUs/m3)	Mean (EUs/m3)	SD	CV
Impinger	115–1700	472	691	1.46	8–46	29	13.6	0.47
Glass fiber filter	42–81	60	17.6	0.29	4–29	14	9.6	0.68
Polycarbonate filter	4–34	16	15.8	0.99	1–6	3	2.17	0.72

TABLE 2

Results of the Spearman Rank Order Correlation Test

Method	Impinger	GF Filter	PC Filter
Impinger (n = 10)	1.00	0.927[a]	0.552
Glass fiber filter (n = 10)	0.927[a]	1.00	0.539
Polycarbonate filter (n = 10)	0.552	0.539	1.00

[a]Significant at the .01 level (two-tailed).

results in sampling for endotoxin-containing aerosols (Gordon, Galanes, & Brosseau, 1992). The liquid impinger sampling method was selected because it does not involve an extraction step during laboratory analysis, which allows the method to be used as a control against the variability seen during analysis when filter collection media are used in endotoxin recovery.

Materials and Methods

Endotoxin samples were collected outdoors, near the base of a 30-inch-high spillway in the primary clarifier of a sewage water treatment facility. This sampling site was selected because it is an environment in which endotoxin concentrations are likely to be relatively high. The impinger sampling train consisted of an SKC® 25-milliliter (25-mL) midget impinger with a standard nozzle, attached to a Gilian® GilAir® personal sampling pump calibrated at a flow rate of 1.60–1.64 liters per minute (L/min). For the collection of endotoxin, each impinger contained 20 mL of pyrogen-free water. Prior to sampling, all impingers were dry-heat sterilized for one hour at 250°C. The sampling trains for the filter sampling methods consisted of either a GF filter (1.0-micron [1.0-µm] pore size, binder-free) or a PC filter (0.4-µm pore size) housed in an SKC 37-millimeter (37-mm) polystyrene filter cassette holder, attached to a Gilian GilAir personal sampling pump calibrated at a flow rate of 2.06–2.10 L/min. Because of the potential for contamination from atomized water in the proximity of the spillway, all filter sampling was performed closed-faced.

Side-by-side endotoxin sampling was performed by positioning a liquid impinger, a PC filter, and a GF filter sampling train (as a triplicate set) 3 inches apart with their inlets 15 inches above the bottom of the spillway, 12 inches from the wall of the falling water, and at 45 degrees relative to the surface of the water. Two sampling runs were performed, each 30 minutes in duration and each consisting of five triplicate sets that collected endotoxin at different locations along the base of the primary clarifier spillway. The two sampling runs resulted in the acquisition of 10 samples for each endotoxin collection method. A time period of three hours elapsed between the two sampling runs. At the time of the sampling, the outdoor air temperature was approximately 24°C and the relative humidity was approximately 35 percent.

The authors extracted endotoxin from the GF and PC filters by placing each filter in a vial containing 5 mL of sterile, pyrogen-free water (at 37–40°C) for 60 minutes and sonicating each vial at 10-minute intervals. The endotoxin in all samples was quantified with the Kinetic Chromogenic Limulus Amebocyte Lysate (LAL) Assay (Nelson Laboratories, Salt Lake City, Utah). This assay places 0.1 mL of either the liquid impinger solution or the filter extract in a microplate along with 0.1 mL of LAL reagent. The mixture is incubated for one hour in a spectrophotometer at 37°C. Spectrophotometer results are compared to control results using standard endotoxin dilutions, and detected endotoxin levels are reported in endotoxin units (EUs). In an effort to determine if interfering materials were present on the filter media or in the impinger solution, tests for enhancement or inhibition were performed as a part of the LAL analytical procedure. The results of these tests showed that the percentage recovery of endotoxin from spiked samples was normal (between 50 and 200 percent), suggesting that no enhancement or inhibition of biological activity had taken place. Blank filters and impinger solutions were analyzed for contamination with endotoxin during each assay and the amount of endotoxin on the blank media was subtracted from associated samples.

Results

The results given in Table 1 show that for both runs the mean amount of endotoxin detected in the impinger samples was two to three times greater than the amounts obtained with GF filters and approximately 10 times greater than the amounts from samples obtained with PC filters.

In an effort to determine the degree of variation for a given sampling method during each run, the method's standard deviation was used to estimate its coefficient of variation (CV). Table 1 shows the relative differences among the estimated CVs. For Run 1, the GF filter method provided a lower CV than the PC filter and impinger methods (0.29, compared with 0.99 and 1.46, respec-

tively). For Run 2, the difference between the CVs was not as pronounced. In Run 2 the impinger method had the lowest CV (0.47), compared with that for the GF and PC filter methods, which had similar CVs (0.68 and 0.72, respectively).

In an effort to determine the presence of a correlation among the results of the three methods, a Spearman rank order correlation test was performed on the data for Run 1 and Run 2 combined. The results of this statistical test are provided in Table 2 and show an apparent correlation between the sampling results obtained with the impinger method and those obtained with the GF filter method.

This non-parametric analysis was performed instead of a Pearson correlation coefficient test because of the small sample size used in this study.

Discussion

The results given in Table 1 show a large decrease between Run 1 and Run 2 in the levels of endotoxin detected with each sampling method. One possible explanation for this observation is that a rainstorm produced approximately ¾ inch of rain during the three hours that elapsed between the runs. It may be that the decrease in the collection of airborne endotoxin was caused by a dilution of the wastewater in the primary clarifier, or it is possible that the rain physically removed endotoxin-contaminated aerosols from the air. The data given in Table 1 also show that the highest endotoxin levels were obtained with the impinger sampling method. This result was expected because the method does not involve an extraction step during laboratory analysis, most likely enhancing the recovery of endotoxin during the analytical procedure. It should be noted, however, that the use of impingers does have limitations. Impinger samplers are often considered inconvenient for use as field-sampling devices. If the impinger is tipped during sampling, the sampling solution inside the impinger may spill, resulting in inaccurate results, or the solution may become contaminated by physical contact with the outside environment. An additional source of contamination can be the required transfer of impinger solutions before and after sampling.

In addition to facility of use in the field, another important aspect in the choice of an endotoxin sampling device is good precision, or high reproducibility of results. Most studies comparing endotoxin aerosol sampling methods have not evaluated the precision of different methods. The CVs estimated in this study provide a comparative measure of precision for each sampling method. Evaluation of the CVs suggests that when sampling takes place during conditions of relatively high levels of endotoxin (Run 1), the GF sampling method provides better precision. In contrast, while sampling is conducted during conditions of relatively low endotoxin levels (Run 2), a smaller difference among the CVs for each method was observed.

Because of the small sample size in this study, the statistically significant correlation found between impinger and GF filter results does not conclusively suggest that endotoxin levels obtained with a glass fiber filter can be directly correlated to those obtained with an impinger. This result does, however, provide the basis for further investigation, which could clarify the appropriateness of applying a correction factor to the results obtained by use of either method, especially when these methods are used to associate sampling results with human health effects.

Conclusions

As with previous research performed to compare the results of different sampling methods for the collection of endotoxin-laden aerosols, the study reported here detected different endotoxin levels when different sampling methods were used. The results show that endotoxin levels were consistently highest with the impinger sampling method, corroborating the theory that the recovery of endotoxin from sampling media is enhanced if extraction is not an element of the analytical procedure. Nevertheless, the fact that the impinger sampling method carries an increased risk of sampling error when used in a field setting, combined with the reproducible results produced by the GF filter sampling method in Run 1, means that this research lends some support to the conclusion by Wood and Jacobs (1997) that GF filters should be designated as the standard method for sampling endotoxin-contaminated aqueous aerosols.

Another important finding of the study reported here is the apparent correlation between the results obtained with the GF and impinger sampling methods. While the limited sample size does not allow for direct conclusions to be drawn from this observation, the finding does validate the need for larger studies to more accurately identify the true variability among these sampling methods and to determine the appropriateness of employing a correction factor when the results are compared.

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Corresponding Author: Dale J. Stephenson, Boise State University, College of Health Sciences, Department of Health Studies, Environmental Health Program, 1910 University Drive, Boise, Idaho 83725-1835. E-mail: dalestephenson@boisestate.edu.

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