

Comparison of Endotoxin Exposure Assessment by Bioaerosol Impinger and Filter-Sampling Methods

CAROLINE DUCHAINE,¹ PETER S. THORNE,^{2*} ANNE MÉRIAUX,¹ YAN GRIMARD,¹
PAUL WHITTEN,² AND YVON CORMIER¹

Centre de Recherche, l'Hôpital Laval, Sainte-Foy, Québec, Canada,¹ and Department of Occupational and Environmental Health, University of Iowa College of Public Health, Iowa City, Iowa²

Received 21 December 2000/Accepted 18 March 2001

Environmental assessment data collected in two prior occupational hygiene studies of swine barns and sawmills allowed the comparison of concurrent, triplicate, side-by-side endotoxin measurements using air sampling filters and bioaerosol impingers. Endotoxin concentrations in impinger solutions and filter eluates were assayed using the *Limulus* amoebocyte lysate assay. In sawmills, impinger sampling yielded significantly higher endotoxin concentration measurements and lower variances than filter sampling with IOM inhalable dust samplers. Analysis of variance for repeated measures showed that this association remained after controlling for other factors such as replicate, sawmill, sawmill operation, wood type, and interaction terms. Endotoxin concentrations in the swine barns were 10-fold higher on average than in sawmills. These samples demonstrated comparable endotoxin concentration estimates for impinger and filter methods although the variability was lower using the impinger method. In both occupational settings, side-by-side replicates were more uniform for the impinger samples than for the filter samples. This study demonstrates that impinger sampling is an acceptable method for quantitation of area endotoxin concentrations. Further, when sampling is performed with impingers for airborne microorganism quantitation, these same impinger solutions can yield valid endotoxin exposure estimates, negating the need for additional filter sampling.

Gram-negative bacteria are found as normal microflora of soils, water, and living organisms. Endotoxins are a major cell wall component of gram-negative bacteria and are ubiquitous in the outdoor and indoor environments. Endotoxins are lipopolysaccharide molecules that contain a lipid region and a long-chain polysaccharide moiety. The lipid region (lipid A) exhibits little variation across genera and imparts the toxicity to endotoxin. The polysaccharide component aids in conformational changes facilitating molecular interaction with cellular receptors. Inhaled endotoxin is recognized as a potent inducer of airway inflammation. Animal inhalation toxicology studies, human exposure studies, and epidemiologic investigations in occupational environments have shown that exposure to endotoxin is associated with pulmonary symptoms, airway bronchoconstriction, recruitment of neutrophils to the airways, and increased release of proinflammatory cytokines including tumor necrosis factor alpha, interleukin-6, and macrophage inflammatory protein 2 (22). There is recent evidence that chronic exposure to endotoxin-containing organic dust is associated with airway remodeling (6). Despite a clear recognition that inhaled endotoxin is an occupational hazard in agricultural settings (8, 12, 19, 20), cotton processing (11), vegetable processing (10, 31), fiberglass manufacturing (16), and metal machining environments (23, 24), there are no established occupational exposure limits in the United States or Canada. This is largely due to the fact that endotoxin exposure assessment methods have not been adequately optimized and validated. Several studies have been conducted in an attempt to

optimize the choice of sampling filter type, filter extraction methods, extraction buffers, and choice of glassware (2, 7, 15, 18, 28). However, no generally accepted protocol has emerged. While there have been studies that address the extraction and analysis of endotoxins from filter samples, there are few studies that have employed impinger sampling for endotoxin exposure assessment, and apparently no studies have systematically compared impinger sampling with filter sampling for measurement of airborne endotoxin.

Impingers such as the All-Glass Impinger-30 (AGI) and the BioSampler were designed specifically for the collection of bacteria and have been demonstrated to be effective and versatile devices for bioaerosol sampling in the laboratory (1, 21, 30) and in the field (12, 13, 17, 26, 27). Bioaerosol impingers collect microorganisms by inducing airborne particles to collide with the agitated surface of the collection fluid. The AGI directs the airstream downward through a single jet forming a vigorous rolling of the fluid, while the BioSampler has three jets that establish a swirling motion of the collection fluid. In both devices, bioaerosol-laden dust is collected from the air into the impinger solution, which is then available for analysis of culturable organisms on various media, of nonculturable organisms by direct count or flow cytometry methods, and of endotoxin by the *Limulus* amoebocyte lysate (LAL) assay or by other methods. Bioaerosol sampling with impingers can be conducted as a short-term area sample of typically 10- to 20-min duration. It can also be performed as a longer, partial-shift sample by combining collection media from serial samples in the same location (12). Thus far, bioaerosol impingers have not proved convenient for personal sampling.

In this study, concentrations of airborne endotoxin in seven swine barns and at multiple locations in eight sawmills were compared using three side-by-side AGI samples and three

* Corresponding author. Mailing address: University of Iowa College of Public Health, Department of Occupational and Environmental Health, Iowa City, IA 52242-5000. Phone: (319) 335-4216. Fax: (319) 335-4006. E-mail: peter-thorne@uiowa.edu.

TABLE 1. Sampling and endotoxin extraction methods for sawmills and swine barns

Type of sampling and location	Sampling method	Flow rate (liters/min)	Mean vol of air sampled (liters)	Extraction ^a	
				Container	Method
Impinger sampling of sawmills and swine barns	AGI, 20.0 ml of pyrogen-free saline	12.5	200	Polypropylene tube	Vortex mixer, 10 min, 20°C
Filter sampling Sawmills	0.8- μ m-pore-size PVC ^b filter, IOM inhalable dust cassette	2.0	862 \pm 158	Polypropylene tube	Sonication bath, 1 h, 20°C
Swine barns	0.8- μ m pore-size PVC filter, closed-face cassette	1.5	361 \pm 3	Borosilicate glass jar	Shaking bath, 16 h, 37°C

^a The extraction solution was pyrogen-free water with 0.04% Tween 80 in each case.

^b PVC, polyvinyl chloride.

side-by-side filter samples. Each sample was analyzed in duplicate using the LAL assay. The basic null hypothesis was that there is no difference in the estimate of endotoxin exposure in either swine barns or sawmills when sampled by liquid impingement or by air-sampling filters. To our knowledge no prior study has addressed this hypothesis. These data allowed computation of the reliability of the duplicate determinations in the LAL assay of the same specimens and the precision of the replicate samples.

MATERIALS AND METHODS

Study design. This study comparing airborne endotoxin measurement methods was nested within two larger exposure assessment studies (3, 5). Both studies included triplicate impinger and filter sampling. All sampling was performed at a height of 1 m with samplers set on a stationary sampling platform. In the first study, seven conventional swine confinement buildings were sampled between June and August in 1997. In the second study, 17 sawmills containing from two to four different work sites (debarking, sawing, planing, and sorting) were sampled between May and November in 1996 and 1997. A total of 51 sites were sampled in the 17 sawmills; however, not all of these were performed in triplicate with both sampling methods. Complete triplicate data with both samplers were available for 18 sites at eight different sawmills and all seven swine barns. Duplicate or triplicate data for either sampler were available for 22 sawmill sites. Endotoxin assays were performed similarly for filter eluates and impinger solutions in both studies as described below.

Exposure evaluation. (i) Impinger sampling. Swine barns and sawmills were sampled with AGI (Ace Glass Inc., Vineland, N.J.) and Gilian Aircon II pumps (Sensidyne, Clearwater, Fla.) operating at a flow rate of 12.5 liters/min for 16 min to yield a sample volume of 200 liters (Table 1). In the swine barns, impinger samples were taken sequentially, while in sawmills impinger samples were taken simultaneously within the filter-sampling period. Pump flow rates were calibrated using a Kurz flow meter (Instruments Inc., Carmel Valley, Calif.). Sterile AGIs were loaded with 20.0 ml of sterile, pyrogen-free saline prior to sampling and were kept on ice after sampling until returned to the laboratory. The solution volumes were measured to evaluate evaporative loss and were brought to 30 ml by the addition of sterile, pyrogen-free saline containing 0.1% Tween 80. This yielded a final concentration of about 0.04% Tween 80. AGI solutions were frozen to await endotoxin measurement. Prior to endotoxin assay, AGI solutions were thawed on ice and vortexed vigorously for 10 min.

(ii) Filter sampling. The methods used for particulate sampling and endotoxin extraction from filters are shown in Table 1. Briefly, 4-h sampling in swine barns was performed using preweighed 37-mm-diameter, 0.8- μ m-pore-size polyvinyl chloride (PVC) filters in closed-face cassettes with SKC 224-44XR personal sample pumps (Dur-Pro, Brossard, Québec, Canada) calibrated at 1.5 liters/min with a Kurz flow meter as previously described (3). Filter sampling in the sawmills was performed for 6 h using preweighed 25-mm-diameter, 0.8- μ m-pore-size PVC filters in IOM inhalable dust cassettes (SKC, Eighty Four, Pa.) with air drawn by SKC 224-44XR personal sample pumps operated at 2.0 liters/min as previously described (5). In both swine barns and sawmills, control filters were brought to the sampling site but not subjected to sampling and were handled and stored in accordance with the same procedure as that for sampled filters. Swine barn filters were then extracted in sterile 60-ml borosilicate glass jars (Fisher Scientific, Montreal, Québec, Canada) in 30 ml of pyrogen-free water containing

0.04% Tween 80 in a shaking bath at 37°C overnight. Sawmill filters were extracted in conical polypropylene tubes in a sonication bath for 1 h. Filter extraction solutions were vortexed vigorously prior to drawing the sample for endotoxin analysis.

Endotoxin measurements. Endotoxin measurements of the extraction solutions were performed in duplicate using the end point chromogenic LAL assay (Associates of Cape Cod, Woods Hole, Mass.) as previously described (3, 5). Briefly, AGI and filter extraction solutions were diluted and an inhibition/enhancement test was performed prior to measurement. Blank filters were extracted for filter controls. Controls for AGI were obtained by washing sterile AGI with sterile, pyrogen-free saline containing 0.04% Tween 80 for several minutes. Control values were subtracted from the sample values.

Statistical methods. All statistical analyses were performed using SAS, version 6.12 (SAS Institute, Cary, N.C.), or BMDP, version 7.0 (BMDP Statistical Software, Los Angeles, Calif.). SAS programs used included PROC FREQ and PROC UNIVARIATE, while BMDP programs included BMDP2V (analysis of variance and covariance with repeated measures) and BMDP5V (unbalanced repeated-measure models with structured covariance matrices). Gravimetric and endotoxin data were plotted, tested, and found to be log-normally distributed. Therefore, all subsequent analyses were performed using the logarithmically transformed data. Geometric means (GM) and geometric standard deviations (GSD) were calculated from Excel databases. In all analyses, *P* values were considered significant at values below 0.05.

RESULTS

Endotoxin concentration GM and GSD are provided in Table 2 for sawmills and swine buildings measured using the impinger method and the filter method. As expected, overall mean concentrations were about an order of magnitude lower in sawmills than in swine barns. Based on data from impinger sampling, the concentration range in sawmills was 207 to 17,063 endotoxin units (EU)/m³ compared to 2,025 to 11,297 EU/m³ in swine barns. Interestingly, the impinger method yielded higher means and lower variances in both sawmills and swine barns.

TABLE 2. Summary of endotoxin data for the complete data set for samples collected in sawmills and swine barns

Location ^a and sample type	<i>n</i>	Endotoxin concn (EU/m ³)				
		GM	GSD	Median	Minimum	Maximum
Sawmill						
AGI	59	740	2.47	485	208	17,063
Filter	62	188	2.83	252	6.2	1,452
Swine barn						
AGI	21	4,385	1.62	4,480	2,026	11,297
Filter	21	3,927	2.65	4,976	729	18,425

^a Eight sawmills and seven swine barns were sampled.

TABLE 3. Endotoxin GM and GSD from duplicate or triplicate sampling in sawmills and swine barns^a

Site ^b	Endotoxin by:								
	Particulates by filter			AGI			Filter		
	n	Concn (mg/m ³)		n	Concn (EU/m ³)		n	Concn (EU/m ³)	
		GM	GSD		GM	GSD		GM	GSD
Sawmills									
1-2	3	0.20	1.25	3	326	1.40	2	275	3.47
1-3	3	1.36	1.10	3	298	1.22	3	339	1.89
1-4	3	1.98	1.38	2	378	1.15	3	163	1.27
2-1	3	1.21	1.11	3	1,313	1.49	3	592	1.18
2-2	3	2.55	1.12	3	398	1.53	3	56	2.91
3-1	3	3.36	1.10	3	1,476	1.49	3	716	1.73
3-2	2	0.99	1.03	2	274	1.20	2	266	1.00
3-3	3	0.61	1.02	2	507	3.54	3	84	2.42
3-4	3	2.87	1.05	2	338	1.09	3	242	1.19
4-1	3	0.71	1.07	3	634	1.61	3	173	3.18
4-2	3	0.93	1.12	3	389	1.59	3	92	3.08
4-4	3	0.96	1.32	3	295	1.15	3	263	2.73
5-1	3	2.83	1.68	3	5,255	2.78	3	745	1.90
5-2	3	2.34	1.62	2	1,048	1.73	3	337	1.95
6-2	3	1.72	2.62	2	1,093	1.71	3	165	1.44
6-3	3	0.071	2.09	3	3,269	3.71	3	8	1.24
7-1	3	0.81	1.22	3	2,131	1.36	3	411	1.22
7-2	2	1.23	2.27	3	2,170	1.36	2	85	10.19
7-4	2	1.52	1.02	2	2,271	1.57	2	54	2.27
8-1	3	0.80	1.22	3	324	1.19	3	472	1.52
8-2	3	3.21	1.14	3	438	1.13	3	306	1.15
8-4	3	4.12	1.09	3	464	1.35	3	151	1.42
Mean			1.35			1.65			2.29
Swine barns									
1	3	1.15	1.45	3	3,534	1.19	3	7,437	1.34
2	3	0.89	1.23	3	3,901	1.30	3	2,882	3.49
3	3	1.24	1.14	3	6,521	1.14	3	11,940	1.46
4	3	1.89	1.26	3	6,478	1.62	3	9,224	1.22
5	3	0.58	1.26	3	4,748	1.64	3	976	1.19
6	3	0.59	1.11	3	2,221	1.16	3	2,974	1.59
7	3	0.69	1.10	3	5,073	1.92	3	2,102	1.36
Mean			1.22			1.42			1.66

^a For sawmills, the GSD for all but 3 of 22 AGI samples were less than 2.0. For the filter samples, the GSD for 8 of 22 exceeded 2.0. For swine barns, the GSD for all but 1 of 14 triplicate samples were less than 2.0.

^b The site numbers for sawmills consist of an arbitrarily assigned sawmill number and a sampling location (1, debarking; 2, sawing; 3, sorting; 4, planing). Swine barns were simply numbered 1 to 7.

Since field locations were sampled simultaneously in duplicate or triplicate, within-site GM and GSD were available for each sampling site and for each method (Table 3). For sawmills these data illustrate that, while airborne particulate measurements determined by filter sampling had a low average GSD, endotoxin assessments from the same filters had a higher average GSD. The endotoxin GSD for replicate impinger samples were lower than those from filters, indicating greater endotoxin measurement precision for the impinger method. The endotoxin values determined from filter and impinger sampling are plotted in Fig. 1 for sawmills and in Fig. 2 for swine barns. Sites were ordered by increasing impinger concentrations. Data in Fig. 1 demonstrate that AGI measurements were higher than filter determinations in nearly all sawmill sites. One set of filter samples (site 6; sorting operation) appeared to be erroneously low, and another (site 7; sawing operation) had an unusually high variance between duplicates. In swine barns neither impinger nor filter samples were consistently higher and the variability was less than in sawmills (Fig. 2).

We performed analysis of variance for repeated measures in

order to explore the observed differences in endotoxin concentrations between sampling methods after adjusting for the influence of other variables. The results of these analyses are presented in Table 4 for sampling data from sawmills and Table 5 for swine barns. The results in Table 4 represent analysis of variance with full data, i.e., inclusion of all sawmill sites for which either two or three replicates were collected ($n = 88$). When triplicate data were available, the first two replicates were arbitrarily selected for analysis. Initial analysis was performed on a full model including the wood type being handled during the sampled operation (hardwoods [oak, birch, or pine] versus softwoods [spruce, fir, or cedar]) and the interaction term between site type and sampler. These were not significant variables and were eliminated to create the reduced model. For the sawmills, significant determinants of endotoxin concentration were sampler (AGI versus filter; $P < 0.0001$) and site type (debarking, sawing, sorting, or planing; $P = 0.02$). None of the within-group factors were significant.

In order to further explore the differences in endotoxin exposure assessment by sampler, an additional analysis was

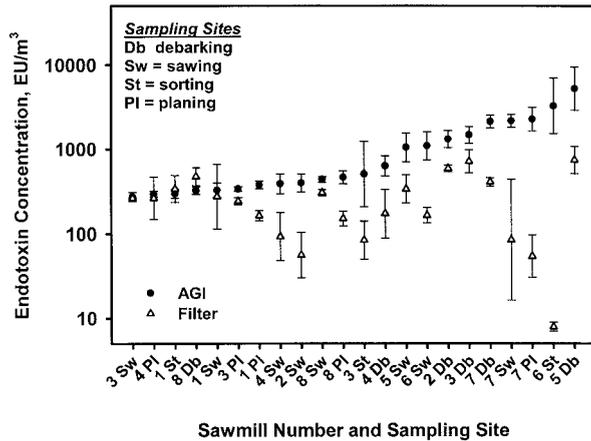


FIG. 1. Endotoxin GM and GSD from duplicate or triplicate samples in the four possible locations (debarking, sawing, sorting, and planing) in the eight sawmills for samples collected using impingers (AGI) and air-sampling filters (filter). Data were ordered according to the AGI value.

performed using the full sawmill data set including endotoxin determinations from sites where AGI and filters were not both available in duplicate or triplicate. This imputed-methods analysis used the model parameters to impute values for empty cells. This allowed the full 193 measured values to be included in the model. The results of this model are provided in Table 6 and are consistent with the previous analysis on the restricted data set. Site type ($P < 0.0001$) and sampler ($P < 0.0001$) were both found to be significant determinants of endotoxin concentration measurements. Wood type and replicate were not significant variables.

Analysis of variance for repeated measures was also performed for data from the swine barns (Table 5). Triplicate determinations were available for all barns with both sampling methods. After accounting for all the components in the model, there was still a significant difference in endotoxin concentration estimates even though none of the components in the model contributed significantly to the variance. For the

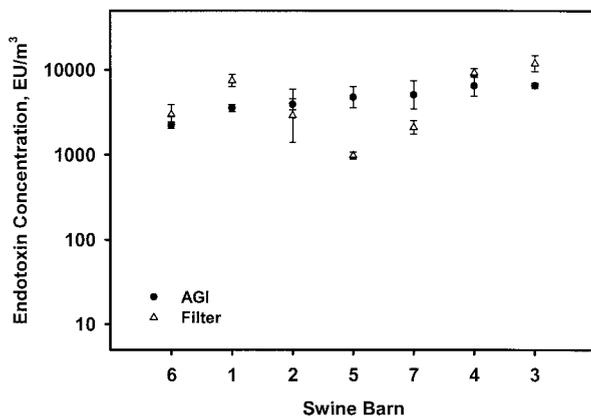


FIG. 2. Endotoxin GM and GSD from triplicate sampling in the seven swine barns for samples collected using impingers (AGI) and air-sampling filters (filter). Data were ordered according to the AGI value.

TABLE 4. Endotoxin concentration data from the sawmills studied using analysis of variance for repeated measures^a

Source ^b	dF ^c	SS ^d	MS ^e	F	P
Grand mean	1	2,683.3	2,683.3	1,392.7	<0.0001
Between groups					
Site type	3	20.91	6.97	3.62	0.02
Sampler	1	43.63	43.63	22.64	<0.0001
Residual	39	75.14	1.93		
Within groups					
Replicate	1	0.52	0.52	1.07	0.31
Rep* site type	3	0.68	0.68	1.39	0.26
Rep* sampler	1	0.006	0.006	0.01	0.91
Residual	39	18.97	0.49		
Total	88	2,844.4			

^a Shown are the results of a reduced model that includes all sites with simultaneous AGI and filter data run in duplicate or triplicate. For sites with triplicate determinations only samples 1 and 2 were included. Wood type ($P = 0.78$) and the interaction between site type and sampler ($P = 0.46$) were not significant and were eliminated from the full model.

^b Grand mean, comparison of data after controlling for all listed parameters and interactions; site type, debarking, sawing, sorting, or planing; sampler, AGI or filter; Residual, error term; replicate, two randomly selected replicate samples with each sampler were used in this analysis; Rep*, interaction terms between the replicates and the site type and sampler.

^c dF, degrees of freedom.

^d SS, sum of squares.

^e MS, mean square.

swine barns there was no difference among the seven barns and there was no difference between the two sampling methods. Furthermore, no interactions were significant.

DISCUSSION

The use of impingers (such as AGI) for bioaerosol sampling is often necessary in occupational settings since this approach allows dilution of the sample prior to culturing of microorganisms. This is not generally possible with samplers that employ impaction on solid media (25). The impinger sample collected for microbial analysis can be readily used for endotoxin quantitation since no additional extraction process has to be performed and since loss of endotoxin activity due to incomplete recovery of endotoxin from the filter substrate is eliminated. A number of studies have examined the efficiencies of various

TABLE 5. Endotoxin concentration data from the swine barns studied using analysis of variance for repeated measures^a

Source of variation	dF	SS	MS	F	P
Grand mean	1	2,914.85	2,914.9	2,641.36	<0.0001
Between groups					
Barn ^b	6	10.58	1.764	1.60	0.29
Sampler	1	0.1275	0.1275	0.12	0.75
Residual	6	6.6213	1.1035		
Within groups					
Replicate	2	0.6357	0.3178	2.04	0.17
Rep* barn	12	3.7876	0.3156	2.03	0.12
Rep* sampler	2	0.1312	0.0656	0.42	0.67
Residual	12	1.8696	0.1558		
Total	42	2,938.61			

^a Terms are as defined for Table 4. Shown are the results of a reduced model. The interaction between barn and sampler was not significant and was eliminated from the full model.

^b Barn, barn number.

TABLE 6. Analysis of sawmill data including all sampled sites using the unbalanced repeated-measures model with structured covariance matrices^a

Source	dF	χ^2	P
Site type	3	38.82	<0.0001
Sampler	1	40.61	<0.0001
Replicate	2	4.59	0.101
Total	193		

^a Terms are as defined for Table 4. In this analysis, 193 measurement values representing 49 measurement sets were included. Neither wood type nor any of the interactions were significant in the full model, so they were removed in this reduced model. Regression equation: Endotoxin concentration = $5.91 + 0.5841 \cdot \text{sawing} - 0.00272 \cdot \text{sorting} - 0.172 \cdot \text{planing} + 0.669 \cdot \text{filter} + 0.0893 \cdot \text{second replicate} - 0.0014 \cdot \text{third replicate}$. Reference variables are debarking for site type, AGI for sampler, and first replicate for replicate. In this analysis Akaike's information criterion was -214.5.

filter media (7, 15, 29), extraction protocols (18, 28), and assay buffers (14, 28) for the recovery and measurement of endotoxins. Similar attention has not been given to alternative sampling approaches. We have used AGI solutions to estimate endotoxin exposures in several studies (3–5) without having fully evaluated the efficiency of this method or having established how the AGI values compare to endotoxin measurements from filter extracts. We are aware of only one paper that discusses the possible use of impingers for endotoxin recovery from air samples, but that paper referred to the midjet impinger and cited its poor collection efficiency for small particles (15). In contrast, several studies have demonstrated that the AGI is highly efficient for collecting particles in the range 0.3 to 5.0 μm (9, 21, 30; T. Pearce, P. S. Thorne, and P. T. O'Shaughnessy, Abstr. 19th Annu. Am. Assoc. Aerosol Res. Conf., abstr. 9A5, 2000). In settings where bioaerosol sampling is performed, it is common to also assess airborne endotoxin. The AGI allows both analyses from the same media and simplifies the sampling burden. Thus, this analysis was undertaken to characterize the performance of AGI for endotoxin measurement compared to filter methods.

The results obtained in this study for sawmills showed that impinger sampling yielded endotoxin concentration estimates significantly higher than those obtained with filter measurements, even after controlling for other factors and without evidence of LAL assay enhancement or inhibition. For swine barns, the two methods led to measurements that were not significantly different. Together these data show that the impinger method does not underestimate endotoxin concentration relative to filter sampling.

There are several possible explanations for finding a difference between impinger and filter methods in sawmills but not in swine barns. First, swine barns typically have more-uniform concentrations than sawmills, thus minimizing the effects of different sampling windows and smaller sampling volumes for impinger samples. Second, the particulate fraction that was sampled differed between sawmills (inhalable dust) and swine barns (total dust); sampling inhalable dust may have reduced the recovery of large particles for filter sampling in sawmills compared to that for filter sampling in swine barns.

We were concerned that differences in particle size selection properties between samplers may have affected the comparison. AGIs generally recover particles smaller than about 12 μm , whereas both filter methods collect larger particles as well.

The IOM collects about 80% of particles less than 10 μm and 50% of particles less than 100 μm . The 37-mm-diameter closed-face cassette used on a stationary sampling platform will collect 70% of particles less than 10 μm and will collect relatively few particles (<10%) above 25 μm . However, since the AGI yield was as high as or higher than that from filters, the improved recovery of endotoxin activity from impingers may have offset the effect of failure to collect larger particles. It may also be the case that the larger particles carry a lower proportion of the endotoxin than the smaller aerosols. Another concern was that the filter extraction method used for swine barn samples but not sawmill samples (pyrogen-free water with 0.04% Tween 80 overnight at 37°C in a shaking bath) could have increased the endotoxin content of the extraction fluid. This method did not appear to have allowed significant gram-negative bacterial growth, since there was no difference between the endotoxin concentrations produced by the two methods in swine barns. In sum, neither of these concerns was perceived as sufficient to invalidate the comparison.

A common objective of environmental sampling is to provide estimates of exposure for studies of adverse health outcomes associated with occupational settings. Environmental concentrations may have high spatial and temporal variability. Since environmental air samples represent a snapshot in time, they may be poor surrogates (i.e., biased estimators) for the actual concentrations they represent. Bias can be reduced by using modeled exposures that are derived from measured values, information on workplace processes, environmental controls, time of day, and other relevant information. Short-term samples are generally more vulnerable to bias due to temporal variation in concentration than longer-term samples. In this study, AGI samples were taken on a sampled volume of 200 liters, whereas filter samples represented mean volumes of 361 liters in swine barns and 862 liters in sawmills. However, AGI samples were taken in just 16 min and could have been heavily influenced by short-term fluctuations in airborne contaminants. Since fluctuations during the sampling period are equally likely to overestimate or underestimate the time-weighted concentration, the bias is random. This is in contrast to filter samples, which systematically underestimate exposures due to poor extraction efficiency. Thus, the best approach for assessing airborne endotoxin concentration may be integrated serial sampling with impingers. Impinger solutions can be pooled for assay of a single solution to represent a time-weighted average or assayed individually, with time weighting of the data. We believe that the analyses presented support the utility of bioaerosol impingers for assessment of concentrations of airborne endotoxin.

ACKNOWLEDGMENTS

The research studies in swine barns and sawmills that provided the data for this study were supported by the Quebec Institute of Research on Occupational Health and Safety (IRSST). The data analyses and manuscript preparation were supported by the University of Iowa, Environmental Health Sciences Research Center (NIH/NIEHS P30 ES05605). C. Duchaine received a fellowship from IRSST and a fellowship from Natural Sciences and Engineering Research of Canada (NSERC).

REFERENCES

1. Buttner, M. P., and L. D. Stetzenbach. 1991. Evaluation of four aerobiological sampling methods for the retrieval of aerosolized *Pseudomonas syringae*.

- Appl. Environ. Microbiol. **57**:1268–1270.
2. **Douwes, J., P. Versloot, A. Hollander, D. Heederik, and G. Doekes.** 1995. Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. *Appl. Environ. Microbiol.* **61**:1763–1769.
 3. **Duchaine, C., Y. Grimard, and Y. Cormier.** 2000. Influence of building maintenance, environmental factors and seasons on airborne contaminants of swine confinement buildings. *Am. Ind. Hyg. Assoc. J.* **61**:56–63.
 4. **Duchaine, C., A. Mériaux, G. Brochu, and Y. Cormier.** 1999. Airborne microflora in Quebec dairy farms: lack of effect of bacterial hay preservatives. *Am. Ind. Hyg. Assoc. J.* **60**:89–95.
 5. **Duchaine, C., A. Mériaux, P. S. Thorne, and Y. Cormier.** 2000. Assessment of particulates and bioaerosols in Eastern Canada sawmills. *Am. Ind. Hyg. Assoc. J.* **61**:727–732.
 6. **George, C. L. S., H. Jin, C. L. Wohlford-Lenane, M. E. O'Neill, J. C. Phipps, P. T. O'Shaughnessy, J. N. Kline, P. S. Thorne, and D. A. Schwartz.** 2001. Endotoxin responsiveness and subchronic grain dust-induced airway disease. *Am. J. Physiol. Lung Cell Mol. Physiol.* **280**:L203–L213.
 7. **Gordon, T., K. Galdanes, and L. Brosseau.** 1992. Comparison of sampling media for endotoxin contaminated aerosols. *Appl. Occup. Environ. Hyg.* **7**:427–436.
 8. **Heederik, D., R. Brouwer, K. Biersteker, and J. S. M. Boleij.** 1991. Relationship of airborne endotoxin and bacteria levels in pig farms with the lung function and respiratory symptoms of farmers. *Int. Arch. Occup. Environ. Health* **62**:595–601.
 9. **Henningson, E. W., I. Fångmark, E. Larsson, and L.-E. Wikström.** 1988. Collection efficiency of liquid samplers for microbiological aerosols. *J. Aerosol Sci.* **19**:911–914.
 10. **Hollander, A., D. Heederik, P. Versloot, and J. Douwes.** 1993. Inhibition and enhancement in the analysis of airborne endotoxin levels in various occupational environments. *Am. Ind. Hyg. Assoc. J.* **54**:647–653.
 11. **Kennedy, S. M., D. C. Christiani, E. A. Eisen, D. H. Wegman, I. A. Greaves, S. A. Olenchock, T. Ye, and P. Lu.** 1987. Cotton dust and endotoxin exposure-response relationships in cotton textile workers. *Am. Rev. Respir. Dis.* **135**:194–200.
 12. **Kullman, G. J., P. S. Thorne, P. F. Waldron, J. J. Marx, B. Ault, D. M. Lewis, P. D. Siegel, S. A. Olenchock, and J. A. Merchant.** 1998. Organic dust exposures from work in dairy barns. *Am. Ind. Hyg. Assoc. J.* **59**:403–414.
 13. **Lange, J. L., P. S. Thorne, and N. L. Lynch.** 1997. Application of flow cytometry and fluorescent in situ hybridization for assessment of exposures to airborne bacteria. *Appl. Environ. Microbiol.* **63**:1557–1563.
 14. **Milton, D. K., H. A. Feldman, D. S. Neuberger, R. J. Bruckner, and I. A. Greaves.** 1992. Environmental endotoxin measurement: the kinetic Limulus assay with resistant-parallel-line estimation. *Environ. Res.* **57**:212–230.
 15. **Milton, D. K., R. J. Gere, H. A. Feldman, and I. A. Greaves.** 1990. Endotoxin measurement: aerosol sampling and application of a new Limulus method. *Am. Ind. Hyg. Assoc. J.* **51**:331–337.
 16. **Milton, D. K., M. D. Walters, S. K. Hammond, and J. S. Evans.** 1996. Worker exposure to endotoxin, phenolic compounds and formaldehyde in a fiberglass insulation manufacturing plant. *Am. Ind. Hyg. Assoc. J.* **57**:889–896.
 17. **Nevalainen, A., J. Pastuszka, F. Liebhaber, and K. Willeke.** 1992. Performance of bioaerosol samplers: collection characteristics and sampler design considerations. *Atmos. Environ.* **26A**:531–540.
 18. **Olenchock, S. A., D. M. Lewis, and J. C. Mull.** 1989. Effects of different extraction protocols on endotoxin analyses of airborne grain dusts. *Scand. J. Work Environ. Health* **15**:430–435.
 19. **Preller, L., D. Heederik, H. Kromhout, J. S. M. Boleij, and M. J. M. Tielen.** 1995. Determinants of dust and endotoxin exposure of pig farmers: development of a control strategy using empirical modeling. *Ann. Occup. Hyg.* **39**:545–557.
 20. **Schenker, M. B., D. Christiani, Y. Cormier, H. Dimich-Ward, G. Doekes, J. Dosman, J. Douwes, K. Dowling, D. Enarson, F. Green, D. Heederik, K. Husman, S. Kennedy, G. Kullman, Y. LaCasse, B. Lawson, P. Malmberg, J. May, S. McCurdy, J. Merchant, J. Myers, M. Nieuwenhuijsen, S. Olenchock, C. Saiki, D. Schwartz, J. Sieber, P. Thorne, G. Wagner, N. White, X. Xu, and M. Chan-Yeung.** 1998. Respiratory health hazards in agriculture. *Am. J. Respir. Crit. Care Med.* **158**:S1–S76.
 21. **Terzieva, S., J. Donnelly, V. Ulevicius, S. A. Grinshpun, K. Willeke, G. N. Stelma, and K. Brenner.** 1996. Comparison of methods for detection and enumeration of airborne microorganisms collected by liquid impingement. *Appl. Environ. Microbiol.* **62**:2264–2272.
 22. **Thorne, P. S.** 2000. Inhalation toxicology models of endotoxin- and bioaerosol-induced inflammation. *Toxicology* **152**:13–23.
 23. **Thorne, P. S., and J. A. DeKoster.** 1996. Pulmonary effects of machining fluids in guinea pigs and mice. *Am. Ind. Hyg. Assoc. J.* **57**:1168–1172.
 24. **Thorne, P. S., J. A. DeKoster, and P. Subramanian.** 1996. Environmental assessment of aerosols, bioaerosols, and airborne endotoxins in a machining plant. *Am. Ind. Hyg. Assoc. J.* **57**:1163–1167.
 25. **Thorne, P. S., and D. Heederik.** 1999. Assessment methods for bioaerosols, p. 85–103. *In* T. Salthammer (ed.), *Organic indoor air pollutants—occurrence, measurement, evaluation.* Wiley/VCH, Weinheim, Germany.
 26. **Thorne, P. S., M. S. Kiekhaefer, P. Whitten, and K. J. Donham.** 1992. Comparison of bioaerosol sampling methods in barns housing swine. *Appl. Environ. Microbiol.* **58**:2543–2551.
 27. **Thorne, P. S., J. L. Lange, P. D. Bloebaum, and G. J. Kullman.** 1994. Bioaerosol sampling in field studies: can samples be express mailed? *Am. Ind. Hyg. Assoc. J.* **55**:1072–1079.
 28. **Thorne, P. S., S. J. Reynolds, D. K. Milton, P. D. Bloebaum, X. Zhang, P. Whitten, and L. F. Burmeister.** 1997. Field evaluation of endotoxin air sampling assay methods. *Am. Ind. Hyg. Assoc. J.* **58**:792–799.
 29. **Walters, M. D., D. K. Milton, L. Larsson, and T. Ford.** 1994. Airborne environmental endotoxin: a cross-validation of sampling and analysis techniques. *Appl. Environ. Microbiol.* **60**:996–1005.
 30. **Willeke, K., X. Lin, and S. A. Grinshpun.** 1998. Improved aerosol collection by combined impaction and centrifugal motion. *Aerosol Sci. Technol.* **28**:439–456.
 31. **Zock, J. P., D. Heederik, and H. Kromhout.** 1995. Exposure to dust, endotoxin and microorganisms in the Dutch potato processing industry. *Ann. Occup. Hyg.* **39**:841–854.