Ann. Occup. Hyg., 2015, Vol. 59, No. 4, 504–513
doi:10.1093/annhyg/meu108
Advance Access publication 22 December 2014

BOHS
The Chartered Society for Worker Health Protection

Endotoxin Deposits on the Inner Surfaces of Closed-Face Cassettes During Bioaerosol Sampling: A Field Investigation at Composting Facilities

Philippe Duquenne[1*], Xavier Simon[1], Valérie Demange[1], Martin Harper[2] and Pascal Wild[1]

1.INRS - Institut National de Recherche et de Sécurité, Aerosols Metrology Laboratory, Pollutants Metrology Division, 1 rue du Morvan CS 60027, Vandœuvre-lès-Nancy Cedex 54519, France
2.NIOSH - National Institute for Occupational Safety and Health, Health Effects Laboratory Division, 1095 Willowdale Road, Morgantown, WV 26505, USA
*Author to whom correspondence should be addressed. Tel: +33-3-83-50-98-75; fax: +33-3-83-50-87-11; e-mail: philippe.duquenne@inrs.fr
Submitted 21 October 2014; revised 5 November 2014; revised version accepted 5 November 2014.

ABSTRACT

A set of 270 bioaerosol samples was taken from 15 composting facilities using polystyrene closed-face filter cassettes (CFCs). The objective was to measure the quantity of endotoxin deposits on the inner surfaces of the cassettes (sometimes referred to as 'wall deposits'). The results show that endotoxins are deposited on the inner surfaces of the CFCs through sampling and/or handling of samples. The quantity of endotoxins measured on inner surfaces range between 0.05 (the limit of detection of the method) and 3100 endotoxin units per cassette. The deposits can represent a large and variable percentage of the endotoxins sampled. More than a third of the samples presented a percentage of inner surface deposits >40% of the total quantity of endotoxins collected (filter + inner surfaces). Omitting these inner surface deposits in the analytical process lead to measurement errors relative to sampling all particles entering the CFC sampler, corresponding to a developing consensus on matching the inhalable particulate sampling convention. The result would be underestimated exposures and could affect the decision as to whether or not a result is acceptable in comparison to airborne concentration limits defined in terms of the inhalability convention. The results of this study suggest including the endotoxins deposited on the inner surfaces of CFCs during analysis. Further researches are necessary to investigate endotoxin deposits on the inner cassette surfaces in other working sectors.

KEYWORDS: air sampling; bioaerosol; closed-face cassette; composting facilities; endotoxins; inner surfaces deposits; wall deposits

INTRODUCTION

Endotoxins are lipopolysaccharides found in the outer membrane of most Gram-negative bacteria and cyanobacteria. They can become airborne by techniques and processes that generate aerosols from materials contaminated by these microorganisms.

Endotoxins are present in the outdoor air at concentrations generally <10 endotoxin units (EU) per cubic meter of air (Madsen, 2006; Duquenne *et al.*, 2013). In occupational environments, exposure levels vary over a relatively wide range and reach several tens and even hundreds of thousands of EU m^{-3}. Exposure to endotoxins has been observed in occupational environments as varied as agriculture and livestock breeding, the agro-food industry, the waste collection and treatment sector, wood processing, and the medical profession (Laitinen *et al.*, 2001; Rylander, 2002; Smit *et al.*, 2005; Harper and Andrew, 2006; Dutil *et al.*, 2009). The inhalation of endotoxins by exposed workers has been linked to respiratory symptoms, such as respiratory tract irritation and thoracic oppression, and more general symptoms, such as fevers and coughing (Rylander, 2002; Sigsgaard *et al.*, 2005; Liebers *et al.*, 2006). Exposure to endotoxins may also modify the response of the organism to allergen exposure (Smit *et al.*, 2010; Basinas *et al.*, 2012). Despite these observations, there is no internationally agreed occupational exposure limit (OEL) for endotoxins.

Given the recognition of an association between exposure to endotoxins and effects on health, the measurement of airborne endotoxins has received particular attention over the past few decades (Spaan *et al.*, 2006; Liebers *et al.*, 2007; Duquenne *et al.*, 2013). The method used most frequently for sampling endotoxins in the air involves the collection of particles on a filter. Collection is generally done on non-pyrogenic fiberglass filters. Sampling of the inhalable fraction of the aerosol is done using a filter holder, such as the closed-face three-piece cassette (CFC), the Button sampler, and the Institute of Occupational Medicine, or IOM sampler, connected with a pump. The protocols used involve the analysis of endotoxins collected only on the filters following elution. The endotoxins likely to be deposited on the inner surfaces of the sampling device are not taken into account in the analytical process. For example, EN 14031 takes the filter deposit to represent the inhalable sampling convention without reference to specific sampler types.

However, several studies have reported dust as well as organic and non-organic pollutants deposits on the inner surfaces of the CFC and the IOM sampler following aerosol sampling (Puskar *et al.*, 1991; Lidén *et al.*, 2000; Demange *et al.*, 2002; Dobson *et al.*, 2005; Soo *et al.*, 2014). In fact, evidence to date indicates that in comparison to several sampler types, the IOM sampler best meets the inhalability convention, including wall deposits by design. Furthermore, field studies indicate that CFC samples with wall deposits better match IOM samples than without (Harper and Demange, 2007). As a result, measurement protocols have been modified to include these deposits in the analysis of samples (INRS, 2003; OSHA, 2006; NIOSH Manual of Analytical Methods, 2013).The occurrence of endotoxin deposits on the inner surfaces of the CFC has not been explored up to now and very few data are available on this issue (Walters *et al.*, 1994; Simon *et al.*, 2011).Thus, the reality of these deposits, their impact on the results of measurements, and whether or not they should be taken into account in the analysis are issues that need to be investigated.

We studied the deposits of endotoxins on the inner surfaces of CFCs when sampling bioaerosols in occupational environments. A field study was carried out at composting facilities with the objectives of assessing the magnitude of these deposits and of providing information on whether they could be important for evaluating exposure to airborne endotoxins.

MATERIALS AND METHODS

Description of composting facilities

Bioaerosol samples were collected from 15 composting facilities (numbered CF1 to C15). The facilities differed by the type of waste treated (sludge from wastewater treatment plants, paper mills, and other; green wastes; household organic waste; residual household waste; fecal matter), the composting process used and the configuration of the installations (see Supplementary Table T1, available at *Annals of Occupational Hygiene* online for information). The intensity of the activity (compost handling, passage of vehicles, etc.) and the meteorological conditions at the time of sampling (not described in this article) could also be different.

Sampling method and strategy

Samples were taken from 2009 to 2012. Stationary (area) samples were collected close to the main composting activities. The sampling devices were placed 1.7 m from the ground, with their air inlets (horizontal) facing the activity being investigated. The number of samples collected per composting facility varied from 5 to 35 depending on the site considered. The facilities were subjected to one (CF1 to 9) or two (CF10 to 15) measurement campaigns during the study.

Sampling of airborne endotoxins was performed by filtration using 37 mm, three-piece polystyrene CFCs (Millipore®, Molsheim, France). The cassettes were mounted with a pyrogen-free fiberglass filter (GF/B glass microfiber filter, Whatman®) as a collection medium and a backup filter of the same type. The filters were heated to 250°C for 120 min beforehand to remove pyrogens and the cassettes were closed using a pneumatic press to avoid leaks. Care was taken to avoid contamination of the inner walls of the cassettes by exogenous endotoxins. The different components of the cassettes were packed in a clean container and kept in clean, dry place before assembly. Likewise, the cassettes were handled and assembled so as to limit contamination (gloves, handling in microbiological safety cabinets). Each CFC was connected to an individual pump (GilAir®; Gilian Instrument Corp.) and sampling was done at a nominal flow rate of 2 l min^{-1}. The flow rate was calibrated before and after sampling using a soap bubble flow meter (Gilibrator; Gilian Instrument Corp.). Duration of sampling ranged from 36 to 210 min (median time: 90 min). After sampling, CFCs were plugged and transported to the laboratory. Temperature and relative humidity of air were measured during sampling with a thermo-hygrometer (Hygropalm-2, Rotronic, France).

Transport and preservation of samples

The samples were transported to the laboratory on the same day or sent by courier overnight to the laboratory for analysis the following day.

Analysis of bioaerosol samples

The samples were delivered to the laboratory as CFCs and the analyses were performed within 48 h following sampling according to the procedures described previously (INRS, 2010; Duquenne *et al.*, 2012). For each sample, the endotoxins were measured both on the filter and from the inner surfaces of the cassette. Controls samples (20 cassettes not used for the sampling) were simultaneously analyzed with the samples to ensure the absence of endotoxins initially on the inner surfaces of the cassettes and on the filters. The latter were used to test all the batches of cassettes used for sampling except those used at one facility (CF15) which were lost. All the equipment and dilution solutions used during experiments were non-pyrogenic and samples were handled in microbiological safety cabinets.

Extraction of endotoxins from filters and inner surfaces of cassettes

For each sample, the fiberglass filter used as collection medium was removed from the sampling cassette, then transferred to a sterile pyrogen-free polypropylene tube (Cellstar tubes®, Greiner Bio-One) containing 10 ml of sterile, pyrogen-free water (PFW; Aqua B. Braun, B. Braun). The second glass fiber filter used as the filter backup was removed from the cassette after which the latter was closed by a pneumatic press. A 10 ml volume of PFW (Aqua B. Braun, B. Braun) was introduced into the cassettes by the inlet. Both the tube and cassette were then shaken for 1 min at 2500 rpm (using a Vortex) after which extraction was performed for 60 min at 2000 rpm (Multi-Reax® shaker, Heidolph®). Extraction was completed by centrifugation at 2000 rpm (Sigma®, 3–18K) for 10 min at 4°C.

LAL analysis of endotoxins in the extracts

The analysis of the endotoxins in the extracts was performed (in duplicate) immediately after extraction by the kinetic-chromogenic Limulus amoebocyte lysate (LAL) using Kinetic-QCL® kits (Lonza Group Ltd). The assay was performed according to the method published by the INRS (2010) and based on the EN 14031 standard (Comité Européen de Normalisation, 2003). The LAL assay kits used for the study came from four different batches with an referent standard endotoxin/control standard endotoxin (CSE) ratio of 10 EU ng^{-1}. The analyses were performed on aliquots of 100 µl diluted and non-diluted extracts distributed on a microtitration plate (96 Well Clear Polystyrene Microplates, Costar®). LAL reagent (100 µl) was added to each well and the microplate was analyzed immediately. The analysis was performed at 37°C using an automated microplate incubator/reader (ELx800 Absorbance Microplate Reader, BioTek®) interfaced with the WinKQCL® software (version 1.20). A standard curve (calibration curve) obtained from a reference endotoxin, *Escherichia coli* (strain O55:B5), was used to determine the concentration of endotoxins in the samples, expressed in EU. PFW was used as the negative control and a CSE solution was used as the positive control. The samples were spiked with a known quantity of endotoxins to detect possible interference. Absence of interference was confirmed if the quantity of added endotoxins found was

between 50 and 200%. The limit of detection (LOD) of the assay was 0.005 EU ml^{-1}. The validation criteria were: absence of interference, a coefficient of correlation >0.98 for the standard curve and a coefficient of variation <10% for the two replicates of samples.

Data analysis

Given the much skewed distribution of concentration of endotoxins in general and in this study in particular, the concentrations are presented on geometric scales. Thus the endotoxins detected on the inner surfaces of cassettes were expressed as a percentage of the total endotoxins (filter + inner surfaces) and are presented as box plots by categorized endotoxin concentration on the filter (categories defined by the following arbitrary cut-points: 10, 50, 90, 200, 500, and 1000 EU m^{-3}). The effect of omitting endotoxins on the inner surfaces in comparison with arbitrary cut-points, was assessed by cross-tabulating the number of exposure measurements based on filter determinations with the number of measurements above the same arbitrary cut-points based on filter + inner surfaces determinations and by presenting a scatter plot of total endotoxin (filter + inner surfaces) levels as a function of endotoxins measured on the filter.

In order to assess whether the fraction of endotoxins on the inner surfaces depended on the sampling sites, temperature, humidity, and the activities, we performed a linear mixed effect regression with this log-transformed fraction of endotoxins as a dependent variable and sampling site (random effect), temperature, humidity, and activities (fixed effects) as independent variables.

RESULTS

A set of 281 samples was collected from the 15 sites visited but only 270 were analyzed (see Supplementary Table T1, available at *Annals of Occupational Hygiene* online). Five samples could not be analyzed and for six others the quantity of endotoxins measured on the filter and on the walls was below the LOD of the method.

Endotoxins collected on filter only

Endotoxins were detected on filters from the LOD of the method to 1200 EU per filter. This corresponds to concentrations of endotoxins measured in the air of the investigated facilities ranging (filter only) from the method's LOD (< LOD) to 6647 EU m^{-3}, with a median close to 18 EU m^{-3} and a geometric mean of 21.7 EU m^{-3}. Nearly 40% of the measured concentrations were <10 EU m^{-3}; 36% were between 10 and 100 EU m^{-3}, 19% between 100 and 1000 EU m^{-3}, and 5% were >1000 EU m^{-3} (Fig. 1A). The analyses performed on control samples showed that endotoxins were not initially present on filters (< LOD).

Endotoxins deposited on inner surfaces of cassettes

The quantity of endotoxins measured range between the LOD of the method and 3100 EU per cassette.

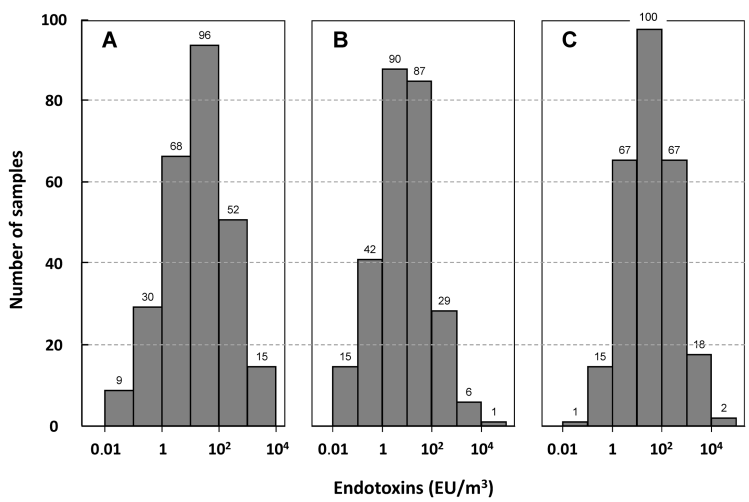

Figure 1 Distribution of samples as a function of the concentration of endotoxins measured in the air of the 15 compositing facilities visited. The results are presented when the calculations are performed by taking into account: (A) the endotoxins collected only on the filter; or (B) the endotoxins deposited only on the inner surfaces of cassettes; or (C) the total endotoxins collected on both.

The measured quantities corresponded to concentrations of airborne endotoxins between the LOD of the method and 17 500 EU m^{-3} with a median close to 8.0 EU m^{-3} and geometric mean of 9.9 EU m^{-3}. The distribution of the concentrations measured, presenting characteristics of a lognormal distribution, is shown in Fig. 1B. For 11 samples, endotoxins were detected on the filter but not on the inner surfaces of the cassette (< LOD). The concentrations measured with the corresponding samples were ≤3.5 EU m^{-3}. On the contrary, for nine other samples endotoxins were detected on the inner surfaces of the cassette but not on the filter (<LOD). The concentrations measured with eight of the corresponding samples were ≤2.0 EU m^{-3}. For the ninth, the concentration was 64.7 EU m^{-3}.

Apart from these specific cases, the presence of endotoxins on inner surfaces of cassettes was observed whatever the concentration of endotoxins measured in the air. The percentages of endotoxins deposited on the inner surfaces of the sampling cassettes are described as box plots according to categorized endotoxin concentration on the filter (Fig. 2). These percentages can be quite large: 34% of the samples considered in our study, the percentage of deposits on the inner surfaces represented >40% of the total endotoxins collected both on the filter and on the inner surfaces. For some samples this rate exceeded 60 and even 80% (i.e. inner surfaces > filter). The fraction of endotoxins deposited on the inner surfaces varied considerably, especially for the endotoxins concentrations (measured with the filter alone) <20 EU m^{-3} (Fig. 2).

The regression analysis showed significant differences of the fraction of endotoxins on the inner surfaces ($P < 0.001$—data not shown) according to the sites but no effect of humidity, temperature, or duration of sampling. On the other hand, the effect of the activities was statistically significant ($P = 0.0008$) with lower relative fractions on the inner surfaces in high exposed activities (screening, mixing).

The analyses performed on the 20 control samples showed that the quantity of endotoxins initially present on the inner surfaces of the cassettes was lower than the LOD of the method (< LOD) for 18 of the samples. For two of them, the quantity of endotoxins present on the walls was measured at 0.06 and 0.10 EU.

Ambient concentrations of total endotoxins (filter + inner surfaces)

The results presented in Fig. 1C show that the concentrations of total endotoxins (filter + deposits on the inner surfaces) measured in the air of the facilities investigated range between 0.05 and 24 150 EU m^{-3}, with a median close to 35 EU m^{-3} and a geometric mean of 31.7 EU m^{-3}. Analysis of the data revealed characteristics of a lognormal distribution and that nearly 31% of the concentrations measured were <10 EU m^{-3}; 37% were between 10 and 100 EU m^{-3}, 25% between 100 and 1000 EU m^{-3}, and 7% were >1000 EU m^{-3} (Fig. 1C).

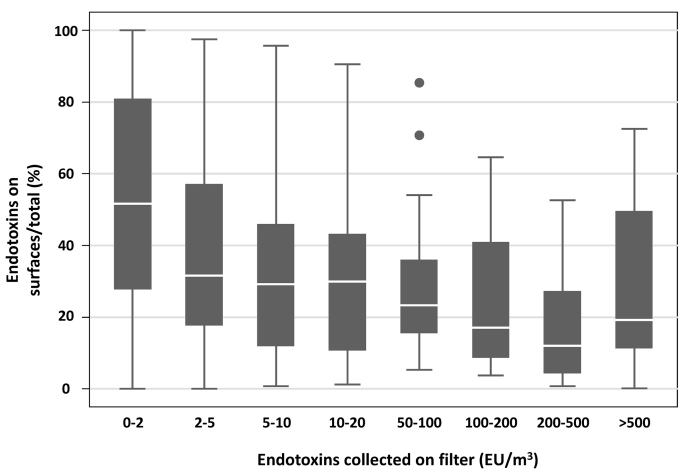

Figure 2 Box plots of proportions of endotoxins deposited on the inner surfaces of cassettes, in comparison to the total (inner surfaces + filter) endotoxins collected.

Incidence of taking into account inner surfaces deposits on the results

We considered the impact of taking into account inner surface deposits in the calculation on the evaluation of the endotoxin concentrations measured. Calculations from data presented in Fig. 2 revealed that the concentrations of total endotoxins (deposited on filter + inner surfaces) were from 1- to 50-fold higher than those measured without taking into consideration the deposits (filter only). The inclusion of the endotoxins deposited on the inner surfaces in the analysis increased the number of samples exceeding a target value. This number increased from 15 to 33% of the samples collected according to the target value chosen (Table 1). This result is remarkable when one considers that a concentration of 1000 EU m^{-3} was exceeded for an additional 33% of samples when the deposits were included in the analysis. No specific value of ratio between concentration of total endotoxins (deposited on filter + inner surfaces) and concentration measured with endotoxins deposited on filter only can be deduced from the data obtained in our study.

Table 1. Impact of inner cassette surface deposits on assessment of measurements of airborne endotoxins. The values represent the number of measurements out of a total 270 samples that exceed a specified target value according to whether the concentrations of total endotoxins (filter + inner surfaces) or those on the filter only are considered. The additional endotoxin on the inner cassette surfaces increases the exceedance by between 15 and 33%.

Concentration (EU m^{-3})	Number of samples	
	Endotoxins on filter only	Total endotoxins (filter + inner surfaces)
>10 EU m^{-3}	163	187
>50 EU m^{-3}	103	119
>90 EU m^{-3}	75	95
>200 EU m^{-3}	43	57
>500 EU m^{-3}	26	31
>1000 EU m^{-3}	15	20

DISCUSSION

Representativeness of the measurements

Our study confirmed the presence of endotoxins in the air of composting facilities and the ambient concentrations we measured corroborate those reported in previous works using similar measurement methods (Darragh *et al.*, 1997; Tolvanen *et al.*, 2005; Duquenne *et al.*, 2012). The facilities selected were characteristic of the diversity of the processes, installations, and the type of wastes treated that can be found in the composting sector in France. In addition, bioaerosol samples were collected during periods when the facilities were operating under normal conditions. It can therefore be considered that the measurements were representative of situations usually found under real exposure conditions in this sector. On the other hand, our measurements were only performed in composting facilities and the results could be different if the measurements had been taken in another working environment. Further tests performed under other conditions are necessary to investigate endotoxin deposit on the inner cassette surfaces in other occupational sectors.

Furthermore, endotoxins were usually not detected on the inner surfaces of the cassettes and on the filters from control samples. The quantities of endotoxins present on the inner surfaces of two control cassettes, measured at 0.06 and at 0.10 EU respectively, were much lower than quantities of endotoxins measured on the inner surfaces of the cassettes after sampling (2.0–166 and 1.93–1200 EU per sample, respectively). Thus it can be considered as having no influence on the results of our study. We were not able to analyze the control samples with the samples taken at facility PF15. Regarding the results obtained for the other batches of control cassettes, we presume that the corresponding cassettes were, initially, not or only slightly contaminated by endotoxins.

Occurrence of endotoxins on inner surfaces of CFCs

The results revealed the presence of particles containing endotoxins on the inner surfaces of the sampling cassettes. Endotoxin deposits on the inner surfaces occurred very frequently (96% of the samples) and could amount to a high percentage (as high as 100%) of the sampled biological particles.

The issue of particle deposits on the inner surfaces of CFC is not new. Indeed, several previous studies highlighted the presence of such deposits when sampling aerosols to perform gravimetric analyses of dust (Puskar *et al.*, 1991; Awan and Burgess, 1996; Soo *et al.*, 2014), analyses of organic compounds (Lafontaine *et al.*, 1999), metals and non-metals (Demange et al., 1990, 2002; Harper and Pacolay, 2006; Harper and Demange, 2007; Hendricks *et al.*, 2009; Lee *et al.*, 2009; Chisholm *et al.*, 2012), and silica (Dobson *et al.*, 2005). These studies showed that the particles deposited on the inner surfaces of the cassette could comprise a significant fraction of the aerosols that enter the sampler. The incidence of particle deposits on the estimation of exposure, the conformity of the sampling devices with sampling conventions, and the definition of the sample itself have been reviewed and discussed (Baron, 2003; Harper and Demange, 2007; Brisson and Archuleta, 2009). Findings regarding certain pollutants led to modifications of protocols so as to ensure the incorporation of the deposits in the analysis (INRS, 2003; OSHA, 2006; NIOSH Manual of Analytical Methods, 2013).

However, this issue has not been reported in the scientific literature on endotoxins (Duquenne *et al.*, 2013). Indeed, we found only one field study highlighting the presence of endotoxins on the inner surfaces of the cassette, though for a very small number of samples (Walters *et al.*, 1994). Another study carried out with experimental bioaerosols produced in laboratory conditions from pure bacterial cultures has also reported similar observations (Simon *et al.*, 2011). In addition, the endotoxin deposits on the inner cassette walls have not been dealt with in the existing reference methods (Duquenne *et al.*, 2013). Our work published here included a large number of samples, therefore making a new contribution to current knowledge on the subject.

Mechanisms involved in particle deposition on inner cassette surfaces

The particles that penetrate into the cassette can be deposited on the inner surfaces during sampling itself, when handling the cassettes after sampling and during their transport to the laboratory. However, the mechanisms involved in deposits on the inner surfaces of aerosol samplers have not been clarified and additional studies are required. Particle bounce, electrostatic forces, inertial impaction, gravitational settling, and the size and density of particles as well as turbulence of air flows in the cassette are probably involved (Blackford *et al.*, 1985). Walters *et al.* (1994) suggest that the relative humidity of the air in their tests affected the quantity of endotoxins deposited in the cassettes. Furthermore, studies carried out on lead showed that there was no qualitative difference in the size distribution of particles deposited on the filter and those on inner surfaces of the cassettes (Lee *et al.*, 2009; Chisholm *et al.*, 2012) and thus no basis for excluding them as material that would not also be inhaled by a worker. A multiple regression analysis from our results (data not shown) did not show any significant effect of temperature and relative humidity on endotoxins deposited on the inner surfaces of CFCs in our study, both when results are expressed in EU m^{-3} ($r^2 = 0.0$; $n = 240$; $P = 0.3727$) and in % ($r^2 = 0.99$; $n = 240$; $P = 0.09$). Operators usually handle cassettes carefully in the field to prevent material from being dislodged from the filter, but transport of cassettes by courier may involve rougher handling that could result in transfer of material from the filter to the inner surfaces of the cassette. However, this typically only occurs with very high dust loadings. Since the aim of our study was not to elucidate these mechanisms or to better define the determinants of endotoxin deposits on the inner surfaces of CFCs, our results do not provide information on this subject.

Significance of inner cassette surface endotoxin deposits on measurement variability and interpretation of concentration levels

Our results show that separating inner cassette surface deposits from analysis when evaluating exposure to endotoxins is a major source of error in measurements relative to sampling all particles entering the CFC sampler, in line with a developing consensus on matching the inhalable particulate sampling convention. In our results the percentage of deposits on the inner surfaces of >30% of samples was >40% of the total sample. Therefore, establishing concentrations measured only on the basis of filter analyses leads to a variable systematic error. Analyses performed by two laboratories on samples assumed to be identical could therefore lead to considerably different analytical results. The inconsistency of ratio between concentration of total endotoxins (deposited on filter + inner

surfaces) and concentration measured with endotoxins deposited on filter only does not allow a correction factor to be used to convert filter-only values.

Our results also show that omitting deposits on the inner surfaces of the CFCs from analysis leads to underestimating the concentrations measured. As yet there is no OEL for airborne endotoxins but several recommendations have been formulated or proposed to facilitate the interpretation of measurement data. Rylander (1997) suggested a 'no effect' level (i.e. absence of inflammation of the respiratory tract) at ~10 ng m^{-3} (~100 EU m^{-3}). He also proposed a level of 100 ng m^{-3} (~1000 EU m^{-3}) for the occurrence of systemic effects and 200 ng m^{-3} (~2000 EU m^{-3}) for the occurrence of symptoms linked to organic dust toxic syndrome. In spite of these recommendations, no regulatory value is available at present. An OEL of 50 EU m^{-3} was proposed as early as 1998 in the Netherlands (Heederik and Douwes, 1997). Finally, in 2001, the Dutch Ministry of Labor raised this value to 200 EU m^{-3}, which was a reference for several years before being abandoned. In July 2010, an expert committee of the Dutch Ministry of Health published a report proposing a new OEL of 90 EU m^{-3} (Dutch Expert Committee on Occupational Standards, 2010). These different values were established on the basis of workplace studies whose measurement protocols did not take into account endotoxins deposited on the inner surfaces of samplers. It therefore appears difficult to draw hasty conclusions from our study on the effects of concentration levels on health. However, our results suggest that work is needed to determine the extent to which the decision to consider a result as acceptable or not can be affected by the inclusion of endotoxins deposited on the inner surfaces of cassettes in calculations.

Possible alternative techniques

Technical measures could be developed to incorporate cassette inner surface deposits in these measurements. Firstly, it may be possible to improve extraction protocol. Elution could be performed in the cassette with possible additional wiping of its inner walls. Elution would be performed separately or in addition to that done for the filter (Harper, 2006; OSHA, 2006) or directly in the cassette containing the filter (INRS, 2003). Another alternative would be to place an ACCU-CAP™ type cartridge in the cassette, which is a solution studied for sampling aerosols intended for non-microbiological analyses (Görner *et al.*, 2010; Kauffer *et al.*, 2010; Lee *et al.*, 2011, Harper and Ashley, 2012, 2013). The cartridge would allow recovering all the particles sampled in a single elution step. The two alternatives involve several technical challenges as the materials used must be compatible with endotoxin sampling and their analysis. In particular, these materials must be pyrogen-free and they must neither absorb the endotoxins nor release substances interfering with LAL analysis. The polystyrene composing the cassette has been shown to be compatible with LAL analysis (Novitsky *et al.*, 1986). It is also the main component of the microtitration plate used during analysis in our study. Nonetheless, the properties of plastic may vary from one product supplier, or batch, to another and their use should be validated systematically. A third approach could consist in replacing the CFC by another sampler with equivalent performances and not subject to deposits of particles on the inner walls of the device. In a recent review of the literature (Duquenne *et al.*, 2013), we established that several devices could be used for sampling airborne endotoxins. For some of them, e.g. the IOM sampler, the deposit of particles on the inner surfaces of the device was highlighted during studies of non-biological aerosols (Liden *et al.*, 2000; Witschger *et al.*, 2004). The implementation of others CIP 10-M and the high-volume electrostatic field sampler remains either less practical than that of the cassette or else they require additional studies and research in order to be validated (Duquenne *et al.*, 2013). The Button sampler, which has limited internal surfaces, maybe a viable candidate but the choice of such a device from among others should be subject to further discussion.

Conclusions

Our study showed that endotoxins deposited on the inner cassette surfaces during sampling and/or when transporting the samples. These deposits can comprise a large and variable proportion of the endotoxins sampled. Our results show that separating inner cassette surface deposits from analysis when evaluating exposure to endotoxins is a major source of error in measurements relative to sampling all particles entering the CFC sampler, in line with a developing consensus on matching the inhalable particulate sampling convention. Our results suggest that the endotoxins deposited on the inner surfaces of the cassettes should be included in analyses. CDC/NIOSH have published

recommendations to address the matter of aerosol sampler wall deposits (Ashley and Harper, 2013). However, additional research is needed to determine whether or not to consider the deposits on the inner surfaces of CFCs for endotoxin exposure and to define alternative technical solutions. Attention should be given to this subject in any project aimed at standardizing measurement methods (Duquenne *et al.*, 2013). Further tests are required to investigate the magnitude of endotoxin deposits on the inner cassette surfaces in other working environments.

SUPPLEMENTARY DATA

Supplementary data can be found at http://annhyg.oxfordjournals.org/.

FUNDING

INRS (Institut National de Recherche et de Sécurité) which is founded by the French National salaried workers' health insurance fund ('http://www.ameli.fr/' \t '_blank' CNAMTS).

ACKNOWLEDGEMENTS

The authors would like to thank Pascal Poirot and Thérèse Nicot of the INRS Process Engineering Department, and Dominique Rousselle and Mathieu Dziurla of the INRS Occupational Epidemiology Department, who participated in collecting the samples. The authors are also grateful to Catherine Coulais and Cécile Piernot for their participation in performing the tests. The authors declare no conflict of interest relating to the material presented in this Article.

DISCLAIMER

Its contents, including any opinions and/or conclusions expressed, are those of the authors.

REFERENCES

Ashley K, Harper M. (2013) Closed-face filter cassette (CFC) sampling-guidance on procedures for inclusion of material adhering to internal sampler surfaces. *J Occup Environ Hyg*; 10: D29–33.

Awan S, Burgess G. (1996) The effect of storage, handling and transport traumas on filter-mounted dusts. *Ann Occup Hyg*; 40: 525–30.

Baron P. (2003) Chapter O - Factors affecting aerosol sampling. *NIOSH Manual of Analytical Methods*. Cincinnati, OH: Centers for Disease Control and Prevention. pp. 184–207. Available at www.cdc.goc/niosh/nmam.

Basinas I, Schlünssen V, Heederik D *et al.* (2012) Sensitisation to common allergens and respiratory symptoms in endotoxin exposed workers: a pooled analysis. *Occup Environ Med*; 69: 99–106.

Blackford DB, Harris GW, Revell G. (1985) The reduction of dust losses within the cassette of the SIMPEDS personal dust sampler. *Ann Occup Hyg*; 29: 169–80.

Brisson MJ, Archuleta MM. (2009) The real issue with wall deposits in closed filter cassettes–what's the sample? *J Occup Environ Hyg*; 6: 783–8.

Comité Européen de Normalisation. (2003) *Workplace atmosphere - Determination of airborne endotoxins (CEN standard EN 14031)*. Brussels, Belgium: CEN.

Chisholm WP, Lee T, Slaven JE *et al.* (2012) Comparison of filter and wall deposits from samplers used to collect airborne lead-containing dusts at field sites. *Aerosol Sci Technol*; 46: 411–8.

Darragh AH, Buchan RM, Sandfort DR *et al.* (1997) Quantification of air contaminants at a municipal sewage sludge composting facility. *Appl Occup Environ Hyg*; 12: 190–4.

Dutch Expert Committee on Occupational Standards. (2010) *Endotoxins: health-based recommended occupational limit*. Publication No. 2010/04OSH. The Hague: The Health Council of the Netherlands.

Demange M, Gendre JC, Herve-Bazin B *et al.* (1990) Aerosol evaluation difficulties due to particle deposition on filter holder inner walls. *Ann Occup Hyg*; 34: 399–403.

Demange M, Görner P, Elcabache JM *et al.* (2002) Field comparison of 37-mm closed-face cassettes and IOM samplers. *Appl Occup Environ Hyg*; 17: 200–8.

Dobson L, Reichmann L, Popp D. (2005) Evaluation of quartz residue on cassette interiors of AIHA proficiency samples. *J ASTM Int*; 2: 1–6.

Duquenne P, Marchand G, Duchaine C. (2013) Measurement of endotoxins in bioaerosols at workplace: a critical review of literature and a standardization issue. *Ann Occup Hyg*; 57: 137–72.

Duquenne P, Simon X, Koehler V *et al.* (2012) Documentation of bioaerosol concentrations in an indoor composting facility in France. *J Environ Monit*; 14: 409–19.

Dutil S, Meriaux A, de Latremoille MC *et al.* (2009) Measurement of airborne bacteria and endotoxin generated during dental cleaning. *J Occup Environ Hyg*; 6: 121–30.

Görner P, Simon X, Wrobel R *et al.* (2010) Laboratory study of selected personal inhalable aerosol samplers. *Ann Occup Hyg*; 54: 165–87.

Harper M. (2006) A review of workplace aerosol sampling procedures and their relevance to the assessment of beryllium exposures. *J Environ Monit*; 8: 598–604.

Harper M, Andrew ME. (2006) Airborne endotoxin in woodworking (joinery) shops. *J Environ Monit*; 8: 73–8.

Harper M, Pacolay B. (2006) A comparison of X-ray fluorescence and wet chemical analysis for lead on air filters from different personal samplers used in a secondary lead smelter/solder manufacturer. *J Environ Monit*; 8: 140–6.

Harper M, Demange M. (2007) Concerning sampler wall deposits in the chemical analysis of airborne metals. *J Occup Environ Hyg*; 4: D81–6.

Harper M, Ashley K. (2012) Preliminary studies on the use of acid-soluble cellulose acetate internal capsules for workplace metals sampling and analysis. *J Occup Environ Hyg*; 9: D125–9.

Harper M, Ashley K. (2013) Acid-soluble internal capsules for closed-face cassette elemental sampling and analysis of workplace air. *J Occup Environ Hyg*; 10: 297–306.

Heederik D, Douwes J. (1997) Towards an occupational exposure limit for endotoxins? *Ann Agric Environ Med*; 4: 17–9.

Hendricks W, Stones F, Lillquist D. (2009) On wiping the interior walls of 37-mm closed-face cassettes: an OSHA perspective. *J Occup Environ Hyg*; 6: 732–4.

INRS. (2003) Fiche METROPOL 003: métaux et métalloïdes. Recueil-Métropol. Available at http://www.inrs.fr/.

INRS. (2010) Fiche METROPOL 089/V02: Endotoxines. Recueil-Métropol. Available at http://www.inrs.fr/.

Kauffer E, Wrobel R, Görner P *et al.* (2010) Site comparison of selected aerosol samplers in the wood industry. *Ann Occup Hyg*; 54: 188–203.

Lafontaine M, Vu-Duc T, Delsaut P *et al.* (1999) Aerosols deposits on the inner cassette walls during Pah sampling: underestimation of the inhaled fraction and of the occupational risk. *Poly Arom Compounds*; 17: 221–8.

Laitinen SK, Kangas J, Husman K *et al.* (2001) Evaluation of exposure to airborne bacterial endotoxins and peptidoglycans in selected work environments. *Ann Agric Environ Med*; 8: 213–9.

Lee T, Chisholm WP, Slaven JE *et al.* (2009) Size distributions of 0.5 to 20 μm aerodynamic diameter lead-containing particles from aerosol sampler walls and filters. *Aerosol Sci Technol*; 43: 1042–50.

Lee T, Harper M, Slaven JE *et al.* (2011) Wood dust sampling: field evaluation of personal samplers when large particles are present. *Ann Occup Hyg*; 55: 180–91.

Lidén G, Melin B, Lidblom A *et al.* (2000) Personal sampling in parallel with open-face filter cassettes and IOM samplers for inhalable dust–implications for occupational exposure limits. *Appl Occup Environ Hyg*; 15: 263–76.

Liebers V, Brüning T, Raulf-Heimsoth M. (2006) Occupational endotoxin-exposure and possible health effects on humans. *Am J Ind Med*; 49: 474–91.

Liebers V, Raulf-Heimsoth M, Linsel G *et al.* (2007) Evaluation of quantification methods of occupational endotoxin exposure. *J Toxicol Environ Health A*; 70: 1798–805.

Madsen AM. (2006) Airborne endotoxin in different background environments and seasons. *Ann Agric Environ Med*; 13: 81–6.

NIOSH Manual of Analytical Methods (NMAM®), 4th edn., DHHS (NIOSH) Publication 94–113, 3rd Supplement 2003–154, update, 2013. Available at http://www.cdc.gov/niosh/docs/2003–154/cassetteguidance.html.

Novitsky TJ, Schmidt-Gengenbach J, Remillard JF. (1986) Factors affecting recovery of endotoxin adsorbed to container surfaces. *J Parenter Sci Technol*; 40: 284–6.

OSHA. (2006) Method ID215, version 2 - Hexavalent chromium. In *OSHA sampling and analytical methods*. Sandy, UT: OSHA, Salt Lake Technical Center. U.S. Department of Labor | Occupational Safety & Health Administration. Available at www.osha.gov/dts/sltc/methods.

Puskar MA, Harkins JM, Moomey JD *et al.* (1991) Internal wall losses of pharmaceutical dusts during closed-face, 37-mm polystyrene cassette sampling. *Am Ind Hyg Assoc J*; 52: 280–6.

Rylander R. (1997) Evaluation of the risks of endotoxin exposures. *Int J Occup Environ Health*; 3: S32–5.

Rylander R. (2002) Endotoxin in the environment–exposure and effects. *J Endotoxin Res*; 8: 241–52.

Sigsgaard T, Bonefeld-Jørgensen EC, Hoffmann HJ *et al.* (2005) Microbial cell wall agents as an occupational hazard. *Toxicol Appl Pharmacol*; 207 (Suppl. 2): 310–9.

Simon X, Duquenne P, Koehler V *et al.* (2011) Aerosolisation of *Escherichia coli* and associated endotoxin using an improved bubbling bioaerosol generator." *J Aerosol Sci*; 42: 517–31.

Smit LA, Heederik D, Doekes G *et al.* (2010) Occupational endotoxin exposure reduces the risk of atopic sensitization but increases the risk of bronchial hyperresponsiveness. *Int Arch Allergy Immunol*; 152: 151–8.

Smit LA, Spaan S, Heederik D. (2005) Endotoxin exposure and symptoms in wastewater treatment workers. *Am J Ind Med*; 48: 30–9.

Soo JC, Lee T, Kashon M *et al.* (2014) Quartz in coal dust deposited on internal surface of respirable size selective samplers. *J Occup Environ Hyg*; 11: D215–9.

Spaan S, Wouters IM, Oosting I *et al.* (2006) Exposure to inhalable dust and endotoxins in agricultural industries. *J Environ Monit*; 8: 63–72.

Tolvanen O, Nykänen J, Nivukoski U *et al.* (2005) Occupational hygiene in a Finnish drum composting plant. *Waste Manag*; 25: 427–33.

Walters M, Milton D, Larsson L *et al.* (1994) Airborne environmental endotoxin: a cross-validation of sampling and analysis techniques. *Appl Environ Microbiol*; 60: 996–1005.

Witschger O, Grinshpun SA, Fauvel S *et al.* (2004) Performance of personal inhalable aerosol samplers in very slowly moving air when facing the aerosol source. *Ann Occup Hyg*; 48: 351–68.