ENDOTOXINS

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1. Introduction

Endotoxin is produced by Gram-negative bacteria, which are common in the environment, particularly in water or in water damaged conditions. It has been associated with many respiratory symptoms and complaints related to the indoor environment.

We recommend that sampling and testing for endotoxin during microbial IAQ investigations and surveys replace air sampling for bacteria. The reasons are two-fold. Air sampling for endotoxin allows for collecting a larger air volume (240 to 1000 L) as well as a longer sample duration (2-8 hours), in contrast with 1-5 minutes (28.3 to 141.5 L) for Andersen sampling. Endotoxin is considered to be the major bioactive agent of Gram-negative bacteria and has been linked to various respiratory symptoms (see below). Measurements of endotoxin have been correlated with IAQ complaints and respiratory diseases in office buildings as well as occupational settings.

2. Why sample for endotoxins?

Endotoxin is fever causing and has many patho-physiological effects associated with Gram-negative bacterial infection and bacteremia. Response to endotoxin exposure varies with dose, site, or route, and rapidity of release into the blood system. Sub-lethal dose exposure causes dramatic changes in human body temperature, in the hematological, immune, and endocrine systems, and in metabolism.

The inhaled endotoxin has been associated with many pulmonary diseases. Endotoxin has been thought to be responsible for the adverse health effects after inhalation of organic dusts. Some inhalation studies showed that endotoxin can cause fever, cough, dyspnea, headache, nose and throat irritation, diffuse aches, nausea, shortness of breath & chest tightness, acute air flow obstruction, and airway inflammation. Endotoxin exposure may also result in low lung function. In the indoor environment, chest tightness, mild fever, and flu-like symptoms experienced by building occupants may be associated with endotoxin exposure.

3. How to sample for endotoxins

Endotoxins may be found in air, water, or carpet dust. The methodology for air, dust, and water sampling and testing for endotoxin is available in the literature. An endotoxin-free filter cassette with a selected membrane filter can be used for air sampling and dust sampling. Water samples are collected with a container that is endotoxin-free and does not bind endotoxin. Details of sampling protocol are available on the next page.

4. Sampling strategy

Although proposed guidelines for airborne endotoxin are available, comparative sampling (indoor vs. outdoors; complaint vs. non-complaint indoor references) is highly recommended. In addition, dust and bulk liquid (most likely water) samples from problem and control buildings should be sampled for comparison and source identification.

5. Select your analytical methods

The current standard analytical method for endotoxin is the *Limulus* amebocyte lysate (LAL) assay. Recent advances also allow LAL reagent to be specific to bacterial LPS (without the interference of glucan). Several variations of LAL methods are available. The gel-clot method is the simplest, least expensive, and has good sensitivity. Turbidimetric assay is considered a "more quantitative measurement" of endotoxin over a range of concentrations. The kinetic chromogenic method is much more accurate, sensitive, and reproducible than the gel-clot or turbidimetric methods. *The kinetic chromogenic method, which is used by EMLab P&K, has become the method of choice for endotoxin analysis.*

6. What do the results mean?

In 1997, Dr. R. Rylander proposed guidelines for "no-effect level" for environmental endotoxins (see table below). The guidelines were developed based on challenge studies and field studies. This set of guidelines are based on samples collected with glass-fiber filters, extracted for 15 minutes to one hour, centrifuged for 10 minutes at 1,000g, and analyzed with the LAL kinetic chromogenic method. It is important to note that exposure levels which cause irritation effects in the respiratory system may be lower that 10 ng/m^3 (also see discussion of granulomatous pneumonitis by Rose et al., 1998).

Disease	Conc. ng/m^3
Airway inflammation	10
Systemic effects	100
Toxic pneumonitis	200

Note: 1 ng endotoxin may be approximately equal to 10 to 15 endotoxin units (EU).

Douwes and Heederik (1997) suggested that an exposure level of 1-20 ng/m^3, based on studies of cotton workers, has an "adverse respiratory health effect", as defined by the American Thoracic Society guidelines.

Most of what we know regarding diseases caused by endotoxin is related to occupational exposures. Environments with exposure to endotoxin include agricultural, industrial, waste processing, and office buildings. Various endotoxin measurements have been reported from these environments. They varied from the below detection limit to greater than 4,000 ng/m^3. Two European studies in office buildings reported endotoxin levels of 0.064-0.018 and 35-254 ng/m^3, respectively. In a large study involving 19 offices and 1355 occupants in the Netherlands, higher levels of Gram-negative bacteria and endotoxin (0.25 ng/m^3 vs. 0.05 ng/m^3) were reported in offices where occupants reported respiratory or pulmonary problems. In a recent report, Reynolds et al. (2001) found that reported symptoms were correlated with indoor endotoxin levels in six mid-western US buildings. Some of the reported levels were below 1 EU/m^3. *Our experience suggests that indoor endotoxin levels over 1 EU/m^3 are rather unusual unless there is water related problem leading to amplification of gram-negative bacteria. Keep in mind that this has not been correlated with any illness or IAQ symptoms.*

Epidemiologic studies, conducted with cotton mill workers by Kennedy et al. (1987) and animal feed workers by Smid et al. (1992), suggested threshold levels below 20 ng/m^3 for both acute and chronic effects. Smid et al. suggested threshold levels of 3-7.5 ng/m^3. In a fiberglass manufacturing facility, Milton et al. found evidence that acute effects on lung function of workers were observed at endotoxin exposure levels as low as 4 to 15 ng/m^3 (geometric mean = 8.4 ng/m^3). More recently, Rose et al. (1998) reported that airborne endotoxin levels of 76 and 28 EU/m^3 (endotoxin levels in pool water were 240 and 150 EU/ml, respectively) were associated with granulomatous pneumonitis (lifeguard lung) in an indoor swimming pool. Life guards working longer hours (31 vs. 23 hours; p = 0.09) were found to have higher incidences of the disease. Therefore, longer exposures to endotoxin levels as low as 28 EU/m^3 may cause significant respiratory and lung diseases. However, until a generally accepted clinical guideline is available, comparisons of results from indoors, outdoors, complaint and non-complaint areas are suggested. Keep in mind that exposure to airborne endotoxin levels as low as 28 EU/m^3 (approximately 2 – 3 ng/m^3) for an extended period may result in significant damage to the respiratory system and the lung.

7. Sources of Gram-negative bacteria and endotoxin

Gram-negative bacteria are ubiquitous in the environment. They are associated with plants and animals, and are found in the oral cavities and intestinal tracts of mammals. They are especially plentiful in water. They have been reported in contaminated ventilation systems and humidifiers. In the swimming pool study (Rose et al., 1998), significant levels of Gram negative bacteria, including *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Pseudomonas* spp., and *Acinetobacter calcoaceticus* were identified. We have observed Gram-negative bacteria in large numbers in water damaged building materials, including fire proofing spray-on, carpet, ceiling tile, drywall, etc.

Endotoxin is produced by Gram-negative bacteria during growth, division, death or lysis. Therefore, where there are Gram-negative bacteria, there is endotoxin. It has been detected in the air, in dust, and in water. Endotoxin exposures are mainly through the air. Endotoxin in dust or in water must be aerosolized to cause the exposure.

8. Control and prevention

Gram-negative bacteria and endotoxin are ubiquitous in environment with water. Controlling water reduces possible growth of Gram-negative bacteria, and hence, the levels of endotoxin. Endotoxins are very stable. Therefore, heat and chemical treatments are not practical. In the event excessive levels of endotoxins are detected, removal of contaminated materials and HEPA vacuuming can reduce endotoxins.

9. References

1. Rylander, R. (edited) 1997. Endotoxins in the environment: A criteria document. Int. J. Occup. And Env. Health, supp. 3(1):S1-S48.

2. Rylander, R. 1994. Endotoxins in "Organic dusts: Exposure, Effects, and Prevention", edited by R. Rylander & R. R. Jacobs. Lewis Publishers.

3. Rose C. S., J. W. Martyny, L. S. Newman, D. K. Milton, T. E. King, Jr., J. L. Beebe, J. B. McCammon, R. E. Hoffman, and K. Kreiss. 1998. "Lifeguard Lung": Endemic granulomatous pneumonitis in an indoor swimming pool. Am J Public Health 88: 1795-1800.

4. Reynolds et al. 2001. Indoor environmental Quality in Six Commercial Office Buildings in the Midwest United States. Appl. Occup. Env. Hygiene 16: 1065-1077.

Sampling protocol for endotoxins

1. Liquid samples

a) Obtain sterile 15ml screw-cap containers that are free of detectable endotoxin for sampling. Obtain an extra container as a field blank.
b) Keep the sampling container closed until it is used. Fill up the container with water.
c) Tightly cap the bottles. Make sure that water will not leak out during shipping and transporting.

2. Bulk dust samples

a) Obtain 3-piece 37-mm closed-face cassettes, preloaded with 0.45 um pore-size filters for sampling. Obtain an extra as a field blank.
b) A sufficient amount of dust is required for analysis, preferably 0.1 g or more.
c) Vacuum with a pump at 10-15 lpm for at least five minutes at the sampling sites.

3. Air samples

a) Obtain 3-piece 37-mm closed-face cassettes, preloaded with 0.45 µm pore-size filters, for sampling. Obtain an extra as a field blank.
b) Sufficient air volume is required for analysis, a minimum of 240 L (based on 2 l/min for 120 min using personal pump) or more (2-4 l/min and up to 1,000 L) is suggested.

4. Chain of Custody (COC)

a) Write the sample number on the container/cassettes and on C-O-C sheet. Use a distinctive number for each sample.
b) Complete all sample information on C-O-C sheet, such as sampling date(s), air volume, time, location, your project or job number, purchase order number(s) for the job, your name, company name, phone and fax number. Keep your own record and send a copy with samples to the laboratory.

5. Shipping samples

a) For liquid samples, place containers in a clean plastic bag then put into an insulated box with blue ice or reusable ice packs to maintain the temperature between 2 to 8 °C. **Do not use ice cubes or dry ice.** Stuff the box with foam chips to provide sufficient cushion and seal the box securely for shipping.

b) Place air and dust samples in plastic bags and then in a cardboard box. Securely seal and tape the bag for shipping.
c) Send samples to the laboratory by overnight express carrier. Call and inform the laboratory. Take holidays into consideration.