THE ENDOTOXIN CRITERIA DOCUMENT: THE RISK EVALUATION

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Abstract

There is now abundant evidence that bacterial endotoxin is related to symptoms and diseases caused by organic dusts. Endotoxin is present in almost all kinds of organic dusts and airborne levels range up to several micrograms /m^3. Experimental studies demonstrate that the effects induced by organic dusts are not related to the dust level. There is thus a need for guidelines on the amount of airborne endotoxin. The Committee on organic dusts of the ICOH has completed work on an endotoxin criteria document. The information assembled in the document demonstrates that endotoxin causes several disease entities in terms of toxic pneumonitis, airways inflammation and possibly chronic bronchitis. The suggested guidelines for toxic pneumonitis is 200 ng/m^3 and for airways inflammation 10 ng/m^3.

Relation and causality

It is clear from the previous review of experimental and epidemiological investigations that a considerable number of studies has now shown a relation between exposure to dusts containing endotoxin and the presence of acute as well as chronic disease symptoms in humans. There is general agreement that organic dusts containing endotoxin have important biological effects and that they cannot be considered as nuisance dusts. There is also general agreement that a dust standard is not sufficient for protection, as the relative amount of endotoxin in different dusts may vary and thus the same dust levels could express differences in risk.

Even if a relation between the amounts of endotoxin and effects on humans has been found, this does not mean that there is causality. To conclude that a causative relationship exist, the following requirements must be met:

1. Endotoxin must be capable of producing the signs and symptoms of the disease when challenges with the pure substance are made in humans.
2. There must be a relation between the prevalence of the symptoms/disease and the endotoxin exposure levels.
3. There must be a decrease of symptoms after reduction in endotoxin exposure.

These requirements are generally met in the different studies reported. Organic dusts contain, however, many biologically potent agents such as bacterial enzymes, tannins, mycotoxins and the mold cell wall constituent (1→3)-β-D-glucan. Theoretically, endotoxin could be a marker for some or several of these agents. Furthermore, it is possible that the effects seen in endotoxin contaminated environments may be the result of a combined exposure to several of the bioactive agents, resulting in additive or synergistic effects. Such effects have been demonstrated between endotoxin and (1→3)-β-D-glucan in animal experiments [Fogelmark *et al* 1994] and between endotoxin and allergens [Hunt *et al* 1994].

On the other hand, experience from inhalation challenge studies as reviewed above, demonstrates that several of the effects seen in environments with endotoxin, can be reproduced by the inhalation of the pure substance. There is also evidence on dose-response relationships in several different environments and the clinical symptoms agree with results from animal exposures using purified endotoxin.

In spite of the limitations discussed earlier, it is thus reasonable to conclude that endotoxin can be used as a marker for disease risk. A review of exposure effects and guidelines for endotoxin exposure will be presented in the following.

Endotoxin exposure levels

The information on criteria and guidelines rely on data from challenge studies using purified endotoxin and field studies where endotoxin was measured in dust samples. Only a few studies have tried to relate the dose of endotoxin in those two settings.

In inhalation experiments using pure endotoxin and cell wall preparations from *Enterobacter agglomerans*, the amount of endotoxin was measured in the two preparations [Rylander *et al* 1989]. Comparing the biological affect in terms of the acute reduction of FEV_1, the Limulus value for the cell wall preparation underestimated the endotoxin content about three times. This suggests that the Limulus method detects only about 1/3 of biologically active endotoxin and that the remainder is present inside fragment of dust particles/bacterial cells , but still able to exert effects when deposited in the lung. If the same relation is present for endotoxin in dusts (where it chiefly appears on the bacteria or fragments thereof) the relation between a challenge dose of pure endotoxin and endotoxin in environmental samples becomes as illustrated in Table 1.

Table 1 shows that a value of 1 µg/m^3 of endotoxin, measured in an environmental sample (Environmental Endotoxin, EE), corresponds to about 10 µg of inhaled pure endotoxin under the conditions described. Obviously this calculation is based on the results of one study only and has several uncertainties but for the purpose of this document, the relationships in Table 2 will be used.

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Endotoxin exposure effects

Background

For a discussion of the various effects seen after exposure to endotoxin, it is useful to synthesize individual symptoms or alterations of clinical test outcomes into syndromes or diseases. Although not always corresponding exactly to the underlying pathology, there are important advantages with this approach, particularly when in communicating with persons with disease. In this context, the disease nomenclature described for organic dusts will be used [Rylander and Jacobs 1994].

Toxic pneumonitis

Toxic pneumonitis (inhalation fever, organic dust toxic syndrome) is an acute disease, characterized by an increase in body temperature, shivering, dry cough, and muscle and joint pains [Von Essen *et al* 1990]. The assembly of symptoms resembles influenza. The disease appears 4 to 6 hours after exposure, but it is short-lived and the symptoms have usually disappeared by the following day. Clinical findings are leukocytosis and an increased number of neutrophils and markers of inflammation in the airways. The disease affects the lung tissue as evidenced by the reduction of alveo-capillary gas transfer [Herbert *et al* 1992] and the restrictive lung function changes reported by Michel *et al* [1996]. It is likely that the macrophage is the major cell orchestrating the cellular inflammation. Inflammatory mediators have been found in the blood after exposure, suggesting a link between the local effects and systemic effects (see below).

After repeated exposures, there is adaptation, and the symptoms of toxic pneumonitis do not reappear until there is an unusually large exposure or there has been a period of absenteeism, such as during holidays.

In relation to organic dusts, there are many generic names such as mill fever, grain workers' fever and printers' fever. These are all toxic pneumonitis. The data on endotoxin as a causative agent are abundant.

Airways inflammation

Airways inflammation is an inflammatory process in the airway epithelium and the underlying mucosa which develops gradually during continuous exposure to endotoxin. There is an increased infiltration of inflammatory cells; in the early and acute stage neutrophils - and in chronic exposure conditions eosinophils and lymphocytes. Major effector cells are the macrophages and epithelial cells which orchestrate the reactivity and movement of other inflammatory cells.

Initially, airways inflammation is experienced as a dry cough and irritation in the nose or throat. This can proceed to a feeling of a "continuous cold", sinus symptoms and wheezing. An increased airway responsiveness may develop. This can be assessed using a methacholine or histamine test [Rylander and Bergström 1993; Carvalheiro *et al* 1995]. Persons with increased airway responsiveness experience subjective symptoms in the airways in dusty environments in general or when strong odors such as perfume are present.

In more advanced stages of the disease, there may be an impairment of lung function, usually measured as the decrease in forced expiratory volume during one second (FEV_1). Lung function can be impaired over the day (workshift) and the maximum fall in peak flow appears 6 to 8 hours after exposure. It can also be permanently decreased below predicted values or have a larger than normal decline with age. Data from longitudinal investigations on workers exposed to endotoxin contaminated dusts suggest that the acute reaction over a workshift is related to an increased risk to FEV_1 decline over several years [Schwartz *et al* 1995; Li *et al* 1995; Glindmeyer *et al* 1991].

The peak flow variations seen in airways inflammation are similar to those seen among workers with occupational asthma. In view of the similarity to asthma, the term "nonallergic asthma" has been suggested to underline its nature as a general inflammatory response, unrelated to sensitization by a specific agent.

It has been debated whether airways inflammation should be recognized as a disease or limited to a description of a symptom. As the condition can lead to decreases in working capacity, general symptoms and need medical treatment, it is justified to consider it a disease. Also, the definition of airways inflammation as a disease entity facilitates the understanding of a major effect induced by endotoxin contaminated dusts and the communication with the persons having the disease, avoiding the more dramatic diagnosis "asthma".

Endotoxin is a causative agent for airways inflammation.

Chronic bronchitis

Chronic bronchitis is characterized by an increased number of goblet cells in the epithelium, enlarged subepithelial glands and increased production of mucus in the airways. The mucus has altered rheological characteristics and is more viscous.

The condition causes a more or less continuous productive cough and shortness of breath as well as an obstructive defect in lung function. In some cases there is increased airway responsiveness. The increased amount of mucus renders the patient more susceptible to microbial infections in the airways.

Clinically, chronic bronchitis is diagnosed as productive cough for three months per year for at least two years. This definition is, however, purely operational, and persons not fulfilling these criteria may also suffer from the functional disturbances described above. The disease is common

among smokers, but nonsmokers exposed to air pollution in the general or occupational environment are also at risk.

The relation between airways inflammation and chronic bronchitis is not fully understood. On the one hand, it may be a continuum, reflecting a larger exposure dose or a longer exposure time. Epidemiological data suggest that endotoxin is a causative agent for chronic bronchitis but other agents may be required for the disease to develop.

Hypersensitivity pneumonitis, allergic asthma

There are no data suggesting that endotoxin is the causative agent for these diseases. It might facilitate the development and persistence of the diseases due to its inflammatogenic and adjuvant properties.

Systemic effects

Endotoxin can cause systemic effects. The classical effect is fever as described above in connection with toxic pneumonitis [Von Essen *et al* 1990]. Fever has been described in a variety of environments contaminated by endotoxin and also after challenge with the pure substance. Other systemic effects after endotoxin exposure are muscle pain, joint pains and excessive fatigue. It is likely that the systemic effects are caused by inflammatory mediators produced in the lung after inhalation of endotoxin and distributed to different parts of the body via the blood [Dunn *et al* 1992; Michel *et al* 1995].

Adjuvant effects

Endotoxin has a strong adjuvant effect on the reaction to antigens and increases the production of antibodies. There is only one study suggesting that this effect is found in endotoxin contaminated environments [Mattsby and Rylander 1986].

Guidelines

General concepts

Experience from the experimental as well as epidemiological studies suggests that the different diseases caused by endotoxin are related to different levels of exposure. There is also evidence for a large inter-individual variation in endotoxin sensitivity. Several investigations suggest that persons with atopy and/or asthma are risk groups which react at lower exposure levels. It is thus necessary to define guidelines both with respect to the particular effect and with respect to the particular group in the population. In the following, the different disease entities as described above will be related to experience in the low exposure range to identify the no reaction threshold. The relation between pure endotoxin and environmental endotoxin (EE) will be used as presented in Table 2.

Toxic pneumonitis

The first recorded experiment on toxic pneumonitis was made by Neal *et al* [1942] who exposed subjects to a filtrate of *Aerobacter cloacae*. No measurements of endotoxin values were made but the levels were probably quite high. Pernis *et al* [1961] produced symptoms of toxic pneumonitis among subjects who inhaled 30-60 ug endotoxin (3-6 µg/m^3 EE).

Results from later human challenge experiments demonstrated that subjective symptoms of toxic pneumonitis developed in healthy, previously unexposed subjects after exposure to 300 µg pure endotoxin (30 µg/m^3 EE) [Rylander *et al* 1989]. No symptoms were found at 20 µg of pure endotoxin (2 µg/m^3 EE) [Rylander *et al* 1989; Herbert *et al* 1992;, Sandström et al 1992, Michel *et al* 1995] or after exposure to 12 µg (1.2 µg/m^3 EE) [Jamison and Lowry 1986]. Symptoms of toxic pneumonitis were not present among a group of healthy volunteers exposed to 0.1 µg/m^3 [Rylander 1996].

Carbon monoxide diffusion can also be looked upon as an effect criterion of toxic pneumonitis. There was a decrease in 13 normal subjects after inhalation of 30 µg endotoxin (3 µg/m^3 EE) [Rylander *et al* 1989; Herbert *et al* 1992]. In the experiments reported by Jamison and Lowry [1986], a slight decrease in transfer factor was found at 12 µg (1.2 µg/m^3 EE). Muittari [1980] reported that carbon monoxide transfer decreased in normal persons exposed to an endotoxin contaminated bath water at a dose of 0.8-4 µg (0.08-0.4 µg/m^3 EE).

These studies suggest that 0.2 µg/m^3 or 200 ng/m^3 as measured with the techniques employed in laboratories with extensive experience from endotoxin analysis in the environment, is a level where toxic pneumonitis will not develop among normal individuals. There are no data on risk groups.

Airways inflammation

Airways inflammation has been evaluated using several different endpoints. A decrease in FEV_1 at 4-6 hours after exposure is one effect criterion. A small decrease in FEV_1 has been found after exposure to 30 µg endotoxin (3 µg/m^3 EE) but not at 20 µg (2 µg/m^3 EE) [Rylander *et al* 1989]. In healthy subjects who inhaled 0.01 µg (0.001 µg/m^3 EE), no decrease in FEV_1 was found [Rylander 1996]. In challenge studies with cotton dust, Castellan *et al* [1987] reported a no reactions threshold of 9 ng/m^3 in cotton dust among persons selected for reactive airways. In an experiment on cotton workers and previously unexposed persons, the threshold value for FEV_1 decreases after a 4 hour exposure was 0.17 µg/m^3 among non-smoking cotton workers and students and 0.08 µg/m^3 among smoking cotton workers [Haglind and Rylander *et al* 1984].

Airway responsiveness is also as a marker for airways inflammation that has been used in several studies on endotoxin. It was increased in normal subjects after inhalation of 300 µg (EE 30 µg/m^3). In cotton workers, airway responsiveness was increased at among those exposed to 0.03 µg/m^3 (Rylander and Bergström 1993].

These studies suggest that 0.01 µg/m^3 or 10 ng/m^3 of EE as measured with the techniques employed will not lead to an increased risk for airways inflammation in normal subjects or subjects with atopy.

Systemic effects

No precise information is available on the presence of joint pains, muscle pains or tiredness in relation to endotoxin levels. It is suggested that a lower guideline as that for toxic pneumonitis can be used (100 ng/m^3).

Adjuvant effects

No data are available which allow the formulation on guidelines for the adjuvant effects.

Guidelines summary

Table 2 gives an overview of the guidelines for endotoxin.

The values for environmental endotoxin refer to the endotoxin analysis techniques used by major research groups with relatively similar assay procedures. This involves sampling of airborne particles on a filter, washing in pyrogenfree water 15-60 minutes and asssaying the amount of endotoxin with the kinetic, chromogenic version of the Limulus assay. As the detailed assay procedures differ between researchers, there is an urgent need to develop standard procedure to assay for endotoxin.

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Table 1. Environmental endotoxin in relation to dose of pure endotoxin, assuming a ventilation of 3 m^3 during an 8 hour work day

Environmental endotoxin	1 µg/m^3
Biologically active amount	3.4 µg/m^3
Inhaled dose 8 hours, equivalent to pure endotoxin	10.2 µg

Table 2. Guidelines for no effect level for environmental endotoxin (EE). The guidelines are based upon persons with a history of atopy or asthma

Disease	**ng/m^3**
Toxic pneumonitis	200
Airways inflammation	10
Systemic effects	100