

Fungal spores - Time for an OEL?

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In 2007 the criteria document *139. Fungal spores* was completed in close cooperation with

***The Nordic Expert Group for Criteria
Documentation of Health Risk from Chemicals***

This document was published in *Arbete och Hälsa 2006* and can be downloaded from Göteborg Universitets website

http://www.medicine.gu.se/avdelningar/samhallsmedicin_folkhalsa/amm/aoh/2006_21/



Criteria documents are comprehensive reviews of the literature on

- Toxicology
 - *in vitro* studies
 - *in vivo* studies
 - human challenge studies
- Epidemiological studies

and includes information on

- Exposure
- Measurement methods
- Microbiology
- Immunology



Scope

- **Fungal and actinomycete spores**
- **Non-infectious outcomes**
- **Working populations**



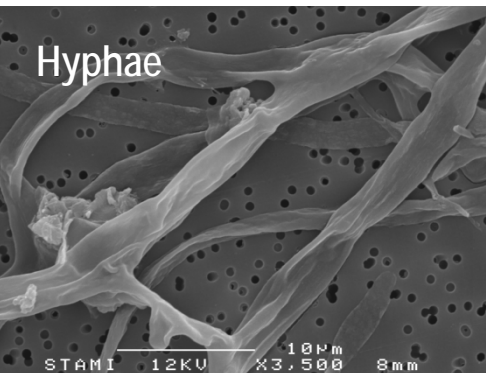
Contents

- Airborne fungal particles
- Mechanisms
- *in vivo* studies
- Human challenge studies
- Epidemiological studies of common indoor and highly exposed working populations
- Conclusions
- Impact until now

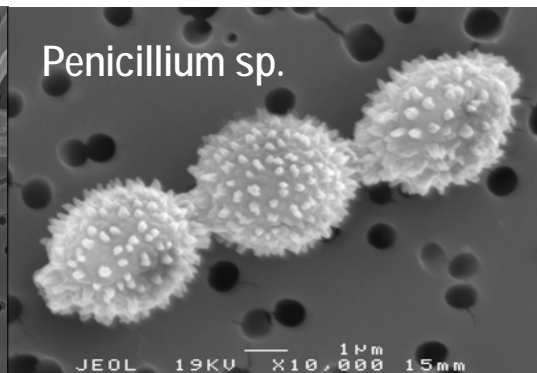


Airborne fungal agents

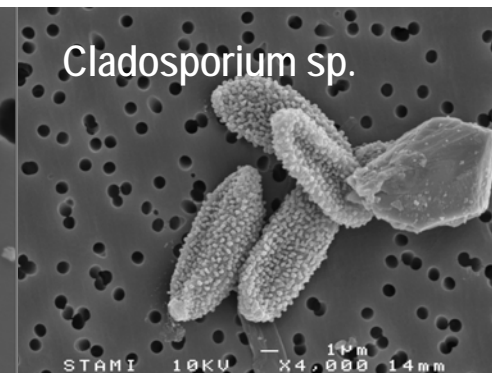
- Spores - single and aggregates
- Hyphae - few data, no established measurement methods
- Many different species - some produce mycotoxins
- Spores and hyphae can be viable or non-viable
- Hyphal fragments have been demonstrated during sporulation



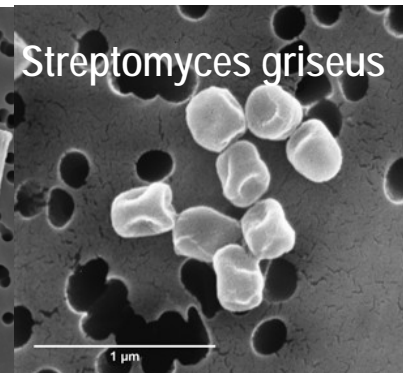
Hyphae



Penicillium sp.



Cladosporium sp.



Streptomyces griseus



Innate immuno-defense – non-allergic inflammation

- **Alveolar macrophages**
 - bind resting spores through toll-like and other receptors
 - phagocytise spores
 - kill phagocytised spores in the lysosomes
 - remove phagocytised spores through the mucociliary clearance
 - release pyrogenic $TNF\alpha$ and IL-6
 - release IL-8 that attracts neutrophils
- **Neutrophils**
 - bind hyphae and germinating spores
 - phagocytise and kill hyphae and germinating spores
 - released fungicidal chemokines that may cause tissue damage

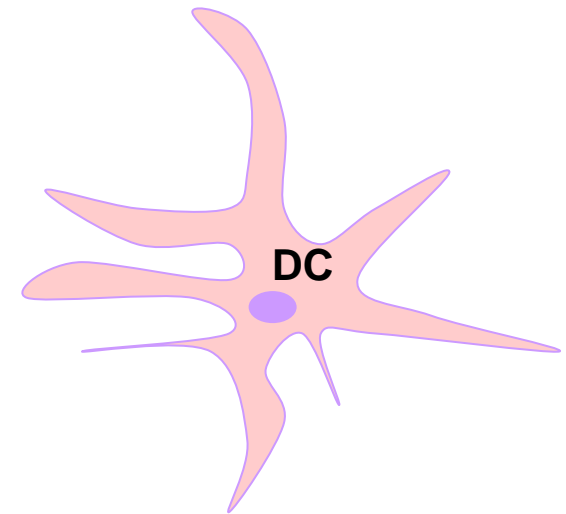


Adaptive immuno-defense

– allergic and non-allergic inflammation

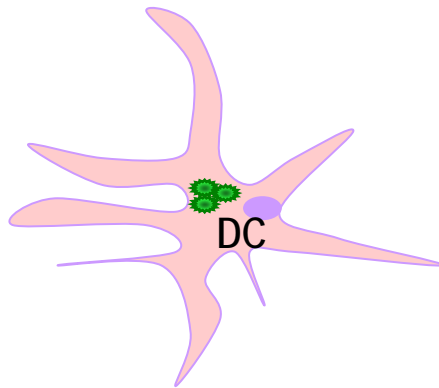
- Dendritic cells

- bind spores and hyphae
- phagocytise spores and hyphae
- migrate from the lung to the lymph nodes
- prime precursor Th cells (pTh)



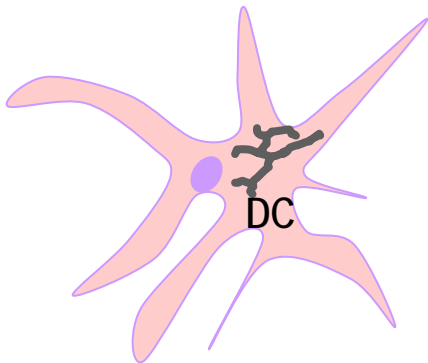


Ingestion of *spores* induces a non-allergic inflammation



- IL-12 production
- specific Th1 and B cells
- specific IgG production
- activation of alveolar macrophages

Ingestion of *hyphae* induces an allergic inflammation



- IL-4, IL-5 and IL10 production
- specific Th2 and B cells
- specific IgE production
- proliferation of eosinophils and mast cells
- activation of eosinophils and mast cells



Few fungal species have been recognised as allergenic, but allergens from few species have been characterised

The mostly prevalent allergenic species are

Cladosporium herbarum

Alternaria alternata

Aspergillus fumigatus

These species are also dominating species in outdoor air

It can therefore not be excluded that other species are allergenic

Kurup VP et al (2000). Respiratory fungal allergy. *Microbes Infect.* 2:1101-1110.

Lacey J (1981). The aerobiology of conidial fungi. In: The biology of conidial fungi. Vol I. T. Cole and W.B. Kendrick, eds., pp. 373–416. Academic Press, New York.



Summary of *in vivo* studies (1)

Single exposure studies

- Mainly transient **non-allergic** responses
- Also **allergic** responses after exposure to *Aspergillus fumigatus* and toxic *Stachybotrys chartarum*

Repeated exposure studies

- Lasting **non-allergic** responses
- **Allergic** responses in 3 of 4 studies
- Fibrosis in one study 90 days after very high exposures to *Saccharopolyspora rectivirgula*



Summary of *in vivo* studies (2)

Both single and repeated exposure studies show

- Dose-effect associations
- Species differences partly dependent on mycotoxin production



One *in vivo* study with repeated exposures to *Penicillium chrysogenum* showed

- non-allergic responses to methanol-killed spores
- allergic responses to viable spores

However, methanol treatment could also have removed mycotoxins from the spores

Cooley JD et al (2000). An animal model for allergic penicilliosis induced by the intranasal instillation of viable *Penicillium chrysogenum* conidia. *Thorax* 55:489-496.



Human challenge by inhalation of fungal spores

Licorish et al (1985) exposed mild asthma patients with specific IgE to the fungus to increasing doses of spores with an inhaler and calculated the dose that induced significant airway obstruction

The doses indicate following *Lowest Observed Effect Levels (LOEL)* when assumed inhaled during 8 hours

<i>Alternaria alternata</i>	$2 \cdot 10^4$ spores/m ³
<i>Penicillium</i> sp.	$1 \cdot 10^4$ spores/m ³

As the patients had specific allergy to these fungi, unsensitised individuals are expected not to respond to these levels

Licorish K et al (1985). Role of *Alternaria* and *Penicillium* spores in the pathogenesis of asthma. *J Allergy Clin Immunol.* 76:819-825.



Human challenge by inhalation of fungal spores

Meyer et al (2005) exposed school employees with sick building syndrome (SBS) to a single spore concentration of two fungi that were prevalent in the school building during 5 min

Airway obstruction, respiratory and systemic symptoms or inflammation were not increased

The doses indicate following *No Observed Effect Levels* (NOEL) when assumed inhaled during 8 hours

<i>Penicillium chrysogenum</i>	$4 \cdot 10^3$ spores/m ³
<i>Trichoderma harzianum</i>	$2 \cdot 10^3$ spores/m ³

Meyer H W t al (2005). Double blind placebo controlled exposure to molds: exposure system and clinical results. *Indoor Air* 15 (Suppl 10):73-80.



Epidemiological studies - short-term effects in farmers, waste handlers and sawmill workers

LOEL*, spores/m³

Effects

1·10⁵ - 2·10⁵

nose and eye irritation

lung function changes

nasal congestion

nasal inflammation (neutrophils)

3·10⁵ - 3·10⁶

cough

1·10⁸ - 4·10⁸

fever attacks in farmers' lung patients

2·10⁹

fever attacks (inhalation fever, ODTs)

* assuming 1 cfu=10 spores

ODTS = organic dust toxic syndrome



Epidemiological studies - long-term effects in farmers, waste handlers, school staff, sawmill-, wood-, cork- and day-care workers

LOEL* , spores/m ³	Effects
1 · 10 ⁵ - 4 · 10 ⁵	bronchitis symptoms, wheeze
5 · 10 ⁵	lung function changes (FEV ₁ , FVC)
8 · 10 ⁶	non-atopic asthma
	decreased atopic asthma
10 ⁷	X-ray changes

* assuming 1 cfu=10 spores



Conclusions

- *in vivo* studies show inflammatory potential of all tested species
- Mycotoxin containing spores and spores from pathogenic fungi were more toxic than most other species
- Human challenge studies indicate a LOEL for common fungi of $>1.10^4$ spores/m³ in healthy subjects
- Epidemiological studies indicate LOELs in various highly exposed populations of $\geq 1.10^5$ spores/m³ for respiratory symptoms, lung function changes and airway inflammation



Time for an OEL?

There seem to be fairly similar effect levels at $1 \cdot 10^5$ spores/m³ (LOEL) for fungal spores except from mycotoxin producing and pathogenic species that are more toxic

- 👉 To my opinion this allows for setting an OEL for fungal spores except mycotoxin producing and pathogenic species



Are these findings valid for common indoor air?

- Relatively few studies were found of populations exposed to common indoor air and only one good (negative) study
- However, the human challenge studies indicate that exposure levels of $2 \cdot 10^3$ - $4 \cdot 10^3$ spores/ m^3 from common indoor fungi are too low to induce a response in sensitive individuals (with SBS)
- The most prevalent species in the office environment were not particularly toxic

Species	Mean rank*
<i>Cladosporium</i> sp.	1.3
<i>Penicillium</i> sp.	1.4
Yeasts	3.0
<i>Aspergillus</i> sp.	3.5

👉 As yet, there is no reason to believe that fungal spores in indoor air have higher toxicity than in highly contaminated environments



Research challenges

- **Exposure assessment of**
 - viable and non-viable spores and hyphae
 - fungal allergens
 - hyphal fragments
 - mycotoxins
- **Species specific exposure assessment**
- **Epidemiological studies of sensitisation and allergic outcomes in high and low exposed populations**
- **Detection of IgE to fungal species**
- **Epidemiological studies of mycotoxin producing and opportunistic pathogenic species**
- **The role of fungal agents in indoor air complaints (spores, hyphae, fragments, mycotoxins, MVOC)**



Impact of this criteria document per 26 august 2008

- NIVA course Mould problems in indoor environments in Danmark



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Letter to the Editor

A health-based criteria document on fungal spore exposure in the working population. Is it relevant for the general population?

- Mouldy house case in Sweden – mycotoxin detected in settled dust
- Direktoratet for Arbeidstilsynet of Norway
not yet on the current list of agents that will be (re)evaluated