Toxicity screening of materials from buildings with fungal indoor air quality problems (*Stachybotrys chartarum*)

Johanning E*, Gareis M2, Yang Chin S3, Hintikka E-L4, Nikulin M5, Jarvis B6, and Dietrich R7

1 Mount Sinai School of Medicine, Dep. Community Medicine, Eastern New York Occupational and Environmental Health Center, Albany, N.Y., Tel. 518 436 5511  
e-mail: johanni2@knick.net  
2 Institute for Microbiology and Toxicology, Federal Meat Research Institute, Kulmbach, Germany.  
3 P&K Microbiology, Cherry Hill, NJ  
4,5 National Veterinary and Food Research Institute, Helsinki, Finland  
6 Dep Chemistry and Biochemistry, University of Maryland, College Park, MD  
7 Institute for Hygiene and Technology of Food of Animal Origin, Veterinary Faculty, University of Munich, Munich, Germany

Abstract:

Samples of building materials visibly contaminated with moisture-related fungi (drywall, fiberglass, wallpaper, wood) were tested with indirect (FFL) and direct (MTT) cytotoxicity screening tests that are particularly sensitive to *Stachybotrys chartarum* toxins. In addition, microscopic, chemical, immunochemical (Roridin A enzyme immunoassay) and mycological culture analyses were performed. In all cases in which building occupants had reported verifiable skin, mucous membrane, respiratory, central nervous system or neuropsychological abnormalities, cytotoxicity was identified. Results of a cytotoxicity screening test of field samples, such as the direct MTT test method, will give investigators of health problems related to indoor air quality problems important toxicity information.

Introduction:

The presence of indoor fungi, such as *Stachybotrys chartarum* (a.k.a. *S. atra*) on building materials has been recognized as an important risk factor for indoor air contamination. Serious adverse health effects in animals and humans associated with intense indoor or occupational exposure to *S. chartarum* and other fungal species (*A. versicolor*, *A. fumigatus*, *Fusarium spp.*, *Trichoderma viride*, *Penicillium spp.*, etc.) have been described and reviewed elsewhere in detail (1-6). High exposure levels of airborne pathogenic fungi in the work environment have been reported among farmers, wood workers, composting waste workers, hospitals and office workers with defective ventilation systems. In recent medical-epidemiological investigations toxic-inflammatory effects
have been found in infants with fatal hemorrhagic pneumonitis (7-9) or in office workers handling moldy paper materials and breathing air contaminated with fungi (10). These effects are thought to be related to toxic metabolites (mycotoxins) produced by certain fungal species, such as those in the genera *Stachybotrys*, *Aspergillus*, *Penicillium*, *Trichoderma* and *Fusarium*. *S. chartarum* is clinically important because it produces biologically very potent mycotoxins, such as trichothecenes (i.e., Satratoxin) and spirolactones, which interfere with protein synthesis, DNA and cellular or the humoral immune system (11). The production apparently depends on certain environmental conditions and the nature of materials or substrates, such as high water content and cellulose (12).

Conventional industrial hygiene exposure assessment has focused generally on speciation (taxonomy) and quantification of culturable (viable) fungi, but rarely on toxicity analysis. Toxicity screening tests have not been available in the past for such investigations. However, such information would be important to improve the understanding of the related pathology and for medical treatment or other intervention strategies.

The goal of our investigation was to evaluate fungal samples taken from water damaged homes of occupants who reported significant health complaints (sentinel cluster investigation) for the presence of toxicity (i.e., mycotoxins) using indirect and direct cytotoxicity screening tests. Some other analytical tests, in addition to conventional bioaerosol sampling, were also done. Other significant sources of indoor air contamination (dust, pesticides, VOCs etc.) were ruled out based on the occupants’ information and walk-through visual inspection of the problem homes or offices by a trained professional.

**Method**

Assessment and documentation of health problems:

Occupants' health complaints and abnormalities related in time and place to occupancy of a water-damaged home or workplace with fungal contamination were verified by an occupational and environmental physician with extensive experience in the assessment of building-related illnesses. A standardized health symptom questionnaire was utilized to detect abnormalities of certain target organs, such as upper and lower airways, eyes, skin and mucous membranes, central nervous system and constitutional complaints (flu-like symptoms, loss of appetite, weight-loss). Selected fungal IgE (immediate type allergy) and IgG (delayed type allergy) antibodies were tested in the building occupants as markers of exposure. Further, lymphocyte enumeration and function tests (T-lymphocyte mitogen proliferation analysis) were done in some cases to detect and verify immune dysfunction and suppression suggestive of fungal exposure. The medical laboratory tests were analyzed by licenced commercial labs following standard quality assurance (QA) procedures (IBT-laboratory, Kansas, Ms., and Specialty Laboratory, Inc., Santa Monica, Ca.). Only important and supportive laboratory findings will be presented, since this is not the focus of this paper. A subgroup of individuals underwent formal, standardized neuro-behavioral evaluation by a clinical psychologist (Detailed results will be presented elsewhere).

**Fungal sampling:**

Culturable fungi were sampled with a conventional impaction air sampler, Andersen N6 (single stage), with a flow rate of 28.3 l/min using a sampling time of 3 min. Culture