

NTP Project Update: *Molds*

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Introduction

Recent natural disasters and accompanying media coverage have heightened awareness of the potential health effects of mold exposure. In May 2004, an Institute of Medicine (IOM) committee concluded (Institute of Medicine, 2004) that there is sufficient evidence of an association between mold and other factors related to damp conditions and several respiratory ailments including upper respiratory tract symptoms, cough, wheeze, hypersensitivity pneumonitis in susceptible persons, and asthmatic symptoms in sensitized persons. However, the IOM committee concluded that there was inadequate or insufficient evidence to make conclusions for many other health outcomes including rheumatologic and other inflammatory diseases, neurological symptoms, cancer, and reproductive effects. Notably, the IOM report indicated that none of these health outcomes met the definition for the category of “limited or suggestive evidence of no association.” The committee also identified numerous research needs and recommended more research in exposure assessment of mold, standardization of definitions (e.g., “dampness”), and laboratory (toxicology) studies in animals to evaluate the effect of chronic exposures to mycotoxins via inhalation.

Nomination History

Stachybotrys chartarum, a mold present in indoor environments, was nominated to the National Toxicology Program (NTP) in 2004 for toxicological studies (<http://ntp.niehs.nih.gov/go/316>). The nomination reflected a broad public concern regarding potential non-infectious, adverse health effects of fungal exposures. The NTP Board of Scientific Counselors and NTP Executive Committee (EC) both endorsed that NTP should consider studying more common indoor fungi such as *Aspergillus* and *Penicillium* and emphasized a need to develop methods for standardized production of fungal organisms and/or specific components and to evaluate potential toxicants at multiple life stages and under varying growth conditions.

In a concept review (Web ref-<http://ntp.niehs.nih.gov/go/21099>) conducted in 2006, NIEHS/NTP scientific staff recommended that toxicological studies with the whole fungal organism be considered, since it is likely that any noninfectious adverse effects observed in the animals would be a result of their exposure to multiple, biologically active fungal components and not solely to specific fungal mycotoxins.

The recommendations from the internal Concept review included:

1. Explore the feasibility of conducting studies of “real-life” exposure scenarios to mimic the conditions found in damp or water-damaged buildings. These studies could be conducted in multiple strains of rodents to address issues of host susceptibility. As much as possible, try and tie these studies in with ongoing NIEHS efforts evaluating health effects in humans following exposure to mold.

2. Convene an expert panel of 15-20 individuals to address the three areas of health effects, biomarkers of exposure and methods of exposure. The purpose of the panel would be to solicit expert input on how to large scale conduct “real-life” exposure rodent studies and the toxicological endpoints to be measured.

Key Issues

There are a number of difficulties inherent in evaluating indoor mold exposures, such as determining the appropriate test article (whole fungal organism or mycotoxin) and selecting the appropriate fungal species and strain for evaluation, toxicological endpoints, testing strategies, and route of exposure. The toxicological study of mold exposures is further complicated by the biology of these organisms, because they have multiple life stages and the physical (i.e. spores, hyphae) and chemical (mycotoxins, proteolytic enzymes) agents that they produce may vary with life stage and growing conditions.

To address these issues, NTP asked scientists familiar with the challenges in mold toxicology to provide individual expert input that would assist NTP in the development of designs and conduct of animal toxicity studies on mold. The scientists met at the NIEHS in March 2007 and were asked to address how NTP might employ exposure scenarios (including test substances, route, duration of exposure) that closely mimic real world human exposure circumstances to reveal the range of biological responses in laboratory animal models and identify potential hazards to human health. The report from this meeting is attached as a separate file.

Proposed Approach

The overall goal of this research project is to investigate the potential for molds found in damp or water damaged buildings to cause systemic toxicity. The specific aims of the proposed studies are to:

- Assess organ system toxicity following inhalation exposure to molds
- Evaluate the available biomarkers of exposure and effect (both general and specific for the organisms to be studied)
- Evaluate the contribution of different organisms to overall health effects by studying individual isolates as well as mixtures

NTP will conduct subchronic studies in rodents using inhalation as the route of exposure. Pilot studies will examine feasibility with the duration of follow-up studies to be determined. We will be collaborating with investigators at NIOSH who are currently developing an animal model for using dry fungal particles and evaluating biomarkers for fungal exposures. Based on the original nomination and the recommendations of the NTP BSC, concept review committee and information group experts, two mixtures and 4 isolates of individual organisms will be tested. As the issues associated with production of large quantities of these organisms in a format suitable for animal exposures are unique, we will establish a new contract for the production of the test materials. Although there will be extensive characterization of the test materials (including an evaluation of relevant mycotoxins, glucans, allergens, particle size, protease activity, colony-forming units, spores, and endotoxin levels) both prior to and during the studies, and some methods development may be required, it is believed that this can be accomplished using existing analytical chemistry contracts. Proposed test materials include:

- Mixed culture of molds from a water damaged building from New Orleans, LA
- Mixed culture of molds from a damp building with reported health effects (sick-building syndrome)
- *Stachybotrys chartarum* isolate 1 (macrocyclic tricothecene chemotype)
- *Stachybotrys chartarum* isolate 2 (atranone chemotype)
- *Aspergillus versicolor*
- *Alternaria alternata*

Neurotoxicity will be evaluated using a functional observation battery, olfactory sensing, and cognitive tests. Particular attention will be paid to the cardiovascular, respiratory, gastrointestinal, and immune systems during histological evaluation as these organs have been reported to be potential targets following exposure to molds.

Significance and Expected Outcome

These studies will provide important information regarding which fungal organisms may be causative agents for human health effects, target organs for fungal toxicity, the utility of biomarkers other than IgE as measures of exposure and effect, and dose-dependent effects with particular emphasis on respiratory, immune, and neurologic endpoints.

References

Institute of Medicine. Damp Indoor Spaces and Health. National Academies Press, Washington, D.C., 2004