



# Implementation of EPA Bioaerosol Research Findings Can Ensure Regulations Based on Sound Science and Can Protect the Health of Asthmatics. Presenter: Stephen J. Vesper (NERL) Contributors: Robert Devlin, Mary Jane Selgrade and Marsha Ward (NHEERL); Nigel Fields (NCER); Ron Williams (NERL); Marc Menetrez (NRMRL); David Peden (UNC)

research and development

## Science Question

Asthma is on the rise around the world. At least 75% of asthma is believed to be associated with allergens of a biological origin. The economic burden from these diseases is now in the billions of dollars each year. This growing problem has resulted in the USEPA asking the following questions:

- What is the effect of bioaerosols on respiratory health, with an emphasis on asthma, especially in sensitive sub-populations like children and the elderly?
- What are the bioaerosols or components of bioaerosols that contribute to asthma incidence and exacerbation?
- What are the typical concentrations of the bioaerosol endotoxin and what effect does the various concentrations of endotoxin have on respiratory health?
- Are there synergistic interactions between bioaerosols and other air pollutants like particulate matter (PM), ozone etc?
- How can we measure the actual exposure to the various bioaerosols/air pollutants involved in asthma?
- How can EPA mitigate the risk and then measure the effectiveness of EPA regulations, guidance, and risk assessments?

## Research Goals

The Goal of this Research is to determine which factors contribute to asthma pathology and to use this information to implement testing, remediation and regulations that reduce the health and financial burdens of asthma.

To achieve this overall goal a number of tasks must be accomplished:

- (1) The factors that contribute to asthma development and exacerbation must be understood. In this program, the focus is on indoor bioaerosol contaminants endotoxins and molds and with their interactions with air pollutants like ozone and PM.
- (2) The occurrence and concentration of bioaerosols must be accurately measured in the indoor and outdoor environment.
- (3) The allergenic proteins that underlie asthma must be identified.
- (4) Strategies must be developed to reduce or eliminate/remediate the problem bioaerosols.
- (5) The results of this research must be implemented and educational materials must be made available to the public.

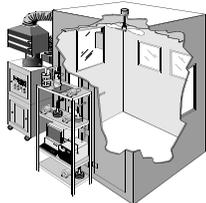


Figure 3. Chamber used for testing anti-microbial agents

## Methods/Approach

### Role of Indoor Molds in Asthma

Molds have been clearly linked to allergy/asthma for many years. We needed to know what molds and at what concentration(s) the disease occurs. In order to understand the role of molds in asthma, a highly standardized method of mold analysis was needed. Extensive sequencing of indoor molds, allowed for the development of quantitative PCR (qPCR) assays for more than 100 molds using a highly standardized, machine based (Figure 1) analysis that takes the human error out of the analysis. This new technology has been and is being used to study molds in dust samples from the homes of asthmatic children around the country.



Figure 1. Sequence Detector Instrument used in qPCR analysis of indoor molds.

### Identifying Mold Allergens

The reagents used by physicians today are not specific enough for the diagnosis of mold allergy. To identify the specific allergenic proteins, soluble mold extracts were instilled into BALB/c mice and asthma like symptoms quantified and the IgE recovered. The soluble fraction from each mold were then separated on 2D gels (Figure 2) and the corresponding IgE inducing proteins identified by MALDI-TOF.

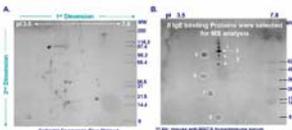


Figure 2. The *Metarhizium anisopliae* mycelium extract proteins were (A) separated by 2-Dimensional Gel Electrophoresis. Subsequently, (B) the IgE binding proteins were identified Western blot analysis

### Controlling Molds

As a collaborative effort (see accompanying poster "Risk Management for Indoor Mold Contamination to Reduce Exposure of Asthmatic Subpopulations"), physical, chemical (anti-microbial agents) and remediation methods were tested to determine if mold problems could be avoided or eliminated. These studies were done in controlled environments (Figure 3) and in water damaged homes in Ohio.

### Role of Endotoxins in Asthma:

Size fractionated endotoxins were measured in both indoor and outdoor residential settings in a time series to determine the time variability in occurrence of this bioaerosol (Figure 4). In order to determine the significance of endotoxin in respiratory function/asthma, thirteen non-allergic healthy volunteers were challenged on separate occasions with escalating doses of endotoxin (Figure 5). Sputum cells and fluid-phase components were recovered 6 hours after challenge. Sputum inflammatory cells were analyzed by means of flow cytometry for mCD14 and CD11b expression and immune function (phagocytosis of IgG-opsonized zymosan particles).

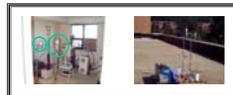


Figure 4. Monitoring equipment used for endotoxin measurements indoors and outdoors



Figure 5. Tests of healthy volunteers (not actual volunteer shown)

## Results/Conclusions

1. Certain species of molds were found to be associated with the homes of asthmatic children.
2. Respiratory exposures to soluble fraction of mold caused responses typical of allergic airway disease in mouse model. Catalogs identified as a mold allergen.
3. Inhalation of relatively high doses of endotoxin induced airway neutrophilia and decreased phagocytosis by airway monocytes, macrophages, and neutrophils.
4. Very low doses of endotoxin, which are not associated with neutrophil influx, enhance response to allergen in the nasal and bronchial airway and enhances phagocytosis, upregulates expression of CD11b and mCD14 and increases IL-13 levels, whereas IFN-gamma levels were significantly decreased.
5. Methods, practices and agents have been tested and proven to reduce the level of bioaerosols.

In conclusion, allergens from specific molds alone or in combination with endotoxins negatively affect respiratory health. The level of endotoxin is important in determining if neutrophilia occurs and the resulting effects on the respiratory airway. The negative health effects can be reduced by using one or a combination of techniques to reduce bioaerosol exposures resulting in the improved respiratory health of sensitive subpopulations.

## Impact and Outcomes

1. The Indoor Environments Division/OAR has developed a number of popular mold documents with over 200,000 hard copies distributed since 2002 and about 100,000 downloads from the website per month
2. Protocols have been developed for bioaerosol control which has resulted in the GSA including UVC Air Treatment as a useful method in controlling bioaerosols in Federal Buildings.
3. Other specific impacts include: a patented mold identification technology (#6,387,652) licensed by 15 companies in the US and European Union.
4. The Office of Pesticide Programs and Toxic Substances is using the developed recommendations for registration of new anti-microbial agents under the Antimicrobial Registration and Efficacy Testing.
5. In conjunction with HUD, "specifications" for cost effective home remediation of molds were developed.

## Future Directions

The bioaerosol team will build on the data obtained thus far by embarking on more comprehensive studies in other cities including Raleigh-Durham, Cincinnati and Detroit. In these studies, the interactions of bioaerosols with a variety of environmental pollutants like diesel exhaust, ozone and PM<sub>2.5</sub> and PM 2.5-10 will be assessed for synergistic impacts on health. These studies will include both residences and schools. Ongoing studies in conjunction with HUD and NIEHS included a national survey of molds in homes as well as studies of ventilation and humidity as asthma triggers. In addition, the relative allergenic potency of various mold proteins will be established. EPA will also be working to disseminate this information about bioaerosols and methods of control and make these new technologies broadly available.

## References

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# Susceptible Subpopulations

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