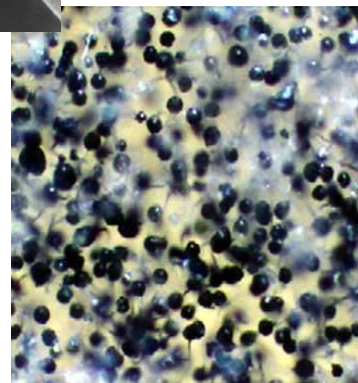
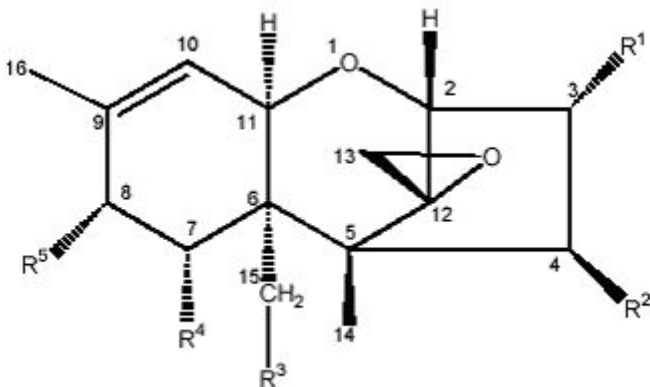
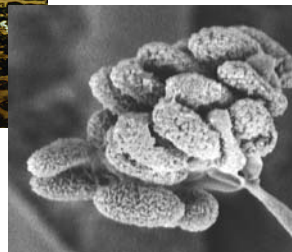
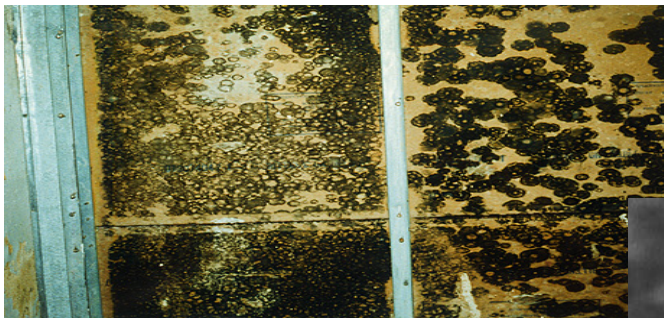




Infectious Disease Epidemiology Section  
Office of Public Health  
Louisiana Dept of Health & Hospitals  
...Your Taxes at Work...

## Stachybotrys chartarum (a.k.a. Stachybotrys atra or alternans)



## **Stachybotrys chartarum**

Greenish black; Sometimes called "black mold"

High moisture environments

Grows well on high cellulose-content materials, wet paper, gypsum board

Degrades cellulose

May produce mycotoxins

## **Trichothecene Toxins**

Group of related chemical compounds

Produced by *Fusarium*, *Cephalosporium*, *Myrothecium*, *Trichoderma*, *Stachybotrys*

### **4 major trichothecenes:**

T-2 toxin,

nivalenol,

deoxynivalenol = DON,

Diacetoxyscirpenol = DAS

Inhibitors of protein synthesis in mammalian cells

### **DON: Deoxynivalenol**

Most common but least toxic also called vomitoxin

Mainly produced by *Fusarium graminearum* & *F. culmorum*

Head blight (also wheat scab)

In world-wide, DON in 57% wheat, 40% maize, 68% oats, 59% barley, 49% rye, 27% rice

Also in flour, bread, breakfast cereals

Large annual variation, from <5-50 µg/kg to > 30 mg/kg.

**Nivalenol** common in Europe, Australia, Asia

**T2 toxin** found in many different products, but often in very low amounts.

### **Food toxin**

Alimentary Toxic Aleukia (ATA) in Russia

Outbreak in Orenburg district during 1942-1947:

100 000 people died

Consumption of *Fusarium* infected wheat kept outdoors

Skin spots, necrotic angina, extreme leukopenia, multiple haemorrhages, bone marrow exhaustion

Isolates of *Fusarium sporotrichioides* & *F. poae* from wheat shown to produce T-2 toxin

Similar symptoms observed in animals

Associated with outbreaks of human gastrointestinal disorders in Japan.

## **The Stachybotrys Episode**

Excellent demonstration of the perils and pitfalls associated with assigning a disease to a fungus and its toxins

Prior to 1993, *Stachybotrys chartarum* known for ability to produce trichothecene mycotoxins – animal syndrome “stachybotrytoxicosis”: leukopenia & hemorrhage leading to death

Jan 1993 to Nov 1994,

Cluster of infants in Cleveland died with an unexplained hemorrhagic lung process of acute onset that was subsequently given the label acute idiopathic pulmonary hemosiderosis (AIPH)

Case control study found majority lived in water-damaged homes

Microbiological investigation suggested toxin-producing *S. chartarum* in case homes

Clinical syndrome not similar to veterinary stachybotrytoxicosis

Other infants heavily exposed to *S. chartarum* failed to develop similar symptoms

Investigation of similar cluster of AIPH in Chicago did not find same linkage

Data again reviewed. Assumptions made during the original investigation challenged and reconsidered

Omitting technical details, bottom line = NO meaningful statistical linkage between *S.s* & AIPH

However, damage done and horse out of the barn. Now an urban legend

## **Bottom-line**

*S. chartarum* produces toxins

Some strains can cause disease in animals

Not at all clear that *Sc* able to produce human disease

*Sc* least common fungus in home environment

Amount of exposure required to produce *Sc* disease estimated to be at least 1,000-fold higher than amounts reported in most environmental surveys

Strauss DC, Wilson SC. Correspondence re Respirable trichothecene mycotoxins can be demonstrated in the air of *Stachybotrys chartarum*-contaminated buildings  
Journal of Allergy and Clinical Immunology

This correspondence is in response to the American Academy of Allergy, Asthma and Immunology position paper recently published in the Journal and entitled "The medical effects of mold exposure." The authors imply that the most important way that trichothecene mycotoxins could get into the human body is via the inhalation of *Stachybotrys chartarum* (SC) conidia. We have recently shown that the number of SC conidia in the air in a SC-infested building is not a good predictor for the amount of macrocyclic trichothecene mycotoxins (MTMs) in the air. This is because the MTMs can exist in the air on fungal fragments free of conidia, so the number of SC conidia found in the air should play only a small role in determining airborne MTM levels. This becomes very important because it has recently been shown that there are 514 times more SC fungal fragments released by this organism than there are SC conidia released. The authors also imply that the idea that the presence of mycotoxins in a building should give rise to an array of nonspecific complaints is "not consistent with what is known to occur when a toxic dose is achieved." This simply is not the case. Indeed, in a report examining the introduction of this type of mycotoxin (a trichothecene) into human beings, the opposite was observed. We know what kinds of symptoms are observed when a simple trichothecene (a preparation of diacetoxyscirpenol, also known as anguidine) is injected into humans. They are (among others) nausea, vomiting, low blood pressure, drowsiness, ataxia, and mental confusion. These symptoms are consistent with those reported by individuals in SC-infested buildings. The authors also state, "...however, potential levels of mycotoxins in nonagricultural air samples are too low to be measured practically with this technology." That may be true regarding the discussed technology; however, we have measured MTMs in the air of nonagricultural buildings. Finally, the authors stated, "Testing for airborne mycotoxins in nonagricultural environments cannot be used to diagnose mold exposure." This is not the case. We have successfully performed airborne testing for MTMs in nonagricultural settings.[6] In fact, we have used an ELISA to measure MTMs in the serum of individuals from SC-infested buildings. In conclusion, we feel that the following statements are true. SC has been shown to grow in buildings where people are having health problems. SC definitely produces MTMs in these situations. These MTMs definitely get into the air in these buildings, where they can be inhaled. They definitely are following, then, is the final question that remains to be answered: do the MTMs get into inhaled by people in these buildings. **The human beings in concentrations sufficient to cause the health problems observed in people in SC-contaminated buildings?**

- 1 Bush R.K., Portnoy J.M., Saxton A., Terr A.I., Wood R.A.: The medical effects of mold exposure. J Allergy Clin Immunol 117. 326-333.2006; Abstract
- 2 Brasel T.L., Douglas D.R., Wilson S.C., Straus D.C.: Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins on particulates smaller than conidia. Appl Environ Microbiol 71. 114-122.2005; Abstract
- 3 Cho S.-H., Seo S.-C., Schmechel D., Grinshpun S.A., Reponen T.: Aerodynamic characteristics and respiratory deposition of fungal fragments. Atmos Environ 39. 5454-5465.2005;
- 4 Murphy W.K., Burgess M.A., Valdivesio M., Livingston R.B., Bodey G.P., Freireich E.J.: Phase I clinical evaluation of anguidine. Cancer Treat Rep 62. 1497-1502.1978; Citation
- 5 Scheel C.M., Rosing W.C., Farone A.L.: Possible sources of sick building syndrome in a Tennessee middle school. Arch Environ Health 56. 413-417.2001; Abstract
- 6 Brasel T.L., Martin J.M., Carriker C.G., Wilson S.C., Straus D.C.: Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins in the indoor environment. Appl Environ Microbiol 71. 7376-7388.2005; Abstract
- 7 Brasel T.L., Campbell A.W., Demers R.E., Fergusen B.S., Fink J., Vojdani A., et al: Detection of trichothecene mycotoxins in sera from individuals exposed to *Stachybotrys chartarum* in indoor environments. Arch Environ Health 59. 317-323.2004; Abstract

Brasel TL. Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins on particulates smaller than conidia. *Appl Environ Microbiol* - 01-JAN-2005; 71(1): 114-22

Highly respirable particles (diameter, <1 microm) constitute the majority of particulate matter found in indoor air. It is hypothesized that these particles serve as carriers for toxic compounds, specifically the compounds produced by molds in water-damaged buildings. The presence of airborne *Stachybotrys chartarum* trichothecene mycotoxins on particles smaller than conidia (e.g., fungal fragments) was therefore investigated. Cellulose ceiling tiles with confluent *Stachybotrys* growth were placed in gas-drying containers through which filtered air was passed. Exiting particulates were collected by using a series of polycarbonate membrane filters with decreasing pore sizes. Scanning electron microscopy was employed to determine the presence of conidia on the filters. A competitive enzyme-linked immunosorbent assay (ELISA) specific for macrocyclic trichothecenes was used to analyze filter extracts. Cross-reactivity to various mycotoxins was examined to confirm the specificity. Statistically significant ( $P < 0.05$ ) ELISA binding was observed primarily for macrocyclic trichothecenes at concentrations of 50 and 5 ng/ml and 500 pg/ml (58.4 to 83.5% inhibition). Of the remaining toxins tested, only verrucarol and diacetylverrucarol (nonmacrocyclic trichothecenes) demonstrated significant binding (18.2 and 51.7% inhibition, respectively) and then only at high concentrations. The results showed that extracts from conidium-free filters demonstrated statistically significant ( $P < 0.05$ ) antibody binding that increased with sampling time (38.4 to 71.9% inhibition, representing a range of 0.5 to 4.0 ng/ml). High-performance liquid chromatography analysis suggested the presence of satratoxin H in conidium-free filter extracts. These data show that *S. chartarum* trichothecene mycotoxins can become airborne in association with intact conidia or smaller particles. These findings may have important implications for indoor air quality assessment.

Brasel TL. Detection of trichothecene mycotoxins in sera from individuals exposed to *Stachybotrys chartarum* in indoor environments. *Arch Environ Health* - 01-JUN-2004; 59(6): 317-23

To date, no study has effectively demonstrated a direct human exposure to mycotoxins in mold-contaminated buildings. Therefore, the authors investigated the presence of trichothecene mycotoxins in sera from individuals exposed to indoor molds (specifically *Stachybotrys chartarum*). Sera from occupants of contaminated (test samples,  $n=44$ ) and uncontaminated (control samples,  $n=26$ ) buildings were analyzed using a competitive enzyme-linked immunosorbent assay (ELISA) highly specific for macrocyclic trichothecenes. Twenty-three samples were significantly different ( $p < 0.05$ ) from normal human serum tested in the same manner, whereas only 1 of the control samples tested positive. Mass spectrometry analysis could not confirm the presence of intact *S. chartarum* macrocyclic trichothecenes. The authors hypothesize that this result was caused by uncharacterized ELISA-reactive metabolic breakdown products. Data from this study suggest that **trichothecene mycotoxins can be demonstrated in the tissues of certain individuals exposed to *S. chartarum* in contaminated buildings.**

**Croft WA; Jastromski BM; Croft AL; Peters HA Clinical confirmation of trichothecene mycotoxicosis in patient urine. *J Environ Biol* - 01-JUL-2002; 23(3): 301-20**

The investigations of four Cases involving mold-contaminated buildings and human reaction to exposure, documents tests of extracted urine containing trichothecene mycotoxins confirming exposure and the diagnosis of mycotoxicosis in humans. In each of four Cases, the urine demonstrated antibiotic activity, sulfuric acid charring, and protein release. Urine was extracted using ethyl acetate 40V/60V[EA]. Extracted mycotoxin spotted on (TLC) displayed color and a range of (rf) between 0.2-0.6 using various solvents. Extract was re-suspended using 50% ethanol V/V to inject mycotoxins into weanling female Sprague-Dawley rats. Degeneration and necrosis of the rat's tissue followed. Koch's Postulates conditions were fulfilled by isolation of the causative agent, the trichothecene mycotoxins and the reproduction of disease. Examination of human tissue within the urine extraction group confirms Koch's Postulates and comparative pathology confirms inhalation Mycotoxicosis, with severe necrosis of the central nervous system and severe scarring within the lungs. Extraction of mycotoxins from human patient urine is a very useful confirmatory test to demonstrate exposure and identify mycotoxicosis. Low concentrations (6%) of sodium hypochlorite were ineffective against the activity of trichothecene mycotoxin. The severity or stages of disease directly correlates the level of exposure or poisoning (Patent Pending).

**Trichothecenes can be found in urine**

**Schothorst RC. Determination of trichothecenes in duplicate diets of young children by capillary gas chromatography with mass spectrometric detection. Food Addit Contam - 01-JAN-2005; 22(1): 48-55**

Trichothecenes are mycotoxins produced by several fungal genera, mainly *Fusarium* species, that can contaminate a wide range of cereals used for human and animal consumption. They are associated with various adverse health effects in animals and humans such as feed refusal, vomiting and immunotoxic effects. A method based on capillary gas chromatography with mass spectrometric detection was developed and .... The final extracts were analysed for trichothecenes by GC-MS. The response was linear in the range tested (1-10 microg kg(-1)). ... Seventy-four food samples from young children collected by 74 respondents in a duplicate diet study were analysed for trichothecenes with the developed method. The mean levels of deoxynivalenol, nivalenol, HT-2 toxin and T-2 toxin were 5.8, 0.3, 0.3 and 0.1 microg kg(-1), respectively. Based on the individual results, dietary intake calculations were made. For deoxynivalenol, the tolerable daily intake of 1 microg kg(-1) body weight was exceeded by nine respondents. For the combined intake of T-2 and HT-2 toxin, the temporary tolerable daily intake of 0.06 microg kg(-1) body weight was exceeded by nine respondents.

Klötzel M. Determination of 12 type A and B trichothecenes in cereals by liquid chromatography-electrospray ionization tandem mass spectrometry. *J Agric Food Chem* - 16-NOV-2005; 53(23): 8904-10

A new sensitive method for the simultaneous determination of 12 trichothecenes (deoxynivalenol, nivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, fusarenon X, T-2 toxin, HT-2 toxin, neosolaniol, monoacetoxyscirpenol, diacetoxyscirpenol, T-2 triol, and T-2 tetraol) by liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) is presented. The development of the method and investigations on the matrix influence on the MS signal are described in particular. The matrix effect was thereby minimized by using an internal standard, a special mobile phase, and specific fragmentation parameters. The sample was extracted with acetonitrile/water (84:16, v/v), and the extract was cleaned up with a MycoSep 227 column. Quantification was based on the internal standard de-epoxy-deoxynivalenol. Calibration curves were linear between 16 and 1600 ng/g, and the limits of detection ranged from 0.18 to 5.0 ng/g. The developed method was applied for the determination of trichothecenes in 120 naturally contaminated wheat and oat samples.

Aldred D - *Toxicol Lett* - 10-OCT-2004; 153(1): 165-71. Prevention strategies for trichothecenes.

Contamination of cereal commodities with mycotoxins represents a significant hazard to consumer health and has thus received increasing attention from food safety authorities and legislators. For trichothecenes, and deoxynivalenol (DON) in particular, the imminent implementation of legislative limits has focused attention on ways to prevent entry of such mycotoxin contaminants into the food and feed chains.

Schlatter J. Toxicity data relevant for hazard characterization. *Toxicol Lett* - 10-OCT-2004; 153(1): 83-9

The present paper summarizes toxicity data relevant for hazard characterization for the trichothecene mycotoxins deoxynivalenol (DON), nivalenol (NIV), T-2 and HT-2 from recent opinions prepared by the European Commission Scientific Committee on Food (SCF) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Details on immunotoxicity, cardiovascular toxicity and co-occurrence of different trichothecenes and other mycotoxins and their possible interactions are considered in separate papers in the present issue as well as other aspects such as mould growth, trichothecenes formation, storage, processing, sampling, analytical measurements, exposure assessment and surveillance. The toxicological profiles of DON, NIV, T-2 and HT-2 are similar. The general toxicity and immunotoxicity in experimental animals, and for NIV also haematotoxicity, are considered to be the critical effects. Tolerable Daily Intakes of 1, 0.7 and 0.06 microg/kg b.w. were established for DON, NIV and the sum of T-2 and HT-2, respectively. The TDI's for NIV, T-2 and HT-2 were made temporary because of deficiencies the database.

Bauer J. Are mycotoxins in food a health hazard? - *Dtsch Tierarztl Wochenschr* - 01-AUG-2004; 111(8): 307-12

The effects of mycotoxins are well known since antiquity. Modern trace analysis show the wide prevalence of mycotoxins in the food chain. Aflatoxins (AFB1, AFB2, AFG1, AFG2), trichothecenes (deoxynivalenol, T-2 Toxin, HT-2 Toxin), zearalenone, fumonisins (FB1, FB2) and ochratoxin A are the most important mycotoxins world wide. Foods of plant origin are normally contaminated more frequently and in higher concentrations than food of animal origin. The mean concentrations of mycotoxins analysed in European foods can be assessed as low. This may be one reason why acute poisonings are rare incidences and why the limits of the tolerable daily intake (TDI) levels are exceeded only very seldom; however the population group "infant" exceed the preliminary TDI-level for trichothecenes. The consequences of a chronic intake of low amounts of mycotoxins is hardly to assess. A participation of the aflatoxins in the pathogenesis of primary liver cancer is considered as certain. Other coherence between the dietary intake of mycotoxins and specific clinical symptoms of man are not convincingly shown till now

Terr, AI. *Ann Allergy Asthma Immunol.* 2003 Aug; 91(2):212-3; Stachybotrys: relevance to human disease.

**LEARNING OBJECTIVES:** Recent public concern about the danger of environmental fungi has focused attention on one particular mold, *Stachybotrys*. The purpose of this review is to examine and critique the published literature on *Stachybotrys* for objective scientific and clinical evidence of disease caused by the presence of this fungal organism in the environment.

**DATA SOURCES:** Data were obtained from all published research and reviews of *Stachybotrys* indexed in MEDLINE since 1966.

**STUDY SELECTION:** The publications used for this review were those that contained information about human health effects of this microorganism. The critique of these publications is the author's. **RESULTS:** *Stachybotrys* is a minor component of the indoor mycoflora, found on certain building material surfaces in water-damaged buildings, but airborne spores are present in very low concentrations. Published reports fail to establish inhalation of *Stachybotrys* spores as a cause of human disease even in water-damaged buildings. A possible exception may be mycotoxin-caused pulmonary hemorrhage/hemosiderosis in infants, although scientific evidence to date is suggestive but not conclusive. Based on old reports ingestion of food prepared from *Stachybotrys*-contaminated grains may cause a toxic gastroenteropathy. No convincing cases of human allergic disease or infection from this mold have been published.

**CONCLUSIONS:** The current public concern for adverse health effects from inhalation of *Stachybotrys* spores in water-damaged buildings is not supported by published reports in the medical literature.