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By Jack Dwayne Thrasher, Dennis H Hooper & Jeff Taber

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# Family of Six, their Health and the Death of a 16 Month Old Male from Pulmonary Hemorrhage: Identification of Mycotoxins and Mold in the Home and Lungs, Liver and Brain of Deceased Infant

Jack Dwayne Thrasher <sup>α</sup>, Dennis H Hooper <sup>σ</sup> & Jeff Taber <sup>ρ</sup>

**Abstract-** The health of a family of six residing in a water-damaged home is presented. The family consisted of the parents (age 29) two boys (ages 8 and 12) and new born fraternal twins (male and female). The parents and two boys developed RADS/asthma and had multiple symptoms including nose bleeds. The fraternal twins experienced respiratory illness that required hospital treatments. The infant girl survived while her brother was found face down, blue in color, lifeless with oral and nasal blood discharge. Pathology demonstrated areas of peribronchial inflammation, intra-alveolar, and numerous hemosiderin laden macrophage (hemosiderosis). Environmental evaluation of the home revealed *Stachybotrys*, *Aspergillus/Penicillium*, *Cladosporium* and *Chaetomium* in various rooms of the home. Mycotoxins detected in the home included Sterigmatocystin, 5 methoxy-sterigmatocystin Roquefortine C, Satratoxin G and H, Roridin E and L-2, isosatratoxin Fas as well as other *Stachybotrys* secondary metabolites. *Aspergillus versicolor* was identified by PCR-DNA analysis in the lungs and brain of the deceased child. Aflatoxin was detected in his lungs, while monocyclic trichothecenes were identified in the lungs, liver and brain. The literature is briefly reviewed on the subject of fungi and their secondary metabolites present in water-damaged homes and buildings.

## I. INTRODUCTION

Water incursion into buildings and homes leads to an increased frequency of upper and lower respiratory disease and abnormal lung function in adults and children (1-12). Spontaneous pulmonary hemorrhage in infants is rare. Known causes in children and adults include environmental tobacco smoke, infections, traumatic injury, e.g. intratracheal tube, cardiac and vascular processes, e.g. von

Willebrand disease (13). Pulmonary hemosiderosis (PH) is the result of chronic and recurrent pulmonary bleeding with the occurrence of hemosiderin laden pulmonary macrophages. Pulmonary hemorrhage associated with water intrusion, *Stachybotrys chartarum*, *Aspergillus*, *Penicillium* and tobacco smoke in Cleveland, Ohio was reported (14-16). Additional cases of PH include a one month-old infant with no environmental tobacco smoke and the presence of *Aspergillus/Penicillium spp*, *Memnoniella*, *Alternaria*, *Cladosporium*, *Chaetomium*, *Torula* and *Stachybotrys*. Roridin L-2, Roridin E and Satratoxin H were identified in a sample from the bedroom closet ceiling (17); PH in a 40 day-old male infant exposed to *Penicillium* and *Trichoderma* species for 2 weeks followed by an acute exposure to tobacco smoke (18); and an infant with pulmonary hemorrhage (19). Finally, *Stachybotrys chartarum* was isolated from the lungs of a 7 year-old male who recovered from pulmonary hemorrhage (20).

Hemolysins are produced by a several species of mold isolated from the homes associated with pulmonary bleeding in the Cleveland homes (21, 22). However, other mold contaminants probably have a role in illnesses associated with mold exposure in water damaged structures. For example, mycotoxins have been demonstrated in the air, dust and building materials contaminated with mold. These include, but not necessarily limited to sterigmatocystin, trichothecenes, aflatoxins, gliotoxin, chaetoglobosum A, Roquefortine C (12, 23-30). In addition, trichothecenes have been identified in the sera of individuals exposed to *Stachybotrys chartarum* (31) while gliotoxin is present in the sera of patients and mice with invasive aspergillosis (32). Trichothecenes, aflatoxins and ochratoxins are present in biopsy and autopsy specimens obtained from mold exposed subjects (33). More recently, trichothecenes, ochratoxins and aflatoxins were reported in the urine, nasal secretions, sinus biopsies, umbilical cord and placenta from a family of five with illnesses associated with a mold

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infested water-damaged home (34). In addition, individuals exposed to mold in their water-damaged homes with chronic fatigue also have the same mycotoxins in their urine (35).

In this communication we report on a healthy family of 6 (nonsmokers) who developed multiple symptoms and health problems (e.g. nasal bleeding, sinusitis, asthma, RADS) following a prolonged exposure to several genera of mold in a water damaged home. Most significantly, fraternal twins were hospitalized with pulmonary bleeding. The female survived but developed RADS. The male twin died from respiratory failure and pulmonary bleeding. Both had *in utero* and neonatal exposure to these molds and their mycotoxins, including *Stachybotrys chartarum*. Real time PCR detected *Aspergillus versicolor* DNA in the brain and lungs of the deceased infant, while an ELISA procedure detected aflatoxins and trichothecenes in the lungs, liver and brain in autopsy materials from the deceased infant. In addition, mycotoxins produced by *Penicillium sp.*, *Asp. versicolor* and *S. chartarum* were detected in bulk and dust samples from the home by LC/MS/MS.

## II. MATERIALS AND METHODS

### a) Description of the Home

The 3 bedroom home was located in Visalia, California. Construction was wood framing, exterior stucco, dry wall interior, asphalt shingles, fireplace, wood sub flooring, crawl space, attic and central air condition. It consisted of the following occupied rooms: Living room with a corner fireplace, play room with a baby crib, infant's bedroom where the twins slept, den immediately adjacent to the infant's bedroom, master bedroom, add on office, kitchen and two bath rooms. Upon moving into the home it was noticed to be in disrepair. The carpet was wet, moldy and falling apart with a musty odor. There was discoloration of ceiling dry-wall indicative of water intrusion, and water damage to the flooring. Occupation of the home began in August 1993 and vacated on November 11, 2001. The home was inspected by the Joseph Company, Fresno, California. The following defects were noted: (1) faulty roofing; (2) increased moisture readings from 30 to 100 %; (3) Ceiling water stains throughout the house; (4) Visual mold growth; and (5) improperly installed shingles which allowed moisture intrusion under the shingles and into the interior of home, e.g attic and wall cavities. The home was eventually razed because of the disrepair, water damage and mold growth.

### b) Mold Testing (Air, swab and Bulk Sampling)

Visual inspections, bioaerosols, bulk and wipe sampling of the home were done under the direction of Jeff Taber, Kings County Public Health Department. Bulk, wipe and air bioaerosols were sent under chain of custody to Aerotech Laboratories (Currently EM Lab P &

K, Phoenix Arizona) to identify mold to at least the Genus level. Air sampling was accomplished using Zeflon Air-0-Cells at room temperature, 3-5 minutes at 130 liters per minute.

### c) Environmental Myco toxin Testing

Bulk and wipe samples were taken from various areas of the home and sent under chain of custody to P-K Jarvis (currently Bureau Veritas North America), Novi, Michigan to test for a variety of mycotoxins produced by *Aspergillus* and *Penicillium spp.* and *Stachybotrys chartarum*. The samples were extracted with methanol, run on LC/MS/MS and analyzed by John Neville, Ph.D.

### d) Autopsy of the Deceased Child

An autopsy was performed by G. Walter, MD, Coroner's Office, Tulare County, California. A second opinion regarding the results of the autopsy and histopathology was done by a pediatric pathologist D. Scharnhorst, M.D., Ph. D, Valley Children's Hospital, Madera, California. Histology slides were only stained with Hematoxylin Eosin. Paraffin embedded and frozen samples of liver, lung and brain of deceased infant were sent to RealTime Laboratories, Carrollton, Texas to test for mycotoxins and the presence of mold DNA.

### e) Hypersensitivity Pneumonitis and Mycotoxin Antibodies:

Serum samples from the surviving members of the family were sent to Aerotech Laboratories (Currently EMLab P & K) to perform the Hypersensitivity Pneumonitis Panel and for the detection of IgE, IgA and IgM antibodies against various molds. Antibodies against aflatoxin, trichothecenes and satratoxin adduct and *Stachybotrys chartarum* in sera of the family were tested by Immunosciences as previously reported (36).

### f) Real Time PCR (RT-QPCR) Mold DNA Analysis

The DNA probes for mold species utilized in the RT-QPCR included species of *Aspergillus*, *Penicillium* and *Stachybotrys chartarum* were developed and patented by Real Time Laboratories, Carrollton, Texas. The RT-QPCR was carried out as published (37). The tissues used for mold DNA and mycotoxins were emulsified and extracted as described (33-35) are briefly reviewed below.

### g) Detection of Mycotoxins in Autopsy Specimens by an ELISA Procedure

25 mg of the lung, liver and brain were received frozen or embedded in paraffin blocks. They were analyzed for aflatoxins (AT), trichothecenes (MT) and ochratoxin A (OTA) using immune affinity columns, and T-2 and HT-2 Ochr Test, (Afla Test® test kits, VICAM, L.P., Watertown, MA) containing specific monoclonal antibodies. The tissues were emulsified in phosphate buffered saline (PBS, 0.9%) and reagent grade methanol (Sigma-Aldrich) in a 1:1 dilution. To disrupt the cells, tissues were bead beaten using silica beads (Sigma-

Aldrich) for 1 minute at the speed of 45 rpm, heated at 65° C for 15 minutes. Samples were centrifuged at 13000 rpm for 2 minutes. 500 µl of cellular extract was placed in a glass tube, and further diluted in PBS prior to testing. All samples were free of paraffin (33-35).

Samples were then applied to an Afla Test® column (VICAM, L.P., Watertown, MA) which contains specific monoclonal antibodies (MT) directed against AT (B1, B2, G1, and G2), OTA) and monocyclic tricho the cenes. Columns were washed twice with reagent grade water (Fisher Health Care, Houston, Texas). The samples were eluted from the column to remove the bound mycotoxins with reagent grade methanol. Fluorochrome developer (AFLATEST® Developer, VICAM) was added to the extracted methanol. All samples were read by fluorometry (Sequoia-Turner Fluorometer, Model 450, which was calibrated using standards supplied by VICAM (Green Filter = 2, and Red Filter = 120). Spiked standards using known amounts of AT B1, B2, G1, and G2 (Trilogy Analytical Laboratory Inc., Washington, Missouri) of human heart tissue were run as validation controls prior to testing (sensitivity of 95% and specificity of 92%). Known controls of mycotoxins (50 ppb, 25 ppb, and 1.25 ppb, Trilogy Analytical Laboratory and Real Time Laboratories, Carrollton, Texas) were run with each test. The eluted solution was then read by fluorometry at 450 Angstroms. The lower and upper limits of detection are

1.0 and 23.0 parts per billion (ppb) calibrators, respectively. Test results are plotted against the standard curve of the calibrations. Results were reported in ppb.

### III. RESULTS

#### a) Family Health

The two adults, nonsmokers, and two older male children were healthy prior to moving into the mold contaminated home. They resided in the home until November/December 2002. The home was razed in early 2002. Within two months of occupancy all members began to experience symptoms and health problems that are summarized in **Tables 1 and 2**. All surviving members developed lung disease and were positive when tested for Hypersensitivity pneumonitis (**Table 2**) and were given the diagnosis of RADS/asthma with prescribed bronchodilators. In addition, all members of the family had reduced RBC hemoglobin (Hb) and were diagnosed as anemic. Nose bleeds and a flu-like illness were other common symptoms (**Table 1**). After moving out of the contaminated home, their health improved, however, they remained symptomatic with the RADS/asthma as well as other symptoms such as fatigue and generally not feeling as well as they did prior to occupation. There was no family history of von Willebrand disease.

**Table 1 :** This table summarizes to health and symptoms of the family members. All members of the household were healthy prior to moving into the contaminated home. The fraternal twins were born after moving into the home

Family Member	Health and Symptoms
Father, Age 29	Nonsmoker, Flu-like symptoms, nausea, blurred vision, headaches, dizziness, fainting spells, excessive weakness, shortness of breath, nose bleeds, inability to concentrate, loss of memory, abnormal PFT RADS. Albuterol.
Mother, Age 29	Nonsmoker, Flu-like symptoms: Fever, chills, fatigue, Pharyngitis, bowel cramping, sinus congestion, coughing, shortness of breath, chest tightness, sneezing, headache, decreased Hb, RBC (anemia) and increased neutrophils. Negative allergy skin testing. Abnormal PFT RADS. Albuterol.
Son, age 10 (Son 1)	Normal well baby Exam. Flu-like symptoms, skin rash, frequent colds, sore throat, fevers, coughing, shortness of breath, vomiting, gastroenteritis, conjunctivitis RADS/asthma, Albuterol
Youngest Son, age 8 (son 2)	Normal well baby exam. Flu-like illness, developmental delay, bilateral OTM, conjunctivitis, chest congestion, sinusitis, headaches. At age 4 diagnosed with developmental delay, delayed speech and language, and at age 6 with autistic spectrum disorder. RADS requiring Albuterol.
Fraternal Twin (female) 18 moths	Normal well baby exam. Symptoms began at approximate 3 months of age: fever, congestion, coughing, hoarseness, shortness of breath, nasal bleeding, , vomiting, patchy pneumonia, increased sed. rate, elevated neutrophils, decreased RBC hemoglobin, anemia, diagnosis of asthma, wheezing, Pulmocort and Albuterol. One hospital stay, recovered, sent home.

Deceased Fraternal Twin (male), 18 months	Normal well baby exam. At home he had Flu-like symptoms, cyanotic episodes, limp, lethargy, sweating, shaky, tonsillitis. OTM, bronchitis, bilateral wheezing, eyes rolling back, decreased RBC hemoglobin, several hospital visits for respiratory difficulties for turning blue, being limp and difficulty breathing. He was found nonresponsive face down in his crib with bloody discharge from nose and mouth. He was pronounced dead on 02/19/2001 and was taken to Country Coroner's Office for autopsy. Bacterial throat cultures, May, 2000 were negative for Strep at 24 hours
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**Table 2 :** This table summarizes the results of the Hypersensitivity Pneumonitis (H.P.) panel performed on the family by Quest Diagnostics as well as their major symptoms. The five surviving members were diagnosed with Hypersensitivity Pneumonitis and Antibodies to mycotoxins

Organism	Mother <sup>1</sup>	Father <sup>2</sup>	Son (1) <sup>3</sup>	Son (2) <sup>4</sup>	Female Twin <sup>2</sup>
<i>M. faeni</i>	+	+	+	+	+
<i>A. pullulans</i>	+	+	-	-	-
<i>A. Alternata</i>	-	+	-	-	-
<i>A. fumigatus</i>	-	+	-	-	-

1. *Mother: H.P., fever, chills, fatigue, H.A.s, diarrhea, Pharyngitis, GERD, SOB, chest and nasal congestion, nose bleed, sneezing, negative allergy testing.*
2. *Father: H.P., asthma, Dizziness, H.A.s, nosebleed, SOB, fatigue, muscle twitching, decreased concentration and memory, skin rashes and petechae. Allergic to dust mites and pollen. SOB, RADS with abnormal PFT*
3. *Eldest Son: Skin rash, fever, nose bleed, coughing, gastroenteritis, conjunctivitis. H.P.*
4. *Son: Developmental delay (autistic spectrum), comprehension and language delay, fever, erythematous skin rashes, diarrhea, coughing, H.A.s, sinusitis, nose bleed SOB, H.P.*
5. *Female Twin: fever, nasal and chest congestion, nose bleed, pneumonia requiring hospitalization, H.P., SOB, RADS requiring Pulmocort and Albuterol*

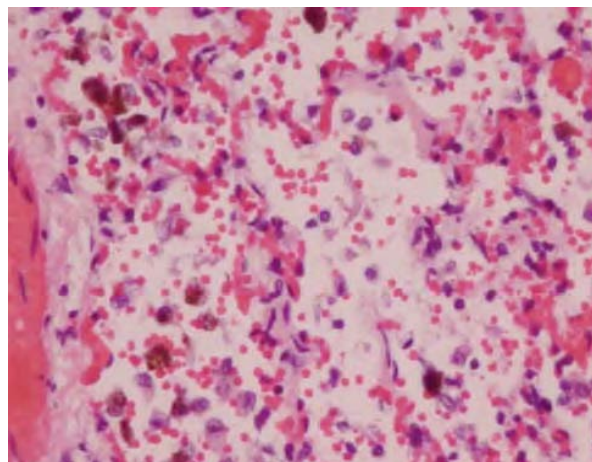
**b) Health of the Fraternal Twins**

The female twin had nasal bleeding, fever, decreased RBC hemoglobin (anemia) coughing and difficulty breathing. She was hospitalized once and released after being stabilized. The male sibling upon arriving home following birth was either had ER visits, frequent physician appointments and was in hospital for severe respiratory problems. At home he was found face down in his crib motionless, blue and with blood coming from the nose and mouth. He was pronounced dead upon arrival at the hospital.

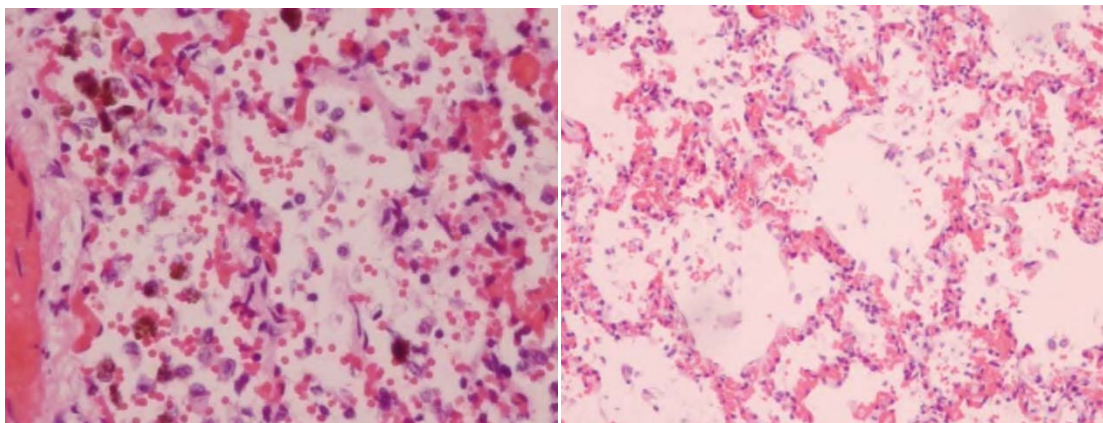
**c) Autopsy of the Deceased**

The following abnormalities were listed in the final autopsy report: (1) liver had mold congestion; (2) Heart had mild hypertrophy without inflammation; (3) Lungs had marked vascular congestion, foci peribronchial inflammation, intra-alveolar blood numerous aggregates of pigment laden macrophages (hemosiderosis) (**Figure 1**). All other organs were normal in appearance.. The cause of death was listed as respiratory failure with pulmonary bleeding and hemosiderosis.

**A. Histology of the Lung (H & E)**



B. Autopsy Slides of Lung (H & E)



**Figure 1:** Lung histology stained with Hematoxylin and Eosin demonstrating markedly congested Alveolar spaces with desquamated cells and blood, hemosiderin-laden macrophages, no inflammation, normal appearing blood vessels and no inflammation

*d) Hypersensitivity Pneumonitis and Antibodies to Mycotoxins*

The father, mother, two boys and the surviving twin were positive with respect to the Hypersensitivity pneumonitis panel (Table 2). The father had positive IgG antibodies to the four molds (*M. Faeni*, *A. pullulans*, *A. alternata* and *A. fumigatus*) followed by the mother (*M. faeni* and *A. pullulans*) and the three children (*M. Faeni*). All experienced symptoms of shortness of breath (SOB), RADS and/or asthma requiring bronchodilators.

Antibodies against *Stachybotrys chartarum* were positive in each family member as follows: Father (IgA and IgG); Mother (IgA); two sons (IgG) and twin daughter (IgG). The data on titers are not shown.

Antibodies to albumin adducts of AT, trichothecenes (MT) and satratoxin (ST) were positive as follows: (1) Father (IgM against AT, MT and SAT); (2) Mother had AT (IgM), MT (IgA and IgM); and Satratoxin (IgA and IgG) antibodies; (3) Sons – eldest had IgA to SAT and the youngest was not tested; (4) Surviving twin

daughter had AT (IgM), MT (IgA and IgM); and SAT IgA and IgG) antibodies. The data on titers against mycotoxins are not shown).

*e) Mold Identification in the Home*

Mold contamination was determined from bulk, wipe and air samples. The results from bulk samples are presented in Table 3. The major molds identified in these samples were *Amerospores*, *Aspergillus/Penicillium*, *Cladosporium*, *Stachybotrys* and *Chaetomium*. Ascospores and Basidiospores were also identified (data not shown). Spore counts per gram of sample were: *Amerospores* (2,308 to 11,536). *Stachybotrys* (20,789 to 28,462), *Cladosporium* (2,308 to 3,077). *Aspergillus/Penicillium* (2,308 to 41,536) and *Chaetomium* (2,308 to 23,308). In addition, *Stachybotrys* and *Chaetomium* were identified in a sample from the carpeting. An air sample from the children's playroom contained *Stachybotrys*.

**Table 3:** This table summarizes the identification of spore types detected in bulk samples obtained from rooms of the home. The samples were examined by Aerotech Laboratories, Inc., Phoenix AZ. The data are expressed as spore counts/gram

Room	Alternaria	Amerospores	Stachybotrys	Cladosporium	Asp/Pen	Chaetomium
Fireplace Floor		8,889				
Living Room N. Wall		7,407				
Addition S. Wall			28,462		17,692	2,308
Addition - Middle		3,077	22,308	3,077		12,308
Addition W. Wall		2,308	13,077	2,308		
Closet Bedroom		11,538			41,536	
Master Bdrm Floor		8,462			2,308	
Master Bdrm Floor		4,615				
Den Closet Ceiling			10,769			
A.C. Filter		308,200		161,000		

*Ascospores and Basidiospores were also present in some rooms of the home. Torula was detected in Addition room W. wall sample. Air spore counts total 1,577 to 2,222 spores/m<sup>3</sup>. Stachybotrys and Chaetomium were not detected in the outdoor air samples. Stachybotrys was detected in air of the children's play room. Stachybotrys and Chaetomium spores were detected in the carpeting.*

f) *Mycotoxin Identification in the Home*

The results for the detection of mycotoxins in bulk samples are presented in **Table 4**. The results are designated as present (at or above detection limit) or not present (not detected). Sterigmatocystin and 5-methoxysterigmatocystin were detected in three rooms, the air conditioning duct and AC filter. Sterigmatocystin was at or above the reporting limit of 20 ng. Chaetoglobosum A, B, and C were not identified.

Roquefortine C was detected in two rooms, while Griseofulvin was not detected in any sample. The most commonly detected mycotoxins were the trichothecenes: Satratoxin H (detection limit of 7.0 ng), Isosatratoxin F, Satratoxin G, Roridin L-2, Isororidin E, and Roridin E. In addition, the *Stachybotrys* metabolites 6B-Hydroxydolaabella MER-5003 Mol. Wt. 47 and Mol. Wt 412 were also present. Standards were not available for several of the mycotoxins (\*\*).

**Table 4 :** This table summarizes the mycotoxins detected in dust samples from various areas of the house. The tests were performed by Jon Neville, PK-Jarvis, Novi, MI

Mycotoxin	Living Room N. Wall	Room Addition W. Wall	Room Addition Middle	NW Bdr m Closet	Living Room Floor at Fireplace	Room Addition SW Wall	Room Addition S. Wall	Room Addition N. wall	Reporting Limit in ng
Sterigmatocystin	--	Present	--	--	--	Present	Present	--	20
5-methoxysterigmatocystin	--	Present	--	--	--	Present	Present	--	**
Chaetoglobosum A	--	NP	NP	NP	--	NP	NP	NP	**
Chaetoglobosum B	--	NP	NP	NP	--	NP	NP	NP	**
Chaetoglobosum C	--	NP	NP	NP	--	NP	NP	NP	**
Griseofulvin	--	NP	--	--	--	NP	NP	--	10
Roquefortine C	--	Present	--	--	--	Present	NP	--	0.4
Satratoxin H	NP	Present	Present	NP	NP	Present	Present	Present	7.0
Trichodermol	NP	NP	NP	NP	NP	NP	NP	NP	**
Trichodermin	NP	NP	NP	NP	NP	NP	NP	NP	**
Isosatratoxin F	NP	Present	NP	NP	NP	Present	Present	Present	**
Satratoxin G	NP	Present	NP	NP	NP	Present	Present	Present	**
Roridin L-2	NP	Present	NP	NP	NP	Present	Present	Present	**
Isororidin E	NP	Present	NP	NP	NP	Present	Present	Present	**
Roridin E	NP	Present	NP	NP	NP	Present	Present	Present	**
Epoxydolabellane A	NP	NP	NP	NP	NP	NP	NP	NP	**
6B-Hydroxydolaabella	Present	Present	Present	NP	Present	Present	Present	Present	**
MER-5003 M. Wt. 470	Present	Present	Present	NP	Present	Present	Present	Present	**
MER-5003 W. Wt. 412	Present	Present	Present	NP	Present	Present	Present	Present	**

Abbreviations: \*\* (standards not available); -- (not tested); NP (not present)

Air Conditioning System: A wipe sample from the air conditioning duct was positive for the presence of Sterigmatocystin, 5-Methoxysterigmatocystin and Roquefortine C. A bulk sample from the A.C. filter was positive for Sterigmatocystin and 5-methoxysterigmatocystin

g) *PCR DNA Test Results of Deceased Tissues*

Real-Time PCR analysis detected *Aspergillus versicolor* in the frozen and paraffin embedded tissues

as follows: Lung (1056 spores/g; Liver (0) and brain (7 spores/gram) **Table 5**).

**Table 5:** This table summarizes the results of PCR DNA testing by EMSL Laboratory Analytical that detected *Aspergillus versicolor* in the lungs, liver and brain of the deceased

Sample	Species <i>Aspergillus versicolor</i>	Spores/g Paraffin embedded tissue
Lung	Present	1066
Liver	Absent	0
Brain	Present	7

*h) Mycotoxins Detected in the Deceased Tissues*

**Figure 2** summarizes the results of the mycotoxin concentrations detected in the lung, liver and brain of the deceased twin. OTA was not detected. AT

was detected at a concentration of 0.2 ppb in the lung. The T-2 Tag that identifies several trichothecenes (e.g. T-2, HT, Acetyl T-2) were as follows: Lung at 4.6 ppb, liver at 4.3 ppb and brain at 0.3 ppb.

- **Reported in ppb**

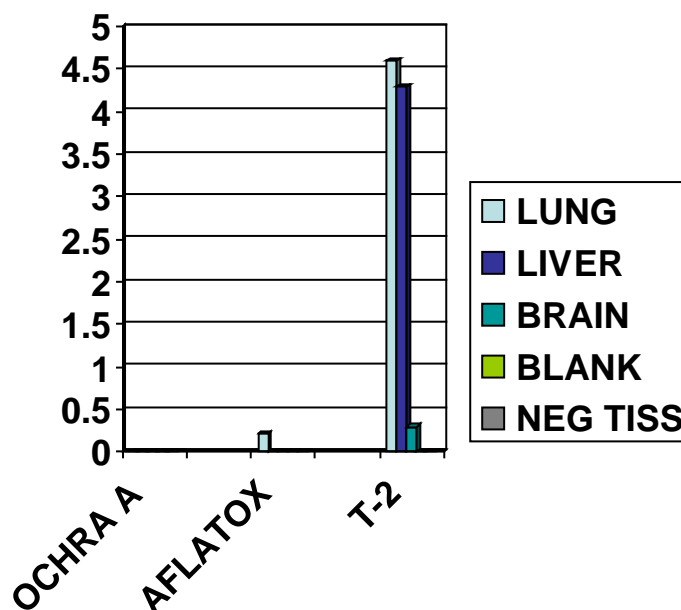
- **T-2 TAG**

- **X Reacts with**

- T-2
- HT@
- Acetyl T-2
- T-2 Triol

- **No X RX with**

- T2 Tetrol
- NIV
- DON



**Figure 2:** Fluorometry Results by Organ. Extracted mycotoxins from tissue with paraffin embedded blocks. Grind Tissue, Blend sample with PEG, Pass through column, Wash with tween and water, Elute Antigen (i.e. T-2) from column with methanol, Use calibrated fluorometer, Results are reported as present in parts billion (ppb)

#### IV. DISCUSSION

The initial literature regarding pulmonary hemorrhaging in infants and mold was limited to the identification of molds, including *S. chartarum*, in the homes of the affected infants. As a result, we have held on to the data presented herein until sufficient information became available in the literature to discuss the health affects observed in this family.

The question is what is the mode of exposure to antigens and toxins in these environments? Bench experiments and results from indoor testing of water damaged homes offer some insight. Bench testing has demonstrated that colonies of mold and bacteria shed particulates (fungal fragments) less than the size of spores in the range of 0.03 to 0.3 microns. The question is what is the mode of exposure to antigens and toxins in these environments? Bench experiments and results

from indoor testing of water damaged homes offer some insight. Bench testing of agar plates with growth of *Stachybotrys*, *Cladosporium*, *Penicillium* and *Aspergillus* has demonstrated that The mechanisms involved in this release includes colony structure, moisture conditions, air velocities and vibration (38-41). The fragments contain antigens, mycotoxins, glucans, endotoxins, exotoxins and a variety of digestive proteins and hemolysins (21, 22, 38, 40, 42,43).The ratio of fungal fragments to spores (F/S) in the indoor in moldy houses has been calculated. The F/S for fragment sizes of 0.03 to 0.3 would be between  $10^3$  to  $10^6$ . These results indicate that the actual contribution of the fungal fragments to the overall exposure may be very high, even much greater than original estimates of 500 times the spore count (40, 41). The aerodynamic characteristics of the fungal fragments apparently have a respiratory deposition 230-250 greater than spores.



With respect to infants, the lower airway deposition is 4-5 times greater than that for adults (41).

The family lived for 8 years in the water damaged home with musty odors and visible mold that resulted from faulty roofing. Inspection and testing of the home led to the identification of elevated *Aspergillus/Penicillium spp*, *Chaetomium*, *Stachybotrys chartarum* among other genera in bulk samples taken from several areas of the home. *S. chartarum* was present in samples taken from the fraternal twins play room (family den) as well as living room (addition walls) (Table 3). The condition of the home was sufficiently serious that the Fresno County Department of Health required the family to move out. The home was eventually razed because of mold contamination and construction defects.

LC/MS/MS detection of mycotoxins demonstrated the presence of *S. chartarum* trichothecenes in bulk samples from areas of the home, including the twin's playroom. Additionally, sterigmatocystin, 5-methoxysterigmatocystin and roquefortine C were also detected in the home and the HVAC system (Table 4). These observations add to the increasing evidence that mycotoxins are present in water-damaged buildings and homes. As such they represent a toxic source of exposure via inhalation as well as oral and skin exposure (12, 17, 23-30, 33-35, 42).

The parents and two older children experienced a chronic flu-like condition with multiple symptoms as summarized in Table 1. These included nasal congestion and bleeding, sinusitis, headaches, fatigue, decreased ability to concentrate and respiratory difficulties diagnosed as RADS/asthma condition requiring bronchodilators. In addition, they had positive antibodies to the hypersensitivity pneumonitis panel (Table 2). It is well recognized that respiratory disease and infections occur in occupants of buildings and homes with water-damage and the presence of mold, bacteria and their secondary metabolites (1-12). The fungi associated with respiratory disease include the genera of *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* (1-12, 44, 45).

The entire family had episodes of nose bleeds. However, the conditions of the twins were more serious leading to hospital stays. It is noteworthy that Stachylysin has been detected in the sera of mice, humans and indoor environment of water damaged homes and buildings (46). In addition, several species of *Aspergillus* and *Penicillium* are known to produce hemolysins and probably siderophores (21, 22, 47-49). Thus, both nasal and pulmonary bleeding may well have been the result of multiple mold hemolysins as well as infection from mold and bacteria. The female twin recovered sufficiently but developed RADS. The fraternal brother was found dead in his crib with bleeding from his nose and mouth. The autopsy revealed pulmonary bleeding and hemosiderosis (Fig. 1). PCR-DNA

demonstrated *Aspergillus versicolor* in his lungs, which produces Versilysin (21, 22). In addition, AT (lungs) and MT (lungs, liver, and brain) were present in the autopsy samples. The observations on the deceased twin, as well as the other members of the family, point towards the recognition of fungi and their secondary on the health of the occupants. The presence of multiple biocontaminants, their complexity of damp indoor spaces as well as microbial particulates ranging from 0.03 to 0.3 microns probably have an impact upon human health that should be taken into account (31, 34, 35, 38-42). Finally, it is becoming increasingly apparent that occupants of water damaged environments have mycotoxins in their serum, urine, nasal cavity and various tissues (31, 33-35). In conclusion, indoor molds resulting from water-intrusion do produce and release fungal fragments (0.03-0.3 microns), multiple species of mold and bacteria, secondary metabolites, nano-particulates and other biocontaminants that most likely impinge upon the health of occupants (23-31, 38-43).

The younger of the two older sons was diagnosed with developmental delay (autistic spectrum disorder) at age 6. Since this child was in the home from infancy the exposure to microbial secondary metabolites in the home may have contributed to this condition. Information in the literature on autistic spectrum disorders suggests that mold and mycotoxin exposure appear to be contributing factors in this neurological disorder (50-53). If the respiratory deposition of fungal fragments that contain mycotoxins is considered, this is a plausible explanation for his neurological condition. A model of the human nasal-sinus cavity has shown that flow patterns in the ethmoid-sphenoid-olfactory area will allow the deposition of nanoparticles into these structures (54). Furthermore, the instillation of trichothecene mycotoxins into the nasal cavities of rodents and Rhesus monkeys causes rhinitis, nasal inflammation, apoptosis of the olfactory sensory neurons, the olfactory bulb and spreads to the brain of rodents (55-58). Furthermore, fine and ultrafine particulates with attached toxins are translocated to systemic circulation by crossing the alveolar membranes and into the brain via the olfactory tract as well as oxidative stress, systemic inflammation associated in cognitive decline. (59 62).

Comments are in order regarding the role or secondary exposure to cigarette smoke. The CDC pointed out that the Cleveland infants had exposure to tobacco smoke in their homes as verified by Dearborn et al (13, 15). The family in this investigation consisted of nonsmokers, but experienced nasal bleeding and the death of one infant from pulmonary hemorrhage. Although, secondary tobacco smoke contains particulates, nicotine and tars, nitrosamines, PAHs, etc., it should be noted that cured tobacco is contaminated with species of *Aspergillus* and *Penicillium spp*, bacteria as well as aflatoxins (63-66). Thus, the potential role of

bacteria, fungi and their toxins present in the environmental cigarette smoke in the Cleveland cases should also be considered. However, the members of the family presented herein were exposed to molds and mycotoxins present in a water-damage home. In addition, *Aspergillus versicolor* DNA and aflatoxins and trichothecenes were detected in the lungs and brain of the deceased infant.

## V. CONCLUSION

The parents and children in this case study were non-smokers. They were exposed to high concentrations of mold spores and mycotoxins present in the indoor environment of their rented home. The parents and siblings experienced multiple health conditions associated with the exposure. With respect to the fraternal twins, the sister developed nasal bleeding, fever, anemia and difficulty with breathing. She recovered sufficiently after being in the hospital and returned home. The male twin died from pulmonary bleeding and failure. PCR-DNA testing revealed *Aspergillus versicolor* in the lungs, liver and brain. Tests for mycotoxins detected aflatoxin in the lungs and trichothecenes in the lungs, liver and brain. Thus, exposure to molds and their secondary metabolites present in a water-damaged indoor environment presents a health hazard to the occupants.

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