June 17, 1999

Report of the CDC Working Group on Pulmonary Hemorrhage/Hemosiderosis

Contents

Memorandum to Associate Director for Science, Centers for Disease Control and Prevention (CDC)

Appendix A - CDC internal working group members

Appendix B - CDC-authored reports and publications

Appendix C - Working group statement on clinical and diagnostic considerations: whether the label "(idiopathic pulmonary) hemosiderosis" should be applied to the clusters of ill infants with pulmonary hemorrhage investigated in Cleveland and Chicago

Appendix D - Working group statement on study design, analytic methods and data management in the association of *Stachybotrys atra* and other fungi with acute idiopathic pulmonary hemorrhage in infants

Appendix E - Working group statement on the association of water damaged homes with acute idiopathic pulmonary hemorrhage in infants

Appendix F - Working group statement on the methods and findings of fungal sampling in the environmental investigation of acute idiopathic pulmonary hemorrhage in infants

Appendix G - Working group statement on the plausibility of the association of *Stachybotrys atra* and acute idiopathic pulmonary hemorrhage/hemosiderosis in infants

Appendix H - Working group statement on whether the clusters of acute idiopathic pulmonary hemorrhage in infants investigated in Cleveland and Chicago could be related to child abuse

Appendix I - Working group statement on the role of the NCEH/Medical Examiner special study to assess the prevalence of pulmonary hemorrhage and hemosiderosis in infant death

Appendix J - Correspondence with Cleveland investigators:
Ruth Etzel, M.D., Ph.D.
Eduardo Montaña Jr., M.D., M.P.H.
Bill Sorenson, Ph.D.
Greg Kullman, CIH, Ph.D.
Terrance Allan, RS, M.P.H.

Appendix K - Text of Dr. Dearborn response to shared working group deliberations on clinical and diagnostic considerations and proposed surveillance case definition: 8/20/98

Appendix L - American Academy of Pediatrics Committee on Environmental Health: Toxic effects of indoor molds

Appendix M - Stachybotrys website (http://gcrc.meds.cwru.edu/stachy)

Appendix N - Tables 5 (Airborne Fungal Concentrations By Filter Methods) and 6 (*S. atra* Concentrations by Filter Method) from:
Sorenson B, Kullman G, Hintz P. NIOSH Health Hazard Evaluation Report. HETA 95-0160-2571, Centers for Disease Control and Prevention, National Center for Environmental Health, U.S. Department of Health and Human Services, Public Health Service, April 1996. ("NIOSH report").
Listing of supplemental "Filter Cassette Samples"provided by Dr. Dearborn

Appendix O - Testimony given at the House Appropriations Subcommittee on Labor, Health & Human Services, & Education on Feb 3, 1998

June 17, 1999

CDC Working Group on Pulmonary Hemosiderosis

Working Group Report on the Review of CDC Investigations into Pulmonary Hemorrhage

Associate Director for Science
Centers for Disease Control and Prevention

Through: Director
Epidemiology Program Office

In November 1997, former CDC Director Dr. David Satcher designated the Epidemiology Program Office (EPO) the lead CIO to coordinate an effort to address unresolved questions in CDC's investigations of pulmonary hemosiderosis in infants in Cleveland and Chicago. EPO was charged with convening a panel of outside experts to examine the evidence and to recommend further activities. A CDC internal working group was to develop an agenda for that panel. Other EPO responsibilities included continuing active surveillance for pulmonary hemosiderosis through sentinel hospitals and medical examiners and investigating new clusters of pulmonary hemosiderosis.

To specify items to be considered by the outside panel, EPO staff agreed the CDC internal working group should conduct a scientific review of major findings and conclusions of the investigations. Specifically, the working group focused on the evidence for a relation between acute pulmonary hemorrhage in infants and living in water-damaged homes with toxigenic *Stachybotrys atra*. This evidence was based largely on the results of the Cleveland investigation, which concluded the following:

- that acute pulmonary hemorrhage in infants was associated with water damage in case homes during the 6-month period preceding the hemorrhage events (Montaña et al.) and
- that infants with the condition were more likely than control infants to live in homes with toxigenic *S. atra* and other fungi in the indoor air (Etzel et al.).

In addition, the group was to address reported clinical and diagnostic concerns of potential relevance to future surveillance efforts.

Several topics were selected by the working group for detailed exploration to cover the important scientific issues and to provide better understanding of the evidence.

The remainder of this memorandum summarizes the working group process and findings and includes recommendations for action. The group's deliberations and findings on specific topics are detailed in Appendices C-I. Topics addressed include clinical/diagnostic considerations (Appendix C), general study design and analytic issues (Appendix D), water damage assessment (Appendix E), fungal sampling (Appendix F), hypothesis plausibility (Appendix G), concerns about child abuse (Appendix H), and surveillance through medical

examiners (Appendix I). Because some issues were relevant to more than one topic, several appendices contain overlapping material. The topic of a surveillance case definition was deferred until the outside panel could help resolve clinical and diagnostic questions.

Process

The working group included CDC scientists with expertise in biostatistics, clinical pulmonology, epidemiology (general, environmental and mycotic), industrial hygiene, microbiology (mycology), and surveillance (Appendix A). Their review focused on evidence and information presented in reports of the Cleveland and Chicago investigations authored by CDC personnel (Appendix B). These documents were supplemented by reports from the literature and technical procedural manuals. Other background information was obtained by correspondence with several Cleveland study investigators (see below) and in consultation with CDC-affiliated pathologists (Appendices H&I). Dr. David Olson, NCEH, provided the Cleveland analytic data set for the working group's review. The review did not cover cases of pulmonary hemorrhage identified after the original Cleveland investigation and which constitute an expanding case series at the Rainbow Babies and Childrens Hospital. The case series, however, should be an important source of data for planning future surveillance and environmental assessment activities. In addition, the review was not intended to generate speculation on alternative hypotheses for which data may not have been gathered or reported. That role was beyond the scope of the working group assessment.

To resolve questions concerning data and methodologic issues not clarified by available documentation, the working group corresponded with several investigators (Appendix J). Individualized questions were prepared for Drs. Ruth Etzel, Eduardo Montaña, Greg Kullman, William Sorenson, and Mr. Terry Allan and mailed June 1, 1998. The working group did not formally send questions to Dr. Dorr Dearborn, the principal clinical investigator, who had already provided detailed insights into clinical concerns. Instead, the working group solicited his comments on case definition and other clinical/diagnostic considerations (Appendix K). Dr. Dearborn concurs with the working group on several points, but there are several differences of opinion that should be addressed by an outside expert panel (see Clinical and diagnostic considerations, below).

In December 1998, a preliminary report of the working group was submitted for review and comment to the Office of the Associate Director for Science, CDC, to the investigators noted above, and to Drs. J. David Miller and Bruce Jarvis. Written comments were received from Drs. Etzel, Sorenson, Dearborn, Miller and Jarvis and Mr. Allan for consideration and response (copies of the preliminary report, the written comments received, and the written CDC response are on file at CDC) and a meeting was held in Atlanta on April 16, 1999 to clarify questions that may not have been resolved in the exchange. Meeting participants included members of the Cleveland investigation team (Mr. Allan and Drs. Dearborn and Etzel) and the Cuyahoga County Health Commissioner, Mr. Timothy Horgan, representatives of the working group (Drs. Baron, Flanders and Sacks), and Drs. Stephen Thacker, Donna Stroup and yourself. This report incorporates information exchanged through comments on the preliminary report and in the April 16 discussions.

Summary of Findings and Conclusions

Clinical and diagnostic considerations (Appendix C)

- Cases reported in Cleveland and Chicago appear distinct from classically described Idiopathic Pulmonary Hemosiderosis (IPH) and may represent a clinical variant.
- The working group views "hemosiderosis" as a pathologic state and considers its use as a disease label to be clinically and epidemiologically non-specific. We propose "acute idiopathic pulmonary hemorrhage (in infants) (AIPH)" to better underscore the limited age range and striking clinical presentation described by the investigators in Cleveland and Chicago.
- The distinctive features warrant separate consideration for purposes of conducting surveillance and exploring possible etiologic factors. (Dr. Dearborn has expressed some differences with the working group opinion. It will be important to understand the clinical and epidemiologic characteristics of the Cleveland series and to assess the diagnostic specificity of bronchoscopic examination for hemosiderin.)
- Further work is needed to assess the importance of AIPH as a public health concern. Defining its clinical spectrum should precede conclusions about its etiologic ties to IPH and related assumptions about the public health importance of IPH.

Role of *Stachybotrys atra* and water damage in AIPH

- The working group concludes the epidemiologic evidence does not provide strong support for the reported association of toxigenic *S. atra* and other fungi with AIPH. Both quantifiable and non quantifiable problems limit the value of the Cleveland study towards that of a descriptive case series, although the findings are interesting and deserve further study. Despite the qualification by Etzel et al. that "further research is needed to determine whether (the) association is causal," published guidelines by the American Academy of Pediatrics Committee on Environmental Health (1998, Appendix L), a website, http://gcrc.meds.cwru.edu/stachy (Appendix M), and congressional testimony (Appendix O) imply a stronger etiologic connection than is justified by the data.

  Quantifiable concerns that influenced the association (Appendix D) include:

  < calculation of mean household *S. atra* levels;
  < assignment of an imputed value;
  < sensitivity of the analysis to a possibly unnecessary match on birth date.

  Each contributed substantially to the reported association. Adjusting for these factors reduced the *S. atra*/disease association towards the null value.

  Non quantifiable, but potentially important biases may have further distorted the *S. atra*/disease association (Appendices D&F) and include:

  < potential differential measurement of exposure;
  < possible selection bias and/or possible confounding by non-fungal correlates of water damage.
- The working group agrees with the impression that case homes had more water damage than control homes (Appendix E), although insufficient descriptive information, undefined specification of "water damage" or other evidence of household moisture, and potential biases limit confirmation and interpretation of the

reported difference.

- Other considerations, not directly related to epidemiologic findings in Cleveland, further limit support for the proposed role and mechanism of *S. atra* or other toxic fungi in the etiology of AIPH (Appendix G):
  - < AIPH does not appear compatible with historical accounts of animal and human illness caused by *S. atra* or related toxigenic fungi;
  - < AIPH does not appear in other flood-prone areas where growth of *S. atra* or other toxigenic fungi might be favored; and
  - < The mold-disease association observed in Cleveland was not observed in the Chicago cluster.
- Plausibility is diminished further by the apparent absence of reports of concurrent illness or symptoms among household members in case homes.
- Finally, technology limitations prevent confirmation of toxin production in the home environment or detection of mycotoxin in human biologic specimens.

Other concerns

- The scientific audience was not adequately cautioned in any of the publications about important limitations of the study and in the data. Furthermore, the working group considers it was an error not to have informed the audience of the extremely high, outlying values from one infant home that not only was sampled at a different time than the other homes in the study, but also strongly influenced the mean concentrations reported for fungi other than *S. atra* (Appendix D, Section IV. D.3.).

Possible role of child abuse

In consultation with CDC pathologists, the working group concluded that child abuse is an unlikely explanation for the Cleveland or Chicago clusters (Appendix H). Follow-up with social service agencies or by screening emergency room records for non-pulmonary visits by these children could provide additional confirmation. The working group considers such effort to be unjustified, however, based on a consistent absence of clinical findings that are more common than pulmonary hemorrhage in child abuse cases.

The need for medical examiner surveillance

Based on findings from an NCEH medical examiner study and a large medical examiner series in North Carolina, and on CDC pathologists' interpretation of the Cuyahoga County Coroners' study, the working group believes there is little rationale for supporting continued forensic review of infant lung tissues as an efficient source of surveillance information about pulmonary hemorrhage (Appendix I).

Recommendations

1. CDC should adopt the official position that the epidemiologic evidence does not provide strong support for the association of *S. atra* or other toxigenic fungi with AIPH. Further study is necessary to confirm the association and/or to identify other responsible factors.
2. CDC should take steps to support and promote public health messages that are consistent with the conclusions of the working group and judicious clinical practice. Public health policies, guidelines and other recommendations motivated by the proposed association of AIPH and *S. atra* should be tempered or revised. Although it is advisable to remediate molds from household environments for a variety of public health and medical reasons, the evidence reviewed by the working group does not provide strong support that it should be done solely on the basis of the proposed *S. atra*/AIPH association.
3. CDC should convene a panel of outside experts to examine all existing data and information relevant to this topic and make recommendations for further activities that will help determine: the public health importance of AIPH; the clinical spectrum and diagnostic criteria for pulmonary hemorrhage/hemosiderosis considered to be of public health concern; the cause of the condition and what factors put infants at risk; and possible interventions. Members of the working group, members of the Cleveland and Chicago investigation teams, and possibly other experts should be asked to participate as invited consultants to the panel, as required.
4. There is no current indication for surveillance for AIPH through the medical examiner or coroner systems (Appendix I).

CDC Working Group on Pulmonary Hemosiderosis

Appendix A

Appendix A

CDC internal working group members

[redacted] Medical Epidemiologist/Epidemiology Program Office

[redacted] Research Microbiologist/National Center for Infectious Diseases

[redacted] DSC/Emory University & National Center for Environmental Health

[redacted] Medical Epidemiologist/National Center for Environmental Health

[redacted] Medical Epidemiologist/National Center for Infectious Diseases

[redacted] Medical Epidemiologist/National Center for Injury Prevention and Control

[redacted] Assistant Director for Public Health (Division of Viral and Rickettsial Diseases)/National Center for Infectious Diseases

[redacted] Research Industrial Hygienist/National Institute for Occupational Safety and Health

Appendix B

Appendix B

CDC-authored reports and publications

EPI-AID/ EPI-95-10 Trip report, Dr. Eduardo Montaña, December 17, 1994.

EPI-AID/ EPI-95-21-1 Trip report, Dr. Clarice Green, January 24, 1995.

CDC. Acute pulmonary hemorrhage/hemosiderosis among infants--Cleveland, January 1993-November 1994. MMWR 1994:43:881-3.

CDC. Acute pulmonary hemorrhage among infants--Chicago, April 1992-November 1994. MMWR 1995:44:67,73-74.

Etzel RA, Montaña E, Sorenson WG, et al. Acute pulmonary hemorrhage in infants associated with exposure to *Stachybotrys atra* and other fungi (draft). Feb. 28, 1997

Green C, Etzel R, Conover C, Wylam M. Idiopathic acute pulmonary hemorrhage among infants in the Chicago area (draft). May 14, 1997

Montaña E, Etzel RA, Allan T, Horgan T, Dearborn D. Environmental risk factors associated with pediatric idiopathic pulmonary hemorrhage and hemosiderosis in a Cleveland community. Pediatrics 1997;99:117-24.

CDC. Update: Pulmonary hemorrhage/hemosiderosis among infants--Cleveland, Ohio, 1993-1996. MMWR 1997;46:33-35.

American Academy of Pediatrics Committee on Environmental Health. Toxic effects of indoor molds. Pediatrics 1998;4:712-4.

Etzel RA, Montaña E, Sorenson WG, et al. Acute pulmonary hemorrhage in infants associated with exposure to *Stachybotrys atra* and other fungi. Arch Pediatr Adolesc Med 1998;152:757-62.

Sorenson B, Kullman G, Hintz P. NIOSH Health Hazard Evaluation Report. HETA 95-0160-2571, Centers for Disease Control and Prevention, National Center for Environmental Health, U.S. Department of Health and Human Services, Public Health Service, April 1996. ("NIOSH report").

Jarvis BB, Sorenson WG, Hintikka EL, et al. Study of toxin production by isolates of *Stachybotrys chartarum* and *Memnoniella echinata* isolated during a study of pulmonary hemosiderosis in infants. Appl Environ Microbiol 1998;64:3620-25.

Appendix C

Appendix C

Working group statement on clinical and diagnostic considerations

Summary statement

" Acute pulmonary hemorrhage/hemosiderosis," "idiopathic pulmonary hemosiderosis," and "acute pulmonary hemorrhage and hemosiderosis," have all been used to label or describe the clusters of ill infants investigated in Cleveland and Chicago.

- The working group views "hemosiderosis" as a pathologic state and considers its use a disease label to be clinically and epidemiologically non-specific. We propose "acute idiopathic pulmonary hemorrhage (in infants) (AIPH)" to better underscore the limited age range and striking clinical presentation as described in Cleveland and Chicago.
- AIPH may be a clinical variant of the conditions classified as idiopathic pulmonary hemosiderosis (IPH), but its distinctive features warrant separate consideration to explore possible etiologic factors.
- Cases labeled as hemosiderosis continue to be identified in Cleveland. A systematic review of their clinical characteristics should be conducted to assess whether they fit AIPH, as presently defined, or reflect more than a single condition.

Rationale

The working group understands "hemosiderosis" to denote the presence of hemosiderin-laden macrophages in the interstitium and alveolar spaces of the lung. Although the term is used interchangeably with pulmonary hemorrhage, the working group views pulmonary hemosiderosis as a pathologic state (Boat) diagnostic for pulmonary bleeding of any type, but not a specific disease, etiology, or pathophysiologic process. Pulmonary "hemorrhage" is considered more clinically descriptive and is preferred over "hemosiderosis" (Boat). Hemosiderin in macrophages, moreover, does not, itself, indicate acute or chronic bleeding; its presence depends on the timing of lung biopsy or bronchoscopy. In Cleveland, serial bronchoalveolar lavage has been applied to the infants from the original cluster and to patients in the ongoing case series to distinguish acute, time-limited pulmonary hemorrhage (defined as hemosiderin present during 2-3 days and 2 weeks of the acute episode) from ongoing, chronic pulmonary hemorrhage (defined as hemosiderin persistent beyond 3 weeks) (Appendix K, Dearborn response). These criteria are based on a clinical study of four infants (Sherman et al.), of which only two were observed long enough to suggest the inference for chronic bleeding. The working group does not agree that a distinction between acute and ongoing/chronic bleeding can be supported by such limited observations.

In children, hemosiderosis may result from primary lung disease or as a secondary complication of cardiac disease or systemic vasculitis (Levy). The most common designation (idiopathic pulmonary hemosiderosis [IPH]) is applied when primary lung disease is unexplained after adequate investigation for etiology. The unexplained clusters in Cleveland and Chicago, however, have different clinical and demographic features than classical IPH described in the literature (Soergel and Sommers). This and textbook descriptions of IPH note its frequent insidious onset, with respiratory and other general symptoms preceding

overt signs of brisk bleeding. IPH is also associated with hypochromic, microcytic anemia, suggesting iron deficiency from chronic blood loss, without evidence of hemolysis. In contrast, all cases in Cleveland and Chicago began with acute onset of bleeding and rapid clinical deterioration. Furthermore, the hematologic work-up of these cases showed normochromic, normocytic anemia, signaling acute blood loss and mild to moderate hemolysis, indicating a prominent microangiopathic component. Finally, classical IPH is reported from infancy through late childhood, with the preponderance of cases occurring during older childhood, whereas the Cleveland and Chicago clusters were infant-specific.

The working group considers that both the acute presentation of bleeding and the infancy are important features that distinguish the Cleveland and Chicago clusters from IPH. We also believe that IPH or other labels that depend on hemosiderin, the "final common pathway"of pulmonary bleeding (Bowman), may blur important clinical and epidemiologic details and promote inclusion of unrelated clinical disorders. We suggest the label "acute idiopathic pulmonary hemorrhage (in infants)" (AIPH) will appropriately highlight the age of those affected and the unique sudden onset of frank bleeding in the Chicago and Cleveland clusters.

At the same time, the working group recognizes that recurrent episodes in some CDC-investigated cases in Cleveland and reported persistence of hemosiderin in others suggest that AIPH may progress to an ongoing or chronic process. Indeed, AIPH may be a variant or special case of the conditions resulting in IPH. Long-term follow-up of cases in the Cleveland cluster and of more recent cases there may shed further light on the natural history of this disorder.

The working group's review has been conducted to assess findings and conclusions of the two 1994-1995 CDC investigations of AIPH that cover the first 10 infants identified in Cleveland and seven infants reported in Chicago. Data gathered from case ascertainment or follow-up after the CDC investigation is beyond the scope of this review. The working group has been informed by Dr. Dearborn (Appendix K) that the Cleveland case series now includes several children who had respiratory symptoms for several days or more before acute presentation and even one child who never had overt hemorrhage. A systematic review of the clinical findings in these and additional cases added to the original series (now numbering 38, including the original 10) would be necessary to adequately describe the clinical spectrum of illness currently identified in Cleveland as AIPH/IPH.

Clarification of the natural history of AIPH and its relation to other clinical presentations of pulmonary hemosiderosis will require careful clinical observation and should not rely solely on monitoring the presence or absence of hemosiderin in specimens obtained at the time of bronchoscopic examination. In the absence of clinical history or direct observation, however, the finding of pulmonary hemosiderin may be useful as a retrospective diagnostic aid and in monitoring the clinical course and therapy of a patient.

References

Boat, TM. Pulmonary hemorrhage and hemoptysis. In: Chernick V, Boat TF, Kendig EL, eds. Disorders of the Respiratory Tract in Children. Philadelphia: WB Saunders Co., 1998:623-33.

Bowman CM. Pulmonary hemosiderosis. In: Loughllin GM, Eigen H, eds. Respiratory Disease in Children. Baltimore: Williams & Wilkins, 1994:417-20.

Levy J, Wilmott R. Pulmonary hemosiderosis. In: Hilman BC (ed): Pediatric Respiratory Disease: Diagnosis and Treatment. Philadelphia: WB Saunders Co., 1993:543-549.

Rosenstein BJ. Hemoptysis. In: Hilman BC, ed. Pediatric Respiratory Disease: Diagnosis and Treatment. Philadelphia: WB Saunders Co., 1993:533-43.

Sherman JM, Winnie G, Thomassen MJ, et al. Time course of hemosiderin production and clearance by human pulmonary macrophages. Chest 1984;86:409-11.

Soergel KH, Sommers SC. Idiopathic pulmonary hemosiderosis and related syndromes. Am J Med 1962;32:499-511.

Appendix D

Appendix D

Working group statement on study design, analytic methods, and data management in the association of *Stachybotrys atra* and other fungi with acute idiopathic pulmonary hemorrhage in infants

I. Background

Analysis of the environmental data from the Cleveland investigation produced several findings reported by Etzel et al. that were central to the association of *Stachybotrys atra* and other fungi with acute idiopathic pulmonary hemorrhage in infants:

- The mean colony count for total viable airborne fungi, over all homes, was substantially higher for case homes (29,227 CFU/m^3) than for control homes (707 CFU/m^3).
- Viable airborne *S. atra* was detected in filter samples in 5/9 (56%) case homes compared with 4/27 (15%) control homes.
- The mean colony count for viable airborne *S. atra* for all case homes (43 CFU/m^3) was substantially higher than the mean colony count for all control homes (4 CFU/m^3).
- The matched odds ratio (OR) for an increase of 10 units in the mean household concentration of viable airborne *S. atra* was 9.8 (95% CI=1.1, $3x10^6$).

Another finding asserted an interaction between environmental tobacco smoke and *S. atra*:

- The matched OR for an increase of 10 units in the mean household concentration of *S. atra* in the presence of environmental tobacco smoke was 21 (95% CI=1.1, $7.5x10^6$).

Following review of the study design, available data, and analytic methods, the working group identified several questions requiring discussion and evaluation. This statement describes the concerns considered by the working group and discusses its findings and conclusions.

II. Summary Statement

The working group's observations regarding study design, data management, and analytic approach raised concerns about the strength of the evidence supporting the association of *S. atra* and other fungi with acute idiopathic pulmonary hemorrhage in infants. The areas of concern include:

- Selection of control population
  - < Controls not selected as described in methods
  - < Potential selection bias
- Over sampling and potential investigator bias
- Possible confounding by correlate of household water damage
- Data management
  - < Calculation of mean *S. atra* concentrations
  - < Assignment of imputed data value
  - < Outlying data values
- Statistical modeling
  - < Sensitivity of the odds ratio
  - < Assessment of tobacco smoke/*S. atra* interaction

Quantitative assessment of the calculation of mean household *S. atra* concentrations and the sensitivity of results to assignment of an imputed value demonstrated that each contributed substantially to the reported association. Adjusting for these concerns reduced the association towards the null value. Other non-quantifiable concerns, including potential bias created by over sampling and simulated household activity, possible unmeasured confounders, and possible self-selection by controls, may also have distorted the association. Unstable results, in part due to scant data, contribute to uncertainty in the study. Further consideration by the working group suggested that reflecting the match on birth date in the analysis was probably unnecessary and contributed to the unstable results. Unmatched analysis led to a weaker association. Finally, concerns about data management and oversight raise important questions about the editing and preparation of data for analysis and presentation.

The working group concludes that although the evidence does not reject the hypothesis, it does not provide strong support for the published association. Although the degree of bias and the true association are difficult to ascertain, both quantifiable and nonquantifiable concerns converge to limit the value of the study towards that of a descriptive case series. Nevertheless, the current findings are interesting and deserve further study.

III. Methods

A. Control selection and enrollment

The methods of Montaña et al. and Etzel et al. indicate that controls were identified from the Rainbow Babies and Childrens Hospital clinic population and from Cleveland birth certificate records. For each case-infant, investigators "generated a list of potential controls from all infants born in Cleveland within 2 weeks of the patient and presently living in an area bearing 1 of the 6 ZIP codes in which all of the patients lived" (Etzel et al.). Prospective parents were contacted by telephone and the first three children whose parents agreed to participate were enrolled.

B. Analysis

Viable airborne fungi were plated out on four different media and colony growth was classified into the following categories: *Aspergillus*, *Cladosporium*, *Penicillium*, *Stachybotrys*, and other, which included all other fungi observed. Concentrations were reported as colony-forming units per cubic meter of air sampled (CFU/m^3). Mean concentrations of fungi in the air were calculated for the homes of cases and controls by dividing the total number of CFU by the number of plates from each home. LogXact was used in conditional (on birth date) logistic regression to calculate a matched OR for a change of 10 units in the mean concentration of viable airborne *S. atra* in the homes of the case-infants compared with the homes of controls. Mean concentration was used as a continuous predictor in the model. To test for interaction with environmental tobacco smoke, a multivariate model that also controlled for the matching was constructed.

IV. Concerns

A. Selection of controls

1. Controls not selected as described in methods - An examination of the analytic data set provided by Dr. David Olson, NCEH, and of a line listing provided by Dr. Dearborn indicated that 13 (48%) of the 27 controls with fungal counts in the data set and 13 (43%) of all 30 controls on the line listing were enrolled from ZIP code areas that did not match the six areas (as reported in Etzel et al.) where case-infants lived (the line listing indicated that cases resided in only five ZIP code areas). Although this appeared to indicate noncompliance with published methods, correspondence with the authors and later discussions in an April 16, 1999 meeting with the Cleveland team indicated all except three controls (study ID #s 12, 15,and 18) were enrolled from neighborhoods that were socioeconomically and geopolitically similar to neighborhoods in which all case infants lived. The working group agreed that the decision to use ZIP codes as the geographic unit for selecting controls may have been unnecessary. Reanalysis of the data after eliminating the three outlying controls did not, itself, alter the published OR (Section V).

2. Potential selection bias - Investigators did not record unsuccessful contact attempts or refusals to assess non-participation among eligible control subjects (Dr. Montaña, interview). Whereas all cases were included, there was substantial opportunity for self-selection (selective nonparticipation) among potential controls. Factors related to child's health, home environment (including flooding and other signs of water damage or other conditions that may correlate with fungal growth), and other social factors could have influenced a decision to participate in the study. Differences observed between case and control infants in birth weight, breast feeding, and household smoking, may reflect selective participation rather than differences related to disease, and suggest that control households may not represent the population from which case-infants were derived. Although these factors may alternatively reflect the risk profile of case patients, the working group could not dismiss self-selection among candidate controls as a possible source of bias in the association of *S. atra* and other fungi with pulmonary hemorrhage.

B. Over sampling and potential investigator bias

As described in the statement on sampling methods (Appendix F, Section IV.A.), the working group observed a systematic pattern of over sampling (80%) for viable airborne fungi in case homes. This was difficult to reconcile with reported assertions that industrial hygienists were blinded to case/control status when sampling homes. The explanation was traced to a single hygienist who suspected he was primarily sampling case homes (although he was never told the case status of any particular home) and who used additional filter cassettes to "ensure that we would not lose an observation point for viable fungi from one of these case homes." This is problematic because case homes predominated in his assignment. Moreover, in view of his intent, the working group could not dismiss the possibility that he may have been more aggressive in his effort to aerosolize fungal spores (simulated household activities, Appendix F). Over sampling and the potential for aggressive aerosolization raises the possibility of biased detection and differential measurement of fungal concentrations.

Apart from analytic and study design concerns, over sampling and investigator bias have important implications for the quality of data described in Appendix F; these factors could potentially explain part or all of the estimated case-control difference in detection or measurement of airborne fungi.

C. Possible confounding by correlate of household water damage

S. atra requires water-saturated, cellulose-based materials for growth in buildings and is not found on dry surfaces. The February 1997 draft of Etzel et al. reported water damage or a plumbing problem in eight of the nine case homes sampled for fungi but in only seven of the 27 matched controls. It further noted that air filter samples detected viable *S. atra* in four of the eight case homes with water damage or plumbing problems compared with three of the seven control homes. Although the numbers are small, this suggests little or no difference in detection of *S. atra* growth between case and control homes with water damage, and suggests that some correlate of water damage, other than *S. atra*, may confound the association. With the very small study size and the known link between water damage and fungal growth, it would have been very difficult to separate the effects of a correlate of water damage and *S. atra* growth in this study.

D. Data management

1. Calculation of mean *S. atra* concentrations - In a telephone conversation, Dr. Sorenson indicated that one of the four culture media, DG18, did not support *S. atra* growth in the laboratory. Values from that medium (always "0") should have been excluded from the calculated mean household *S. atra* concentrations. The February 1997 draft of Etzel et al. also states "The . . . . medium *(DG18)* is not appropriate for *S. atra* because the available water of this medium is less than that required for normal growth and sporulation of the species." This was not in the published manuscript, however, where the reported mean values for study households were based on all four media (Table 6 [*S. atra* Concentrations by Filter Method] of Sorenson et al. [the NIOSH report]; Appendix N). Recalculated means, based only on the three supportive media, increased the overall mean for case homes from 43 (as published) to 62 CFU/m^3 and for control homes from 4 to 5 CFU/m^3. Substituting the recalculated means into the logistic model reduced the matched OR from 9.8 to 5.5 (Section V).

2. Assignment of imputed data value - In the analysis, one case-infant (# 8) was assigned a(n imputed) value, defined as "half the limit of detection . . . when *(colonies are)* detected but too few to count (divided by the number of plates)" for the mean household concentration of airborne *S. atra* (Table 5, Etzel et al.). This infant is represented as "patnum=9" in the analytic data set, which records only the mean concentrations used in the analyses. For the analysis, an imputed value ("4") was substituted for the original value ("0") coded for that infant (laboratory records [1]

[1]Sampling data for case-infant #8 are not included in the NIOSH report since investigators were not permitted to enter the home (Site 9A) until several months after the field investigation. Data, based on samples collected later and processed with samples from subsequent case households, were provided by Dr. Dearborn, and are

provided by Dr. Dearborn confirm *S. atra* was detected, but not quantified, on one plate). The OR by matched analysis was very sensitive to the substitution. After correcting mean household *S. atra* concentrations, replacing the original value "0" reduced the OR from 5.5 to 1.9 (Section V). Sensitivity to a relatively small change in a single data entry is a concern for two additional reasons. First, because the sample was collected and tested several months after other study samples, "detection" of the *S. atra* colony may have been influenced by sampling techniques, laboratory or environmental conditions, or detection criteria that were not applied to specimens collected at the time of the field investigation. Second, use of an imputed value to estimate average concentration in the presence of nondetectable values (Hornung and Reed) may not be justifiable when the proportion of observations with nondetectable values is greater than 50 %, as in this study.

3. Outlying data value - Table 2 (Unmatched Analysis of Filter Samples) in Etzel et al. reports that case homes had a mean total airborne fungal concentration of 29,227 CFU/m^3. This is several-fold higher than the mean reported in Table 7 (Airborne Fungal Concentrations by Case Status Using Filter Methods) of the NIOSH report (3300 CFU/m^3). This did not include data for the infant identified as #8 in Etzel et al. and is much higher than the concentration observed in any single agar plate (19,870 CFU/m^3) recorded in Table 5 of that report. The analytic data set, recording data for case-infant #8 as "patnum=9," had mean household concentrations of non-*Stachybotrys* fungi that were several orders of magnitude higher than the means recorded for the other eight case homes. For "patnum=9", for instance, the data set records the mean *Aspergillus* concentration as 203,214 CFU/m^3 (consistent with the mean for agar-specific values on the list from Dr. Dearborn, in Appendix N), which contrast with mean concentrations for the other eight patients, range: 32-2580 CFU/m^3; it records the mean *Cladosporium* concentration as 11,760 CFU/m^3, contrasting with mean concentrations for other patients, range: 0-530 CFU/m^3; it records the mean "other" fungi concentration as 29,500 CFU/m^3, contrasting with mean concentrations for other patients, range: 60-3830 CFU/m^3; and, finally, it records the mean total (sum of the individual means) fungi concentration as 247,000 CFU/m^3, contrasting with concentrations, range: 290-10,100 CFU/m^3. In a letter commenting on the provisional working group report, Dr. Sorenson verified there were no laboratory or transcription errors. At the same time, he offered no explanation for the extreme values. The working group believes that airborne concentrations of non-*Stachybotrys* fungi in the home of case-infant #8 may have been much higher than in other study infant homes due to differences in sampling methods or laboratory conditions, because this home was investigated several months after the others (values for non-*Stachybotrys* fungi in post-study homes sampled at the same time as that of infant #8 were also exceedingly high). The extreme values were influential in the difference reported between case and control homes for non-*Stachybotrys* fungi and should have been acknowledged by the authors and shared with the readers.

---

included in Appendix N.

E. Statistical modeling

1. Sensitivity of the odds ratio - Section IV.D.2 notes the sensitivity of the OR to a small change in the value of a single data entry. In addition, the working group thought that, in retrospect, reflecting the match in the analysis should be unnecessary. In fact, the OR was also highly sensitive to the match on birth date, declining from 9.8 to 1.5 when matching was not reflected in the analysis (Section V). This should not be due to confounding, because birth date should not influence exposure to *S. atra* measured during the investigation[2]. This sensitivity to relatively small changes in the data or to the choice of analytic strategy indicates an unstable OR, perhaps as a result of the small amount of information in the study.

2. Assessment of tobacco smoke/S. atra interaction - The authors note an "interaction with environmental tobacco smoke" based on results of a multivariate model. The actual logistic model is not described in Etzel et al., but Dr. Olson indicated the model included a main-effect term for *S. atra* and a *S. atra*/tobacco smoke interaction term, but without a main-effect term for tobacco smoke. Application of the non-hierarchical model was dictated by the small amount of data. According to Dr. Olson, the interaction term from the multivariate model would exhibit instability comparable to that of the *S. atra* main effect term in the univariate model and would also be similarly influenced by both quantifiable and nonquantifiable problems recognized in the study design and the data.

[2] In fact, birth date did not correlate with the corrected mean household concentrations of S. atra, as recorded in the data; correl. coef. = 0.2, p=0.4

V. Repeat analyses

The working group applied both matched (conditional on birth date) and unmatched logistic models in a stepwise reanalysis of the data first, eliminating controls from outside the geographic range of cases (Section IV.A.1); next, applying corrected mean household *S. atra* concentrations (section IV.D.1.); and, finally, replacing the imputed value with the original value "0" (Section IV.D.2.). Elimination of subjects outside the geographic location of cases had no impact on the published (9.8) OR, but subsequent corrections in matched analysis reduced the association towards the null value. Moreover, the table illustrates the sensitivity of the published OR to the choice of analytic strategy, as described in Section IV.E.1.

Correction (Stepwise)	OR (95% CI) matched analysis	OR (95% CI) unmatched analysis
None	9.8 (1.1-3x10^6)	1.5 (1.1-2.5)
Remove three controls enrolled from outside case geographic range	9.8 (1.1-3x10^6)	1.5 (1.1-2.5)
Correct mean household *S. atra* concentrations	5.5 (1.1-7x10^4)	1.3 (1.1-2.0)
Replace imputed value with "0"	1.9 (1.1-3x10^4)	1.3 (1.1-1.9)

VI. Conclusion

Several problems related to study design, data management, and analysis were explored by the working group and raise concerns about the association of *Stachybotrys atra* and other fungi with acute idiopathic pulmonary hemorrhage in infants reported in Etzel et al.

Quantitative assessment of the miscalculated mean *S. atra* concentrations and the sensitivity of results to assignment of an imputed value demonstrated that each contributed substantially to the reported association. Adjusting for these factors reduced the association towards the null value. Eliminating controls enrolled from outside the region occupied by case patients had no effect on the reported OR.

The working group is concerned about the imputed value for several reasons. First, the sensitivity of the model to a relatively small change in value suggests uncertainty about the accuracy of an OR based on the imputed data. Second, the imputation was done for a single case household whose exposure data were gathered much later than the data from other study households. Non-*Stachybotrys* airborne fungal concentrations in this and other (non-study) households sampled at the same time were several orders of magnitude higher than concentrations observed in samples taken earlier from other study homes, suggesting that sampling methods or laboratory or environmental conditions may not have been comparable. Finally, in the method applied to estimate average concentration in the presence of

nondetectable values (Hornung and Reed), it is not appropriate to impute a value when the proportion of nondetectables is greater than 50 %, as in this study.

The unmatched analysis also suggest a weak association. Based on the assumption that birth date does not confound the association, analyses that did not reflect the match on birth date produced a substantially smaller OR (1.5) than published (9.8) and one that was more robust to subsequent adjustment.

Other potential biases, including selection bias, differential measurement of exposure (over sampling by air filtration and investigator bias), and possible unmeasured confounders (e.g., correlates of water damage), were not amenable to quantitative assessment, but may have influenced the association. These potential problems could have been discussed by the authors as possible limitations to the study. In particular, and apart from other concerns discussed in this statement, the working group is concerned that possible differential measurement between case and control homes (caused by the inadvertent unblinding of an investigator) may have contributed to a substantial portion of the reported association.

The reported interaction between *S. atra* and environmental tobacco smoke may be misleading for the same reasons that lead to concern about the estimate of the *S. atra* main effect: instability caused by scant data, the reflection of the matching in the analytic strategy, and the influence of other quantifiable (calculation of mean household values and substitution with an imputed value) and nonquantifiable (potential bias) concerns.

Finally, concerns about data management and oversight with regard to calculation of household means, the assignment of an influential imputed value, and failure to acknowledge the influence of extreme values from a single home raise important questions about the editing, preparation, and interpretation of data and findings of the Cleveland investigation.

The working group maintains the evidence does not provide strong support for the published association. Although the degree of bias and the true association are difficult to ascertain, both quantifiable and nonquantifiable concerns converge to limit the value of the study towards that of a descriptive case series. Nevertheless, the findings are interesting and deserve further study.

References

Etzel RA, Montaña E, Sorenson WG, et al. Acute pulmonary hemorrhage in infants associated with exposure to *Stachybotrys atra* and other fungi. Arch Pediatr Adolesc Med 1998;152:757-62.

Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg 1990; 5:46-51.

Montaña E, Etzel RA, Allan T, Horgan T, Dearborn D. Environmental risk factors associated with pediatric idiopathic pulmonary hemorrhage and hemosiderosis in a Cleveland community. Pediatrics 1997;99:117-24.

Sorenson B, Kullman G, Hintz P. NIOSH Health Hazard Evaluation Report. HETA 95-0160-2571, Centers for Disease Control and Prevention, National Center for Environmental Health, U.S. Department of Health and Human Services, Public Health Service, April 1996. ("NIOSH report").

Appendix E

Appendix E

Working group statement on the association of water damaged homes with acute idiopathic pulmonary hemorrhage in infants

I. Background

A key finding in the Cleveland investigation, reported by CDC *(MMWR* 1997) and by Montaña et al., was the association of "major water damage (as a result of chronic plumbing leaks or flooding)" in case homes during the 6-month period preceding the pulmonary hemorrhage events (OR=16.3; 95% CI=2.6-inf.). Concluding that environmental risk factors may contribute to acute idiopathic pulmonary hemorrhage in infants, the investigators proposed that water damage, or something associated with water damage, was the primary risk factor. Notably, water damage was not observed or implicated as a possible risk factor in the Chicago outbreak.

Following review of available data from the Cleveland and Chicago outbreaks, the working group identified several questions about the methods and findings concerning the role of water damage in acute idiopathic pulmonary hemorrhage. This statement describes the issues discussed and evaluated by the working group and discusses its findings and conclusions.

II. Summary statement

The working group concludes there is insufficient descriptive information or documentation of water damage to interpret reported differences between case and control homes. The working group has the following specific concerns:

- **No standardized definition of water damage:** The working group is uncertain about what constitutes "water damage" and about the nature or extent of water damage in many case homes and in all control homes.
- **No standardized protocol applied to inspect for visible signs of water damage:** The working group is uncertain if all homes were inspected completely and if observations were comparably ascertained and recorded.
- **Questions about water damage and leaks added after formal interview:**. The working group is uncertain if the supplemental information was completely ascertained for both case and control homes.
- **Potential biases are not addressed:** The reported methods and other working group findings suggest that selection and investigator bias could have distorted the association.
- **Contradictory reporting of water damage:** Montaña et al. reports water damage/flooding in all ten case homes, whereas a draft of Etzel et al. reports water damage/flooding in eight of the nine case homes that underwent fungal sampling.

In the absence of more detailed descriptive and quantitative data, the report of the Cleveland investigation of acute idiopathic pulmonary hemorrhage supports only an impression that there was more water damage in case homes than in control homes. In addition, potential selection and investigator biases, not addressed by the authors, may have influenced the association. Contradictory reporting of water damage by two principal investigators raises questions about communication among the authors.

III. Background information on household water damage ascertainment

An exploratory walk-through of seven case homes, not described in published accounts, revealed unanticipated "extensive" water damage or flooding in several, but not all, case homes (Montaña interview). Observations included flooded basements, some with free-standing sewage, and water stains on the walls or ceilings. Until then, investigators had been considering the hypothesis that illness was related to organophosphate pesticide poisoning.

During a subsequent visit (described in Montaña et al. as "the initial home visit" to distinguish it from a later visit to collect samples for environmental fungi), all case and control homes were inspected by local registered sanitarians for pesticides and other toxic substances while a medical team conducted interviews with a parent or primary caretaker. Sanitarians and interviewers were "unblinded" (aware of case/control status) during that visit. According to Montaña et al., the interviews were conducted by a pediatrician and included questions about "any home water damage, caused either by plumbing problems, roof leaks, or flooding" in the 6-month period preceding the case-infants' hospital admission for pulmonary hemorrhage. Montaña et al. further state, "while the interview was being conducted, ... the sanitarian evaluated the general condition of the home and recorded any visible signs of water damage to the physical structure."

IV. Issues

- Definition of "water damage" - The working group was unable to determine what constitutes "water damage" or "severe water damage." No definition is offered in the *MMWR* (1997) report or by Montaña et al. Dr. Montaña confirmed that no strict definition was used to assign water damage status to the study homes, and he could not be certain that similar criteria were applied from home to home.
- Descriptive information - The working group observed that published reports from the Cleveland investigation do not include descriptions of the flooding, water damage and other home leaks, or of the mold growth evidence. A research microbiologist from NIOSH visited seven case homes in December 1994 and noted that water damage varied from a small area of wet plaster on a bathroom ceiling in one home to flooded basements in several others (Lonon, M. December 14, 1994 memorandum to Dr. Ruth Etzel, attached). There is no descriptive information available to the working group about water damage in homes of control children.
- Inspection protocol for observing and recording visible signs of water damage - The working group was concerned about the completeness of home inspections. No standardized inspection protocol was applied to the inspection for visible signs of water damage. In his telephone interview, Dr. Montaña was not certain if comparable inspections were conducted from home to home. The working group is uncertain if all homes were inspected completely and if observations were comparably ascertained and recorded.
- Visual inspection not conducted as reported - Responses by Mr. Terry Allen to questions about the methods and findings of the visual inspection for water damage during the "initial" visit indicated some misunderstanding about the nature and timing of this component of the investigation. The working group pursued some of these questions

with Dr. Montaña and learned that the visual inspections were not performed during the structured interviews, as reported in Montaña et al. (last sentence, Section III, above), but rather during the later visit for fungal sampling.

- Interview information about leakage, water damage and flooding - The survey instrument used for the household interview in Cleveland (EPI-AID Trip report, Dr. Montaña) contained only the following questions about flooding, water damage, or plumbing leaks:

  "Did you experience flooding or leaking in the summer months?"

  "Did you have sewage backup in your house?"

  The working group could not reconcile these questions with the information reported about sources or timing of the household water damage. Dr. Montaña's interview indicated that five additional questions were asked in a subsequent visit by a nursing team member to assess home water damage. Dr. Montaña did not participate in the follow-up and could not verify whether supplementary information was completely ascertained for both case and control homes and whether the questions were asked in a comparable manner to each group.
- Potential biases - The working group noted the authors did not address potential sources of selection and investigator biases that may have played a role in distorting the association of water damage with pulmonary hemorrhage. Dr. Montaña indicated that investigators did not keep records of unsuccessful attempts to contact potential enrollees or of refusals by those contacted. Differences observed between case and control infants in birth weight, breast feeding and household smoking, may reflect selective participation rather than differences related to disease, and suggest that control households may not represent the population from which case-infants were derived (alternatively, these factors may truly reflect the risk profile of case patients). Eligible control homes may not have participated if their homes were flooded or had unrepaired damage from past water problems. Without information about success of telephone contact or refusal rate, the working group could not dismiss a possible role of selection (selective nonparticipation) bias in the association of water damage with pulmonary hemorrhage. Because neither the interviewers nor the sanitarians inspecting for water damage were blinded to case/control status, the working group could not dismiss a possible role of investigator bias in identifying or recording evidence of water damage.
- Contradictory information concerning water damage - There may be disagreement between Drs. Montaña and Etzel on the number of water-damaged case homes. Whereas Montaña et al. reported water damage in the homes of all ten case-infants, the February 1997 draft of Etzel et al. reported water damage in only eight of the nine case homes sampled. Dr. Montaña (interview) indicated he did not understand why one case home had not been linked with water damage in the Etzel et al. draft and affirmed his position that all 10 case homes had water damage. No information concerning water damage was mentioned in the published Etzel et al.

V. Conclusion

The working group concludes there is insufficient documentation and description of water damage to interpret the reported difference between case and control homes. Although the working group agrees the reports and correspondence with the investigators support an impression that case homes experienced more water damage than control homes, substantial concerns about the data gathering methods and the findings remain.

- A standardized definition of "water damage" was not applied from inspection site to inspection site, and the working group is uncertain regarding the definition of "water damage" or "severe water damage" in this context. The working group is uncertain about the extent and location of water damage in many of the case homes and in all control homes;
- A standardized inspection protocol was not applied to the assessment of visible signs of water damage, raising questions regarding whether all homes were inspected completely and comparably;
- Interview questions about water damage and leaks were added after the formal interview; the working group has no assurance that supplemental information was ascertained completely and comparably for both case and control homes;
- The association could be distorted by potential biases introduced through self-selection of controls or by systematic biases in the methods interviewers and inspectors used to make observations or to record information. These potential biases were not acknowledged or discussed by the investigators;
- Contradictory information concerning water damage underscores the working group concern about the definition of water damage and raises questions about communication of findings and interpretations among investigators.

Attachment:

References

CDC. Update: Pulmonary hemorrhage/hemosiderosis among infants--Cleveland, Ohio, 1993-1996. MMWR 1997;46:33-35.

EPI-AID/ EPI-95-10 Trip report, Dr. Eduardo Montaña, December 17, 1994.

Etzel RA, Montaña E, Sorenson WG, et al. Acute pulmonary hemorrhage in infants associated with exposure to *Stachybotrys atra* and other fungi. Arch Pediatr Adolesc Med 1998;152:757-62.

Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg 1990; 5:46-51.

Montaña E, Etzel RA, Allan T, Horgan T, Dearborn D. Environmental risk factors associated with pediatric idiopathic pulmonary hemorrhage and hemosiderosis in a Cleveland community. Pediatrics 1997;99:117-24.

December 14, 1994

Miriam Lonon, Ph.D., Research Microbiologist, MRB, DPSE, NIOSH

Investigation of Acute Pulmonary Hemorrhage/Hemosiderosis Among Infants

Ruth Etzel, M.D., Ph.D., Branch Chief
Air Pollution and Respiratory Health Branch, DEHHE, NCEH
Through: Henry Falk, M.D., Director, DEHHE, NCEH_____
Laurence J. Doemeny, Ph.D, Acting Director, DPSE, NIOSH_____
Chief, MRB, DPSE_____

Thank you for the opportunity to participate in the NCEH investigation of idiopathic pulmonary hemosiderosis (IPH) at Rainbow Babies and Children's Hospital in Cleveland. As agreed in our telephone conversation of December 7, 1994, I accompanied the EIS Officer, Dr. Eduardo Montana, on site visits to seven of nine case homes to conduct a preliminary "walk-through" examination of the houses. The purpose of our visits was primarily to look for evidence of gross microbial (fungal) contamination and to take bulk samples of contaminated materials for further analysis. We also took cellophane tape samples of obvious fungal growth for direct microscopic examination. This preliminary examination was similar to the standard NIOSH site visit made by industrial hygienists as the first step in the performance of a Health Hazard Evaluation (HHE). Concurrent with our investigation, there was also a NIOSH-assisted investigation of the case homes plus control homes (3 for each case home) for the presence of organic pesticides.

My observations concerning the homes we examined are as follows:

This home was a two story house (plus attic and basement) that had been divided into two apartments. The had occupied the second floor. ,ut we were still able to gain entry.) The house was of frame construction with the interior finished in drywall, plaster, and wood-grain paneling. The inside of the house was dirty and the family's belongings were still scattered about. There was a

small deck on the roof of the front porch, accessed from the living room. A small dog was kept there and the area was littered with dog feces, much of it in small weathered pellets. No overt contamination was found in any of the rooms in the living area. The basement was accessed via the back stairs. The wall in the basement stair well was wet from a leaking pipe and there was a sooty black growth in the area wet by the leak. The appearance of the material was consistent with that of the fungus, Stachybotrys. Tape samples were taken and later microscopic examination confirmed that it was indeed Stachybotrys. The basement, though now mostly dry and somewhat dusty, showed signs of prior flooding. There was a water mark of approximately six inches on the wall. I was told that there had been flooding this past summer. At this time, the only water appeared to be plumbing leaks, one of which had resulted in rotting of the wood in some of the steps. The air intake for the furnace was at floor level and the filter was dirty. I was told that the baby's crib had been directly over a heating duct from this furnace.

This home was not a house, but a second story apartment unit in an apartment complex. The unit was small, but very clean and well kept. Heat was provided by a steam radiator near the baseboard and there was a wall unit air conditioner. The air conditioner was reported to be new, installed during the late summer to replace an old, non-working unit. There was no evidence or reported history of water incursion, save a chronic leak over the tub in the bathroom, presumably from the bathtub in the unit directly above. The ceiling has just been replastered, but the plaster was wet and already crumbling. Samples were cut from this material, but no mold growth was evident here or any place else in the apartment.

This home was attached to a row of store fronts in a business area. The apartment, entered from the alley, was behind and above a T-shirt shop. The furnace was in the basement and had to be accessed through the shop. (This necessitated a second visit to this site.) The apartment was clean, but in very poor condition. There were stains from roof leaks, but I was told that the roof had been replaced during the summer. The living room (where the baby slept on the couch with his mother) had been recently

repainted. The occupants were in the process of converting the attic to living space and the bedrooms were all upstairs. In lieu of insulation, the spaces between the wall studs were stuffed with old newspaper and were being covered with scraps of drywall and/or cardboard. Some of the paper showed creosote stains and there was a phenolic smell about it. Some of it was yellowed and brittle, suggesting that it had been in place for some time. The paper was dry and there was no evidence of microbial growth (perhaps because of the creosote). There was chalk dust on the paper, probably the result of drywall finishing in the area. The baby's crib was in this area, but the baby reportedly only slept in it for one night, the night he became ill. Samples of the cardboard and paper were taken for further analysis. The basement, entered later through the T-shirt shop, had some water on the floor. This appeared to be due to a water leak from the pipes going to the kitchen in the apartment. Even though there was particle board immediately under the leak, I found nothing to suggest Stachybotrys contamination. There was gray-greenish mold growth, however, and microscopic examination of tape samples suggested the presence of Penicillium. The proprietor of the shop told us that water in the basement was a perennial problem. Bulk samples were taken for further analysis.

The house was a neat Cape Cod style dwelling in a middle-class neighborhood. The mother and baby lived in a room in the finished basement. Although there were reports of past water problems, the basement appeared to be dry. The furnace was in the adjoining room, which was also used for laundry, sewing, and storage. There were what appeared to be asbestos panels on the wall and ceiling surrounding the furnace. The ceiling tiles in the living area had dark stains on them, but these did not appear to be fungus. Likewise, tape samples of the stains revealed nothing. No evidence of fungal growth was found. ,,

This was a frame house similar in style and age to the Again, the dwelling had been converted to two apartments. The (and a large dog) occupied the upper unit. The apartment appeared to be very crowded with furniture and belongings. Even the bathroom

was doubling as a closet, with so many clothes on hangers hung on the shower rod that the tub was almost obscured. Several of the rooms, reportedly sleeping quarters of family members, were padlocked and a thorough inspection of all the rooms was not possible. Narrow back stairs led up to the attic and down to the basement. The stair wells, going up and down were partially blocked by boxes, bags of aluminum cans, toys, and trash. The attic was one long room with windows at each end. The windows at both ends of the room were broken and rain was blowing in. The room was full of stored furniture, clothing, and other belongings, and it was difficult to move about, but there was no evidence of mold. The basement, two levels down, was similarly filled. Picking our way through, we found the furnace, standing in raw sewage from a leak from the bathroom pipe and surrounded by a crumbling heat shield of what appeared to be asbestos. There was evidence of earlier flooding and tape samples from the ruined drywall yielded Stachybotrys. However, unlike the growth found in the this was dry and probably not actively growing, though the spores could certainly remain toxic. Samples were taken, but care was taken to minimize disturbance of the material and aerosolization of the spores. (Actively growing Stachybotrys, such as that found in the is wet and sticky and does not readily release its spores.)

This is the home of the child who died. The family was not anxious to have us back, but agreed to allow us into the basement and then into the bedroom the child had shared with his mother. Again, the age and architecture of the house resembled that of the though this one appeared still to be a single-family home. The basement was very dirty, though currently dry. The reported that it, too, had been flooded during the summer. Stachybotrys was found on a piece of gypsum board that had been used to close off the ash door of an old flue. (The flue appears now to be used as a means to vent the water heater and the furnace.) The fungus was dry and care was used in taking both tape and bulk samples. There was a vent in the baby's room, but we were unable to access it. A similar vent in the bathroom was uncovered and found to contain dirt, hair, and other debris.

This home, which is also the same style and vintage as the was very clean and attractively furnished. I was told that, at Dr. Dearborn's suggestion, gave the house an especially thorough cleaning before the child returned from her last stay in the hospital. (This was the home of This cleaning included removal of jars of water placed in the ducts to humidify the air during the winter months. The basement was also very clean and there was no water problem save a leak in a cracked laundry tub. Stachybotrys was found on the underneath side of a scrap of paper-backed vinyl floor covering used as a rug in front of the washer, near the leaking laundry tub.

Summary:

Stachybotrys was found in four of the seven case homes I visited. All four of these homes were of the same style and approximate age and, with the exception of the all were within the same general area of the city. All had water in the basement at some time during the past year. It is difficult to evaluate the significance of the presence of this fungus in these homes. I believe that control homes of the same style and vintage and with a similar history of water incursion might also contain evidence of similar contamination. The relationship of Stachybotrys in the environment to respiratory exposure of building occupants is poorly understood. Presence does not necessarily equal exposure, especially if the fungus is wet. The link between respiratory exposure to spores and health effects is even less well-defined. All strains of Stachybotrys are not toxigenic and toxigenic strains do not produce the toxin under all environmental and physiological conditions.

Finally, the clinical picture, as it was related to me, does not fit well with that reported for trichothecene toxicosis, particularly with respect to the blood profile. Although there are many different trichothecenes, a general hallmark of trichothecene poisoning is pronounced leukopenia and severe thrombocytopenia. Patients are also likely to experience arrhythmatic heartbeat and neurological symptoms. I was told that the differential blood counts and clotting times for these children was normal. While hemorrhage from the gut is often the result of animals' ingestion of contaminated feeds, there is no clear association between inhalation and pulmonary hemorrhage noted in the literature.

Recommendations:
I do not recommend extensive air sampling using the so-called "viable" methods, which depend on cultivating the organism from air samples collected on growth medium. If the spores are viable, they are likely to be too wet and sticky to become airborne. If they are dry enough to be released, they may also be non-viable and therefore would not be detected by methods that depend on cultivation.

Because of serious scientific and ethical considerations, NIOSH investigators generally do not perform "aggressive" sampling in indoor environments. I most definitely do NOT recommend aggressive sampling in this case. Aggressive sampling consists of disturbing the environment so as to deliberately cause materials to become airborne. As this amounts to deliberate contamination of the air in these homes, the families would have to be moved out. Since many of these homes are not single family dwellings, presumably the relocation would include those individuals in neighboring apartments as well. It must be remembered that many of these buildings may be contaminated with many particulates other than spores, including lead, asbestos, and potentially harmful bacteria. How would clean up of the home be addressed after aggressive sampling? Quiescent sampling is generally recommended as a prelude to aggressive sampling. In this case, it must also follow aggressive sampling to establish when the levels have returned to "baseline". What levels of fungi (not to mention all other contaminants) would be considered acceptable for the resumption of habitation? (There are no standards or exposure limits for fungi.)

If sampling is done, it is important to remember that toxin may be found in nonviable as well as viable spores. For the collection of total particulates, including all spores, other methods, such as sampling on filter cassettes may be more suitable. These samples can be extracted from the filters and the extracts can be tested for cytotoxicity. Spores can also be collected using spore traps and the samples examined for possible enumeration and identification. (However, it is difficult to positively identify fungi without observing the morphology of their spore-bearing structures.) In addition, although it is difficult to associate current environmental conditions, including the presence of fungi, with exposures that may have taken place months ago, acute-phase serum from patients may be tested for evidence of trichothecenes.

In conclusion, I have seen no evidence to suggest that these children are any more at risk for mycotoxicosis than are other children exposed to the same conditions, -- conditions that are ubiquitous in this area. There are many fungi and they are capable of producing dozens of mycotoxins. Initiation of an extensive sampling regimen for one such organism without regard for other contaminants in the environment does not seem warranted. It is, of course, possible that these children have some physiological predisposition that makes them vulnerable to these toxins, but I know of nothing in the literature to suggest this. However, there is evidence that some ethnic populations manifest a genetic predisposition to certain pesticides, and that exposure to these substances may result in pulmonary hemorrhage. I do not know what levels of pesticides have been measured in these homes, but I found it remarkable that in none of the homes I visited, even those which were filthy, and even in the basements and attics, did I ever see any evidence whatsoever of arthropods. I saw no roaches, no spiders, and no water bugs.

Miriam K. Lonon, Ph.D.

Appendix F

Appendix F

Working group statement on the methods and findings of fungal sampling in the environmental investigation of acute idiopathic pulmonary hemorrhage in infants

I. Background

Environmental sampling in the investigation of acute idiopathic pulmonary hemorrhage (AIPH) in Cleveland has led to the published conclusion that infants with acute pulmonary hemorrhage were more likely than control infants to live in homes with toxigenic *Stachybotrys atra* and other fungi in the indoor air (Etzel et al.). This was supported by the following findings:

- Viable *S. atra* was detected equally in surface samples from 4/7 (57%) case and 10/19 (53%) control homes, but the mean sample concentration was substantially higher in case homes ($20x10^6$ CFU/gm) than in control homes ($.007x10^6$ CFU/gm).
- Airborne *S. atra* spores were detected in 7/9 (78%) case vs 9/28 (32%) control homes.
- Viable airborne *S. atra* was detected in filter samples in 5/9 (56%) case vs 4/27 (15%) control homes and the mean colony counts for total fungi (and *S. atra*) were substantially higher in case homes than control homes. The matched odds ratio for a change of 10 units in the mean home concentration of *S. atra* was 9.8 (95% CI=1.1, $3x10^6$). No other individual fungal species was associated with case homes in matched analysis.
- *S. atra* was identified as spores, viable surface contamination, or as viable air filter contamination in 8/9 (89%) case homes vs 16/28 (57%) control homes (reported OR=4.9, 95% CI= 0.5, 255.6).
- Potent tricothecene mycotoxins were identified in isolates from both case and control homes in Cleveland (Jarvis et al.).

Despite a negative association in the Chicago investigation (viable *S. atra* detected in 0/6 case vs 6/20 control homes) and a qualifier by Etzel et al. that "further research is needed to determine whether this association is causal," findings from Cleveland have been advanced in the media, in guidelines for Toxic Effects of Indoor Molds (American Academy of Pediatrics Committee on Environmental Health, 1998, Appendix L) and on the website, http://gcrc.meds.cwru.edu/stachy, (Appendix M), to imply a stronger connection between *S. atra* or other molds and AIPH in infants than may be justified by the data. Moreover, testimony to a House Appropriations subcommittee (Appendix O), stating "this newly recognized environmental hazard . . . is killing young infants in our communities," is neither qualified nor fully justified by the data.

The working group reviewed available documents and communicated with the investigators to assess the fungal sampling methods and the quality of exposure data from the Cleveland investigation. This statement describes the concerns considered by the working group in assessing the sampling data and discusses its findings and conclusions.

II. Summary Statement

Sampling studies in Cleveland confirm that viable/dormant *S. atra* and other fungi were present in the homes of several case-infants and non-ill control infants. However, data quality may be compromised by potential bias and other factors, and may have led to a false association of *S. atra* with AIPH. The potential bias and other factors include:

- **Over sampling of case homes and potential investigator bias:** An "unblinded" industrial hygienist accounted for the collection of a greater number of air filter samples for viable fungi from case homes than from control homes. It is possible this investigator may also have used different sampling techniques than his counterparts that may have enhanced concentrations of viable fungi in surface and air samples taken from case homes.
- **Simulated household activity may have differed from home to home:** Simulated activity conducted to enhance the bioaerosol sampled from each home is difficult to standardize, may have resulted in atypical bioaerosols, and may promote differential specimen collection.
- **Association possibly confounded by a correlate of water damage:** The study could not separate the effect of *S. atra* from water damage. An analysis restricting comparison to only water-damaged case and control homes did not demonstrate a substantial difference in detection of viable *S. atra*.
- **Sampling techniques are not useful for quantitative comparison:** Surface and air sampling methods to detect fungi in this study are not intended for quantitative assessment of personal exposure or for comparing microbial concentrations from home to home.
- **Delayed collection of environmental samples:** Collection of air and surface samples weeks or months after diagnosis of AIPH introduce uncertainty in the relevance and validity of results caused by seasonal and temporal variability in growth and viability of fungal contaminants. If the measurement error is non-differential, any bias would be toward the null value, an argument in favor of the investigators' hypothesis.
- **Lack of direct evidence that recovered *S. atra* was toxigenic in home environments:** Laboratory demonstration of toxin production by household isolates of *S. atra* may not be sufficient evidence of toxin production in the home environment.

These considerations detract from the association of *S. atra* with the AIPH. Although findings of *S. atra* in Cleveland case homes is suggestive, the working group concludes that an association with AIPH has not been established. The working group recognizes that delayed sample collection could not be avoided and area sampling methods are only imperfect surrogates for exposure assessment. Although not ideal for quantitative assessment, the sampling methods are useful for hypothesis formation and provisional interpretation. At the same time, the working group notes that over sampling and the potential for differential measurement and other biases, including confounding, raise questions about the reported association. The working group does not reject a possible relationship between *S. atra* and AIPH in Cleveland. Based on concerns raised by the sampling procedures, however, it believes that possible biases could offer alternative explanations for differences observed between case and control households.

III. Fungal Sampling Methods

As described in Etzel et al., "from December 11 to December 19, 1994, industrial hygienists unaware of case or control status of the homes performed fungal sampling to look specifically for the presence of *S. atra* in the air and on surfaces." Samples were collected from nine case and 27 control homes. According to the NIOSH report (Sorenson et al.), "NIOSH investigators were blinded as to case status until sampling and analysis were completed."

Air samples were collected by area sampling methods to detect *S. atra* spores and to determine the quantity of viable airborne fungi in rooms where infants were reported to have spent the most time. At each home, sampling was preceded by simulating household activities to release dust from ventilation systems and home surfaces. These activities included vacuuming and walking on carpets and tapping or pounding on furnace ducts.

To detect airborne spores, conidia were collected by total dust sampling on cellulose ester membrane filters at a rate of 1.0 or 2.0 liters per minute for a sampling period of 6 - 8 hours. Each sample was scanned with bright-field microscopy by two independent microscopists to identify the presence of *S. atra* spores. Spores were scored by each microscopist as "present" (score = 2), "possible" (score = 1), or "none observed" (score = 0), based on comparison with a standard reference slide. The microscopists' combined score was used to judge the presence of *S. atra* in each sample. *S. atra* was defined as present if the combined score (sum) was ≥ 2. According to Dr. Sorenson, this is not the traditional method for detecting spores, but rather an adaptation of a method for assessing airborne asbestos fibers (NIOSH Method 7400). The method did not use staining to aid identification and did not distinguish between viable and non-viable fungal spores.

Air samples for viable fungi were collected by using the CAMNEA method on polycarbonate filters at a flow rate of 2.0 LPM for approximately 1 hour (range: 42 - 414 minutes, median: 67 minutes). Viable fungi were enumerated by culturing serial dilutions of filter washings on various media. Concentrations were reported as colony forming units per cubic meter of air sampled (CFU/m^3).

Surface samples for viable fungi were collected from areas with suspected (or visible) mold growth or from settled dust by scraping material from those surface materials into sterile centrifuge tubes or plastic bags, or by vacuuming dust with a battery operated sampling pump onto cellulose ester filters. Surface samples were taken from locations throughout the homes, including basements, especially from sites with obvious signs of mold growth; samples were not taken from homes without suspected or visible evidence of mold growth, sampling locations were not predetermined by protocol and the determination of visible growth was not standardized from house to house.

IV. Concerns

A. Sampling validity

1. General sampling -

- Timing of collection - In most homes, sampling was performed several months or more after illness. The samples are not likely to reflect exposure at the time of illness. Airborne fungal spores vary with change in environment and season (Levetin) and are also unpredictable from day to day (AIHA). Delays of weeks or months may result in less relevant data for quantitative comparison, and possibly unreliable data for qualitative comparisons. The measurement errors are probably non-differential, however, resulting in bias probably toward the null value, away from the association.
- Over sampling and investigator bias - Both the NIOSH report and Etzel et al. assert that industrial hygienists collecting air and surface samples for fungi were blinded to case status. Tables 5 (Airborne Fungal Concentrations By Filter Methods) and 6 (*S. atra* Concentrations by Filter Method) of the NIOSH report (Appendix N) show systematic air filter over sampling in case homes. The tables document two air filter cassettes for 7/8 case homes sampled vs 1/27 control homes (a ninth case home, site 9A, was not listed in the NIOSH tables because it was sampled several months after the field investigation was completed. It is included as case-infant #8 in Etzel et al.). Only one air sample was collected from all other homes. Each cassette was plated on four different media, producing four observations for homes with one air sample and eight observations for homes with two samples. Overall, 60 observations were recorded for eight case homes (7.5 per home) and 112 were recorded for 27 control homes (4.1 per home), an 80% over sampling of case homes. Dr. Kullman's response to working group questions provided an apparent explanation:

  > After told by an investigator that he "would be sampling a number of the case homes", Dr. Kullman "put two nucleopore filter samples in some of the sampling baskets for homes (*he*) was scheduled to sample to help ensure that (*he*) would not lose an observation point for viable fungi from one of these case homes" (Dr. Kullman, written response to Dr. Snider, 6/12/98, Appendix J). Although not specifically informed of case status, his assignments included mostly case homes, accounting for the over sampling distribution.

  Over sampling increases the probability of detecting airborne fungi, raising questions about the qualitative findings (# of case/control homes with *S. atra* observed). Moreover, Dr. Kullman's intent suggests he could have been more aggressive than other investigators in his effort to aerosolize fungal spores (see below, simulated household activity) and to collect surface samples, resulting in differential misclassification of both airborne and surface fungal concentrations. Neither the working group nor Dr. Kullman could make an objective assessment of this, however.
- Possible confounding by correlate of water damage - *S. atra* requires water-saturated, cellulose-based materials for growth in buildings and is not found on dry surfaces (Flannigan and Miller). The 1997 prepublication draft of Etzel et al.

reported water damage or a plumbing problem in eight of nine case homes that were sampled but in only seven of the 27 matched controls. It further noted that air filter samples detected viable *S. atra* in four of the eight case homes with water damage or plumbing problems compared with three of the seven control homes. Although the numbers are small, this suggests little or no difference in detection of *S. atra* growth between case and control homes with water damage, and suggests that some correlate of water damage/saturation, other than *S. atra*, may confound the association. With the very small study size and the known link between water damage and fungal growth (*S. atra* does not grow effectively on dry surfaces), it would have been very difficult to separate the effects of a correlate of water damage and *S. atra* growth in this study.

2. Air sampling -

- Area air sampling - In practice, area air sampling is useful for qualitative evaluation of contamination in a general exposure area (e.g., a room). Because quantitative observations vary from sample to sample, even when collected near the time of exposure (Leidel, et al.; Perkins), area sampling is not reliable for comparing concentration levels. Although a convenient surrogate for estimating contamination in the infant's breathing zone, even timely area air sampling would not meet the current standard for measuring personal airborne exposure (Leidel, et al.; Perkins). The Occupational Safety and Health Administration recommends the use of breathing zone apparatus when collecting samples for quantitative evaluation of personal airborne exposures. This application was neither feasible nor practical in Cleveland.
- Simulated household activity - Simulated household activity creates an artificial "worst case" exposure scenario to improve detection of potential air contaminants. Although it may not typically represent the natural aerosol, it is useful in qualitative evaluations of individual buildings (Morey). Simulation is not easily standardized or replicated from home to home, and adds uncertainty to comparisons based on area sampling. Comparisons also may be complicated by differences in furniture and carpeting, by differences in heating and air conditioning equipment, or by availability of a vacuum cleaner. Finally, simulated activity is subject to investigator bias and, in Cleveland, could have been performed more aggressively by the investigator who anticipated visits to case homes. We cannot assess inter-investigator differences or whether this may have accounted for differential measurement in case homes; however, the potential for bias introduces substantial uncertainty about the validity of case/control differences. The working group is not aware that such simulated activity has previously been applied in studies comparing multiple buildings.
- Overall, the working group concludes that air sampling methods in this investigation were not sufficiently robust or standardized to assess concentration differences between homes. The working group is particularly concerned about the potential for simulated household activity to contribute to differential exposure misclassification.

3. Conidia detection -

- Subjectivity - Table 3 (*Stachybotrys atra* Spore Counts from Air Samples) of the NIOSH report shows considerable agreement by the microscopists on the presence of *S. atra* spores in air samples. Of 36 slides reviewed, they agreed completely on 19, differed by one level ("2" vs "1" or "1" vs "0") on 13, and differed by two levels ("2" vs "0") on only four. At the same time, of 15 slides with spores detected (score sum $\geq$2), only three scored "2" by both evaluators and four scored "2" by one and "1" (possible) by the other. The remaining eight slides with detected spores included four with scores of "2" and "0" (none observed) (two cases and two controls) and four with both scores only "1" (two cases and two controls). The working group questions the validity of a scoring method that produces 8/15 (>50%) positive results either when one of the two microscopists could not detect spores or when both considered their presence as only "possible". It does not believe this liberal interpretation was differentially applied to samples from case or control homes, however.
- Investigator bias - For reasons cited above, differences in *S. atra* spore detection could have been influenced by biased household activity simulation.

4. Surface sampling -

- Surface vs airborne exposure - Surface samples cannot reliably predict airborne contamination or individual exposure (Perkins). Although a fungus may be detected growing on a surface, the release of fungal spores into the air is highly variable and is dependent upon several environmental factors, including temperature, relative humidity, light, and air movement (Levetin).
- Quantitative comparison - In general, surface samples are collected from visible fungal growth to determine the biodiversity of fungal growth in buildings and to guide remedial procedures (American Industrial Hygiene Association; Martyny et al.). They are not intended for discriminating quantities from one building to another. Analytical laboratories typically report surface sample values in CFU/g to approximate the fungal mass to be eliminated and the intensity of clean-up required.
- Lack of standardized protocol and potential bias - Surface sampling was largely guided by visual observation, possibly influenced by investigator impression, which may introduce bias. Records of visible mold growth in the homes and the physical relationship between the surfaces sampled and the infant's breathing zone are also subject to bias.
- Reproducibility - The procedure for collecting surface samples is difficult to reproduce (Martyny et al.). Both differences in surface texture and intra/inter-investigator differences in collection technique and efficiency would complicate the comparison of quantitative information.

B. Toxigenicity of S. atra

The evidence that the *S. atra* obtained from case and control homes were "toxigenic" is indirect, based on production of satratoxins G, H and other mycotoxins by the isolates in the laboratory (Jarvis et al.). This may not be sufficient to infer toxin production in the home environment, since mycotoxin production depends on many factors and is not a necessary attribute of all *S. atra* growth (Hendry and Cole). Future development of a method to assay mycotoxin in small-mass household (environmental) samples may provide more direct evidence of *S. atra* toxicity in the home setting. Although Etzel et al. reported toxin production only from case home isolates, the same toxins were also produced from control home isolates (Jarvis et al 1998).

V. Conclusion

From available data and other information communicated by the investigators, the working group concludes that studies of surface and air samples in Cleveland confirm the presence of *S. atra* and other fungi in homes of several case-infants and non-ill controls. Several considerations detract from the Cleveland findings that associate *S. atra* with AIPH, however.

First, over sampling and aggressive sampling may have played a critical role in the association. Fungal sampling was not strictly blinded and the strategy of an investigator assigned mostly to case homes differed from others. His over sampling by air filtration may have increased the probability of detecting airborne *S. atra* spores in case homes. In non quantifiable ways, moreover, his efforts to aerosolize contaminants and collect surface samples may have been more aggressive than those of other investigators. This possible source of differential measurement between case and control homes favors both detection and elevated concentrations of viable airborne *S. atra* in case homes and is the most compelling reason to question the association from the viewpoint of sample collection. The working group considers it possible that investigator bias may have contributed to a substantial portion of the reported association.

Second, it cannot be assumed that simulated household activity would replicate typical bioaerosol conditions within each home. The procedure is subject to both intra- and inter-investigator variability and results may be complicated by differences in furnishings or heating/air conditioning systems between homes. Because this procedure is not standardized or easily reproduced, it is a potential source of differential measurement error if an investigator was unblinded.

Third, the association may have been confounded by unidentified correlates of water damage, a precondition for growth of *S. atra*. Although the numbers were relatively small, a comparison restricted to water-damaged case and control homes failed to demonstrate a substantial difference in detection of viable *S. atra*. Thus, *S. atra* may only be an incidental finding, secondarily associated with moisture and water-damaged homes in this region. It is possible that other factors related to water damage may be etiologically linked with the

illness.

Fourth, surface and air sampling methods in this investigation are not intended for quantitative assessment of personal exposure or to compare microbial concentrations between homes. The methods assess qualitative indicators of potential exposure and are generally applied to determine biodiversity of indoor microbial contamination, to identify specific organisms, and to monitor remediation. Although concentration differences observed between case and control homes can support qualitative findings and suggest a consistent pattern, they may not indicate accurate magnitudes of difference. Surface sampling offers the least reliable comparison due to human and mechanical limitations on reproducibility and the non-standardized approach to selecting surfaces for sampling. Moreover, surface contaminants do not reflect airborne contamination. Whereas air sampling procedures are easier than surface sampling to standardize, their results are also variable and make comparative interpretation difficult. Furthermore, area air concentrations are surrogate measures, at best, for individual breathing zone exposures.

Fifth, because airborne fungal spores vary with change in environment and season, and possibly from day to day, samples collected weeks or months after diagnosis of AIPH may not reflect pre-illness exposure and would be of little value for quantitative comparison. Sampling delays that were non-differential, however, would have biased results, if at all, toward the null value.

Finally, because there was no validated, reliable assay to measure mycotoxin from small mass samples, there was no direct evidence for toxin production by *S. atra* in the home environments. The assertion that *S. atra* isolated from the homes in this investigation were "toxigenic" was based only on the indirect evidence of toxin production by isolates under laboratory conditions. This may not constitute sufficient evidence of toxin production under natural (household environment) conditions because since mycotoxin production depends on many factors and is not a necessary by-product of *S. atra* growth. The working group is not aware of published data supporting a direct correlation.

The working group recognizes the investigation was conducted retrospectively under difficult conditions that did not permit ideal assessment of exposure to airborne fungi. Delayed sample collection was unavoidable and area sampling is only an imperfect surrogate for exposure assessment. Although not ideal for quantitative assessment, the sampling methods are useful for hypothesis formation and the provisional interpretation that case homes may have greater quantities of *S. atra* than control homes. This should have been accompanied by a more complete discussion of the study limitations, however, along with considerations of plausibility. The working group does not believe an association of *S. atra* and illness can be confidently established in light of the over sampling and the potential for differential measurement and other biases, including confounding. These concerns raise questions about the reported association. Although the working group does not reject the possibility of an association between *S. atra* and AIPH in Cleveland, it believes that potential biases could offer alternative explanations for differences observed between case

and control households.

References

American Academy of Pediatrics Committee on Environmental Health. Toxic effects of indoor molds. Pediatrics 1998;4:712-14

American Industrial Hygiene Association. Field Guide for the Determination of Biological Contaminants in Environmental Samples. Fairfax, VA: American Industrial Hygiene Association Publications; 1996.

Etzel R, Montaña E, Sorenson W, et al. Acute Pulmonary Hemorrhage in Infants Associated with Exposure to *Stachybotrys atra* and Other Fungi. Arch Pediatr Adolesc Med 1998; 152:757-62.

Flannigan, B. Miller, JD: Health Implications of Fungi in Indoor Environments — An Overview. In: Samson RA, Flannigan B, Flannigan ME, Verhoeff AP, Adan OC, and Hoekstra ES, eds. Health Implications of Fungi in Indoor Environments. Amsterdam, the Netherlands: Elsevier Science Publications; 1994:3-28.

Hendry KM, Cole EC. A review of mycotoxins in indoor air. J Toxicol Environ Health 1993; 38:183-98.

Jarvis BB, Sorenson WG, Hintikka EL, et al. Study of toxin production by isolates of *Stachybotrys chartarum* and *Memnoniella echinata* isolated during a study of pulmonary hemosiderosis in infants. Appl Environ Microbiol 1998;64:3620-25.

Leidel NA, Busch KA, Lynch JR. Occupational Exposure Sampling Strategy Manual. Cincinnati, OH: National Institute for Occupational Safety and Health; 1977: 1-77.

Levetin E. Fungi. In: Burge HA, ed. Bioaerosols. Boca Raton, FL: Lewis Publishers; 1995: 87-120.

Martyny, JW, Martinez, KF, Morey, PR. Surface Sampling. In: Macher JM, ed.. Bioaerosols Assessment and Control. Cincinnati, OH: American Conference of Governmental Industrial Hygienists (in press): 121-7.

Morey, PR. Bioaerosols in the Indoor Environment — Current Practices and Approaches. In: Weeks DM, Gammage RB, eds. The Practitioner's Approach to Indoor Air Quality Investigations. Akron, OH: American Industrial Hygiene Association; 1990: 51-72.

Perkins JL: Modern Industrial Hygiene. New York, NY: Van Nostrand Reinhold; 1997: 301-7.

Sorenson B, Kullman G, Hintz P. NIOSH Health Hazard Evaluation Report. HETA 95-0160-2571, Centers for Disease Control and Prevention, National Center for Environmental Health, U.S. Department of Health and Human Services, Public Health Service, April 1996. ("NIOSH report").

Appendix G

Appendix G

Working group statement on the plausibility of the association of *Stachybotrys atra* and acute idiopathic pulmonary hemorrhage/hemosiderosis in infants

I. Summary statement

Overall, the working group considers that the proposal to link *S. atra* in the causal chain with acute idiopathic pulmonary hemorrhage in infants (AIPH) lacks the strength of historical support for the following reasons:

- Disease allegedly caused by *S. atra* or related fungi in humans and animals does not resemble AIPH.
- Pulmonary hemorrhage occurred in the Cleveland infants without reports of concurrent illness or other symptoms related to *S. atra* among household members; reports of *S. atra* or other toxigenic fungus-associated disease or symptoms frequently specify multiple cases within groups of exposed persons.

In addition, other evidence to support the hypothesis is currently lacking in the following areas:

- Mycotoxin was not identified at the site of exposure (technology unavailable).
- *S. atra* was not detected in case homes in the Chicago investigation.
- AIPH has not been observed in other regions with flooding or excessive home moisture.
- The proposed mechanism (i.e., protein synthesis inhibition 6 weakened endothelial basement membrane 6 capillary fragility 6 stress hemorrhage) is conceptual and is not directly supported by evidence from human disease or experimental animal models.

II. Background

The association of AIPH with *Stachybotrys atra* and other fungi in home air (Etzel et al.) was qualified by the authors who cited several study limitations and acknowledged that "further research is needed to determine whether this association is causal." Nevertheless, guidelines addressing Toxic Effects of Indoor Molds (American Academy of Pediatrics Committee on Environmental Health, 1998, Appendix L) and the website, http://gcrc.meds.cwru.edu/stachy (Appendix M), imply a stronger connection between *S. atra*/other molds and AIPH than is justified by the data. Moreover, an investigator's testimony to a House Appropriations subcommittee (Appendix O), that "this newly recognized environmental hazard . . . is killing young infants in our communities," is neither qualified nor fully justified by the data.

In this statement, the working group reviews evidence from the literature and from the Cleveland and Chicago investigations that address the plausibility of the hypothesis that *S. atra* is causally linked with AIPH. The working group broadly interprets plausibility to include elements previously distinguished by Hill (1965) as coherence (consistent with known facts), biologic plausibility (reasonable mechanism), and supportive experimental evidence.

III. Origin of hypothesis

In the Cleveland investigation trip report (12/17/94), Dr. Eduardo Montaña noted that "the hypothesis *(acute pulmonary hemorrhage among infants in Cleveland resulted from environmental exposure to a fungus)* was initially generated after discussions with Dr. Vera Anna Hofmeister Ph.D., a Brazilian epidemiologist, who reported a similar illness among infants in southern Brazil." In a communication to Dr. Dixie Snider, Dr. Ruth Etzel indicated the illness "in Brazil may have been linked to mycotoxins." *Stachybotrys atra* became targeted after its spores were microscopically identified in samples of mold taken from surfaces of case-infant homes in Cleveland. According to a televised interview with Dr. Etzel, neither the illness in Brazil nor its connection to mycotoxins were ever verified.

The hypothesis was pursued after a literature search identified reports of hemorrhagic illness in horses and non-hemorrhagic disease in humans attributed to *Stachybotrys,* and after a NIOSH mycologist "verified" that "black slime collected in the home of one of the infants . . . was *S. atra*." (Dr. Etzel e-mail communication to Dr. Wagner, Director, DRDS, NIOSH, 12/9/94, appended to G. Kullman correspondence, Appendix J). The referenced e-mail suggested "the illness was probably caused by a mycotoxin" and specifically requested "help in measuring *Stachybotrys* and satratoxin in the air of the homes of the case babies . . . as soon as possible, because that will allow us to make the best argument for its etiologic role in these infants' illness." The "a priori hypothesis was that infants with pulmonary hemorrhage were more likely than controls to live in homes where *S. atra* was present" (Etzel et al.). The proposed mechanism begins with inhalation of *S. atra* spores containing potent mycotoxins, possibly trichothecene satratoxins G and H, "among the most potent protein synthesis inhibitors known." Protein synthesis inhibition during endothelial basement membrane formation would promote "capillary fragility and . . . stress hemorrhage." Very young infants may be unusually susceptible because of rapid lung growth and cell proliferation. Supporting evidence included both natural and experimental observations in animals of hemorrhage and anemia associated with trichothecene exposure from *S. atra.*

IV. Support for hypothesis

A. Conformity with previously reported disease?

1. Disease in animals- In a report to the Academy of Science USSR in 1942, Drobotko described stachybotryotoxicosis as an enzootic disease of horses in the Ukraine, characterized by hemorrhagic diathesis, agranulocytic leukopenia, and necrotic mucosal ulceration (Drobotko). Illness was traced to ingestion of hay heavily contaminated with the saprophytic mold, *S. atra*. In fatal cases, postmortem findings included widespread hemorrhages affecting particularly the subcutaneous tissue, pleura, pericardium and endocardium, gastrointestinal serosa and mucosa, spleen and lymph nodes. The lungs of these animals were "intensely congested, edematous, emphysemic and frequently contained hemorrhages" (Forgacs). Parenteral challenge with *S. atra* mold extract produced agranulocytic leukopenia, death, and postmortem histopathologic changes typical of naturally acquired disease.

Disease has also been reproduced by feeding *S. atra* to horses, calves, sheep, swine, mice, guinea pigs, rabbits, dogs, chicks, a hippopotamus and bison; direct *S. atra* mold and toxin exposure in rabbits, horses and cattle provoked a skin reaction (Forgacs). Intra-nasal challenge with toxic and nontoxic *S. atra* spores produced bronchiolar/alveolar inflammation in mice, along with observations of spores in histopathologic lung sections (Nikulin et al. 1996,1997). The toxic spore experiments produced severe inflammatory changes within bronchioles and alveoli and hemorrhage in alveoli, and nontoxic spores caused milder lung inflammation (Nikulin et al. 1996, 1997). In these experiments, however, no histologic changes were detectable in the spleen, thymus or intestines, leading the investigators to suggest that toxin is carried either inside or outside the *S. atra* spore to the lower respiratory tract as a "respiratory propagule" where it can cause local tissue damage (Nikulin et al. 1996, 1997). In guinea pigs, pulmonary challenge with *S. atra* resulted in "catarrhal-desquamative inflammation in the tracheal and bronchial mucosa, and epitheloid degeneration, focal bronchopneumonia, areas of serous hemorrhagic interstitial inflammation, and atelectasis and compensatory emphysema" together with degenerative changes in the heart, liver and kidneys (Samsonov). In other animal studies, Soviet investigators introduced aerosolized spores and mycelia fragments of various fungal species (including *S. atra)* resulting in local and systemic effects, as well as pathologic changes in various tissues, particularly the vital organs, indicating a systemic effect of toxins following absorption in the lungs. There was no evidence of fungal proliferation in the bronchopneumonic foci, however (Forgacs). The pathologic changes noted in other organs, particularly in the liver, spleen, kidneys and heart, were consistent with a direct toxic induction (Forgacs).

Mycotoxins produced by *S. atra* are capable of producing immunosuppression and inflammation in the gastrointestinal and pulmonary systems (Fung). Experiments on mice, rats, guinea pigs and pigs, have shown that inhalation exposure to aerosolized T-2 mycotoxin particles caused minimal or no change in the respiratory tract, but necrosis of spleen, thymus, and lymphoid tissues, and of the intestinal epithelium (Creasia et al.1987, 1990, Marrs et al., Pang et al.). These changes were similar to those following experimental administration by the parenteral route.

2. Disease in humans - Localized or systemic toxic manifestations have been described in persons exposed to aerosols of toxic strains of *S. alternans* in the Ukraine (Drobotko). Alimentary toxic aleukia, a condition affecting humans and described in the 1930s in the Soviet Union, is clinically similar to equine stachybotryotoxicosis. The condition was attributed to consumption of bread made from overwintered grain contaminated with the toxin-producing mold, *Fusarium sporotrichoides*. Ingestion resulted in oropharyngeal pain and hyperemia, pancytopenia, elevated temperature, a severe hemorrhagic diathesis and, occasionally, death. The disease affected rural populations but has been rarely reported since World War II. The specific nature of the toxin responsible is unknown. In contrast to alimentary toxic aleukia, persons exposed to *S. atra*-infected hay used for fuel or

bedding developed seroexudative dermatitis, catarrhal or exudative pharyngitis, and rhinitis with bloody exudate (Mirocha). Experimental rubbing of *S. atra* on the skin and a boiled culture of the mold in healthy subjects provoked a local reaction with occasional necrosis. Some patients exposed by the dermal route or by inhalation of *S. atra* mold dust also developed mild leukopenia (Drobotko).

In the United States, prolonged household exposure to toxic *S. atra* mold is believed to have caused nonspecific symptoms in five household residents, whereas subsequent exposure to workers cleaning duct work in the home resulted in respiratory distress and skin irritation (Croft et al.). Exposure to *S. atra* in an office setting resulted in central nervous system complaints, upper and lower respiratory tract symptoms, eye and skin irritation, and chronic fatigue (Johanning et al. 1993, 1996). Impaired immune function, in addition to generalized fatigue, has been attributed to *S. atra* exposure in a Canadian hospital (Mainville).

3. Conclusion - The working group's review of animal and human health effects caused by *S. atra* and other toxic molds does not suggest a clinical link with pulmonary hemorrhage/hemosiderosis, based on historic data. In animals, pulmonary hemorrhage has been reported in context with generalized bleeding diatheses, not as solitary manifestations or even as the most prominent hemorrhagic manifestation. Also, there are no current reports of *S. atra* exposure resulting in human illness consisting only of pulmonary hemorrhage, and a review of published articles dealing with the effects of *S. atra*-related mycotoxin on human health (Fung) does not cite pulmonary hemorrhage as a sole clinical manifestation. In addition to the striking clinical discrepancy between AIPH in Cleveland and reports of animal and human health effects attributed to *S. atra* in the literature, the working group notes that the illness in Cleveland occurred only among infants, without reports of concurrent illness or symptoms within groups of exposed persons, such as household members.

B. Corroboration by investigation?

Mycotoxin in the home setting - Etzel et al. and Jarvis et al. noted that the *S. atra* strains isolated from Cleveland homes were toxigenic, producing satratoxins G and H and several other trichothecene mycotoxins in the laboratory. Whereas Etzel et al. reported toxin production by case home isolates only, Jarvis et al. reported toxin from both case and control home isolates. Cytotoxicity produced by case isolates was similar to that produced by controls (Jarvis et al.). Because mycotoxin production depends on many factors and is not a necessary by-product of *S. atra* growth (Hendry and Cole), evidence of toxigenicity in the laboratory may not be sufficient to establish toxin production in the home environment. Assay methods had not been developed for assessing mycotoxin in small mass environmental samples and it was not possible to gather direct evidence of *S. atra* mycotoxin in the home settings. This may be possible with recent methodologic developments (Dearborn communication).

C. Other observational or experimental evidence to support proposed mechanisms?

1. Stachybotrys in home settings - *Stachybotrys* fungi occur in soil, worldwide (Forgacs) and *S. atra* grows as a mold, requiring adequate moisture and available cellulose-based substrate. The notion that unusual conditions gave rise to the Cleveland outbreak is based on survey findings suggesting that *S. atra* is an uncommon contaminant of indoor environments (Kozak and Gallup). *S. atra* may be more common than portrayed in that study, however, which was conducted in southern California in homes without basements. The study neither specifically targeted *S. atra* nor assured inclusion of homes with moist/humid conditions.

2. Disease in other flooded areas - To the working group's knowledge, clusters of AIPH in infants have not occurred in other regions of the country that have experienced notable (newsworthy) flooding or where excessive home moisture is a problem.

3. Findings in Chicago - The investigation in Chicago did not find an association between AIPH and *S. atra*. In fact, *S. atra* was not detected in any of the Chicago case homes but was observed in six of twenty control homes.

4. Pathophysiology of pulmonary hemorrhage - Dr. Dearborn notes, "Conceptually, local release of toxins during rapid endothelial basement membrane formation could lead to fragile capillaries, which are subsequently at risk for stress hemorrhage" (Dearborn communication). A review of the mechanisms and role of pulmonary capillary stress failure in lung and heart disease outlines several conditions that may involve capillary stress failure (West and Mathieu-Costello). This report mentions pulmonary edema and/or hemorrhage as a pathophysiologic consequence of severe capillary wall disruption. However, no evidence is cited to suggest a role of mycotoxin in pulmonary capillary stress failure or to suggest stress failure as the physiologic basis of idiopathic pulmonary hemorrhage/hemosiderosis. Pathologic features of idiopathic pulmonary hemosiderosis (intra-alveolar hemorrhage, hemosiderin-laden macrophages, and varying degrees of interstitial fibrosis) are nonspecific, and the diagnosis is only made by exclusion of other causes of diffuse alveolar hemorrhage by careful clinical assessment, serology and immunopathology (Leatherman). Ultra-structural studies of lung biopsies of patients with idiopathic pulmonary hemosiderosis have revealed the endothelial cell and/or its basement membrane of the capillary wall as the major site of damage (Corrin et al.), but failed to implicate a specific cause of damage. These findings provide no specific evidence in support of mycotoxin-induced lung damage as a cause of idiopathic pulmonary hemorrhage/hemosiderosis. The working group has found no other evidence to support the concept proposed by Dr. Dearborn.

V. Summary

The hypothesis that AIPH in Cleveland was associated with mycotoxin-producing *S. atra* in their home environments and was caused by inhaled exposure to mycotoxins is difficult to support given the findings of the working group and reports currently in the literature on *S. atra* and other toxigenic fungi. For example, inhalation of *S. atra* has not been well documented as a cause of disease in humans; disease caused by *S. atra* has not been previously reported in infants who may have been exposed in settings associated with illness

in adults; pulmonary hemorrhage has not been a marked feature of the disease reported to be caused by *S. atra* or other toxic molds; and the case-infants' clinical syndrome did not conform to previous reports of disease caused by *S. atra* in animals or humans. Furthermore, mycotoxins have not yet been demonstrated directly in environmental samples, although the working group acknowledges that technical methods for direct detection of mycotoxin in the home environment were unavailable to the investigators. The absence of evidence of AIPH in other regions with flooding or excessive home moisture and the negative association observed in Chicago further reduce the evidence for of a causal role of *S. atra***.** In conclusion, without historical support and without direct confirmation of exposure to mycotoxin, the working group considers the hypothesis to be viable but only at a conceptual stage.

References

American Academy of Pediatrics Committee on Environmental Health. Toxic Effects of Indoor Molds. Pediatrics 1998;101:712-14.

Corrin B, Jagushi M, Dewar A, et al. Fine structural changes in idiopathic pulmonary hemosiderosis. J Pathol 1987;153:249-56.

Creasia DA, Thurman JD, Jones LJ III, et al. Acute inhalation toxicity of T-2 mycotoxin in mice. Fundam Appl Toxicol 1987;8:230-5.

Creasia DA, Thurman JD, Wannemacher RW, Bunner DL. Acute inhalation toxicity of T-2 mycotoxin in the rat and guinea pig. Fundam Appl Toxicol 1990;14:54-9.

Croft WA, Jarvis BB, Yatawara CS. Airborne outbreak of trichothecene toxicosis. Atmosph Environ 1986;20:549-52.

Dearborn DH. Pulmonary hemorrhage in infants and children. Curr Opinion Pediatr 1997;9:219-24.

Drobotko VG. Stachybotryotoxicosis a new disease of horses and humans. Am Rev Soviet Med 1945;2:238-42.

EPI-AID/ EPI-95-10 Trip report, Dr. Eduardo Montaña, December 17, 1994.

Etzel RA, Montana E, Sorenson WG, et al. Acute pulmonary hemorrhage in infants associated with exposure to *Stachybotrys atra* and other fungi. Arch Pediatr Adolesc Med 1998;152:757-62.

Forgacs J. Stachybotryotoxicosis. In: Kadis S, Ciegler A, Aji S. eds. Microbial Toxins Vol.8. New York, NY: Academic Press; 1972.

Fung F, Clark R, Williams S. *Stachybotrys*, a mycotoxin-producing fungus of increasing toxicologic importance. Clin Toxicol 1998;36:79-86.

Hendry KM, Cole EC. A review of mycotoxins in indoor air. J Toxicol Environ Health 1993;38:183-98.

Hill AB. The environment and disease: association or causation? Proc R Soc Med 1965;58:295-300.

Jarvis BB, Soreson WG, Hintikka EL, et al. Study of toxin production by isolates of *Stachybotrys chartarum* and *Memnoniella echinata* isolated during a study of pulmonary hemosiderosis in infants. Appl Environ Microbiol 1998;64:3620-25.

Johanning E, Morey PR, Jarvis BB. Clinical-epidemiological investigation of health effects caused by *Stachybotrys atra* building contamination. Proc Indoor Air 1993;1:225-30.

Johanning E, Biagini R, Hull D, et al. Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. Int Arch Occup Environ Health 1996;68:206-18.

Kozak PS, Gallup J. Endogenous mold exposure: Environmental risk to atopic and nonatopic patients. In: Gammage RV, Kay SV, eds. Indoor air and human health. Chelsea, Mich: Lewis Publishers; 1985:149-67.

Leatherman JW, Davies SF, Hoidal JR. Alveolar hemorrhage syndromes: diffuse microvascular lung hemorrhage in immune and idiopathic disorders. Medicine (Baltimore) 1984;63:343-61.

Mainville C, Auger PL, Smorgawlewica D, et al. Mycotoxins et syndrome d'extreme fatigue dans un hospital. In: B Petterson and T. Lindvall eds. Healthy Buildings. Stockholm, Sweden: Swedish Council for Building Research; 1998.

Marrs TC, Edington JA, Price PN, Upshall DG. Acute toxicity of T2 mycotoxin to the guinea pig by inhalation and subcutaneous routes. Br J Exp Pathol 1986;67:259-68.

Mirocha CJ. Stachybotryotoxicosis. In: Shimoda W. ed. Rubratoxin, Sterigmatocystin and Stachybotrys Mycotoxins; Proceedings of Conference on Mycotoxins in Animal Feeds and Grains Related to Animal Health. Rockville, Maryland: U.S. Department of Commerce, National Technical Information Service; June 13, 1980.

Mycotoxicoses of Man and Plants: Mycotoxin Control and Regulatory Aspects. In: Wyllie TD, Morehouse LG. eds. Mycotoxic fungi, Mycotoxins, Mycotoxicoses. An Encyclopedic Handbook. Vol 3. New York, NY: Marcel Dekker, Inc; 1978.

Nikulin M, Reijula K, Jarvis BB, Hintika EL. Experimental lung mycotoxicosis in mice induced by *Stachybotrys atra*. Int J Exp Pathol 1996;77:213-8.

Nikulin M, Reijula K, Jarvis BB, et al. Effects of intranasal exposure tot spores of *Stachybotrys atra* in mice. Fund Appl Toxicol 1997;35:182-8.

Pang VF, Lambert RL, Felsberg PJ, et al. Experimental T-2 toxicosis in swine following inhalation exposure: effects on pulmonary and systemic immunity and morphologic changes. Toxicol Pathol 1987;15:308-19.

Samsonov PF, Samsonov AP. In: Bilai VL, ed. Mycotoxicoses of Man and Agricultural Animals. Ukr. S.S.R., Kiev: Izd Akad Nauk; 1960:140-151.

West JB, Mathieu-Costello O. Stress failure of pulmonary capillaries: role in lung and heart disease. Lancet 1992;340:762-7.

Appendix H

Appendix H

Working group statement on whether the clusters of acute idiopathic pulmonary hemorrhage in infants investigated in Cleveland and Chicago could be related to child abuse

Child abuse has been proposed as an alternative hypothesis for the Cleveland and Chicago outbreaks. With the assistance of CDC-affiliated pathologists Randy Hanzlick and Gib Parrish, the working group considered this alternative explanation for the cluster and concluded it was unlikely for the following reasons:

- The clinical and pathological pictures appear inconsistent with child abuse. Intracranial and intra-abdominal injury are the most common manifestations of child abuse, with signs of asphyxia (which could include pulmonary hemorrhage) much less common. A cluster of recurrent, nonfatal asphyxia resulting in repeated pulmonary hemorrhage with no other major symptoms or reported physical findings is unlikely on a frequency basis to be a cluster of child abuse. It would be unlikely for a cluster of physically abused infants to present with pulmonary hemorrhage alone as a result of attempted asphyxia in the absence of other signs of physical abuse, particularly intracranial and intra-abdominal injury.
- Child abuse typically occurs in a repetitive pattern. Accordingly, case-households might be expected to have prior social service agency contacts or emergency department visits for injury. These precursors were not noted in the reports the group reviewed.

Although the child abuse cluster hypothesis could be further investigated by reviewing hospital records and files of social service agencies that deal with child abuse, the working group does not recommend devoting the resources necessary to conduct these reviews. In the group's judgement, the probability is too low that such a review would produce sufficient definitive information on enough of the cases in the outbreak to outweigh the absence of clinical evidence of child abuse.

Appendix I

Appendix I

Working group statement on the role of the NCEH/Medical Examiner special study to assess the prevalence of pulmonary hemorrhage and hemosiderosis in infant death

The deaths of three Cleveland infants from pulmonary hemorrhage (one in the cluster investigated in December 1994 and two among an additional 11 cases identified during the next 2 years) prompted a coroner's investigation into the role of pulmonary hemorrhage in infant deaths, particularly those diagnosed as sudden infant death syndrome (SIDS) (*MMWR* 1997). A retrospective review by the Cuyahoga County Coroner of 117 SIDS deaths in the county during January 1993-December 1995 identified six children with evidence of extensive hemosiderin-laden macrophages in lung tissue. The findings were interpreted to suggest that major pulmonary hemorrhage had preceded death; clinical reports for five infants indicated that some symptoms of pulmonary hemorrhage were present before death.

To assess the concern that pulmonary hemorrhage might underlie death in a substantial portion of children diagnosed with SIDS, NCEH initiated a special study/surveillance project with medical examiner (ME) colleagues: Prospective Prevalence Study of Pulmonary Hemorrhage & Hemosiderosis among Infants who have Died in the United States, to determine the prevalence of pulmonary hemorrhage among infants who die suddenly and to quantify "normal" levels of pulmonary hemosiderin in the population of sudden infant deaths. Concurrently, the study also sought to assess the presence of fungal spores in the lungs of these infants as supportive evidence for the role of a toxigenic fungus in pulmonary hemorrhage. Selected MEs submitted post mortem tissues to CDC for examination for evidence of hemorrhage and fungal spores. Enrollment began in August 1996.

With the assistance of CDC-affiliated pathologists Randy Hanzlick and Gib Parrish, the working group considered the value of pursuing the study. Specifically, the working group sought to answer the following questions: What is the rationale for conducting ME surveillance for pulmonary hemorrhage/hemosiderosis among infants who die? What evidence suggests that children are dying from this syndrome without manifesting clinical evidence of bleeding? What is the evidence that this contributes to SIDS?

As of July 1998, specimens had been received for 60 of the originally anticipated 150 deaths; only 23 case tissues have been examined (although the study was slated to extend through July 1998, participation by collaborating MEs appears to have declined, and no case materials had been submitted in the several months prior to July). More than 50% of the cases submitted were classified as SIDS. No iron deposits and no fungi had been observed in pulmonary tissue specimens from the 23 cases examined. Assuming the cases examined to date are representative of ME cases in general, binomial calculations suggest that seeing no iron deposits or fungi in 23 trials implies a 95% confidence level upper limit of 15% for the occurrence of pulmonary hemosiderin or fungi in the lungs of infant deaths examined by the forensic system. Although the confidence interval based on that work is broad, a large series of pediatric autopsy investigations in North Carolina, reported at the 1998 annual meeting of

the National Association of Medical Examiners, demonstrated iron-containing macrophages in only four (3.6%) of 110 SIDS deaths (attached). Overall, the findings to date do not exclude the hypothesis that pulmonary hemorrhage may contribute to some deaths previously categorized as SIDS. At the same time, they support the notion that pulmonary hemorrhage, hemosiderin-laden macrophages, or fungi are not commonly present in infants diagnosed by MEs with SIDS or other causes of non-traumatic death.

Furthermore, the pathologists consulting with the working group did not believe the earlier Cuyahoga County Coroners' study demonstrated sufficient pathologic and clinical evidence to attribute pulmonary hemorrhage as the cause of death in the six children with "extensive" pulmonary hemosiderin. In addition, the working group questions whether at least some of the five children with pre-mortem clinical evidence of pulmonary hemorrhage should have been classified as SIDS cases. Thus, the interpretation of reported findings that stimulated the NCEH study may have been erroneous.

Based on all the above, the working group believes there is little evidence that continuing forensic review of infant lung tissues for pulmonary hemosiderin/fungi would serve as an efficient source of surveillance information about pulmonary hemorrhage in infants. Should surveillance efforts for this uncommon condition be implemented, they should initially target illness in specific high-risk groups or geographic areas.

The working group's pathologist consultants believe there may be some clinical and pathological value in continuing the current study to determine baseline postmortem levels of pulmonary blood/hemosiderin. In particular, they believe that the current study could explore methodologic issues in forensic pathology that may possibly advance the understanding of hemosiderin in infant post mortem lung specimens and ultimately aid in the post mortem surveillance of acute idiopathic pulmonary hemorrhage. Although it might assist forensic pathologists to distinguish rare or uncommon cases of fatal idiopathic pulmonary hemorrhage among children who die from otherwise unexplained causes, the working group does not believe it should serve as, or be mistaken for, a mechanism for conducting public health surveillance.

Attachment:

Reference

CDC. Acute pulmonary hemorrhage among infants--Chicago, April 1992-November 1994. MMWR 1995:44:67,73-74.

FREQUENCY OF PULMONARY HEMOSIDEROSIS IN EASTERN NORTH CAROLINA

Celia Jackson MD PGY-2, MGF Gilliland MD, Professor, East Carolina University, School of Medicine, Department of Pathology and Laboratory Medicine, Greenville, North Carolina

Pulmonary hemosiderosis has been attributed to air-borne fungi in water-damaged homes in studies of a cluster of cases in infants and children in Cleveland, Ohio in 1994. Updates from Cleveland continue to confirm the association between water-damaged homes and pulmonary hemorrhage in infants. We have searched for such emerging infectious agents in the coastal plain of North Carolina which is subject to intermittent flooding.

Pulmonary tissue of 206 infants and young children whose deaths had been investigated from 10/78 through 09/96 was retained at East Carolina University School of Medicine in Greenville, NC. Ages ranged from premature newborns to 49 months. 110 deaths were attributed to Sudden Infant Death Syndrome. New blocks received microscopic analysis of hematoxylin and eosin stain as well as Prussian blue for iron and Gomorri methenamine silver for fungal organisms. Twenty infants and children had iron-containing macrophages. Sixteen of them had underlying illnesses but four were originally diagnosed as Sudden Infant Death Syndrome. Only one case had fungi but it had no iron. It was not originally diagnosed as SIDS. No clustering was identified. The pulmonary hemosiderosis found in this rural area may not be similar to that found in clusters in larger cities. Additional studies are needed to further define the epidemiology of pulmonary hemosiderosis as an emerging infectious disease.

Key words: emerging Infectious Agents, Pulmonary Hemosiderosis, SIDS3

Name:
Address:
City:

Appendix J

Copy of Complete Redacted Document

Available From The

CDC/ATSDR Freedom of Information Act Office

Appendix K

Copy of Complete Redacted Document

Available From The

CDC/ATSDR Freedom of Information Act Office

Appendix L

American Academy of Pediatrics Committee on Environmental Health
Toxic Effects of Indoor Molds

Appendix M

Appendix M

Stachybotrys website http://gcrc.meds.cwru.edu/stachy/default.htm

Appendix N

Appendix N

From the NIOSH report (Sorenson et al.):

Table 5 (Airborne Fungal Concentrations By Filter Methods) and Table 6 (*S. atra* Concentrations by Filter Method)

Listing of supplemental laboratory data provided by Dr. Dearborn:

"Filter Cassette Samples" (see data for Site 9A)

The appended Tables 5 and 6 from Sorenson et al.[3] ("NIOSH Report") document household air filtration sampling data from case and control homes of children investigated during (home #'s 1-53) and after (home #'s $\geq$ 54) the Cleveland outbreak investigation. Analyses reported in the NIOSH Report include the data from case homes identified after the outbreak investigation. Those homes (#'s $\geq$ 54) were not part of the initial cluster, however, and are not included in the tables or analyses reported by Etzel et al. Of the ten original cluster cases included in Etzel et al., only nine had household environmental investigations. Of those nine case homes, only eight appear with airborne fungi concentrations in Tables 5 and 6 of the NIOSH report (home #'s 1, 3, 4, 5, 6, 7, 39 and 50; these #'s do not correspond with case-infant #'s reported in Etzel et al.). All control homes represented in these tables are matched to the outbreak-investigated cases whose homes were sampled.

The left-hand column, "SAMPLE", indicates the identity of the filters used to capture airborne fungi. The second column, "HOME" is the household identification and the third column, "CASE", indicates case (=Y)/control (=N) status. Each filter was plated-out on four media, labeled in the column "MEDIA" as 2M, CELL, DG18, and RBS. For HOME #39, each filter appeared to be plated twice on each medium. The working group was able to determine that only the first result listed for each medium under HOME #39 had been used in the Etzel et al. analysis (consistent with the # of plates used for filter specimens from all other homes); the working group, therefore, omitted the second listing in its assessment.

The tables show only the counts for total fungi (table 5) and *S. atra* (table 6) on the individual plates. The counts from individual plates for each of the other fungi reported in Etzel et al., *Aspergillus*, *Cladosporium*, *Penicillium* and "Other" (includes all other fungi observed), are not displayed in the NIOSH report but are summarized (along with total fungi and *S. atra*) as mean household concentrations in the analytic data set.

Because the ninth home included in the environmental investigation was not sampled until several months after the field investigation, data from this home were not available for inclusion in the NIOSH report, but were included (as case-infant #8) in the tables and analyses reported by Etzel et al. Summary data for that case infant (mean household concentrations) were originally identified by the working group as "patnum=9" in the analytic data set, along with summary means for the other eight case-infant homes and the 27 matched control homes. The agar (media)-specific values for individual fungi identified in that home (site 9A, only) are recorded on the attached listing labeled "Filter cassette samples", provided by Dr. Dearborn. At least one colony of *S. atra* was detected on cellulose agar medium, but the quantity was too small for enumeration.

---

[1]Sorenson B, Kullman G, Hintz P. NIOSH Health Hazard Evaluation Report. HETA 95-0160-2571, Centers for Disease Control and Prevention, National Center for Environmental Health, U.S. Department of Health and Human Services, Public Health Service, April 1996. ("The NIOSH report").

TABLE 5
AIRBORNE FUNGAL CONCENTRATIONS BY FILTER METHODS
CONCENTRATIONS IN CFU/m^3

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	TOTAL FUNGI[1]
CA39	1	Y	2M	1666.7
CA39	1	Y	CELL	333.3
CA39	1	Y	DG18	2555.6
CA39	1	Y	RBS	1555.6
CA45	1	Y	2M	1555.6
CA45	1	Y	CELL	555.6
CA45	1	Y	DG18	2888.9
CA45	1	Y	RBS	2555.6
CA35	3	Y	2M	1388.9
CA35	3	Y	CELL	648.1
CA35	3	Y	DG18	1018.5
CA35	3	Y	RBS	2500
CA40	3	Y	2M	648.1

TABLE 5 (Continued)
AIRBORNE FUNGAL CONCENTRATIONS BY FILTER METHODS
CONCENTRATIONS IN CFU/m^3

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	TOTAL FUNGI[1]
CA40	3	Y	CELL	833.3
CA40	3	Y	DG18	1111.1
CA40	3	Y	RBS	2037
CA26	4	Y	2M	384.6
CA26	4	Y	CELL	0
CA26	4	Y	DG18	512.8
CA26	4	Y	RBS	256.4
CA32	5	Y	2M	1388.9
CA32	5	Y	CELL	694.4
CA32	5	Y	DG18	416.7
CA32	5	Y	RBS	1250
CA43	5	Y	2M	555.6
CA43	5	Y	CELL	416.7
CA43	5	Y	DG18	416.7
CA43	5	Y	RBS	694.4
CA16	6	Y	2M	317.5
CA16	6	Y	CELL	79.4
CA16	6	Y	DG18	79.4
CA16	6	Y	RBS	396.8
CA27	6	Y	2M	0
CA27	6	Y	CELL	0
CA27	6	Y	DG18	158.7
CA27	6	Y	RBS	79.4
CA2	7	Y	2M	2000
CA2	7	Y	CELL	444.4
CA2	7	Y	DG18	1222.2
CA2	7	Y	RBS	1111.1
CA25	7	Y	2M	1333.3

TABLE 5 (Continued)
AIRBORNE FUNGAL CONCENTRATIONS BY FILTER METHODS
CONCENTRATIONS IN CFU/m^3

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	TOTAL FUNGI[1]
CA25	7	Y	CELL	888.9
CA25	7	Y	DG18	1333.3
CA25	7	Y	RBS	1555.6
CA13	10	N	2M	126.3
CA13	10	N	CELL	0
CA13	10	N	DG18	0
CA13	10	N	RBS	151.5
CA37	10	N	2M	0
CA37	10	N	CELL	0
CA37	10	N	DG18	25.3
CA37	10	N	RBS	50.5
1214	11	N	2M	0
1214	11	N	CELL	0
1214	11	N	DG18	208.3
1214	11	N	RBS	625
CA8	12	N	2M	0
CA8	12	N	CELL	0
CA8	12	N	DG18	138.9
CA8	12	N	RBS	138.9
CA48	13	N	2M	2361.1
CA48	13	N	CELL	1388.9
CA48	13	N	DG18	3194.4
CA48	13	N	RBS	1944.4
CA1	16	N	2M	2272.7
CA1	16	N	CELL	1363.6
CA1	16	N	DG18	2424.2
CA1	16	N	RBS	1969.7
CA12	18	N	2M	416.7

TABLE 5 (Continued)
AIRBORNE FUNGAL CONCENTRATIONS BY FILTER METHODS
CONCENTRATIONS IN CFU/m³

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	TOTAL FUNGI[1]
CA12	18	N	CELL	277.8
CA12	18	N	DG18	694.4
CA12	18	N	RBS	416.7
CA24	19	N	2M	0
CA24	19	N	CELL	0
CA24	19	N	DG18	666.7
CA24	19	N	RBS	0
CA30	20	N	2M	0
CA30	20	N	CELL	277.8
CA30	20	N	DG18	277.8
CA30	20	N	RBS	0
1213	22	N	2M	694.4
1213	22	N	CELL	416.7
1213	22	N	DG18	555.6
1213	22	N	RBS	694.4
CA38	23	N	2M	0
CA38	23	N	CELL	0
CA38	23	N	DG18	46.9
CA38	23	N	RBS	0
CA36	24	N	2M	769.2
CA36	24	N	CELL	128.2
CA36	24	N	DG18	769.2
CA36	24	N	RBS	769.2
CA46	25	N	2M	1500
CA46	25	N	CELL	0
CA46	25	N	DG18	833.3
CA46	25	N	RBS	666.7
CA47	26	N	2M	92.6

TABLE 5 (Continued)
AIRBORNE FUNGAL CONCENTRATIONS BY FILTER METHODS
CONCENTRATIONS IN CFU/m^3

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	TOTAL FUNGI[1]
CA47	26	N	CELL	0
CA47	26	N	DG18	0
CA47	26	N	RBS	0
CA49	28	N	2M	714.3
CA49	28	N	CELL	238.1
CA49	28	N	DG18	714.3
CA49	28	N	RBS	476.2
CA14	32	N	2M	277.8
CA14	32	N	CELL	138.9
CA14	32	N	DG18	0
CA14	32	N	RBS	0
CA11	33	N	2M	301.2
CA11	33	N	CELL	60.2
CA11	33	N	DG18	120.5
CA11	33	N	RBS	261
CA42	34	N	2M	119
CA42	34	N	CELL	119
CA42	34	N	DG18	119
CA42	34	N	RBS	238.1
CA7	35	N	2M	105.5
CA7	35	N	CELL	63.3
CA7	35	N	DG18	105.5
CA7	35	N	RBS	84.4
CA29	36	N	2M	2179.5
CA29	36	N	CELL	2307.7
CA29	36	N	DG18	2948.7
CA29	36	N	RBS	2564.1
CA5	37	N	2M	277.8

TABLE 5 (Continued)
AIRBORNE FUNGAL CONCENTRATIONS BY FILTER METHODS
CONCENTRATIONS IN CFU/m^3

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	TOTAL FUNGI[1]
CA5	37	N	CELL	0
CA5	37	N	DG18	416.7
CA5	37	N	RBS	277.8
CA18	38	N	2M	6111.1
CA18	38	N	CELL	2500
CA18	38	N	DG18	6527.8
CA18	38	N	RBS	7083.3
CA31	39	Y	2M	10384.6
CA31	39	Y	2M	10256.4
CA31	39	Y	CELL	4871.8
CA31	39	Y	CELL	6410.3
CA31	39	Y	DG18	8717.9
CA31	39	Y	DG18	13461.5
CA31	39	Y	RBS	11282.1
CA31	39	Y	RBS	13461.5
CA6	39	Y	2M	15256.4
CA6	39	Y	2M	13461.5
CA6	39	Y	CELL	4487.2
CA6	39	Y	CELL	0
CA6	39	Y	DG18	13333.3
CA6	39	Y	DG18	8974.4
CA6	39	Y	RBS	12435.9
CA6	39	Y	RBS	19871.8
CA22	41	N	2M	0
CA22	41	N	CELL	0
CA22	41	N	DG18	138.9
CA22	41	N	RBS	0
CA44	42	N	2M	897.4

TABLE 5 (Continued)
AIRBORNE FUNGAL CONCENTRATIONS BY FILTER METHODS
CONCENTRATIONS IN CFU/m^3

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	TOTAL FUNGI[1]
CA44	42	N	CELL	256.4
CA44	42	N	DG18	384.6
CA44	42	N	RBS	128.2
CA21	43	N	2M	238.1
CA21	43	N	CELL	119
CA21	43	N	DG18	357.1
CA21	43	N	RBS	476.2
CA41	50	Y	2M	0
CA41	50	Y	CELL	104.2
CA41	50	Y	DG18	104.2
CA41	50	Y	RBS	208.3
CA9	50	Y	2M	520.8
CA9	50	Y	CELL	312.5
CA9	50	Y	DG18	312.5
CA9	50	Y	RBS	208.3
1216	51	N	2M	277.8
1216	51	N	CELL	138.9
1216	51	N	DG18	138.9
1216	51	N	RBS	277.8
1235	52	N	2M	925.9
1235	52	N	CELL	1203.7
1235	52	N	DG18	2314.8
1235	52	N	RBS	1296.3
1249	53	N	2M	119
1249	53	N	CELL	0
1249	53	N	DG18	0
1249	53	N	RBS	0
CA20	0054A	Y	2M	0

TABLE 5 (Continued)
AIRBORNE FUNGAL CONCENTRATIONS BY FILTER METHODS
CONCENTRATIONS IN CFU/m³

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	TOTAL FUNGI[1]
CA20	0054A	Y	CELL	222.2
CA20	0054A	Y	DG18	222.2
CA20	0054A	Y	RBS	0
CA23	0054A	Y	2M	111.1
CA23	0054A	Y	CELL	111.1
CA23	0054A	Y	DG18	111.1
CA23	0054A	Y	RBS	222.2
CA15	0054B	Y	2M	8055.6
CA15	0054B	Y	2M	7870.4
CA15	0054B	Y	CELL	4537
CA15	0054B	Y	CELL	6018.5
CA15	0054B	Y	DG18	10092.6
CA15	0054B	Y	DG18	11111.1
CA15	0054B	Y	RBS	9907.4
CA15	0054B	Y	RBS	10185.2
CA28	0054B	Y	2M	11574.1
CA28	0054B	Y	2M	10185.2
CA28	0054B	Y	CELL	5648.1
CA28	0054B	Y	CELL	6481.5
CA28	0054B	Y	DG18	11481.5
CA28	0054B	Y	DG18	7407.4
CA28	0054B	Y	RBS	10277.8
CA28	0054B	Y	RBS	12037
1233	0055A	Y	2M	1222.2
1233	0055A	Y	CELL	444.4
1233	0055A	Y	DG18	2444.4
1233	0055A	Y	RBS	666.7
1258	0055A	Y	2M	666.7

TABLE 5 (Continued)
AIRBORNE FUNGAL CONCENTRATIONS BY FILTER METHODS
CONCENTRATIONS IN CFU/m³

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	TOTAL FUNGI[1]
1258	0055A	Y	CELL	111.1
1258	0055A	Y	DG18	888.9
1258	0055A	Y	RBS	555.6
1208	0055B	Y	2M	370.4
1208	0055B	Y	CELL	277.8
1208	0055B	Y	DG18	1111.1
1208	0055B	Y	RBS	370.4
1225	0055B	Y	2M	1481.5
1225	0055B	Y	CELL	740.7
1225	0055B	Y	DG18	1759.3
1225	0055B	Y	RBS	1111.1

[1] Concentrations below the lower limit of Quantification, approximately 70 CFU/m³, are recorded as 0.
CFU/m³=Colony forming units per cubic meter of air.

TABLE 6
S. ATRA CONCENTRATIONS BY FILTER METHODS
AIR CONCENTRATIONS IN CFU/m³

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	*S. ATRA* (CFU/m³)[1]
CA39	1	Y	2M	444.4
CA39	1	Y	CELL	0
CA39	1	Y	DG18	0
CA39	1	Y	RBS	333.3
CA45	1	Y	2M	222.2
CA45	1	Y	CELL	111.1
CA45	1	Y	DG18	0
CA45	1	Y	RBS	111.1
CA35	3	Y	2M	0
CA35	3	Y	CELL	0
CA35	3	Y	DG18	0
CA35	3	Y	RBS	0
CA40	3	Y	2M	0
CA40	3	Y	CELL	0
CA40	3	Y	DG18	0
CA40	3	Y	RBS	0
CA26	4	Y	2M	0
CA26	4	Y	CELL	0
CA26	4	Y	DG18	0
CA26	4	Y	RBS	128.2
CA32	5	Y	2M	277.8
CA32	5	Y	CELL	0
CA32	5	Y	DG18	0
CA32	5	Y	RBS	277.8
CA43	5	Y	2M	0
CA43	5	Y	CELL	0
CA43	5	Y	DG18	0
CA43	5	Y	RBS	138.9

TABLE 6 (Continued)
S. ATRA CONCENTRATIONS BY FILTER METHODS
AIR CONCENTRATIONS IN CFU/m³

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	*S. ATRA* (CFU/m³)[1]
CA16	6	Y	DG18	0
CA16	6	Y	RBS	0
CA27	6	Y	2M	0
CA27	6	Y	CELL	0
CA27	6	Y	DG18	0
CA27	6	Y	RBS	0
CA2	7	Y	2M	0
CA2	7	Y	CELL	0
CA2	7	Y	DG18	0
CA2	7	Y	RBS	0
CA25	7	Y	2M	0
CA25	7	Y	CELL	0
CA25	7	Y	DG18	0
CA25	7	Y	RBS	0
CA13	10	N	2M	0
CA13	10	N	CELL	0
CA13	10	N	DG18	0
CA13	10	N	RBS	25.3
CA37	10	N	2M	0
CA37	10	N	CELL	0
CA37	10	N	DG18	0
CA37	10	N	RBS	0
1214	11	N	2M	0
1214	11	N	CELL	0
1214	11	N	DG18	0
1214	11	N	RBS	0
CA8	12	N	2M	0
CA8	12	N	CELL	0

TABLE 6 (Continued)
S. ATRA CONCENTRATIONS BY FILTER METHODS
AIR CONCENTRATIONS IN CFU/m^3

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	*S. ATRA* (CFU/m^3)[1]
CA8	12	N	DG18	0
CA8	12	N	RBS	0
CA48	13	N	2M	0
CA48	13	N	CELL	138.9
CA48	13	N	DG18	0
CA48	13	N	RBS	0
CA1	16	N	2M	0
CA1	16	N	CELL	0
CA1	16	N	DG18	0
CA1	16	N	RBS	151.5
CA12	18	N	2M	0
CA12	18	N	CELL	0
CA12	18	N	DG18	0
CA12	18	N	RBS	0
CA24	19	N	2M	0
CA24	19	N	CELL	0
CA24	19	N	DG18	0
CA24	19	N	RBS	0
CA30	20	N	2M	0
CA30	20	N	CELL	0
CA30	20	N	DG18	0
CA30	20	N	RBS	0
1213	22	N	2M	0
1213	22	N	CELL	0
1213	22	N	DG18	0
1213	22	N	RBS	0
CA38	23	N	2M	0
CA38	23	N	CELL	0

TABLE 6 (Continued)
S. ATRA CONCENTRATIONS BY FILTER METHODS
AIR CONCENTRATIONS IN CFU/m^3

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	*S. ATRA* (CFU/m^3)[1]
CA38	23	N	DG18	0
CA38	23	N	RBS	0
CA36	24	N	2M	0
CA36	24	N	CELL	0
CA36	24	N	DG18	0
CA36	24	N	RBS	0
CA46	25	N	2M	0
CA46	25	N	CELL	0
CA46	25	N	DG18	0
CA46	25	N	RBS	0
CA47	26	N	2M	0
CA47	26	N	CELL	0
CA47	26	N	DG18	0
CA47	26	N	RBS	0
CA49	28	N	2M	0
CA49	28	N	CELL	0
CA49	28	N	DG18	0
CA49	28	N	RBS	0
CA14	32	N	2M	0
CA14	32	N	CELL	0
CA14	32	N	DG18	0
CA14	32	N	RBS	0
CA11	33	N	2M	0
CA11	33	N	CELL	0
CA11	33	N	DG18	0
CA11	33	N	RBS	0
CA42	34	N	2M	0
CA42	34	N	CELL	0

TABLE 6 (Continued)
S. *ATRA* CONCENTRATIONS BY FILTER METHODS
AIR CONCENTRATIONS IN CFU/m^3

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	*S. ATRA* (CFU/m^3)[1]
CA42	34	N	DG18	0
CA42	34	N	RBS	0
CA7	35	N	2M	0
CA7	35	N	CELL	0
CA7	35	N	DG18	0
CA7	35	N	RBS	0
CA29	36	N	2M	0
CA29	36	N	CELL	0
CA29	36	N	DG18	0
CA29	36	N	RBS	0
CA5	37	N	2M	0
CA5	37	N	CELL	0
CA5	37	N	DG18	0
CA5	37	N	RBS	0
CA18	38	N	2M	0
CA18	38	N	CELL	0
CA18	38	N	DG18	0
CA18	38	N	RBS	0
CA31	39	Y	2M	0
CA31	39	Y	2M	0
CA31	39	Y	CELL	128.2
CA31	39	Y	CELL	0
CA31	39	Y	DG18	0
CA31	39	Y	DG18	0
CA31	39	Y	RBS	512.8
CA31	39	Y	RBS	641
CA6	39	Y	2M	0
CA6	39	Y	2M	0

TABLE 6 (Continued)
S. ATRA CONCENTRATIONS BY FILTER METHODS
AIR CONCENTRATIONS IN CFU/m^3

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	*S. ATRA* (CFU/m^3)[1]
CA6	39	Y	CELL	256.4
CA6	39	Y	CELL	0
CA6	39	Y	DG18	0
CA6	39	Y	DG18	0
CA6	39	Y	RBS	256.4
CA6	39	Y	RBS	0
CA22	41	N	2M	0
CA22	41	N	CELL	0
CA22	41	N	DG18	
CA22	41	N	RBS	
CA44	42	N	2M	
CA44	42	N	CELL	0
CA44	42	N	DG18	0
CA44	42	N	RBS	0
CA21	43	N	2M	0
CA21	43	N	CELL	0
CA21	43	N	DG18	0
CA21	43	N	RBS	0
CA41	50	Y	2M	0
CA41	50	Y	CELL	0
CA41	50	Y	DG18	0
CA41	50	Y	RBS	0
CA9	50	Y	2M	0
CA9	50	Y	CELL	0
CA9	50	Y	DG18	0
CA9	50	Y	RBS	0
1216	51	N	2M	0
1216	51	N	CELL	0

TABLE 6 (Continued)
S. ATRA CONCENTRATIONS BY FILTER METHODS
AIR CONCENTRATIONS IN CFU/m^3

HETA 95-0160
National Center for Environmental Health

SAMPLE	HOME	CASE	MEDIA	*S. ATRA* (CFU/m^3)[1]
1216	51	N	DG18	0
1216	51	N	RBS	0
1235	52	N	2M	0
1235	52	N	CELL	0
1235	52	N	DG18	0
1235	52	N	RBS	92.6
1249	53	N	2M	0
1249	53	N	CELL	0
1249	53	N	DG18	0
1249	53	N	RBS	0

0009A	PC90	RBS	666666.7	83.9	3.6	0.0	0.0	12.5	
0009A	PC90	CELL	595238.1	86.0	8.0	0.0	0.0	6.0	S. ATRA (1 OR MORE? COLS ON 1/1)
0009A	PC90	DG18	940476.2	75.9	3.8	1.3	0.0	19.0	4 Wallemia
0009A	PC90	2M	619047.6	80.8	5.8	3.8	0.0	9.6	
0009A	1246	RBS	29761.9	88.0	2.0	0.0	0.0	10.0	
0009A	1246	CELL	20833.3	88.6	8.6	0.0	0.0	2.9	
0009A	1246	DG18	40476.2	75.0	0.0	0.0	0.0	25.0	3 Wallemia
0009A	1246	2M	29166.7	83.7	0.0	0.0	0.0	16.3	
0009B	PC10	RBS	31547.6	94.3	0.0	1.9	0.0	3.8	at least 3 spp of Aspergillus
0009B	PC10	CELL	27976.2	87.2	6.4	0.0	0.0	6.4	at least 3 spp of Aspergillus
0009B	PC10	DG18	45238.1	61.8	1.3	2.6	0.0	34.2	at least 3 spp of Aspergillus, 11 Wallemi
0009B	PC10	2M	32142.9	81.5	0.0	9.3	0.0	9.3	at least 3 spp of Aspergillus
0009B	1221	RBS	11904.8	82.0	3.0	1.0	0.0	14.0	at least 3 spp of Aspergillus
0009B	1221	CELL	8214.3	89.9	2.9	2.9	0.0	4.3	at least 3 spp of Aspergillus
0009B	1221	DG18	13809.5	78.4	0.0	0.0	0.0	21.6	at least 3 spp of Aspergillus, 6 Wallemia
0009B	1221	2M	840000.0	86.9	4.8	0.0	0.0	8.3	at least 3 spp of Aspergillus
0058A	PC10	RBS	0.0	ERR	ERR	ERR	ERR	ERR	
0058A	PC10	CELL	0.0	ERR	ERR	ERR	ERR	ERR	
0058A	PC10	DG18	128.2	0.0	0.0	0.0	0.0	100.0	
0058A	PC10	2M	0.0	ERR	ERR	ERR	ERR	ERR	
0058A	1240	RBS	128.2	100.0	0.0	0.0	0.0	0.0	
0058A	1240	CELL	0.0	ERR	ERR	ERR	ERR	ERR	
0058A	1240	DG18	128.2	100.0	0.0	0.0	0.0	0.0	
0058A	1240	2M	512.8	0.0	50.0	50.0	0.0	0.0	
0058A	PC84(	RBS							
0058A	PC84(	CELL							
0058A	PC84(	DG18							
0058A	PC84(	2M							
0058B	1243	RBS	ERR	ERR	ERR	ERR	ERR	ERR	
0058B	1243	CELL	ERR	ERR	ERR	ERR	ERR	ERR	
0058B	1243	DG18	ERR	ERR	ERR	ERR	ERR	ERR	
0058B	1243	2M	ERR	ERR	ERR	ERR	ERR	ERR	
0058B	PC10	RBS	ERR	ERR	ERR	ERR	ERR	ERR	

Filter Cassette Samples*

SITE	Cst#	Medium	Vol (M3)	CFU/M3	Asp/M3	Clad/M3	Pen/M3	Stachy/M3	Other/M3
0009A	PC90	RBS	0.14	6.7E+05	5.6E+05	2.4E+04	0.0E+00	0.0E+00	8.3E+04
0009A	PC90	CELL	0.14	6.0E+05	5.1E+05	4.8E+04	0.0E+00	0.0E+00	3.6E+04
0009A	PC90	DG18	0.14	9.4E+05	7.1E+05	3.6E+04	1.2E+04	0.0E+00	1.8E+05
0009A	PC90	2M	0.14	6.2E+05	5.0E+05	3.6E+04	2.4E+04	0.0E+00	6.0E+04
0009A	1246	RBS	0.14	3.0E+04	2.6E+04	6.0E+02	0.0E+00	0.0E+00	3.0E+03
0009A	1246	CELL	0.14	2.1E+04	1.8E+04	1.8E+03	0.0E+00	0.0E+00	6.0E+02
0009A	1246	DG18	0.14	4.0E+04	3.0E+04	0.0E+00	0.0E+00	0.0E+00	1.0E+04
0009A	1246	2M	0.14	2.9E+04	2.4E+04	0.0E+00	0.0E+00	0.0E+00	4.8E+03
0009B	PC105	RBS	0.14	3.2E+04	3.0E+04	0.0E+00	6.0E+02	0.0E+00	1.2E+03
0009B	PC105	CELL	0.14	2.8E+04	2.4E+04	1.8E+03	0.0E+00	0.0E+00	1.8E+03
0009B	PC105	DG18	0.14	4.5E+04	2.8E+04	6.0E+02	1.2E+03	0.0E+00	1.5E+04
0009B	PC105	2M	0.14	3.2E+04	2.6E+04	0.0E+00	3.0E+03	0.0E+00	3.0E+03
0009B	1221	RBS	0.14	1.2E+04	9.8E+03	3.6E+02	1.2E+02	0.0E+00	1.7E+03
0009B	1221	CELL	0.14	8.2E+03	7.4E+03	2.4E+02	2.4E+02	0.0E+00	3.6E+02
0009B	1221	DG18	0.14	1.4E+04	1.1E+04	0.0E+00	0.0E+00	0.0E+00	3.0E+03
0009B	1221	2M	0.14	8.4E+05	7.3E+05	4.0E+04	0.0E+00	0.0E+00	7.0E+04
0058A	PC106	RBS	0.13	0.0E+00					
0058A	PC106	CELL	0.13	0.0E+00					
0058A	PC106	DG18	0.13	1.3E+02	0.0E+00	0.0E+00	0.0E+00	0.0E+00	1.3E+02
0058A	PC106	2M	0.13	0.0E+00					
0058A	1240	RBS	0.13	1.3E+02	1.3E+02	0.0E+00	0.0E+00	0.0E+00	0.0E+00
0058A	1240	CELL	0.13	0.0E+00					
0058A	1240	DG18	0.13	1.3E+02	1.3E+02	0.0E+00	0.0E+00	0.0E+00	0.0E+00
0058A	1240	2M	0.13	5.1E+02	0.0E+00	2.6E+02	2.6E+02	0.0E+00	0.0E+00
0058B	12-9	RBS	0.11	4.9E+04	3.3E+04	0.0E+00	8.2E+03	0.0E+00	8.2E+03
0058B	12-9	CELL	0.11	7.3E+04	3.0E+04	3.3E+03	1.7E+04	1.3E+04	1.0E+04
0058B	12-9	DG18	0.11	6.7E+04	5.4E+04	0.0E+00	3.2E+03	0.0E+00	9.5E+03
0058B	12-9	2M	0.11	4.9E+04	1.6E+04	0.0E+00	2.7E+03	1.1E+04	1.9E+04
0058B	PC98	RBS	0.11	3.0E+04	6.4E+03	0.0E+00	8.5E+03	6.4E+03	8.5E+03
0058B	PC98	CELL	0.11	1.4E+05	3.2E+04	2.3E+04	9.1E+03	4.5E+03	6.8E+04
0058B	PC98	DG18	0.11	6.1E+04	2.1E+04	3.0E+03	1.2E+04	0.0E+00	2.4E+04
0058B	PC98	2M	0.11	2.6E+04	7.9E+03	2.0E+03	2.0E+03	2.0E+03	1.2E+04
0059	12-1	RBS	0.11	2.8E+06	1.9E+06	6.2E+04	2.3E+05	6.2E+04	6.2E+05
0059	12-1	CELL	0.11	1.7E+06	1.3E+06	3.2E+04	1.3E+05	6.4E+04	1.3E+05
0059	12-1	DG18	0.11	1.6E+05	1.1E+05	4.8E+03	4.8E+03	0.0E+00	3.4E+04
0059	12-1	2M	0.11	1.5E+05	8.9E+04	0.0E+00	3.3E+04	0.0E+00	2.3E+04
0059	PC104	RBS	0.12	1.6E+05	9.9E+04	9.4E+03	1.4E+04	4.7E+03	3.3E+04
0059	PC104	CELL	0.12	1.7E+05	1.2E+05	0.0E+00	2.4E+04	0.0E+00	2.9E+04
0059	PC104	DG18	0.12	1.0E+05	6.0E+04	3.8E+03	1.5E+04	0.0E+00	2.3E+04
0059	PC104	2M	0.12	1.3E+05	8.6E+04	8.6E+03	0.0E+00	0.0E+00	3.9E+04
0060	PC95	RBS	0.12	2.0E+04	6.7E+03	3.3E+03	1.7E+03	0.0E+00	8.3E+03
0060	PC95	CELL	0.12	3.5E+03	3.5E+03	0.0E+00	0.0E+00	0.0E+00	0.0E+00
0060	PC95	DG18	0.12	2.0E+04	1.8E+04	0.0E+00	0.0E+00	0.0E+00	1.7E+03
0060	PC95	2M	0.12	2.7E+04	1.4E+04	1.9E+03	1.9E+03	1.9E+03	7.8E+03
0060	PC96	RBS	0.12	5.0E+04	1.8E+04	0.0E+00	2.6E+03	7.9E+03	2.1E+04
0060	PC96	CELL	0.12	3.6E+04	2.2E+04	4.4E+03	0.0E+00	2.2E+03	6.7E+03
0060	PC96	DG18	0.12	5.0E+04	3.4E+04	0.0E+00	0.0E+00	0.0E+00	1.6E+04
0060	PC96	2M	0.12	5.6E+04	1.4E+04	0.0E+00	0.0E+00	0.0E+00	4.2E+04
0061	PC101	RBS	0.12	3.3E+05	1.0E+05	0.0E+00	1.4E+05	0.0E+00	9.5E+04
0061	PC101	CELL	0.12	2.6E+05	1.3E+05	2.4E+04	5.4E+04	0.0E+00	5.4E+04
0061	PC101	DG18	0.12	1.2E+06	4.8E+05	3.9E+04	2.2E+05	0.0E+00	4.8E+05
0061	PC101	2M	0.12	4.2E+05	1.8E+05	4.6E+04	1.5E+05	0.0E+00	4.6E+04
0061	PC108	RBS	0.12	2.9E+05	1.2E+05	1.3E+04	1.2E+05	0.0E+00	4.5E+04
0061	PC108	CELL	0.12	1.8E+05	8.5E+04	0.0E+00	6.5E+04	0.0E+00	3.0E+04
0061	PC108	DG18	0.12	4.1E+05	2.1E+05	3.0E+04	3.0E+04	0.0E+00	1.4E+05
0061	PC108	2M	0.12	2.1E+05	6.5E+04	1.6E+04	7.6E+04	0.0E+00	5.4E+04

Filter cassettes numbered 12-4, 12-43, PC 84, PC86, PC87, PC103, and PC 112 were blanks and were eliminated from this report

*I received none higher than 61

Appendix O

OUTBREAK OF PULMONARY HEMORRHAGE AMONG CLEVELAND INFANTS

[testimony given at the House Appropriations Subcommittee on Labor, Health & Human Services & Education on Feb 3, 1998]

Dorr G. Dearborn, PhD, MD
Associate Professor of Pediatrics
Case Western Reserve University
Rainbow Babies & Childrens Hospital

A previously rare disorder, acute pulmonary hemorrhage, has been diagnosed in 38 infants in the Cleveland area in the past five years. This serious disorder causes infants to cough up blood and usually requires intensive care measures to save them. Fourteen of these infants have died, including nine originally thought to be Sudden Infant Death Syndrome. Thirty infants were African American, all of whom lived in a limited geographic area of eastern metropolitan Cleveland, an area of older housing stock. This area corresponds directly with Congressman Stoke's district.

An investigation of this outbreak led by the CDC has linked this disease to exposure to a toxic mold called *Stachybotrys*, which was found in the infants' homes. This mold requires water- saturated wood products to grow, and appears to have occurred secondary to chronic basement flooding or from chronic plumbing/maintenance problems. Once die source of water damage is corrected, the mold can sometimes be removed with a bleach solution; however, more extensive abatement's often necessary.

Stachybotrys, while not a common mold, known to have a wide distribution. We are aware of a total of 122 cases of acute pulmonary hemorrhage in 'infants nation-wide over the past five years. The rapidly growing lungs of young infants appear to be especially vulnerable to the toxins made by this mold. The CDC investigation also found that environmental tobacco smoke was frequently a trigger precipitating the acute bleeding.

This is an emerging disease. We need to act now to learn how to prevent it and how to treat it. I urge you to provide new supplemental funds to both the CDC and NIH to address this problem.

CDC needs an additional $3 million in order to:

1. Assist the NE Ohio health agencies who have already started a Pulmonary Hemorrhage Prevention Program.
2. Further investigate the Cleveland area to discern why we are having so many cases; also other cities on the Great Lakes where the cases seem to be clustering.
3. Mount a national epidemiological investigation to determine if other infants with acute pulmonary hemorrhage also have toxic fungi in their homes.
4. Develop rapid methods to detect and quantify airborne toxic fungi, such as *Stachybotrys*. This information is needed in order to make objective public health decisions especially regarding the safety of infants remaining in water-damaged homes. New molecular biology methods are needed to replace current culturing techniques which are not adequate because the toxicity of fungal spores lasts much longer than spore viability.

NIH-NIEHS needs an additional $2 million to initiate research on:

1. Blood or urine tests to detect recent exposure to toxic fungi. These are needed in order to better distinguish this disorder from other causes of lung bleeding, and to more completely understand how the level of fungal toxins relates to human disease processes.
2. The role of several different fungal toxins in producing human disease. This especially needs to be delineated in infant animal models.
3. Why these infants continue to have low grade lung bleeding even after removal from their toxic environments; how to treat this continued bleeding and how to stop their continued vulnerability to fatal hemorrhage.
4. The relationship of this disorder to the fatal mechanisms of SIDS.

I urge you to help us attack this newly recognized environmental hazard that is killing young infants in our communities. These fiscal requests are a crucial initial empowerment.