FINAL PROGRESS REPORT
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CHRYSOTILE AND LUNG CANCER: TIME-RELATED EFFECTS AND POOLED ANALYSIS
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LIST OF TERMS AND ABBREVIATIONS

95% CI	95% confidence interval
PCM	Phase contrast microscopy
RR	Rate ratio
SMR	Standardized mortality ratio
TEM	Transmission electron microscopy

ABSTRACT

Background. Although all forms of asbestos are currently classified as human carcinogens, there is ongoing controversy about the risks associated with the chrysotile form. The purpose of this project was to strengthen quantitative risk assessments for chrysotile exposure. The project aimed to: conduct pooled analyses of the association of lung cancer with exposure to chrysotile asbestos in previously-enumerated cohorts of asbestos textile workers in North Carolina and South Carolina; evaluate exposure-time-response relationships between chrysotile asbestos and lung cancer mortality with several alternative models, and conduct analyses applying biologically-based models of carcinogenesis.

Methods. Men and women who worked ≥30 days in production departments in any of 4 asbestos textile plants in North Carolina or South Carolina and were employed between 1-01-1950 and 31-12-1973 in NC or 1 -01-1940 and 31-12-1965 in SC were eligible for inclusion. This cohort was followed for mortality through the end of 2003. Cumulative exposure to asbestos fibers was estimated from over 9000 historical dust samples using data from our previous research. Fiber numbers and dimensions were determined by transmission electron microscope (TEM) analysis of 160 filters collected from the 1960s to the 1980s. Department-specific exposures were estimated using regression models and adjustment factors. The mortality of the study population relative to national and state populations was estimated using the NIOSH modified lifetable program to estimate standardized mortality ratios (SMRs). Associations of lung cancer mortality with cumulative fiber exposures were evaluating using Poisson regression to estimate mortality rate ratios (RRs) adjusted for age, sex, race and calendar year.

Results. The study included 6,136 workers, contributing 218,631 person-years of observation and 3,356 deaths. Mortality from all causes and all cancers was significantly higher than expected. The SMR for lung cancer (SMR = 1.93, 95% CI 1.73-2.15). The relative rate for lung cancer was 1.11 (95% CI 1.06-1.16) at 100 fiber-years/ml compared with 0 fiber-years/ml. Stratification showed different effects in SC (RR = 1.65, 95% CI 1.42-1.92) than in NC (RR = 1.12, 95% CI 1.06-1.19). Exposure to fibers throughout the range of length and diameter was significantly associated with increased risk of lung cancer. The best fitting models were those for fibers >5 μm long and <0.25 μm in diameter. The greatest magnitude of association with lung cancer was seen for fibers 5-10 μm long and <0.25 μm in diameter (RR approx. 1.04 per intra-quartile range, $p < 0.001$). When indicators of mean fiber length and diameter were modeled simultaneously, length was positively associated with lung cancer while diameter was negatively associated.

Conclusions. The findings of this study add to the evidence that the chrysotile form of asbestos is carcinogenic to humans. Our findings also support the hypothesis that long, thin fibers are more carcinogenic than shorter, wider fibers, although exposures to fibers of all sizes were associated with lung cancer. Heterogeneity in risk observed in previous, separate studies of the NC and SC cohorts does not appear to be a result of the use of different analytical methods or inclusion criteria.

SECTION 1

Highlights/Significant Findings

- Lung cancer risk increases quantitatively with greater fiber length and smaller fiber diameter. Specific fiber sizes associated with increased risk are difficult to identify for technical reasons. The strongest evidence of increased risk is for fibers less than 0.25 µm in diameter and greater than 5 µm long. It is noteworthy that fibers in this size range can be classified as nanoparticles and may have properties in common with some engineered nanomaterials.

- Lung cancer mortality increased an average of about 11% per 100 fiber-years/ml in a combined cohort of asbestos textile workers from North Carolina and South Carolina cohorts. This finding was statistically significant.

- The exposure-response slope was statistically significant for the two cohorts we studied, but was steeper in South Carolina than in North Carolina. Our analysis suggests that differences in cohort definition or analytical methods are unlikely to explain this apparent variation between cohorts. Differences in exposure levels, fiber-size distributions or data quality are potential explanations.

Translation of Findings

The findings of this study add to the existing evidence that the chrysotile form of asbestos is carcinogenic to humans. Our results support continuing regulation of chrysotile as a carcinogen.

Our data further suggest that the risk of cancer increases with the amount of exposure to long, thin fibers. The standard method currently used to monitor fiber exposure does not measure fiber length and often fails to detect very thin fibers, so we recommend the adoption of methods capable of measuring fiber length and diameter for research and regulatory purposes. Development of rapid, low-cost methods for making these measurements is also desirable.

If fiber length and diameter were measured on a regular basis, long, thin fibers could be given priority for regulation and exposure reduction. It is important to recognize, however, that exposure to fibers of all sizes was significantly associated with the risk of cancer in our research. We found no indication that a safe fiber-size fraction exists.

Outcomes/Relevance/Impact

Potential impact on occupational safety and health.

1. We observed statistically significant, quantitative increases in the risk of lung cancer with increasing exposure to fibers in asbestos textile industries using chrysotile asbestos. This finding supports continuing regulation of all forms of asbestos as human carcinogens.

2. We also found that the risk of cancer increases as the length of fibers that workers are exposed to increases and their diameter decreases. In other words, risk increases with the amount of exposure to long, thin fibers. This finding suggests that reducing exposures long, thin fibers should be a high priority. We found no evidence that shorter or wider fibers are harmless, however: exposure to fibers of all sizes was significantly associated with the risk of lung cancer in our research.

Implications for future research and development

1. Our finding that the risk of lung cancer varies with the length and diameter of the fibers to which workers are exposed indicates a need for further research on these relationships. The dependence of cancer risk on fiber size should be investigated in other groups of workers exposed to asbestos. Our findings may also be relevant for other types of fibers. Some of the fibers we found were most strongly associated with increased risk of lung cancer were less than 1 μm in diameter and can therefore be classified as nanoparticles. These findings support the need for accelerated research on the health risks of engineered nanoparticles.

2. Although we found clear evidence of carcinogenicity in both of the cohorts we studied, the level of risk varied between plants in spite of the similarity of the cohorts and the application of identical analytical methods. Studies of other cohorts exposed to chrysotile would help to resolve any remaining uncertainty about the quantitative level of risk associated with exposure.

3. The method we used to determine fiber length and diameter by transmission electron microscopy yielded useful results, but was time consuming and costly. Development of rapid, low-cost methods for characterizing fiber length, diameter and number would facilitate more widespread assessment of these aspects of fiber exposure in research and for regulatory purposes.

4. Our statistical research on efficient methods for modeling the association of health outcomes with fiber dimensions also suggests a need for further research on statistical methods for handling complex, multidimensional exposure data.

SECTION 2

Scientific Report

Background

This project is a renewal of our successful research on lung cancer exposure-response and new exposure indicators in a cohort of asbestos textile workers from North Carolina. The project's overall aim is to strengthen quantitative risk assessments for chrysotile exposure. Despite limits on the production and use of all forms of asbestos in the United States, a large amount remains in the environment, and large quantities continue to be produced and used elsewhere in the world. Most of this asbestos is chrysotile. Hence, if exposure to chrysotile leads to an increased risk of cancer, this widely-used form of asbestos has the potential to remain an important cause of disease worldwide for years to come. The ability to establish appropriate policy regarding occupational and environmental exposures to asbestos depends upon valid estimates of the hazardousness of chrysotile. Quantitative risk assessments for chrysotile exposure are therefore important both for science and for policy.

The cohort of asbestos textile workers from North Carolina that was the basis for our original work on this project [1, 2] and the cohort of South Carolina asbestos textile workers studied by NIOSH [3, 4] are arguably the best sources of data currently available for investigating the health consequences of long-term exposure to chrysotile asbestos. These cohorts have been studied intensively and together they include over 6000 production workers exposed to chrysotile with little or no concomitant exposure to amphibole asbestos. Over 9000 historical dust measurements are available for these workers, and we have already characterized asbestos exposures for both cohorts using both standard PCM methods and a newer approach utilizing transmission electronic microscopy (TEM) [5-7].

Before our work with the North Carolina asbestos textile cohort, virtually the only lung cancer exposure-response data available for assessing the risks arising from exposure to chrysotile were derived from studies of asbestos miners and millers and workers at three asbestos textile plants, including the one in South Carolina and two others where workers were exposed to amphibole asbestos, as well as to chrysotile. Meta-analyses published prior to our work suggest considerable heterogeneity in the strength of the association of lung cancer with exposure to chrysotile as measured by PCM [8, 9]. In these analyses, the exposure-response slope for the South Carolina asbestos textile cohort is notably steeper than for other cohorts exposed exclusively to chrysotile, suggesting a need both for more data and for further research to investigate sources of heterogeneity among the studies.

Interest in the hypothesis that differences in the shape and size of the asbestos fibers to which workers in different industries and plants are exposed could explain a portion of this heterogeneity was one of the motivations for our work with the North Carolina cohort. To evaluate this hypothesis, we characterized the distributions of fibers in the North Carolina and South Carolina plants [5, 7]. In analyses of the data from the North Carolina cohort, we observed an association of lung cancer and cumulative fiber exposure intermediate between the South Carolina asbestos textile cohort and earlier studies of miners and millers [2]. While lower in magnitude compared to the South Carolina cohort, this observation reduced the overall heterogeneity in the association of lung cancer and chrysotile fibers measured by PCM methods. Our analysis of the association of lung cancer with fibers of varying size measured

with TEM fibers suggested associations of increased risk with exposure to long, thin fibers largely consistent with findings from analysis of the South Carolina workers [1, 10].

The similarities between the North Carolina and South Carolina cohorts, including the similarity of the plants and production process, the era in which they operated, and the region of the US in which they were located, suggest that the two cohorts could be pooled to improve statistical power and to further investigate heterogeneity. We anticipated that pooled analyses of the Carolina asbestos textile cohorts would eliminate heterogeneity due to differences in analytical methods and allow us to evaluate the consistency of effect estimates; derive an overall effect estimate, and provide greater opportunity to investigate the effects of fiber size and morphology on risk estimates.

We also proposed to investigate alternative models for time-related effects using the pooled data. Different models of the effects of temporal variables lead to substantially different projections of lifetime risk. The additional analyses we proposed involve the application of empirical and biologically-based models that can provide insights into latency effects. Such effects are another potential explanation for heterogeneity in risk among cohorts exposed to chrysotile.

Specific Aims

The original aims of the project were to:

1. Evaluate exposure-time-response relationships between chrysotile asbestos and lung cancer mortality using the following methods: a) exposure time-window methods; b) bilinear latency modeling; c) sigmoid latency modeling, and d) cubic B-spline latency modeling.

2. Conduct analyses that apply the following biologically-based models of carcinogenesis: a) the Armitage-Doll model, and b) the Moolgavkar two-stage clonal expansion model.

3. Conduct pooled analyses of chrysotile asbestos exposures and associations of lung cancer mortality with exposure using workers in the North Carolina Asbestos Textile Study and the Charleston, South Carolina asbestos textile worker study.

As a result of a 25% budget reduction imposed at the time of the award, we focused our efforts on Aim 3, the most time-consuming aim with the greatest direct impact on the assessment of risk. Analysis of the pooled data focused on investigating differences in exposure-response for lung cancer using PCM and TEM exposure metrics and established epidemiologic methods.

Methods

Data for the North Carolina cohort were collected during the original phase of this project and are described in a previous publication by our group [2]. Data for the South Carolina cohort were obtained through a request to NIOSH; details of the cohort are described by Hein [4]. For this project, we included workers after 30 days of employment in a production job during the qualifying period for the plant where they were hired (1940-65 in South Carolina, 1950-73 in North Carolina) and followed them through 2001 for South Carolina or 2003 for North Carolina.

The combined cohort included 6,136 workers, contributing 218,630.8 person-years of observation and 3,356 deaths.

For analysis of the relationship of lung cancer and conventional measures of cumulative asbestos fiber exposure based on PCM methods, we used estimates of exposure for the individual cohorts, as published previously [5, 6]. For analysis of the variation of risk with fiber size, we reassessed and re-analyzed fiber data obtained by TEM for both cohorts. Details of the methods and results of this exposure assessment are presented in a paper we published this year [11]. Briefly, TEM was used to estimate the distribution of fibers for combinations of plant and department in categories defined by diameter (4 categories) and length (6 categories). This assessment was performed on a stratified random sample of 160 historical dust samples captured on membrane filters collected in surveys of the study plants in 1964-1971. The TEM fiber-counting protocol was based on the ISO direct-transfer method and data reduction and derivation of size-specific exposure estimates followed the procedure described by Dement [7]. The bivariate fiber diameter/length distributions from TEM were used to estimate size-specific fiber exposures with adjustment factors. Adjustment factors were developed for each length-diameter category and applied to a matrix of plant-, department- and time-specific fiber concentrations determined by the standard phase-contrast microscopy (PCM) method to produce fiber size-specific estimates of exposure. As summary measures of fiber length and diameter, we used the indicators of mean length and diameter we developed in our previous work in North Carolina [1]

Standardized mortality ratios (SMR) were estimated with the NIOSH lifetable program using both national and state reference rates. Exposure-response analyses were based on deaths with any mention of lung cancer on the death certificate. Lung cancer mortality rates were modeled by Poisson regression as in previous internal analyses of the North Carolina cohort, with the fit of the models evaluated by the Akaike Information Criterion (AIC). Model fitting was carried out using Stata 10 and R for Mac OS X.

The complexities of fitting models to multi-dimensional data describing fiber length, diameter and concentration also led us to investigate alternatives to conventional dose-response models. To facilitate the simultaneous modeling of these 3 dimensions, we investigated propose a spline-based partially linear Poisson single-index model using *B*-splines to approximate the unknown regression function. A direct and consistent variance estimation method based on least squares estimation is also proposed. We conducted a Monte Carlo study to evaluate the finite sample performance of the proposed spline method and fit the model to empirical data from the North Carolina cohort.

Results and Discussion

Pooled Analyses.

All work on this aim was successfully completed. Results of the exposure assessment have been published (Dement et al., 2011 in the publication list) and abstracts on the epidemiologic findings have been submitted for presentation at the 2011 EPICOH conference. Full-length papers on those results are in preparation and will be submitted in May 2011. The results for this aim are summarized below.

When compared to national or state populations, mortality from all causes and all cancers in the combined cohort was higher than expected. Mortality from lung cancer was notably elevated (SMR = 1.93, 95% CI 1.73-2.15).

Exposures to dust were notably higher in North Carolina than in South Carolina. Exposure-response analyses based on cumulative exposure to fibers measured by PCM indicated a relative rate for lung cancer of 1.11 (95% CI 1.06-1.16) at 100 fiber-years/ml compared with 0 fiber-years/ml. Stratification by state showed different effects in SC (RR = 1.65, 95% CI 1.42-1.92) than in NC (RR = 1.12, 95% CI 1.06-1.19).

Analysis of the association of lung cancer with fiber size showed that exposures to fibers throughout the range of length and diameter were significantly associated with increases in risk. However, because of strong correlations between exposure indicators, the specific categories of fiber size that best predict risk are difficult to identify. The best model fits were obtained for fibers >5 μm long and <0.25 μm in diameter, while the greatest magnitude of association with lung cancer was seen for fibers 5-10 μm long and <0.25 μm in diameter (excess RR about 4% per intra-quartile range, $p < 0.001$). When indicators of mean fiber length and diameter were modeled simultaneously, length was positively associated with lung cancer while diameter was negatively associated. There was no noteworthy interaction between indicators of fiber length and diameter and state.

The findings from analysis of a combined cohort of asbestos workers in North Carolina and South Carolina suggest that mortality from lung cancer increases about 11% per 100 fiber-years/ml. The effect is modified by state, with a stronger association in North Carolina than in South Carolina. This finding is compatible with the results of separate analyses of data from the two cohorts published previously, but those studies used different eligibility criteria to define the cohort and applied different analytical methods. Our analysis of the combined cohort allows those factors to be ruled out as explanations for heterogeneity between cohorts. The age and length of follow-up of the cohorts are also similar and are unlikely to be significant sources of heterogeneity. Potential explanations that remain include the higher dust levels in North Carolina, differences in fiber-size distributions, unmeasured co-exposures, and differences in the completeness and quality of work history and exposure data.

Our findings from analyses of TEM fiber-size data support the toxicological hypothesis that long, thin fibers have higher carcinogenic potency compared to shorter and wider fibers. The large amount of data available from the combined cohort allowed us to improve on separate analyses of the Carolina cohorts and provided improved statistical power. Notably, in contrast to our analysis of conventional exposure indicators obtained with PCM, state did not appear to modify the effects of fiber length or diameter. This observation suggests that different fiber-size distributions may account for some of the heterogeneity in dose-response relations based on standard, PCM-based, estimates of fiber concentration as used in our previous studies and those of other cohorts.

Other Studies

The spline method proposed in our statistical studies shows good theoretical properties and desirable finite sample performance. The spline estimator achieves the possible optimal rate of convergence when the underlying true function is sufficiently smooth. Moreover, the estimates of coefficients are asymptotically normal and efficient. The spline method is robust to the selection of knots. When the proposed method is applied to data on lung cancer and TEM fibers

from the North Carolina cohort, the interpretation is compatible with the results we obtained with more conventional methods, but models the multidimensional data with fewer parameters. As a The proposed method provides a useful approach in the application to semiparametric models that can be applied in other settings. Results of this work have been submitted for publication and were presented at the ENAR conference in March 2011.

Conclusions

Our findings suggest the following major conclusions:

1. Statistically significant increases in the risk of lung cancer with increasing cumulative exposure to fibers measured by conventional methods are observable in both of the Carolina asbestos textile cohort, but there is evidence of heterogeneity, with stronger effects in South Carolina than in North Carolina. These differences do not appear to be related to the use of different analytical methods or inclusion criteria.

2. Lung cancer risk increases with greater fiber length and smaller fiber diameter. This finding is not dependent on plant or state and may be a generalizeable phenomenon that should be examined in other cohorts exposed to asbestos.

3. Assessments of asbestos exposure for research and regulation should include determinations of fiber length and diameter using TEM or other methods.

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Publications

Published

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Richardson DB, MacLehose RF, Langholz B, Cole SR. Hierarchical latency models for dose-time-response associations. Am J Epidemiol. 2011 Mar 15;173:695-702.

Lu M, Loomis D. Sieve Likelihood Estimation of Partially linear Poisson Regression with Single-Index Model. Abstract presented at International Biometrics Society Eastern North American Region meeting (ENAR) , Miami FL, March 2011.

Submitted

Elliott L, Loomis D, Dement J, Hein M, Richardson D, Kuempel E, Stayner L. Lung Cancer Mortality in a Combined Cohort of North Carolina and South Carolina Asbestos Textile Workers. Abstract submitted for presentation at the 2011 EPICOH Conference, Oxford, England, September 2011.

Loomis D, Dement J, Elliott L, Richardson DB, Kuempel E. Longer, thinner fibers are associated with increased lung cancer mortality among asbestos textile workers. Abstract submitted for presentation at the 2011 EPICOH Conference, Oxford, England, September 2011.

Lu M, Loomis D. Sieve likelihood estimation of partially linear Poisson regression with single-index model. Submitted to Statistics in Medicine.

In Preparation

Loomis D, Dement J, Elliott L, Richardson D, Kuempel E, Stayner L. Increased lung cancer mortality among asbestos textile workers is associated with exposure to long, thin fibers.

Elliott L, Loomis D, Dement JM, Hein MJ, Richardson DB, Stayner L. Lung cancer mortality in North Carolina and South Carolina asbestos textile workers.

Inclusion of gender and minority subjects

See enrollment table below.

Program Director/Principal Investigator (Last, First, Middle): Loomis, Dana

Targeted/Planned Enrollment Table

Study Title: CHRYSOTILE AND LUNG CANCER: TIME-RELATED EFFECTS AND POOLED ANALYSIS

Total Planned Enrollment: 6136

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino			
Not Hispanic or Latino	2,419	3,717	6136
Ethnic Category: Total of All Subjects *	2,419	3,717	6,136
Racial Categories			
American Indian/Alaska Native			
Asian			
Native Hawaiian or Other Pacific Islander			
Black or African American	123	994	1,117
White	2,052	2,376	4428
Racial Categories: Total of All Subjects *	2,419	3717	6136

Inclusion of children

During the period covered by this historical cohort study, it was commonplace for individuals younger than 21 years to be employed in southern textile mills. The study included all qualifying workers without respect to age: 1861 workers were hired at ages less than 21 years and contributed 3681 person-years of observation before the age of 21. However, because of the study's long follow-up period, individuals who began employment could also contribute person-time as adults and even the study's youngest subjects would have been adults at the time the data were collected. Because none of the subjects were children at the time the study was done and there was no direct contact with human subjects, there were no special provisions for the protection of children.