Longitudinal Assessment of Respiratory Symptoms and Pulmonary Function Among Flavor Manufacturing Workers

Stella E. Hines, M.D.

Candidate for the Master of Science in Public Health degree

April 2009

Thesis Committee

Candidate: Stella E. Hines, M.D.

Degree: Master of Science in Public Health

Institution: The University of Colorado at Denver

Members:

Cecile Rose, MD, MPH – Primary mentor

Gary Zerbe, PhD – Thesis Committee Chair

John Martyny, PhD, CIH

Mike VanDyke, CIH (ex-officio)

Disclaimer: National Jewish Health received compensation for consultation from individual flavor manufacturing companies.

Abstract

Background: Flavor industry workers exposed to diacetyl in butter flavorings are at risk for bronchiolitis obliterans, manifested mainly by accelerated declines in FEV1 on spirometry associated with worsening respiratory symptoms. We analyzed longitudinal spirometry and symptoms in flavor workers to assess possible work-related fixed airways disease. **Methods:** 191 workers participated in medical surveillance using serial questionnaires and spirometry. Task-specific diacetyl exposures were measured using NIOSH methods 2557. We used the general linear mixed model to evaluate longitudinal change in FEV1 & respiratory symptoms, then stratified overall changes by smoking status and analyzed by four different exposure variables: job category; reported use of diacetyl,; work with powders, liquids or spray-dried flavors; and cumulative diacetyl exposure. **Results:** Decline in FEV1 (adjusted for height, race and gender) was not significantly different between production and non-production workers (-40.40 v. -40.67 ml/yr, p=0.95), even when stratified for smoking. In the overall group of flavor workers, we found no significant differences in FEV1 based on reported frequency of diacetyl use, work with different flavor formulations, or cumulative diacetyl exposure. However, never and former smoker production workers in the high cumulative exposure group had greater declines in FEV1 (-41.77 ml/yr and -44.00 ml/yr) compared to current smoker production workers (-29.44 ml/yr, p=0.02 for former v. current smokers). Among participants working in flavor manufacturing for two years or less, workers in the high cumulative diacetyl exposure group did have a greater rate of decline in FEV1 (-65.14 ml/yr) compared to medium (-42.75 ml/yr, p=0.0166) and low exposure groups (-46.69 ml/yr, p=0.0151). There were few differences in respiratory symptoms or asthma diagnosis among any of the exposure groups. Only for the symptom chest tightness was the yearly prevalence increasing for production workers and workers reporting use of diacetyl, compared to non-production workers (p=0.0069) and those not reporting use of diacetyl (p<0.0001). **Conclusions: W**e found no significant differences in rate of FEV1decline and rate of change in respiratory symptoms over time among flavor workers characterized by four different workplace exposure variables. However, more recently hired flavor workers in the high exposure group did have significantly greater declines in FEV1 compared to the low exposure group, suggesting that regular medical surveillance for work-related lung disease is important, particularly in this subset of flavor workers. We also found that currently smoking flavor workers in high diacetyl exposure categories may have a slower

rate of lung function decline compared to non-smokers in the high exposure group, suggesting a "protective" effect from smoking relative to flavor chemical effects.

Introduction

Bronchiolitis obliterans (BO) was first reported among workers in the butter-flavor microwave popcorn industry in 2002 (1). Subsequent epidemiologic investigation of microwave popcorn workers demonstrated elevated rates of airflow limitation on spirometry and respiratory symptoms including cough and asthma, particularly in non-smokers (2). Environmental sampling of the workplace found elevated concentrations of diacetyl, a ketone used to impart butter flavoring (3). Diacetyl, in addition to multiple other volatile chemicals, is used throughout the flavor and fragrance industries Two cases of BO were reported in 2006 among flavor manufacturing workers in California (4). Three additional cases were reported in production workers from a Dutch diacetyl manufacturing company, providing further support for the role of diacetyl exposure in risk for occupational BO (5).

In response to these findings, the trade association representing many flavor companies developed a respiratory health program for its members incorporating exposure monitoring, respiratory protection and medical surveillance. In a cross-sectional evaluation of more than 600 U.S. flavor manufacturing workers participating in this program, we reported higher than expected prevalences of airflow limitation and cough among younger, non-smoking flavor production workers (6). Through exposure monitoring, we also found that exposures to diacetyl-containing powder formulations and heated liquids were associated with higher airborne diacetyl concentrations (7).

While cross-sectional studies of flavor (6), diacetyl production (5) and microwave popcorn manufacturing workers (8) have shown that exposure to butter flavoring chemicals containing diacetyl is associated with higher than expected rates of respiratory symptoms and fixed airflow limitation, change in pulmonary function over time has not been reported. Moreover, there is little information regarding the levels of diacetyl exposure associated with work-related lung disease. Thus, medical surveillance of at-risk workers is an important component of lung disease prevention in manufacturing workplaces using diacetyl. Longitudinal assessment of pulmonary function may identify respiratory problems before a worker's spirometry becomes abnormal. Assessment of rate of change in pulmonary function over time is an important tool in medical surveillance of workers as this may be the first clue to an exposure-related problem.

The primary objective of this investigation was to assess changes over time in spirometry and respiratory symptoms in flavor industry workers characterized by diacetyl exposure and by job category. We anticipated that flavor industry workers with greater exposure to diacetyl, based on questionnaire responses assessing diacetyl exposure frequency and on workplace exposure monitoring data, would have greater decrements in pulmonary function and report more symptoms over time compared to workers with lower exposure to diacetyl. Furthermore, we expected that non-smoking flavor production workers would be more likely to experience diacetyl-mediated respiratory effects, and that smoking status in general would modify our findings.

Methods

Study setting: This prospective clustered cohort study occurred at seven flavor manufacturing companies participating at least twice in a medical surveillance program for flavor workers between 2004-2007. The frequency of surveillance as well as the interval between screening tests varied between companies.

Medical surveillance: Medical surveillance included a symptom/exposure questionnaire and spirometry. Questions were adapted from the American Thoracic Society standardized respiratory symptom questionnaire (9). Questionnaires were completed by individual workers and reviewed with a physician. Based upon questionnaire responses regarding job tasks, participants were grouped into four categories for analysis: Production, Lab/Quality Control (QC), Warehouse, and Administration. For dichotomous analysis, Lab/QC, Warehouse and Administration workers were grouped as non-production workers and compared to production workers. We grouped participants based on reported tobacco histories into one of three categories: current smoker, former smoker, or never smoker (<10 Packs).

Spirometry: Spirometry was performed by two different groups of NIOSH-certified technicians following American Thoracic Society guidelines, with quality assurance review by an experienced pulmonologist. (10) One spirometry technician group used a flow-based spirometer interfaced to a computer utilizing Jaeger software version 4.65. The second spirometry technician group used the EasyOne Diagnostic 2.5 hand held spirometer. We evaluated FEV1, FVC, and FEV1/FVC ratio, and did not assess post-bronchodilator spirometry.

Exposure sampling and variables: Mean and short-term peak diacetyl samples were collected during "worst case exposure" production processes, that is, those flavor production processes most likely to lead to highest diacetyl exposures as determined by the production supervisor or company manager. Personal and area monitoring was performed using NIOSH method 2557 using personal sampling pumps that were pre-and post-use calibrated daily to a flow rate of approximately 150 cc/min. All samples were shipped on ice via overnight mail to an American Industrial Hygiene Association accredited laboratory for analysis (7). Further details on sampling methods are been described elsewhere (7).

In addition to comparing production to non-production flavor workers, we analyzed three different markers of workplace exposure to diacetyl, based on questionnaire response alone and on a combination of workplace sampling results for diacetyl and questionnaire responses. We analyzed outcomes according to reported use of diacetyl (yes or no). An individual cumulative exposure variable was calculated for each participant based on the product of mean diacetyl level by company (ppm) per job task multiplied by reported frequency of diacetyl use and by years of exposure. Then, each participant was categorized into an exposure group as follows: low, medium, or high exposure. We also analyzed exposure based on workers' reports of using flavor chemicals in powdered, liquid or spray dried forms, as concern has been raised that exposure to powdered flavor chemicals increases this risk for obstructive lung disease (7).

Analysis plan: The general linear mixed model was used to assess longitudinal changes in FEV1 in this flavor cohort. This model provides accurate and meaningful assessments of longitudinal change in pulmonary function by accounting for inter-subject and intra-subject sources of variability. It expands on the ordinary linear regression model by allowing one to incorporate lack of independence between observations and to model more than one error term (11). This makes the model extremely helpful in analyzing longitudinal data and in assessing unbalanced repeated measures.

Based on initial model development, main effects of age, height, gender, and race were adjusted for in all subsequent models, as well as the interactions between age and race and between height and gender using an unstructured covariance matrix that permitted slopes (change in FEV1) and intercepts to vary from subject to subject. We stratified the overall longitudinal change among workers by smoking status, and then by cumulative diacetyl exposure, or by job type (production versus non-production).

For example, to compare production and nonproduction workers with respect to FEV1 decline longitudinally, we employed the linear mixed model,

$$FEV1ijk = (\alpha_i + a_{ij}) + (\beta_i + b_{ij})t_{ijk} + \gamma(x_{ijk} - \bar{x}) + e_{ijk}.$$

Here i=1 for the production worker group; i=2 for the nonproduction worker group. $FEV1ijk$ is the FEV1 measured on subject j in group i at time t_{ijk}, and x_{ijk} represents one or more covariates measured on subject j in group i at time t_{ijk}. α_1 is the intercept, β_1 is the slope, and $FEV1_1(t) = \alpha_1 + \beta_1 t$ is the mean curve for production workers at time t evaluated at the average value $\bar{x}$ of the covariates. It is plotted in Figure 1. α_2 is the intercept, β_2 is the slope, and $FEV1_2(t) = \alpha_2 + \beta_2 t$ is the mean curve for nonproduction workers at time t evaluated at the average value $\bar{x}$ of the covariates. It too is plotted in Figure 1.

The a_{ij} are subject specific random deviations in the intercepts; the b_{ij} are subject specific random deviations in the slopes; their inclusion permits us to acknowledge variation between subjects and distinguish it from variation within subjects. The e_{ijx} are time specific random deviations on a subject that permit us to acknowledge within subject variability. We assume that the a_{ij} and b_{ij} are bivariately normally distributed and independently of the e_{ijk} which are assumed univariately normally distributed.

We estimated and tested the significance of the following quantities, all adjusted for covariates. β_1 is the change in FEV1 in th production worker group in one year; β_2 is the change in FEV1 in the nonproduction worker group in one year. $\beta_1 - \beta_2$ is the difference in changes in FEV1 in one year, or difference in rates of change. $[\alpha_1 + \beta_1 t] - [\alpha_2 + \beta_2 t]$ is the difference in mean FEV1 (production – nonproduction) at time t. It corresponds to the vertical distance between the two lines in Figure 1 at time t.

We used a generalized linear model with repeated measurements to characterize change in respiratory symptoms over time, analyzing the overall longitudinal change by cumulative diacetyl exposure and job type and stratifying by smoking status. In a very analogous manner to compare production and nonproduction workers with respect to Prevalence of Productive Cough change longitudinally over time on study, we employed a generalized linear mixed model (a logistic regression model with random effects) to a dichotomous assessment Y_{ijk} (Y_{ijk}=1 if Productive Cough is present, Y_{ijk}=0 otherwise) for the jth subject in the ith group at time t_{ijk}. Y_{ijk} is assumed to have a Bernoulli distribution (Binomial with n=1) with parameter P_{ijk} = the probability of a Productive Cough. We further assume,

$$\log it(P_{ijk}) = \ln\left(\frac{P_{ijk}}{1-P_{ijk}}\right) = (\alpha_i + a_{ij}) + \beta_i t_{ijk} + \gamma(x_{ijk} - \bar{x}).$$

Here i=1 for the production worker group; i=2 for the nonproduction worker group. P_{ijk} is the probability of a Productive Cough for subject j in group i at time t_{ijk}, and x_{ijk} represents one or more covariables measured on subject j in group i at time t_{ijk}. α_1 is the intercept, β_1 is the slope, $\alpha_1 + \beta_1 t$ is the logit at time t, and $P_1(t) = \dfrac{\exp(\alpha_1 + \beta_1 t)}{1+\exp(\alpha_1 + \beta_1 t)}$ is the <u>prevalence</u> at time t for production workers evaluated at the average value $\bar{x}$ of the covariates. α_2 is the intercept, β_2 is the slope, $\alpha_2 + \beta_2 t$ is the is the logit at time t, and $P_2(t) = \dfrac{\exp(\alpha_2 + \beta_2 t)}{1+\exp(\alpha_2 + \beta_2 t)}$ is the <u>prevalence</u> at time t for for nonproduction workers evaluated at the average value $\bar{x}$ of the covariates.

The a_{ij} are subject specific random deviations in the intercepts; the slopes are not permitted to vary due to software and convergence limitations. Inclusion of the random a_{ij} permits us to acknowledge variation between subjects and distinguish it from variation within subjects.

We estimated and tested the significance of the following quantities, all adjusted for covariates. β_1 is the log odds ratio for the Prevalence of Productive Cough for production workers at time t relative to Production workers at time (t-1); β_2 is the log odds ratio for the Prevalence of Productive Cough for Nonproduction workers at time t relative to Nonproduction workers at time (t-1). $\beta_1 - \beta_2$ is the difference in the above two log odds ratios, a measure of how much faster one group's prevalence is increasing over time.

$P_1(t) = \dfrac{\exp(\alpha_1 + \beta_1 t)}{1 + \exp(\alpha_1 + \beta_1 t)}$ is the prevalence at time t for production workers;

$P_2(t) = \dfrac{\exp(\alpha_2 + \beta_2 t)}{1 + \exp(\alpha_2 + \beta_2 t)}$ is the prevalence at time t for for nonproduction workers.

$[\alpha_1 + \beta_1 t] - [\alpha_2 + \beta_2 t] = \ln\left\{ \dfrac{P_1(t)/[1 - P_1(t)]}{P_2(t)/[1 - P_2(t)]} \right\}$ is the log odds ratio for the Prevalence of Productive Cough for Production workers at time t relative to nonproduction workers at time t.

All analyses were performed using SAS® software version 9.2, using the SAS procedures MIXED to analyze change in FEV1 and GENMOD to analyze change in symptoms (12). Descriptive statistics were used to examine summary data for the cohort. For analysis of reported exposure to flavoring powders, liquids or spray drying processes, if a worker ever reporting working with powder, “ever powder” was considered true. The same approach was used for work with liquids or with spray drying.

After the overall cohort evaluation, we then analyzed the results for only those workers who had worked for two years or less in their current job. We used this approach in order to evaluate for the presence of a “healthy worker effect” (13), which would indicate that the workers employed for a shorter time period might have more symptoms or change in lung function and may not yet have left the flavor workplace, compared to an older, survivor working population perhaps not susceptible to the health effects in question. This analysis is important since the reported latency of onset of diacetyl-related symptoms and health effects may be as short as a few months, and averaged 1.5 years in the sentinel cohort of microwave production workers (14)

Results

Longitudinal FEV1 Analysis

Table 1 shows demographic characteristics of 191 participating flavor workers. The majority were male never smokers with an average employment in the industry of approximately six years. The mean length of time between first and last spirometry evaluation was 16.2 months, ranging from 2.6 months to 34.5 months.

Adjusted rate of decline in FEV1 for production workers was similar to non-production workers (-40.40 ml/yr vs. -40.67 ml/yr) (Figure 1). There were no significant differences overall in rate of decline in FEV1 among workers analyzed according to smoking status. Never smokers declined at -41.87 ml/yr, former smokers at -45.12 ml/yr, and current smokers at -35.39 ml/yr. (Appendix, Table 5) We did see a trend towards greater decline in FEV1 in production workers who were never smokers (-44.77 ml/yr, p=0.1384) and former smokers (-47.34 ml/yr, p=0.077) compared to current smokers (-35.34 ml/yr), but these trends did not reach statistical significance. (Table 2a)

Rate of FEV1 decline in workers reporting work with diacetyl was similar to that of workers reporting not having worked with diacetyl (-39.87 ml/yr versus -43.39 ml/yr, p = 0.219.). (Appendix, Table 6) Table 2a shows that there were no overall differences in FEV1 change over time in workers, stratified by smoking, who reported no diacetyl exposure compared to those who reported current exposure to diacetyl in their jobs.

When change in FEV1 was evaluated according to low, medium or high cumulative diacetyl exposure group, we found no significant differences between rates of decline in any of the exposure groups (-43.72 ml/yr, -42.77 ml/yr, -38.95 ml/yr respectively, p=0.2883). (Appendix, Table 7) When stratified by smoking status, current smokers in the high exposure category had a significantly lower rate of decline in FEV1 compared to the rate of decline in former smokers (p=0.0201) (Table 2a).

We analyzed change in FEV1 according to ever use of powders, liquids or spray drying. There were no significant differences in rate of change in FEV1 in those who had ever worked with these materials/processes compared to those who had not. (Appendix, Table 8)

Analysis of workers with two years or less of work in flavor manufacturing

We re-analyzed these same variables including only data from 35 workers reporting two or fewer years of work in flavor manufacturing. Production workers in this category had a greater decline in FEV1 (-56.66 ml/yr) compared to non-production workers (-43.33 ml/yr), although this difference was not statistically significant (p=0.187). (Appendix, Table 9) Among workers with a shorter duration of employment in the industry, current smokers had a significantly greater rate of decline in FEV1 (-70.45 ml/yr) compared to both former (-20.36 ml/yr, p=0.0079) and never smokers (-44.65 ml/yr, p=0.0087) (Appendix, Table 10) When the newer workers were analyzed by production status and stratified by smoking category, the current smokers in both production (-73.33 ml/yr) and non-production (-73.46 ml/yr) had significantly higher rates of decline in FEV1 than did the never and former smokers (Table 2b). Thus, once stratified we found no clear effect from exposure, apart from smoking, in production workers compared to non-production workers with shorter duration of employment.

We also examined effects of other exposure variables on longitudinal spirometry in these more recently hired flavor workers. There were no significant differences in rate of FEV1 decline according to reported diacetyl exposure (-47.56 ml/yr for reported diacetyl vs. -46.03 ml/yr for no diacetyl, p=0.81). (Appendix, Table 11) When workers were analyzed by diacetyl exposure and stratified into smoking groups, current smokers in both the reported diacetyl use and cumulative diacetyl exposure groups showed a greater decline in FEV1 compared to the never and former smokers, again showing no exposure effect in these stratified groups apart from smoking (Table 2b). This difference was significant for the current smoker group reporting diacetyl exposure compared to the diacetyl-exposed never smokers (p=0.0093), and the workers not reporting diacetyl exposure who were never (p=0.0371) and former smokers (p=0.0278).

Notably, workers with fewer than two years of flavor industry employment who were in the category of high cumulative diacetyl exposure had a significantly greater decline in FEV1 (-65.14 ml/yr) compared to both the medium (-42.75 ml/yr, p=0.0166) and low exposure groups (-46.69 ml/yr, p=0.0151). (Appendix, Table 12) When stratified by smoking status, no specific trends were observed in decline in FEV1 and associated diacetyl or smoking exposures (Table 2b).

We analyzed these newer workers according to reported ever or never use of or reported exposure to powders, liquid or spray dried flavor ingredients and processes. Surprisingly, workers who reported never having worked with flavor powders had a greater decline in FEV1 (-73.47 ml/yr) compared to workers who had ever worked with powders (-26.15 ml/yr, p=0.0054). (Appendix, Table 13) A similar trend was seen for workers who had never worked with liquids (-59.31 ml/yr vs. -28.38 ml/yr), although this difference was not significant. (p=0.0599). There were no significant differences between those ever or never working with spray drying processes (-57.37 ml/yr vs. -227.1 ml/yr, p=0.09).

Analysis of longitudinal respiratory symptoms and diagnoses

We analyzed self-reported respiratory symptoms and diagnoses for change in prevalence over time according to the three exposure variables. Each analysis was adjusted for age, race, gender and smoking status, unless convergence criteria were unable to be achieved. In those cases, the model was adjusted for as many covariates as possible but still allowing convergence. Specifically, “dry cough”, “productive cough”, “chest tightness”, “dyspnea” (a variable that includes three possible responses for shortness of breath: any report of shortness of breath, shortness of breath when walking fast on level ground or walking up a hill, or shortness of breath when walking with others at an ordinary pace on level ground), “wheeze” and previous diagnosis asthma were evaluated. In analyses where the model converged with inclusion of smoking as an adjustment term, we then stratified the primary analysis into smoking-related strata.

Our model measured rate of change in prevalence of a symptom or diagnosis, reported as the log odds ratio of the symptom relative to the previous year. Thus, a decrease in yearly prevalence of a symptom, reported as a negative value, implies a beneficial effect. A positive value denotes an increase in yearly prevalence, which implies a harmful effect.

We found no significant differences in yearly rate of change in prevalence of dry cough, productive cough or asthma diagnosis for production versus non-production workers,

workers reporting diacetyl exposure versus no diacetyl exposure, those reporting use of different diacetyl formulations, or those in high, medium or low exposure categories (Table 3).

For the summary variable “dyspnea”, there were no significant differences in yearly rate of change in prevalence between production (-0.27) and non-production workers (-0.84, p=0.1956) (Table 3). When stratified into smoking categories, currently smoking non-production workers had a significantly greater decrease in yearly prevalence (-2.59) compared to the more modest decrease in yearly prevalence for the currently smoking production workers (-0.15, p=0.0242) (Table 4). There were no significant differences in yearly rate of change in prevalence of dyspnea between workers reporting diacetyl exposure (-0.28) and those reporting no diacetyl exposure (-0.55, p=0.38) (Table 3). When stratified into smoking categories, currently smoking workers reporting no diacetyl exposure had a significantly greater decrease (-1.7758) in yearly prevalence of dyspnea compared to the more modest decrease in yearly prevalence for the currently smoking workers reporting diacetyl exposure (-0.1805, p=0.017) (Table 4). There were no significant differences in yearly rate of change in prevalence of dyspnea according to categories of high (-0.18), medium (-0.18) or low (-0.20) cumulative exposure to diacetyl) (Table 3).

Yearly rate of change in prevalence of chest tightness increased significantly more for the production workers (0.42) compared to the non-production workers (-0.56), where the yearly rate of change was decreasing (p=0.0069) (Table 3). Similarly, workers reporting diacetyl exposure demonstrated a significantly greater increase in yearly prevalence of chest tightness (0.28) compared to workers reporting no diacetyl exposure (-1.41), whose yearly rate of change in prevalence decreased ($p<0.0001$) (Table 3). Change in prevalence of chest tightness per year was increasing for the high exposure workers (0.37) and decreasing over time for the medium (-0.39) and low exposure groups (-1.46), however none of these rates of change were significantly different from each other (Table 3).

There were no significant differences in yearly rate of change in prevalence of wheezing for production versus non-production workers, even when stratified into smoking and into different exposure categories (Tables 3 and 4). Yearly prevalence of wheeze decreased for workers with and without reports of diacetyl exposure who were never smokers, and increased in the former and current smokers in both diacetyl categories (Table 4). Never smoker workers with no reported diacetyl exposure had a significant decrease in yearly prevalence of wheeze (-1.25) compared to the more modest decrease among never smoker workers who did report diacetyl exposure (-0.15, $p=0.0296$) (Table 4).

We found no differences in rates of symptoms of dry cough, dyspnea or wheeze based on questionnaire reports of ever working with powders, liquids or spray drying. This was true even when analyzed according to smoking strata for the ever powder or ever liquid analyses (results not shown).

Discussion

At the request of a number of flavor manufacturing companies using chemicals associated with risk for lung disease, we helped implement prevention programs that included longitudinal medical surveillance. Medical surveillance can be an important component of occupational disease prevention, particularly in circumstances where safe levels of exposure to a chemical as well as efficacy of primary prevention strategies such as engineering controls and respiratory protection remain uncertain. Though this effort was not designed at the outset to test research hypotheses, we were interested in whether these programs could identify accelerated declines in lung function and excessive respiratory symptoms in flavor workers characterized by exposures that, based on previously published reports, convey increased risk for work-related airways disease.

We found no significant differences among participating flavor workers in rates of FEV1decline according to job type, reported use of diacetyl, cumulative diacetyl exposure group, or by report of ever working with flavor chemical powders, liquids, or spray-drying processes. Overall smoking-adjusted rates of decline in FEV1 fell within those expected based on age (15, 16).

Unlike many chronic occupational lung diseases, the average latency period for diacetyl-associated BO is reported to be less than two years (14). With this in mind, we analyzed longitudinal findings in the subset of workers employed for two years or less to evaluate for a potential survivor effect in the larger cohort with a longer duration of employment. For these newer workers, those in the high exposure group showed a significantly greater decline in FEV1 compared to the medium or low exposure groups. This finding suggests that there may be an accelerated rate of FEV1 decline in newer workers in the high diacetyl exposure category, and supports the need for regular longitudinal medical surveillance in recently hired flavor workers with diacetyl exposure.

Not surprisingly, in the more recently hired workers, we found that smokers, regardless of workplace exposure, had significantly greater declines in FEV1 than did non-smoking workers in either the high or low diacetyl exposure groups. This finding is consistent with epidemiologic data showing that smokers have at least twice the rate of decline in FEV1 compared to non-smokers. (17,18)

There were few significant differences in the longitudinal rates of change of most reported respiratory symptoms and diagnoses in the different exposure groups. We did find that reports of chest tightness increased over time for flavor production workers compared to non-production workers and in diacetyl-exposed versus non-exposed workers.

Our study has several limitations. First, the mean length of time between first and last surveillance observations was only 16 months. Since the accuracy of predicted slope (as a parameter of rate of spirometric decline) from longitudinal data increases with length of study, our findings may not reflect trends that might emerge from a more extended period of observation.

We found only a few significant differences in this study, though there were some intriguing trends. Had there been a larger number of participants, we may have found more significant differences, particularly in the smaller subgroups such as the workers employed for less than two years. Based on the number of workers participating in our study, we were able to calculate what difference would be necessary between group-specific mean FEV1 slopes in order to achieve 80% power, based on an alpha of 0.05. These differences were quite modest in many cases, and even with our limited number of participants the differences in the means were not large enough to achieve significance. For example, analyzing change in FEV1 for 82 production workers compared to 109 non-production workers, based on an alpha of 0.05, we had 80% power to detect a difference in mean slopes of 12 ml/yr based on a standard error of 0.004238 between the two slopes. These slopes differed by less than 1 ml/yr (40.67 ml/yr for production workers versus 40.40 ml/yr for nonproduction workers); thus, we know that this difference truly is not significant, and the lack of a detectable difference was not secondary to power limitations. . Thus, except for the analysis of more recently hired flavor workers, we were not limited by inadequate statistical power in detecting significant differences; these differences truly did not exist.

Third, all of our exposure assessments depended on workers' self reports of diacetyl exposure, with opportunities for misclassification. There are often hundreds of chemicals used in flavor manufacturing, some only occasionally and in small quantities. (7) It is likely that some workers, particularly those not involved directly in flavor mixing, were not always aware when diacetyl was used in the production processes. For the analyses involving work with powders, liquids or spray-drying techniques, we had no information about duration, intensity or frequency of exposure to these specific processes and formulations. To truly clarify the question of whether powder, spray dried or liquid chemicals pose greater threats to respiratory health over time, a more thorough assessment of exposure would be necessary. Moreover, some non-production workers may have previously worked in production areas, and moved into lower exposure jobs because of significant respiratory symptoms, thus biasing our findings toward the null.

Of note, the companies participating in the medical surveillance program were simultaneously implementing engineering controls and respiratory protection programs to better control flavor chemical exposures and reduce lung disease risks. It is possible that there were no significant differences in most longitudinal rates of change in spirometry and respiratory symptoms because these preventive interventions had effectively diminished risks to flavor workers. Moreover, because of increasing attention focused on potential risks associated with use of diacetyl, flavor companies may have decreased their use of the chemical over the time of data collection, thus limiting longitudinal effects.

We did note a significantly accelerated rate of decline in FEV1 among both of the non-smoker groups compared to current smokers in the high-exposure category. This observation is similar to cross-sectional findings in both microwave popcorn and flavor manufacturing workers, where non-smoking production workers had significantly higher prevalences of airflow limitation compared to current-smokers. (2, 6) Lung diseases that are more likely to occur in non-smokers include the granulomatous diseases like hypersensitivity pneumonitis, chronic beryllium disease, and sarcoidosis. In the original nine microwave popcorn cases of BO, of the three with published reports of their lung biopsies, two demonstrated granulomas. (14) In limited animal studies of diacetyl-exposed mice, though granulomas were not seen on lung pathology, fibrohistiocytic proliferation occurred at junctions of terminal bronchioles and alveolar ducts (19). Obliterative bronchiolitis and organizing pneumonia involving the distal airways is a common feature of hypersensitivity pneumonitis. It is possible that the pathogenic mechanisms of inflammatory granulomatous lung diseases that are more likely to occur in non-smokers are overlapping with those of flavor-related lung disease.

Conclusions

While cross-sectional evaluations have reported higher-than-expected rates of airflow limitation and respiratory symptoms in microwave popcorn and flavor manufacturing workers exposed to diacetyl, we found no significant differences in rate of FEV1decline and rate of change in respiratory symptoms over time among flavor workers characterized by different exposure variables. However, more recently hired flavor workers in the high exposure group did have significantly greater declines in FEV1 compared to the low exposure group, suggesting that regular medical surveillance for work-related lung disease is important, particularly in this subset of flavor workers. We also found that currently smoking flavor workers in the high diacetyl exposure group may have a slower rate of lung function decline compared to non-smokers in this group, suggesting a “protective” effect from smoking relative to flavor chemical effects. Our findings support the implementation of targeted medical surveillance programs for flavor workers potentially at risk for exposure related lung diseases, particularly for recently hired workers.

TABLES

Table 1. Characteristics of 191 workers participating in longitudinal medical surveillance from 7 flavor companies

Mean age in years (+SD)	40.1 ± 10.4
Number (%) of females	53 (27.3 %)
Race/ethnicity	
White	69 (35.6%)
Hispanic	74 (38.1%)
Black	11 (5.7%)
Asian	31 (16.0%)
Other	9 (4.6%)
Mean duration of employment in years (+SD)	5.95 ± 5.61
Smoking status (Number (%))	
Current smokers	40 (20.6%)
Former smokers	37 (19.1%)
Never smokers	117 (60.3%)
Job category (Number (%))	
Production workers	81 (42.4%)
Lab/QC workers	62 (32.5 %)
Warehouse/shipping workers	32 (16.7 %)
Administration	16 (8.4 %)
Mean interval in months between first-last spirometry test (±SD)	16.19 ± 7.43

Table 2a. Yearly change in FEV1 (ml/yr) for never, former, and current smokers among 191 flavor manufacturing workers, adjusted for gender, height and race.

	Never smokers	**Former smokers**	**Current smokers**
Exposure variable	**ml/yr (n)**	**ml/yr (n)**	**ml/yr (n)**
Job Type			
Production	-44.77 (35)	*-47.34 (20)	-35.34 (27)
Non-production	-39.16 (82)	-43.75 (15)	-44.11 (12)
Reported use of diacetyl			
Yes	-41.77 (74)	-40.71 (21)	-36.11 (28)
No	-42.66 (43)	-54.02 (14)	-37.92 (11)
Cumulative diacetyl exposure group			
High	-41.77 (45)	*-44.00 (19)	-29.44 (26)
Medium	-44.75 (27)	-40.99 (3)	-51.72 (2)
Low	-46.20 (45)	-50.93 (13)	-36.43 (11)

** Statistically significant difference between Former-smoker Production worker v. Current-smoker Production worker, p=0.047; and Former-smoker High exposure v Current-smoker High exposure p=0.0201*

Table 2b. Yearly change in FEV1 (ml/yr) for never, former, and current smokers among 35 flavor manufacturing workers employed <2 years , adjusted for gender, height and race.

	Never smokers	**Former smokers**	**Current smokers**
Exposure variable	**ml/yr (n)**	**ml/yr (n)**	**ml/yr (n)**
Job Type			
Production	-49.94 (7)	+ 7.9 (3)	-73.33 (2)
Non-production	-52.67 (17)	-29.49 (3)	-73.46 (3)
Reported use of diacetyl			
Yes	*-42.30 (14)	-22.29 (2)	-68.08 (3)
No	-44.86 (10)	-26.77 (4)	-62.73 (2)
Cumulative diacetyl exposure group			
High	-63.55 (2)	0 (0)	–44.10(2)
Medium	-35.89(11)	-27.46 (1)	-93.40 (1)
Low	-51.86 (11)	-34.73 (4)	-58.89 (2)

**Significant difference between Never-smoker diacetyl reported v. current-smoker diacetyl reported p=0.0093; Of note, the difference between never-smoker High exp and current-smoker High exp was not significant, p=0.1640*

Table 3. Yearly rate of change in prevalence of respiratory symptom/diagnosis (*) for different exposure variables, adjusted for race, gender, age, and smoking, as model would allow

	Log odds ratio relative to previous year					
Exposure variable	**Dry cough**	**Productive cough**	**Chest tightness**	**Wheeze**	**Dyspnea**	**Asthma**
Job Type						
Production	0.1335	-0.3645	0.4219	-0.1272	-0.2743	-0.0723
Non-production	-0.2359	-0.4145	-0.5624	0.2905	-0.8424	-0.4033
p-value	*0.4445*	0.8972	***0.0069***	*0.3680*	*0.1956*	*0.1801*
Reported use of diacetyl						
Yes	-0.1205	-0.3612	0.2841	0.0154	-0.2575	-0.2719
No	0.2969	-0.4741	-1.4132	0.0204	-0.5474	-0.0669
p-value	*0.5818*	0.7774	**<*.0001***	*0.9918*	*0.3825*	*0.3041*
Cumulative diacetyl exposure group						
High	0.0154	-0.3394	0.3698	0.0290	-0.1819	-0.1919
Medium	-0.6729	-0.4123	-0.3901	-0.2051	-0.1799	-0.9987
Low	0.1158	-0.4353	-1.4574	0.0621	-0.1972	0.1036
p-value	NS	NS	NS	NS	NS	**

* Data expressed as log odds ratio of symptom/diagnosis compared to previous year. Positive log odds ratio means symptom prevalence is increasing; negative log odds ratio means symptom prevalence is decreasing

***Asthma: Low v Medium exposure p= 0.0269*

NS=No significant differences between groups

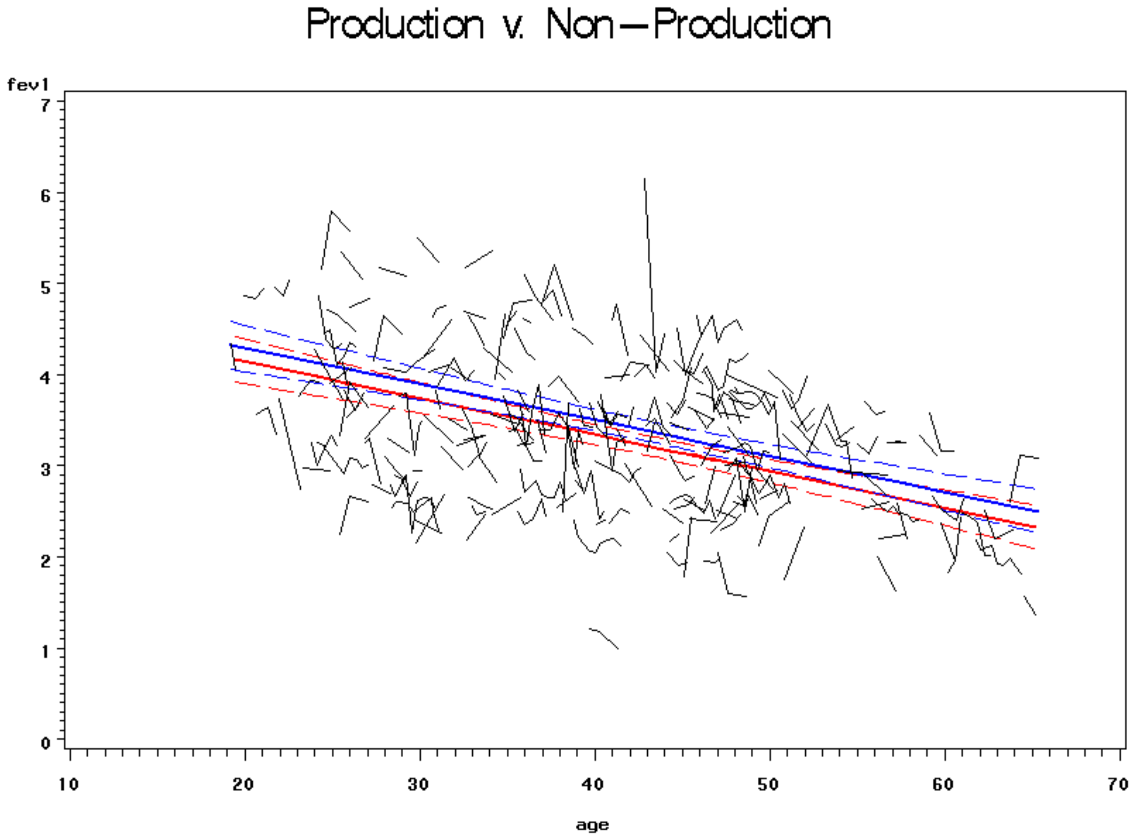
Table 4. Yearly rate of change in prevalence of symptom (*) expressed for different smoking groups by exposure comparisons, adjusted for race, gender, age

Symptom	**Log odds ratio relative to previous year**		
Exposure variable	**Never Smokers**	**Former Smokers**	**Current Smokers**
DRY COUGH			
Job type			
Production	-0.0096	0.2978	0.1122
Non-production	-0.3486	-0.1080	-0.2512
p-value	*0.4797*	*0.6567*	*0.7071*
Reported use of diacetyl			
Yes	-0.2761	0.0005	-0.0860
No	-0.2155	0.8211	0.2851
p-value	*0.9360*	*0.6354*	*0.7695*
DYSPNEA			
Job type			
Production	-0.2768	-0.3920	-0.1541
Non-production	-0.1016	0.1621	-2.5877
p-value	*0.6437*	*0.3878*	***0.0242***
Reported use of diacetyl			
Yes	-0.2756	-0.3165	-0.1805
No	0.1023	0.0312	-1.7758
p-value	*0.3498*	*0.5950*	***0.0170***
WHEEZE			
Job type			
Production	-0.5836	0.1267	0.0754
Non-production	-0.2041	0.7762	0.2995
p-value	*0.5606*	*0.4875*	*0.7600*
Reported use of diacetyl			
Yes	-0.1482	0.0721	0.1223
No	-1.2545	1.0248	0.2909
p-value	***0.0296***	*0.3618*	*0.7988*

* Data expressed as log odds ratio of symptom/diagnosis compared to previous year. Positive log odds ratio means symptom prevalence is increasing; negative log odds ratio means symptom prevalence is decreasing

FIGURES

Figure 1: Spaghetti plot shows that production workers and non-production workers have similar rates of decline in FEV1 over time.



Legends for figures

Figure 1:

Non-Production slope -40.67 ml/yr

Non-Production 95% confidence intervals

Production slope -40.40 ml/yr

Non-Production 95% confidence intervals

References:

1. Parmet AJ, Von Essen S. Rapidly progressive, fixed airway obstructive disease in popcorn workers: A new occupational pulmonary illness? *J Occup Environ Med* 2002;44:212-218.
2. Kreiss K, Gomaa A, Kullman G, Fedan K, Simoes E, Enright PL. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. *NEJM* 2002;347:330-338.
3. Kullman G, Boylstein R, Jones W, Piacitelli C, Pendergrass S, Kreiss K. Characterization of respiratory exposures at a microwave popcorn plant with cases of bronchiolitis obliterans. *J Occupational and Environmental Hygiene* 2005;2:169-178.
4. CDC. Fixed obstructive lung disease among workers in the flavor-manufacturing industry. *MMWR* 2007;56:389-393.
5. Van Rooy FG, Rooyackers JM, Prokop M, Houba R, Smit LA, Heederik DJ. Bronchiolitis obliterans syndrome in chemical workers producing diacetyl for food flavorings. *Am J Respir Crit Care Med* 2007;176:498-504.
6. Rose C, Van Dyke M, Ellison M, Towle M, Martyny J. Respiratory symptoms and airflow limitation in flavor manufacturing workers. *Am J Respir Crit Care Med* 2007;175:A17.
7. Martyny JW, Van Dyke MV, Arbuckle S, Towle M, Rose CS. Diacetyl exposures in the flavor manufacturing industry. *J Occup Environ Hyg*. 2008; 5:679-88.
8. Kanwal R, Kullman G, Piacitelli C, Boylstein R, Sahakian N, Martin S, Fedan K, Kreiss K. Evaluation of flavorings-related lung disease risk at six microwave popcorn plants. *J Occup Environ Med* 2006;48:149-157.
9. Ferris B. Epidemiology standardization project. *Am Rev Respir Dis* 1978;118:1-53.
10. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CPM, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G and Wanger J. Standardization of spirometry. ATS/ERS task force: Standardization of lung function testing. Ed. Brusasco V, Crapo R and Viegi. *Eur Respir J* 2005; 26:319-338.
11. Cnaan A LN, Slasor P. Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. *Statistics in medicine* 1997;16:2349-2380.
12. SAS Institute Inc. 2004. *SAS/STAT® 9.1 User's Guide*. Cary, NC: SAS Institute Inc.
13. McMichael AJ. Standardized mortality ratios and the "healthy worker effect": Scratching beneath the surface. J Occup Med. 1976; 18(3):165-8.
14. Akpinar-Elci M, Travis WD, Lynch DA, Kreiss K. Bronchiolitis obliterans sydrome in popcorn production plant workers. *Eur Respir J* 2004;24:298-302.
15. Sherrill DL, Lebowitz MD, Knudson RJ, Burrows B. Continuous longitudinal regression equations for pulmonary function measures. *Eur Respir J* 1992;5:452-462.

16. Knudson RJ, Lebowitz MD, Holberg CH, Burrows B. Changes in the normal maximal expiratory flow-volume curve with growth and aging. *Am Rev Respir Dis* 1983;127:725-734.
17. Hankinson JL, Bang KM. Acceptability and reproducibility criteria of the american thoracic society as observed in a sample of the general population. *Am Rev Respir Dis* 1991;143:516-521.
18. Glindmeyer HW, Diem JE, Jones RN, Weill H. Noncomparability of longitudinally and cross-sectionally determined annual change in spirometry. *Am Rev Respir Dis* 1982;125:544-548.
19. Morgan DL, Flake GP, Kirby PJ, Palmer SM. Respiratory toxicity of diacetyl in C57BL/6 mice. Toxicol Sci. 2008; 103:169-80.

Appendix – Supplementary Tables

Table 5. Yearly change in FEV1 (ml/yr) for never, former, and current smokers among 191 flavor manufacturing workers, adjusted for gender, height and race.

	Never smokers (n)	**Former smokers (n)**	**Current smokers (n)**	***p-value***
Decline in FEV1 (ml/yr)	-41.87 (117)	-45.12 (35)	-35.39 (39)	0.2266

Table 6. Yearly change in FEV1 (ml/yr) for workers reporting diacetyl use versus not reporting diacetyl use among 191 flavor manufacturing workers adjusted for gender, height, race and smoking.

	Workers reporting diacetyl use (n)	**Workers not reporting diacetyl use (n)**	**p-value**
Decline in FEV1 (ml/yr)	-39.87 (123)	-43.39 (68)	0.2190

Table 7. Yearly change in FEV1 (ml/yr) by Cumulative Diacetyl Exposure group among 191 flavor manufacturing workers, adjusted for height, gender, race, smoking status

	Low (n)	**Medium (n)**	**High (n)**	**p for overall differences**
Decline in FEV1 (ml/yr)	-43.72 (69)	-42.77 (32)	-38.95 (90)	0.2883

No significant differences between any strata

Table 8. Yearly change in FEV1 (ml/yr) among 191 workers reporting ever use of powdered, liquid or spray dry flavoring ingredients versus never use, adjusted for height, gender, race and smoking

	Yes (n)	**No (n)**	**p-value**
Ever Powder	-40.63 (70)	-44.32 (121)	0.6276
Ever Liquids	-42.23 (93)	-38.83 (98)	0.6368
Ever Spray Drying	-39.86 (17)	-41.02 (174)	0.9436

Table 9. Yearly change in FEV1 (ml/yr) for production versus non-production workers among 35 flavor manufacturing workers in job for 2 years or less, adjusted for gender, height, race and smoking.

	Production (n)	**Non-production (n)**	**p-value**
Decline in FEV1 (ml/yr)	-56.66 (12)	-43.33 (23)	0.187

Table 10. Yearly change in FEV1 (ml/yr) for never, former, and current smokers among 35 flavor manufacturing workers in job for 2 years or less, adjusted for gender, height and race.

	Never smokers (n)	**Former smokers (n)**	**Current smokers (n)**	***p-value***
Decline in FEV1 (ml/yr)	-44.65 (24)	-20.36 (6)	-70.45 (5)	0.2283

Table 11. Yearly change in FEV1 (ml/yr) for workers reporting diacetyl use versus not reporting diacetyl use among 35 flavor manufacturing workers in job for 2 years or less, adjusted for gender, height, race and smoking.

	Workers reporting diacetyl use (n)	**Workers not reporting diacetyl use (n)**	**p-value**
Decline in FEV1 (ml/yr)	-47.56 (19)	-46.03 (16)	0.8078

Table 12. Yearly change in FEV1 (ml/yr) by Cumulative Diacetyl Exposure group among 35 flavor manufacturing workers in job for 2 years or less, adjusted for height, gender, race, and smoking

	Low	**Medium**	**High**	**p for overall differences**
Decline in FEV1 (ml/yr)	-49.87 (17)*	-39.54 (13)**	-65.02 (5)	0.0116

**Low versus high cumulative diacetyl exposure (p=0.0256); **Medium versus high cumulative diacetyl exposure (p=0.0037)*

Table 13. Yearly change in FEV1 (ml/yr) among 35 workers in job for 2 years or less reporting ever use of powdered, liquid or spray dry flavoring ingredients versus never use, adjusted for height, gender, race and smoking

	Yes	**No**	**p-value**
Ever Powder	-26.15 (14)	-73.47 (21)	0.0054
Ever Liquids	-28.38 (13)	-59.31 (22)	0.0599
Ever Spray Drying	-22.71 (3)	-57.37 (32)	0.0942