

# CHEST<sup>®</sup>

THE CARDIOPULMONARY  
AND CRITICAL CARE JOURNAL

FOR PULMONOLOGISTS, CARDIOLOGISTS, CARDIOTHORACIC SURGEONS,  
CRITICAL CARE PHYSICIANS, AND RELATED SPECIALISTS

**Induced Sputum Evaluation in Microwave Popcorn Production Workers**  
Muge Akpınar-Elci, Kimberly J. Stemple, Paul L. Enright, John V. Fahy, Toni A.  
Bledsoe, Kathleen Kreiss and David N. Weissman  
*Chest* 2005;128;991-997  
DOI: 10.1378/chest.128.2.991

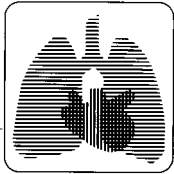
**This information is current as of August 24, 2006**

The online version of this article, along with updated information and services, is  
located on the World Wide Web at:

<http://www.chestjournal.org/cgi/content/full/128/2/991>

CHEST is the official journal of the American College of Chest Physicians. It has been published monthly since 1935. Copyright 2005 by the American College of Chest Physicians, 3300 Dundee Road, Northbrook IL 60062. All rights reserved. No part of this article or PDF may be reproduced or distributed without the prior written permission of the copyright holder. ISSN: 0012-3692.

A M E R I C A N C O L L E G E O F  
 C H E S T  
P H Y S I C I A N S



# occupational and environmental lung disease

## Induced Sputum Evaluation in Microwave Popcorn Production Workers\*

Muge Akpınar-Elci, MD, MPH; Kimberly J. Stemple, MPH; Paul L. Enright, MD; John V. Fahy, MD; Toni A. Bledsoe, MS; Kathleen Kreiss, MD; and David N. Weissman, MD

**Objective:** Severe airways obstruction and bronchiolitis obliterans have been reported in microwave popcorn production workers and attributed to inhalation of flavoring agents. We investigated whether exposure to flavoring agents is associated with airways inflammation in popcorn production workers.

**Methods:** Fifty-nine workers with high exposures and 22 patients with low exposures to flavoring vapors completed a questionnaire, spirometry, and sputum induction. Sputum cell counts were categorized as “high” if greater than (and “low” if less than or equal to) the median cell counts of a healthy external control group (n = 24). We compared high- and low-exposure groups as well as all workers with control subjects.

**Results:** Neutrophil concentrations in nonsmoking workers were significantly higher than those of the healthy nonsmoking control group ( $p < 0.05$ ). The smoking-adjusted odds ratio for high neutrophil count ( $> 1.63 \times 10^5/\text{mL}$ ) was 3.8 (95% confidence interval, 1.3 to 11.5) in the high-exposure group compared with the low-exposure group. Sputum interleukin-8 and eosinophil cationic protein levels were higher in high-exposure workers than in low-exposure workers ( $p < 0.05$ ). For the worker group, mean values of FEV<sub>1</sub> percentage of predicted and FEV<sub>1</sub>/FVC percentage of predicted were  $> 95\%$ . There were no relationships between sputum characteristics and the presence of airways obstruction.

**Conclusions:** High exposure to popcorn flavoring agents is associated with neutrophilic airway inflammation in popcorn production workers. These data provide further evidence that popcorn production workers face a significant occupational hazard through exposure to flavoring agents. (CHEST 2005; 128:991–997)

**Key words:** airway inflammation; bronchiolitis obliterans; cytokines; diacetyl; flavoring; occupation

**Abbreviations:** BOS = bronchiolitis obliterans syndrome; ECP = eosinophil cationic protein; IL = interleukin; NIOSH = National Institute for Occupational Safety and Health; OR = odds ratio

The history of popcorn dates back many centuries. It is believed that popcorn originated in Mexico; the oldest ears of corn ever found in Mexico were about 6,000 years old. In the late 1800s, popcorn was

sold all across America by street vendors using steam- or gas-powered poppers. In 1907, electric-powered corn poppers made their catalog debut. Some of the popcorn flavorings used during this era

\*From the Division of Respiratory Diseases Studies (Drs. Akpınar-Elci, Enright, Kreiss, and Weissman) and Health Effects Laboratory Division (Ms. Bledsoe), Centers for Disease Control and Prevention/National Institute for Occupational Safety and Health, Morgantown, WV; National Institutes of Health/National Institute of Allergy and Infectious Diseases (Ms. Stemple), Bethesda, MD; and Division of Pulmonary and Critical Care Medicine and Cardiovascular Research Institute (Dr. Fahy), University of California, San Francisco CA.

Manuscript received January 25, 2005; revision accepted March 10, 2005.

Reproduction of this article is prohibited without written permission from the American College of Chest Physicians ([www.chestjournal.org/misc/reprints.shtml](http://www.chestjournal.org/misc/reprints.shtml)).

Correspondence to: Muge Akpınar-Elci, MD, MPH, NIOSH Division of Respiratory Diseases Studies, Field Studies Branch, Mail Stop H-2800, 1095 Willowdale Rd, Morgantown, WV 26505; e-mail: [melci@cdc.gov](mailto:melci@cdc.gov)

included orange and lemon juice, rose, peppermint, honey, vanilla, molasses, and sugar. Microwave heating was first used in the 1940s.<sup>1</sup> Today, contemporary products such as microwave popcorn are made using a variety of chemicals as flavoring agents, to which popcorn production workers can be exposed.

The National Institute for Occupational Safety and Health (NIOSH) recently reported on a microwave popcorn production plant where nine former workers showed severe fixed airways obstruction. Two of the former workers underwent biopsies and had findings compatible with bronchiolitis obliterans.<sup>2</sup> Current workers reported excessive rates of respiratory symptoms and had high rates of airways obstruction.<sup>3</sup> The popcorn production plant produces both flavored microwave popcorn and unflavored kernel popcorn. Inhalation exposure to a flavoring mixture was implicated as an etiologic agent. Diacetyl (2, 3-butanedione), a ketone with butter flavor characteristics, was the predominant compound.

Since exposure to a flavoring mixture was associated with severe airways obstruction, we hypothesized that exposure would also be associated with airway inflammation. To test this hypothesis, we measured airway inflammation by assessment of induced sputum obtained from workers in the microwave popcorn production plant. Induced sputum analysis is a reproducible, valid, and noninvasive method for studying airway inflammation.<sup>4</sup> Use of induced sputum has been studied extensively in asthma and to a somewhat lesser degree in COPD.<sup>5</sup> It has been shown that induced sputum can be used to study interstitial lung diseases such as pneumoconioses, sarcoidosis, and nongranulomatous interstitial lung diseases.<sup>6,7</sup> Induced sputum has also been studied in occupational lung diseases.<sup>8-12</sup> By using this technique, we measured inflammatory cells, interleukin (IL)-8, and eosinophil cationic protein (ECP), and we tested for an association between these sputum characteristics and exposure to flavorings, the presence of respiratory symptoms, and abnormal lung function.

## MATERIALS AND METHODS

### *Study Population*

This study was conducted in a microwave popcorn plant in November 2001. Of 149 current workers, 140 participated in the survey. Of the 140 participants, 32 were excluded from sputum induction: 27 subjects had medical contraindications from  $\beta$ -blocker use ( $n = 8$ ), cardiac problems ( $n = 5$ ), recent operation ( $n = 2$ ), recent pneumothorax ( $n = 1$ ), pregnancy ( $n = 2$ ), or postbronchodilator baseline FEV<sub>1</sub> < 60% of predicted ( $n = 9$ ). Five subjects were excluded because they had worked at the plant for < 1 month. Common cold and other upper airways infectious diseases were among exclusion criteria, but according to the pretest questionnaire none of the

participants had any infectious diseases before starting the induced sputum procedure. Of the 108 participants undergoing sputum induction, analyzable induced sputum samples were obtained from 81 subjects. Sixteen workers failed to produce sputum, 8 subjects produced inadequate samples, and 3 workers experienced a decline in FEV<sub>1</sub> during induction sufficient to halt the procedure. The mean age ( $\pm$  SD) of participant workers with analyzable sputum samples was 33.8  $\pm$  11.9 years. Forty-nine workers were male, and 38 workers reported current smoking. There were 59 workers in the high-exposure group, with a history of high flavoring exposure in mixing, microwave packaging, maintenance, and quality control. A low-exposure group included 22 workers from office, polyethylene packaging, warehouse, and outside sections of the plant. Diacetyl exposure over the preceding year for the two exposure groups differed by up to two orders of magnitude (Fig 1). Normal control values for sputum cell counts were obtained from a previously characterized group of 24 healthy, never-smoking, nonasthmatic subjects (41.6% men; mean age, 33.8  $\pm$  9.3 years) participating in airway research at the University of California, San Francisco.

### *Questionnaire*

After obtaining written informed consent (approved by the NIOSH Human Studies Review Board), trained interviewers administered a standard questionnaire.<sup>13</sup> Additional questions were added for irritation, systemic and respiratory symptoms, and occupational information. Data were collected on demography, allergic and respiratory symptoms, family and personal history of respiratory and allergic diseases, smoking, and occupational history including previous work history.

### *Spirometry*

Trained NIOSH technicians followed American Thoracic Society recommendations for spirometry prior to inducing sputum.<sup>14</sup> The test results were compared to the lower limit of normal values from the National Health and Nutrition Examination Survey III reference values<sup>15</sup> to identify workers with abnormal spirometry results.<sup>16</sup> Airways obstruction was defined to be the combination of FEV<sub>1</sub> and FEV<sub>1</sub>/FVC percentage of predicted below the lower limit of normal. We calculated FEV<sub>1</sub> slopes if workers had more than two spirometry measurements between November 2000 and August 2003. During this period of 2.75 years, there were a total of eight spirometry tests offered to these workers.

### *Sputum Induction*

Participants inhaled 3% saline solution for a total of 12 min from an ultrasonic nebulizer (model 65; DeVilbiss; Somerset, PA). This nebulizer generates particles with a mean mass median diameter of < 4  $\mu$ m, with an output of 6 mL/min. To reduce salivary contamination of induced sputum samples, we had the subjects spit out saliva into separate containers just prior to attempts at coughing up sputum. Subjects began quiet breathing on the mouthpiece and every 2 min were asked to take the mouthpiece out, spit all saliva into a saliva cup, replace the mouthpiece, take a full breath, hold their breath, remove the mouthpiece, and cough the full breath out along with any sputum into the sputum cup. Halfway through the test (after 6 min on the nebulizer), FEV<sub>1</sub> was measured and compared to baseline. If FEV<sub>1</sub> fell below 80% of the baseline FEV<sub>1</sub>, another FEV<sub>1</sub> maneuver was obtained to confirm the drop. If the FEV<sub>1</sub> remained below 80%, the test was stopped and the participant was administered an inhaled bronchodilator.

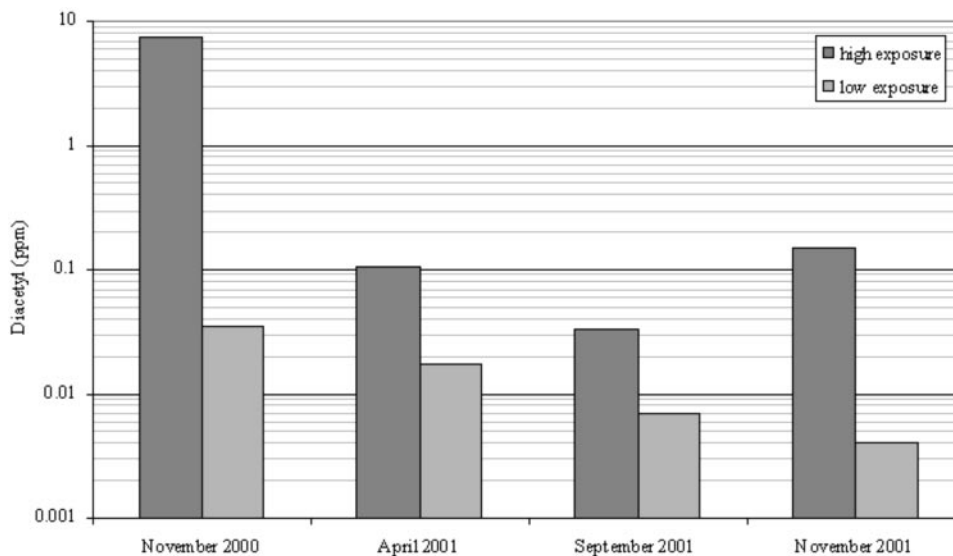


FIGURE 1. Mean diacetyl exposure by high- and low-process categories by sampling months, November 2000 to November 2001. High-exposure workers performed mixing, microwave packaging, quality control, and maintenance. Low-exposure workers performed office work, unflavored kernel popcorn handling, warehouse work, and outside area work.

### Sputum Processing

Sputum samples were collected, stored on ice, and processed within 2 h of collection. A standardized method for processing whole sputum was used.<sup>17,18</sup> After collection, sputum volume was determined. Sputum was liquefied by addition of an equal volume of 0.1% dithiothreitol in saline solution (10% Sputolysin; Behring Diagnostics; Somerville, NJ) followed by incubation on a shaking water bath at 37°C for 15 min. Cell concentration in liquefied sputum was determined by mixing equal volumes of diluted homogenized sputum and Turks solution and counting cells in a hemocytometer. To determine sputum differential cell counts, homogenized sputum was diluted in saline and cytopspins prepared using a cytocentrifuge (Cytospin II; Shandon Scientific; Sewickley, PA). Cytopspins were fixed in alcohol and stained (Diff-Quik; Baxter Scientific Products; Miami, FL). For cell differentiation, 500 cells per sample were examined. Cell differentials exclusive of squamous cells were determined by microscopic examination of stained cytopspins. Concentrations of various cell types (cell counts) were calculated as the product of total cell concentration times percentage cell type. Remaining homogenized samples were centrifuged at 1,037g for 5 min. The supernatant was aspirated and stored in aliquots at -70°C for later analysis of selected soluble constituents.

### Biochemical Assays

IL-8 concentrations in homogenized sputum supernatants were determined using a commercially available fluorometric bead array assay (Upstate Biotechnology; Lake Placid, NY) read on a Luminex-100 system (Luminex Corporation; Austin, TX). ECP concentrations in homogenized sputum supernatants were determined using a commercially available fluorescence enzyme immunoassay (Pharmacia ImmunoCAP; Pharmacia Diagnostics; Kalamazoo, MI).

### Industrial Hygiene Evaluation

From November 2000 through November 2001, the NIOSH performed four industrial hygiene surveys that consisted of an

inspection of the entire microwave popcorn production process and air sampling for butter-flavoring chemicals. Quantitative area sampling and personal air sampling were performed for diacetyl. Diacetyl was collected on carbon molecular sieve tubes at a flow rate ranging from 30 to 150 mL/min and was analyzed quantitatively by gas chromatography according to NIOSH method 2557.<sup>19</sup>

### Statistics

Since sputum cell counts from the healthy control group were not normally distributed, we determined median values (neutrophils,  $1.63 \times 10^5$ /mL; eosinophils, 0.0/mL; macrophages,  $3.88 \times 10^5$ /mL; and lymphocytes,  $0.11 \times 10^5$ /mL) and used these values as cutoff points to categorize cell counts into two groups: high and low. Since current smoking is known to affect cell count,<sup>20</sup> we adjusted our analysis for associations between exposures, symptoms, or lung function and airways inflammation for smoking status using the Mantel-Haenszel approach. We did not adjust for age and gender, since preliminary analyses showed that these factors were not associated with the results. We compared the IL-8 and ECP results stratified by gender, symptoms, spirometry, current smoking status, and exposure group using Mann-Whitney *U* tests. Differences were considered significant at a level of  $p < 0.05$ . Analyses were performed using statistical software (version 11.01; SPSS; Chicago, IL).

## RESULTS

Among popcorn production workers mean FEV<sub>1</sub> percentage of predicted and mean FEV<sub>1</sub>/FVC percentage of predicted were both > 95% (Table 1). Obstruction was present in 4 of 59 high-exposure workers and 2 of 22 low-exposure workers (Table 2). There were no significant differences between the high-exposure and low-exposure groups for spirometric values. The proportion of individuals with obstruction was not significantly increased in smokers.

**Table 1—Demographic Characteristics of Workers and Healthy External Control Subjects\***

Characteristics	Workers		Healthy External Control Subjects (n = 24)
	High-Exposure Group (n = 59)	Low-Exposure Group (n = 22)	
Age, yr†	31.3 ± 11.5	40.6 ± 10.4	33.8 ± 9.3
Male gender	54.2	77.3	41.6
Current smoker	52.5	31.8	0.0
Tenure, yr†	2.9 ± 4.0	7.7 ± 5.7	Not applicable
FEV <sub>1</sub> % predicted	95.9 ± 13.0	95.0 ± 14.6	104.3 ± 12.8
FEV <sub>1</sub> /FVC % predicted	96.6 ± 9.9	96.0 ± 9.5	99.6 ± 11.2

\*Data are presented as mean ± SD or %.

†Significant difference between high-exposure and low-exposure groups ( $p < 0.01$ ).

There was no significant difference on sputum sampling volume between groups. Overall percentages of sputum cell types among popcorn workers were as follows: neutrophils,  $69.6 \pm 23.1$ ; eosinophils,  $0.4 \pm 0.8$ ; macrophages,  $24.5 \pm 20.2$ ; and lymphocytes,  $0.4 \pm 0.5$ . When we evaluated the distribution of sputum absolute cell counts as shown in Figure 2, we observed a significantly higher neutrophil concentration in nonsmoking workers compared with the healthy nonsmoking control group ( $p < 0.05$ ).

We calculated smoking-adjusted odds ratios (ORs) to assess relationships between various symptoms or work characteristics and high sputum cell counts (Table 3). The high-exposure group had an increased risk for a high neutrophil count (OR, 3.8; 95% confidence interval [CI], 1.3 to 11.5). However, the presence of phlegm, chest tightness, wheezing attack, fever, chills, skin, nasal or eye irritation symptoms, physician-diagnosed asthma and COPD, or obstructive spirometry results were not associated with elevated sputum cell counts. Although we did not find any significant relationship between FEV<sub>1</sub>

slopes and sputum inflammatory markers, workers with the high neutrophilic count and low neutrophilic count showed mean 40 mL/yr and 25 mL/yr decreases in mean FEV<sub>1</sub> slopes, respectively ( $p > 0.05$ ).

In this report, we analyzed absolute cell counts since they were most closely associated with cytokine levels. We observed significant correlations between IL-8 and absolute neutrophil count (Pearson  $r = 0.33$ ,  $p = 0.003$ ) and between ECP and absolute eosinophil count (Pearson  $r = 0.80$ ,  $p < 0.001$ ).

Sputum IL-8 and ECP were significantly higher in the high-exposure group of workers when compared to the low-exposure group ( $p < 0.05$ ) [Table 4]. Since we observed a gender difference in IL-8 levels, we compared IL-8 levels in high-exposure and low-exposure groups of workers by adjusting with gender in general linear models. The difference between high-exposure workers (estimated marginal mean IL-8, 2,726.8; 95% CI, 2,092.9 to 3,444.3) and low-exposure workers (estimated marginal mean IL-8, 1,277.7; 95% CI, 627.1 to 2,157.6) remained significant ( $F = 6.755$ ,  $p = 0.011$ ). Elevated levels of

**Table 2—Median Percentage of Sputum Cell Types of Popcorn Production Workers and External Control Subjects by Smoking Status and Spirometric Obstruction\***

Variables	Workers				External Control Subjects (n = 24)
	High-Exposure Group (n = 59)		Low-Exposure Group (n = 22)		
	With Obstruction	Without Obstruction	With Obstruction	Without Obstruction	
Smokers	n = 1	n = 30	n = 1	n = 6	
Neutrophils	85.6	66.7	72.9	55.2	
Eosinophils	0.5	0.3	0.0	0.0	
Macrophages	12.6	25.9	22.8	39.2	
Lymphocytes	0.2	0.4	0.0	0.5	
Nonsmokers	n = 3	n = 25	n = 1	n = 14	
Neutrophils	31.9	83.9	92.3	76.9	30.1
Eosinophils	0.5	0.0	0.0	0.0	0.0
Macrophages	32.4	13.1	3.3	12.6	49.0
Lymphocytes	0.4	0.2	0.3	0.0	1.0

\*Data are presented as %. Cell differentials do not add up to 100% because epithelial cells are not shown.

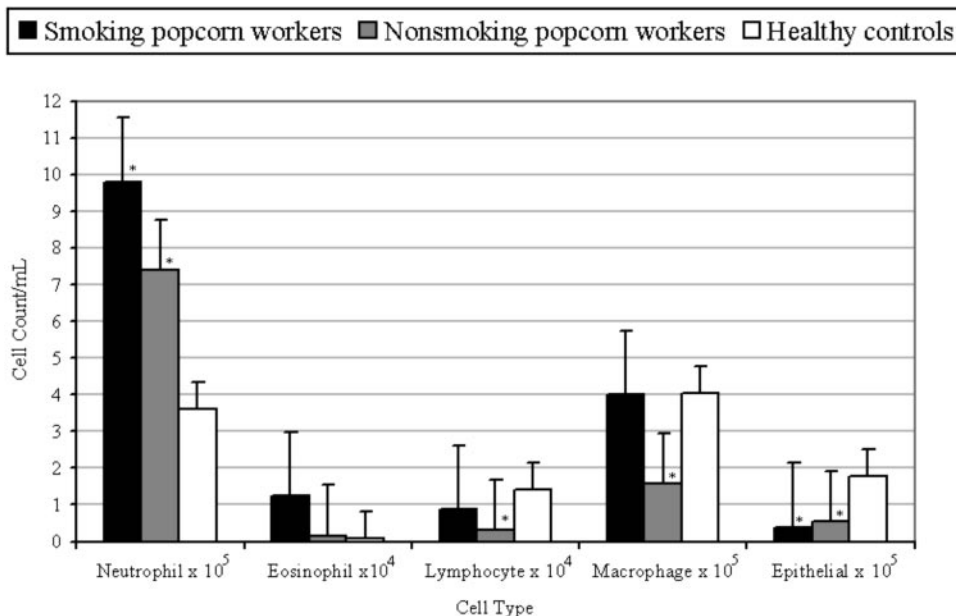


FIGURE 2. Mean sputum absolute cell counts and SEs are shown with an error bar of popcorn production workers by smoking status compared to an external healthy control group. \*Statistically significant ( $p < 0.01$ ) difference compared with the healthy control group.

sputum IL-8 were associated with symptomatic complaints of phlegm and fever (data not shown). We did not observe any relationship between respiratory or other symptoms and ECP. There were no significant differences between nonsmoking and smoking workers in sputum levels of IL-8 or ECP.

## DISCUSSION

Previous publications<sup>2,3</sup> showed that inhalation exposure to an artificial flavoring mixture used in producing microwave popcorn was implicated as causing disease. In this study, we evaluated if airway inflammation was associated with flavoring exposures among popcorn production workers by using in-

duced sputum, and we found an association between flavoring exposures and neutrophilic inflammation.

Our analysis showed that high exposure to popcorn flavoring agents is significantly associated with neutrophilic airway inflammation in popcorn production workers. Absolute neutrophil counts were higher in nonsmoking popcorn production workers than in a nonsmoking healthy external control group. Even after controlling for smoking, the relationship between high exposure to flavoring agents and neutrophilic inflammation remained significant. This further analysis supported the evidence that popcorn production workers are prone to a significant occupational hazard through exposure to flavoring agents. Even though exposure levels were lowered by engi-

**Table 3—Smoking-Adjusted Mantel-Haenszel ORs and 95% CIs for Selected Symptoms and Other Variables With High Cell Counts per Milliliter in 81 Popcorn Production Workers\***

Characteristics	Prevalence, %	High Neutrophils, OR (95% CI)	High Eosinophils, OR (95% CI)	High Macrophages, OR (95% CI)	High Lymphocytes, OR (95% CI)
<b>Symptoms</b>					
Cough	35.8	1.2 (0.4–3.89)	1.8 (0.7–4.7)	1.4 (0.4–4.6)	0.5 (0.1–1.7)
Shortness of breath	35.8	1.0 (0.3–2.8)	2.6 (1.0–6.8)	1.1 (0.3–3.4)	0.4 (0.1–1.4)
Wheezing	25.9	1.3 (0.4–4.5)	2.4 (0.8–6.9)	1.6 (0.5–5.4)	0.2 (0.0–1.3)
Flu-like aches	44.4	1.0 (0.4–2.8)	1.9 (0.7–4.7)	1.2 (0.4–3.8)	1.7 (0.5–5.2)
High-exposure group	72.8	3.8 (1.3–11.5)	1.8 (0.6–5.0)	5.4 (0.6–45.3)	2.4 (0.5–11.9)
Airways obstruction	7.4	1.8 (0.2–17.5)	1.5 (0.3–8.2)	1.0 (0.1–9.0)	1.2 (0.1–10.8)

\*Higher than median cell count of external healthy control subjects.

**Table 4—Median Induced-Sputum ECP and IL-8  
Results in Popcorn Production Workers**

Variables	ECP, $\mu\text{g/L}$	IL-8, $\text{pg/mL}$
Gender		
Male	42.4	2,777*
Female	44.4	1,508
Smoking		
Yes	43.6	2,168
No	43.9	1,511
Exposure groups		
High	48.1*	2,164*
Low	22.3	1,162
Airways obstruction		
Yes	38.1	1,871
No	43.3	1,871

\*Mann-Whitney *U* test,  $p < 0.05$ .

neering controls in April 2001, the chronic effect of previous exposures might have continued.

Neutrophilic airways inflammation is a potentially important underlying factor in development of the bronchiolitis obliterans syndrome (BOS) previously documented in workers at this plant.<sup>2,3</sup> Increased BAL neutrophils have been associated with the BOS occurring after lung transplantation.<sup>21</sup> Elevated BAL fluid neutrophil percentages as well as levels of the granulocyte activation markers myeloperoxidase and ECP appear to be early signs of development of BOS in lung transplant recipients.<sup>22</sup> Beeh et al<sup>23</sup> reported that total sputum cell counts and percentage of neutrophils were increased in chronic transplant rejection patients, and concluded that sputum neutrophilia reflects an underlying inflammatory process involved in the pathogenesis of chronic rejection, promoting inflammation, tissue damage, and airway wall remodeling.

An unexpected finding in our study was the lack of a significant relationship between neutrophilic inflammatory markers in sputum and spirometric airways obstruction. Several factors might have accounted for this finding. For medical safety reasons, we excluded the nine most severe obstructive cases from undergoing induced sputum collection. This is the major limitation of field studies. Although we keep basic medical emergency and ambulatory needs handy, it is not possible to provide intensive medical care in the field. This prevented us from including severe obstructive cases in to the study. Three additional participants did not complete sputum collection due to  $> 20\%$  decreases in FEV<sub>1</sub>. After these exclusions, there were only six obstructive cases in our study group, which might have been insufficient to show a significant relationship between inflammation and airways obstruction. In addition, our study was cross-sectional and so would

not detect a relationship between airways inflammation and obstruction if they occur at different times in the pathophysiologic process of the disease. This conclusion is consistent with our finding that workers with elevated neutrophil counts showed a somewhat higher FEV<sub>1</sub> decline in the slope analysis than the low-neutrophil count group. Finally, it is possible that obstruction in popcorn workers is at least in part due to processes other than neutrophilic airways inflammation.

Among popcorn production workers, sputum IL-8 and ECP concentrations were higher in the high-exposure group than the low-exposure group. IL-8 was recognized as a key chemokine to induce neutrophil recruitment and activation.<sup>24</sup> The sputum concentration of IL-8 appeared to be closely associated with the degree of airflow obstruction in patients with COPD and may serve as a marker in evaluating the severity of airways inflammation, which is a risk factor for COPD.<sup>25</sup> Although ECP has been regarded as an eosinophil-specific protein, current evidence suggests that it is not a unique marker for eosinophils. Proteins immunochemically indistinguishable from ECP are present in peripheral blood neutrophils. High levels of sputum ECP have been observed in subjects without associated sputum eosinophilia. Finally, there is a direct correlation between sputum ECP levels and sputum neutrophils in normal individuals.<sup>26</sup> Thus, elevated sputum ECP levels in our high-exposure workers are consistent with neutrophilic airways inflammation.

In conclusion, analysis of induced sputum showed that high exposure to artificial flavorings in this plant was associated with neutrophilic airways inflammation. This observation provided further support for previous reports suggesting an occupational hazard through exposure to flavoring agents in popcorn production workers.

ACKNOWLEDGMENT: We thank J. Liu, H. Wong, G. Kullman, K. Fedan, D. L. Freeland, J. Taylor, and D. Spainhour for their help.

## REFERENCES

- 1 Smith AF. A social history of popcorn in America. In: Smith AF, ed. A social history of popcorn in America. Chapel Hill, NC: University of North Carolina, 1999; 645–684
- 2 Akpınar-Elci M, Travis WD, Lynch DA, et al. Bronchiolitis obliterans syndrome in popcorn production plant workers. *Eur Respir J* 2004; 24:298–302
- 3 Kreiss K, Goma A, Kullman G, et al. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. *N Engl J Med* 2002; 347:330–338
- 4 Pavord ID, Sterk PJ, Hargreave FE, et al. Clinical applications of assessment of airway inflammation using induced sputum. *Eur Respir J Suppl* 2002; 37:40S–43S
- 5 Djukanovic R, Sterk PJ, Fahy JV, et al. Standardised methodology of sputum induction and processing. *Eur Respir J Suppl* 2002; 37:1S–2S

- 6 Fireman E. Induced sputum: opening a new window to the lung. *Sarcoid Vasc Diffuse Lung Dis* 2001; 18:263–271
- 7 Olivieri D, D'Ippolito R, Chetta A. Induced sputum: diagnostic value in interstitial lung disease. *Curr Opin Pulm Med* 2000; 6:411–414
- 8 Campo P, Lummus ZL, Bernstein DI. Advances in methods used in evaluation of occupational asthma. *Curr Opin Pulm Med* 2004; 10:142–146
- 9 Fireman E, Goshen M, Ganor E, et al. Induced sputum as an additional tool in the identification of metal-induced sarcoid-like reaction. *Sarcoidosis Vasc Diffuse Lung Dis* 2004; 21: 152–156
- 10 Heldal KK, Halstensen AS, Thorn J, et al. Airway inflammation in waste handlers exposed to bioaerosols assessed by induced sputum. *Eur Respir J* 2003; 21:641–645
- 11 Lerman Y, Schwarz Y, Kaufman G, et al. Case series: use of induced sputum in the evaluation of occupational lung diseases. *Arch Environ Health* 2003; 58:284–289
- 12 Von Essen SC, Scheppers LA, Robbins RA, et al. Respiratory tract inflammation in swine confinement workers studied using induced sputum and exhaled nitric oxide. *J Toxicol Clin Toxicol* 1998; 36:557–565
- 13 Ferris BG. Epidemiology Standardization Project (American Thoracic Society). *Am Rev Respir Dis* 1978; 118:1–120
- 14 American Thoracic Society. Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med* 1995; 152:1107–1136
- 15 Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999; 159:179–187
- 16 American Thoracic Society. Lung function testing: selection of reference values and interpretative strategies. *Am Rev Respir Dis* 1991; 144:1202–1218
- 17 Fahy JV. A safe, simple, standardized method should be used for sputum induction for research purposes. *Clin Exp Allergy* 1998; 28:1047–1049
- 18 Gershman NH, Wong HH, Liu JT, et al. Comparison of two methods of collecting induced sputum in asthmatic subjects. *Eur Respir J* 1996; 9:2448–2453
- 19 NIOSH manual of analytical methods. 4th ed. Washington, DC: US Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, 1994; publication No. 94–113
- 20 Lensmar C, Elmberger G, Sandgren P, et al. Leukocyte counts and macrophage phenotypes in induced sputum and bronchoalveolar lavage fluid from normal subjects. *Eur Respir J* 1998; 12:595–600
- 21 Zheng L, Walters EH, Ward C, et al. Airway neutrophilia in stable and bronchiolitis obliterans syndrome patients following lung transplantation. *Thorax* 2000; 55:53–59
- 22 Riise GC, Andersson BA, Kjellstrom C, et al. Persistent high BAL fluid granulocyte activation marker levels as early indicators of bronchiolitis obliterans after lung transplant. *Eur Respir J* 1999; 14:1123–1130
- 23 Beeh KM, Kornmann O, Lill J, et al. Induced sputum cell profiles in lung transplant recipients with or without chronic rejection: correlation with lung function. *Thorax* 2001; 56: 557–560
- 24 Park HS, Jung KS, Hwang SC, et al. Neutrophil infiltration and release of IL-8 in airway mucosa from subjects with grain dust-induced occupational asthma. *Clin Exp Allergy* 1998; 28:724–730
- 25 Yamamoto C, Yoneda T, Yoshikawa M, et al. Airway inflammation in COPD assessed by sputum levels of interleukin-8. *Chest* 1997; 112:505–510
- 26 Leigh R, Belda J, Kelly MM, et al. Eosinophil cationic protein relates to sputum neutrophil counts in healthy subjects. *J Allergy Clin Immunol* 2000; 106:593–594

**Induced Sputum Evaluation in Microwave Popcorn Production Workers**  
Muge Akpinar-Elci, Kimberly J. Stemple, Paul L. Enright, John V. Fahy, Toni A.  
Bledsoe, Kathleen Kreiss and David N. Weissman  
*Chest* 2005;128:991-997  
DOI: 10.1378/chest.128.2.991

**This information is current as of August 24, 2006**

<b>Updated Information &amp; Services</b>	Updated information and services, including high-resolution figures, can be found at: <a href="http://www.chestjournal.org/cgi/content/full/128/2/991">http://www.chestjournal.org/cgi/content/full/128/2/991</a>
<b>References</b>	This article cites 24 articles, 10 of which you can access for free at: <a href="http://www.chestjournal.org/cgi/content/full/128/2/991#BIBL">http://www.chestjournal.org/cgi/content/full/128/2/991#BIBL</a>
<b>Permissions &amp; Licensing</b>	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: <a href="http://www.chestjournal.org/misc/reprints.shtml">http://www.chestjournal.org/misc/reprints.shtml</a>
<b>Reprints</b>	Information about ordering reprints can be found online: <a href="http://www.chestjournal.org/misc/reprints.shtml">http://www.chestjournal.org/misc/reprints.shtml</a>
<b>Email alerting service</b>	Receive free email alerts when new articles cite this article sign up in the box at the top right corner of the online article.
<b>Images in PowerPoint format</b>	Figures that appear in CHEST articles can be downloaded for teaching purposes in PowerPoint slide format. See any online article figure for directions.

