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Airway obstruction related to diacetyl exposure at microwave popcorn production facilities

J.E. Lockey*,#, T.J. Hilbert*, L.P. Levin*, P.H. Ryan*, K.L. White¶, E.K. Borton*, C.H. Rice*, R.T. McKay* and G.K. LeMasters*

ABSTRACT: **Obstructive lung diseases including bronchiolitis obliterans have been reported among microwave popcorn production employees. Butter flavourings including diacetyl have been associated with these findings.**

The present study was initiated at four microwave popcorn production plants to determine if exposure to diacetyl was associated with decrements in pulmonary function. Comprehensive diacetyl exposure assessment was undertaken for all job tasks. Spirometry was conducted for 765 full-time employees between 2005 and 2006. Outcomes included decrement in forced expiratory volume in one second (FEV_1) % predicted, airway obstruction and persistent decline in FEV_1.

Inclusion in the high-exposure group (mixers) prior to respirator use was associated with a significantly decreased FEV_1 % pred in non-Asian and Asian males at -6.1 and -11.8% pred, respectively, and an eight-fold increased risk for airway obstruction. Cumulative diacetyl exposure $\geqslant$0.8 ppm-yr caused similar results. No significant impact was seen in nonmixers or between current diacetyl exposure and persistent decline in FEV_1.

Unprotected exposure as a mixer to butter flavouring including diacetyl resulted in decrements in FEV_1 (% pred) and increased airway obstruction. Control of employee exposure to butter flavouring additives is warranted in regard to both short-term peak and 8-h workday exposure.

KEYWORDS: **Airway obstruction, bronchiolitis obliterans, diacetyl, food flavourings, microwave popcorn**

AFFILIATIONS
Depts of *Environmental Health and #Internal Medicine (Pulmonary Division), University of Cincinnati College of Medicine, Cincinnatio, OH, and
¶IHI Environmental, Salt Lake City, UT, USA.

CORRESPONDENCE
J.E. Lockey
Dept of Environmental Health and Internal Medicine (Pulmonary Division)
University of Cincinnati
3223 Eden Ave ML 0056
Cincinnati OH
45267-0056 USA
E-mail: James.Lockey@uc.edu

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Diacetyl (2,3-butanedione) is a water soluble volatile di-ketone that readily enters a vapour phase when heated and is a natural constituent of numerous foods including wine and beer. The flavour manufacturing industry produces concentrated diacetyl formulations, which are used in the food production industry as a butter flavouring additive such as in microwave popcorn. In 1995, an estimated 96,000 kg of diacetyl were used in the food industry [1].

Food flavourings have been associated with clinical findings consistent with bronchiolitis obliterans (BO), an uncommon form of irreversible airway obstruction involving the respiratory bronchioles that has been previously linked to various exposures in occupational settings [2]. Studies have shown that exposure to food flavourings have been related to BO in two mixing room workers at a food manufacturer (bakery) by the National Institute of Occupational Safety and Health (NIOSH) in 1986 [3], in five workers involved with food flavouring manufacturing by Lockey *et al.* [4] in 2002 and in a spice process technician by Alleman and Darcey [5] in 2002.

Food flavourings with diacetyl used in microwave popcorn were implicated in May 2000 when eight workers formerly employed in a microwave popcorn production plant were reported to the Missouri Department of Health for BO [6, 7]. This finding in workers was further investigated through inhalational studies of rats exposed to an atmosphere of heated butter flavouring for 6 h that contained 203–371 ppm diacetyl. At these exposure levels in rats, necrosis of nasal and main stem bronchial epithelium occurred [8]. A more recent study of mice exposed to atomised liquid diacetyl at 100 ppm for 6 h·day^{-1}, 5 days·week^{-1} for 12 weeks resulted in peribronchiolar lymphocytic inflammation [9]. These studies confirm that butter flavouring or diacetyl can injure the airways.

In 2002, an index case clinically consistent with BO was identified by J.E. Lockey in a mixing

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room employee at a ConAgra microwave popcorn production plant (Marion, OH, USA). Pulmonary function tests (PFTs) demonstrated a fixed severe reduction in forced expiratory volume in 1 s (FEV_1) and a normal diffusing capacity. High-resolution computed tomography (HRCT) demonstrated mild bronchiectasis and heterogeneous air trapping on expiratory views. A subsequent health hazard evaluation by NIOSH in 2003 [10] identified fixed airflow obstruction in two employees who mixed oil and butter flavouring. Mean area diacetyl exposure in the mixing room was 1.26 ppm. Because of these findings and concern for accelerated rates of loss of pulmonary function, a company-wide exposure and pulmonary surveillance study was undertaken of all current employees involved with microwave popcorn production in four United States ConAgra plants (Marion, OH; Rensselaer, IN; Hamburg, IA; and Edina, MN, all USA). The present study represents the results for current employees across the four plant sites and presents evidence of an exposure–response relationship in the current worker population.

METHODS

Subjects

Employment rosters from four ConAgra microwave popcorn production plants included 765 current full-time employees between February 2005 and January 2006. Informed consent was obtained and pulmonary and work history questionnaires were administered by trained interviewers. Height and weight were measured, and spirometry was conducted on-site by the research team. Testing took place over three sessions averaging 4.8 months apart (range 3.7–5.6 months). Four employees were not tested due to significant cardiovascular disease or pneumonia, and four had unusable tests. In total, 11 office employees and 21 employees reporting asthma prior to employment and currently taking asthma medication were removed from the analysis. Hence, the overall analysis included 725 employees, 56% employees had three test sessions, 26% had two and 18% had one test session.

Exposure assessment and metrics

Each plant has several work areas. In the mixing room area, salt and butter flavourings including diacetyl are added to heated vegetable oil to form a butter flavouring slurry mixture. Mixing rooms have been enclosed since initial production at plants 1, 3 and 4 while plant 2 was enclosed in early 2003. The separate ventilation systems were originally designed to keep each mixing room under negative pressure. The mixing rooms were confirmed to be under negative pressure in 2005. The slurry mixture is piped to the filler station in the general production area, where operators oversee the addition of the slurry mixture to the microwaveable bags containing corn kernels. Other plant areas include quality assurance for testing samples, a maintenance workshop, a warehouse and shipping/receiving and offices.

Exposure to diacetyl was assessed through personal breathing zone monitoring at plant 1 in March 2003 as part of the NIOSH health hazard evaluation as discussed previously. ConAgra began a comprehensive exposure monitoring programme including all plants in February 2005. Sampling duration was typically 7–9 h, and all personal breathing zone samples were collected on the lapel outside of any respiratory protection. Of the 765 current full-time employees, 407 (53%) were sampled one or more times (646 total personal breathing zone samples). These samples characterise exposure across all four plants for all 16 job tasks. When multiple workers performed identical job tasks, representative workers were selected to provide personal samples with an effort to sample differing workers each time. The sampling was conducted using Anasorb CMS sorbent tubes (SKC Inc., Eighty Four, PA, USA) according to NIOSH method 2557 with analysis by gas chromatography with a flame ionisation detector [11]. Air flow was set nominally at 150 $cm^3 \cdot min^{-1}$. Analyte breakthrough did not occur in any sample. The limit of detection (LOD) was 0.007 ppm. Sampling was conducted at all four plants from February 2005 to February 2007. For plant 1, additional ventilation controls were implemented in June 2006; however, only samples collected prior to that date were included in the present analysis.

Workplace exposures were defined using four different exposure metrics. The first metric was total employment duration defined as time worked in any microwave popcorn production facility. The second metric was total duration of time worked after the introduction of diacetyl around January 1, 1994 at the four plants.

For the third metric, employees were categorised into five exposure groups based on job title and tasks: nonmixer, pre-powered air-purifying respirator (PAPR) mixer, PAPR mixer, intermittent pre-PAPR mixing room and quality assurance. The nonmixer group consisted of employees with tasks not requiring entry into the mixing room or entry was infrequent and was defined as $\leqslant$30 $min \cdot month^{-1}$. Nonmixer employees worked in the general production area, warehouse or shipping/receiving areas. The mixer group consisted of employees who spent ~50% of their time in the mixing room and the remainder in nonmixer areas. Since April 2003, all employees entering mixing rooms have been required to wear PAPRs fitted with organic vapour/high efficiency cartridges (3M^{TM} Bumpcap L501-GVP-441; 3M, St Paul, MN, USA). Therefore, mixers were divided into two groups, those ever working as a mixer prior to PAPR usage (pre-PAPR mixer) or those working as a mixer only after required use of a PAPR (PAPR mixer). A fourth group categorised as intermittent pre-PAPR mixing room included those spending >30 $min \cdot month^{-1}$ within the mixing room as mechanics or supervisors. The fifth group categorised as quality assurance included employees involved with popping, on average, 50 bags of microwave popcorn per day.

The fourth exposure metric was individual cumulative diacetyl exposure (ppm-yr), calculated by multiplying each employee's duration of employment by the mean diacetyl level within their respective exposure group. PAPR mixers were assigned a derived exposure estimate (mixing room mean diacetyl level divided by a respirator protection factor) for the half day spent in the mixing room using a PAPR and one-half of nonmixer mean plant specific exposure for the remainder of the work-day. Using a conservative respirator protection factor of 25 [12], employees wearing a PAPR while in the mixing room were assumed to inhale diacetyl concentrations similar to corresponding nonmixing room exposures. As no industrial hygiene records prior to 2003 were available, earlier exposure levels were assumed to be the same as current levels. In total, 10 employees had prior nonmixing room exposure to diacetyl at nonstudy microwave popcorn facilities. They were assigned the mean nonmixer group exposure of all four study plants for the duration of the current study outside employment.

Spirometry

Spirometry was performed by technicians who completed a NIOSH approved spirometry training programme using SensorMedics dry rolling seal spirometers (Cardinal Health, Yorba Linda, CA, USA) and Occupational Marketing Inc. spirometry testing software (Houston, TX, USA). Testing was conducted in accordance with recommendations of the American Thoracic Society (ATS)/European Respiratory Society guidelines [13]. Prior to all testing sessions, spirometers were calibrated and hoses were checked for leaks. Nose clips were used and subjects were seated during testing, except for obese participants who were tested in a standing position. All spirometric tests were reviewed for test quality by R.T. McKay. Subjects' occupational histories were updated at the time of each PFT session and included employment duration and job categorisation.

Age, height, sex and race adjusted spirometric reference values generated from National Health and Nutrition Examination Survey (NHANES) III data, including a 6% adjustment for Asians, were used for percent predicted values and lower limits of normal (LLN) [14, 15]. Plant 4 had a large number of employees originating from several Asian countries who often needed an interpreter during testing.

Health outcomes

Three primary pulmonary outcomes were evaluated. The first outcome, FEV1 and forced vital capacity (FVC) % predicted, was derived from each employee's best overall PFT. Best PFT was defined as the test with the greatest sum of FEV1 and FVC from any of the three test dates during the first surveillance year. The second primary outcome was the presence or absence of an obstructive pattern defined by having a FEV1/FVC ratio and FEV1 less than the LLN [15]. The third outcome evaluated a subset of employees for a rapid decline in lung function over a 12-month interval. A rapid decline was defined as an employee who participated in all three test sessions and demonstrated a progressive net decline in FEV1 of $\geqslant$8% or 330 mL [16]. This short-term repeated measure of a clinically significant decline in FEV1 was adopted from the recommendations of a NIOSH study. This approach is more conservative than the ATS recommended $\geqslant$15% year-to-year FEV1 decline [17]. The inclusion of only those employees with three test sessions increases the precision by decreasing the variability inherent with repeat spirometry testing and matches the minimal number of tests (three to 11 spirometry tests over 5 yrs) included in the NIOSH analysis of repeat measure of FEV1 [16].

Statistical methods

Cross-sectional associations were evaluated for lung function and all four exposure metrics including total production duration, diacetyl production duration, categorical cumulative diacetyl exposure and the five job exposure groups. Lung function was measured by FEV1 % pred and FVC and analysed by multiple linear regression. All regression models were stratified by race (non-Asian/Asian) and sex and adjusted for body mass index (BMI), current smoking status (yes/no) and cumulative pack-yrs smoked. Logistic regression analyses were performed to investigate associations between each exposure variable and prevalence rates of an obstructive pattern. Odds ratios (ORs) adjusted for BMI, current smoking status and pack-yrs were calculated. The ratio of the number of observed cases of an obstructive pattern in the cohort to the number of expected cases, based upon NHANES III as described by KREISS *et al.* [7] was calculated and adjusted for age (<40 and $\geqslant$40 yrs) and smoking (current/former/never).

RESULTS

Personal breathing zone sampling indicated that mean diacetyl levels were similar across the 16 job titles and the subsequent five exposure groups at each plant, with the exception of pre-PAPR mixer groups. Therefore, all nonmixer employees were assigned the arithmetic mean of exposure measurements for all nonmixer jobs combined in each respective plant: 0.031, 0.074, 0.027 and 0.014 ppm for plant 1, 2, 3 and 4, respectively (table 1). Pre-PAPR mixers were assigned plant specific sampled mean diacetyl values of 0.678, 0.348, 0.057 and 0.860 ppm for plant 1, 2, 3 and 4, respectively while PAPR mixer values were calculated at 0.029, 0.044, 0.015 and 0.024 ppm for plant 1, 2, 3 and 4, respectively. Office area samples were below the LOD, and production employees with some historic office work were assigned a value of LOD/2 [18] for the corresponding time period. In calculating cumulative

TABLE 1 Summary results of personal breathing zone diacetyl (ppm) sampling used in cumulative exposure analysis

Plant	Nonmixer group			Mixer group		
	Arithmetic mean ± SD	Arithmetic mean of lognormally distributed data# (95% estimate)¶	Geometric mean of log-transformed data# (GSD)	Arithmetic mean ± SD	Arithmetic mean of lognormally distributed data# (95% estimate)¶	Geometric mean of log-transformed data# (GSD)
1	0.031 ± 0.046	0.030 (0.096)	0.018 (2.737)	0.678 ± 0.664	1.143 (4.421)	0.293 (5.204)
2	0.074 ± 0.124	0.154 (0.513)	0.014 (8.961)	0.348 ± 0.586	0.922 (2.797)	0.059 (10.399)
3	0.027 ± 0.123	0.025 (0.050)	0.001 (16.289)	0.057 ± 0.065	0.070 (0.260)	0.029 (3.809)
4	0.014 ± 0.033	0.012 (0.045)	0.003 (5.013)	0.860 ± 1.048	2.968 (9.493)	0.230 (9.595)

GSD: geometric SD. #: maximum likelihood estimate; ¶: the point at which 95% of the measurements are below this concentration.

exposure, sampled mean values were used for comparison with previously published studies. The range of estimated cumulative diacetyl exposure across all four plants by the five exposure groups are presented in figure 1. Based on this distribution, cumulative diacetyl exposure was dichotomised into high (⩾0.8 ppm-yrs) and low (<0.8 ppm-yrs) categories. Summary statistics for the diacetyl exposure data are also presented as the arithmetic mean of the lognormally distributed data with the 95th percentile, the value at which 95% of the measurements are below this concentration [19, 20]. Diacetyl exposure was also calculated using the geometric mean and geometric SD. The maximum likelihood estimate was used to calculate both of these alternate methods in order to address nondetectable values [21, 22]. Exposure estimates using each of these methods are presented in table 1.

Table 2 describes the study population by categories of race (non-Asian/Asian) and sex. Non-Asian workers were predominantly Caucasian and younger than Asian workers for both males (39.1 and 44.9 yrs, respectively) and females (41.6 and 48.0 yrs, respectively). In general, Asian males and females had higher production duration, in both total years and after the introduction of diacetyl. Employment in the pre-PAPR mixer group was more common in Asian males (13.5%) compared with non-Asian males (6.0%; table 2). No Asian females were reported to have worked as a pre-PAPR mixer or PAPR mixer. Asian females had the lowest FVC and FEV_1 % pred followed by Asian males, possibly as a result of using unrepresentative predicted values for Asian employees (table 2) [23]. The prevalence of an obstructive pattern was highest in non-Asian females (6.7%) and non-Asian males (6.0%; table 2).

Analyses using mixed linear models for both total microwave popcorn production duration and production duration since the introduction of diacetyl were conducted. No consistent significant association was found with FEV_1 or FVC % pred for any group (data not shown).

The association between job exposure groups and FVC and FEV_1 % pred was examined in non-Asian and Asian males and

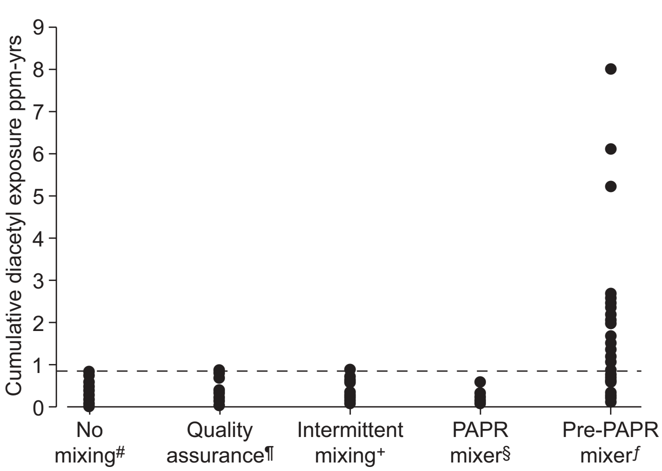

FIGURE 1. Range of estimated cumulative mean diacetyl exposure by exposure group. PAPR: powered air-purifying respirator. – – – –: 0.80 ppm-yrs. #: n=518; ¶: n=27; +: n=121; §: n=20; ƒ: n=39.

non-Asian females. Asian females were excluded from this analysis as none were employed as mixers. Employment as a pre-PAPR mixer was significantly associated with a decrease in FEV_1 % pred at -6.1% and -11.8% pred for non-Asian and Asian males, respectively (table 3). No significant association was observed with pre-PAPR mixer for FVC or FEV_1 % pred in non-Asian females or FVC % pred in non-Asian and Asian males. As mixer exposures from plant 3 were much lower (0.057 ppm) in comparison to mixers from other plants (table 1), the analysis was repeated with the removal of plant 3 pre-PAPR mixers (three males and two females). Findings of the analysis remained unchanged. Also, there was no significant decrease in FEV_1 or FVC % pred in the intermittent pre-PAPR mixing group or those ever employed in quality assurance.

A summary of the cross-sectional analysis of dichotomised cumulative diacetyl exposure (⩾0.8 and <0.8 ppm-yrs) on FEV_1 and FVC for non-Asian males and females and Asian males (non-Asian females ⩾0.8 ppm-yrs) is presented in table 4. In the ⩾0.8 ppm-yrs cumulative diacetyl exposure category, 25 (81%) out of 28 workers worked as pre-PAPR mixers. Among non-Asian and Asian males there was a significant association between the higher diacetyl cumulative exposure category and the decrease in FEV_1 % pred of -10.3% and -12.7% pred, respectively. Next, three additional cumulative exposure metrics were evaluated by comparing those in the upper 95th percentile to all others. These exposure metrics were the sampled mean, arithmetic mean of log normal fit and the geometric mean. Significant decrements in FEV_1 % pred persisted with non-Asian males at -8.6%, -8.6% and -7.8%, respectively. Asian males remained at -12.7% for all three metrics. Another analysis examined three categories of cumulative diacetyl exposure: <0.4 ppm-yrs, ⩾0.4 and <0.8 ppm-yrs and ⩾0.8 ppm-yrs. Only the highest exposure level (⩾0.8 ppm-yrs) for non-Asian and Asian males demonstrated a significant decline in FEV_1 % pred (data not shown).

There was no overall significant increase in obstructive pattern by age and smoking categories based on expected rates from NHANES III data (table 5). However, non-Asian male employment in the pre-PAPR mixer exposure group, was associated with an eight-fold increased risk of an obstructive pattern (95% confidence interval (CI) 2.26–29.24; table 6). Removal of the lower exposed pre-PAPR mixers from plant 3 elevated the risk to 10-fold (95% CI 2.7–37.6; data not shown). Employment as a PAPR mixer was also associated with a significant increase in risk for an obstructive pattern OR 5.7, 95% CI 1.23–26.24). After removing employees with a pre-employment history of asthma (regardless of current medication use), a significant association remained in the pre-PAPR mixer group, but not in the PAPR mixer group (table 6). There were insufficient numbers to evaluate Asian males and all females.

In order to evaluate consistency, the increased risk for an obstructive pattern was further explored with other exposure metrics. Workers were categorised into ⩾0.8 ppm-yrs cumulative diacetyl exposure compared with all others to investigate an obstructive pattern for non-Asian males. A logistic regression model adjusting for pack-yrs, current smoking status and BMI showed that higher cumulative diacetyl exposure (⩾0.8 ppm-yrs) was significantly associated with

TABLE 2 Descriptive statistics of study subjects# included in cross-sectional pulmonary function test (PFT) analyses stratified by race and sex

Characteristic	Non-Asian males¶	Asian males	Non-Asian females+	Asian females
Subjects n	400	52	208	65
Age yrs	39.1 (18.0–71.0)	44.9 (19.0–68.0)	41.6 (18.0–69.0)	48.0 (24.0–67.0)
Smoking§ pack-yrs	19.9 (0.2–87.0)	13.3 (0.5–68.0)	18.4 (0.2–72.5)	8.1 (1.3–15.0)
Current smoking %	42.8	40.4	50.5	1.5
Production durationƒ yr	6.1 (<0.1–30.7)	13.2 (<0.1–21.0)	6.5 (<0.1–21.8)	14.0 (0.7–19.6)
Production duration diacetyl## yr	5.3 (<0.1–12.1)	9.7 (<0.1–12.1)	5.9 (<0.1–12.1)	10.5 (0.7–12.1)
Cumulative diacetyl exposure ppm-yrs	0.2 (<0.1–2.7)	1.0 (<0.1–8.0)	0.3 (<0.1–1.4)	0.1 (0.1–0.2)
Cumulative diacetyl exposure ⩾0.8 ppm-yrs %	3.8	13.5	2.9	0
Exposure groups %				
Nonmixer	62.0	73.1	81.2	97.0
Intermittent pre-PAPR mixing room	26.7	11.5	3.4	1.5
Pre-PAPR mixer	6.0	13.5	3.9	0.0
PAPR mixer	4.0	1.9	1.4	0.0
Quality assurance	1.3	0.0	10.1	1.5
FVC % pred	100.1±12.1	92.9±11.3	100.0±13.7	87.8±11.6
FEV1 % pred	97.2±13.8	91.9±11.6	96.8±14.8	90.0±11.9
Obstructive PFT pattern %	6.0	3.9	6.7	3.1

Data are presented as mean (range) or mean±SD, unless otherwise stated. PAPR: powered air-purifying respirator; FVC: forced vital capacity; % pred: % predicted; FEV1: forced expiratory volume in 1 s. #: all manufacturing facilities, n=725; ¶: 90.5% Caucasian; +: 97.1% Caucasian; §: data reported for current and former smokers only; ƒ: mean years of employees working in popcorn production including production prior to the introduction of diacetyl; ##: mean years of employees working in popcorn production after the introduction of diacetyl.

an obstructive pattern (OR 9.2, 95% CI 2.29–36.75; data not shown). As with the FVC and FEV1 % pred outcome, the cumulative exposure metrics of arithmetic (sampled) mean, arithmetic mean of lognormal fit and geometric mean were evaluated by comparing those in the upper 95th percentile to all others, significant ORs were 6.8, 6.2 and 9.2, respectively (data not shown). Hence, the findings remained consistent for an obstructive pattern. In contrast, no significant association was observed between production duration or diacetyl production duration and an obstructive pattern.

TABLE 3 Cross-sectional analysis of exposure groups and forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) in non-Asian males and females and Asian males#

Variable	Non-Asian males		Asian males		Non-Asian females	
	FEV1 % pred	FVC % pred	FEV1 % pred	FVC % pred	FEV1 % pred	FVC % pred
Subjects n	400	400	52	52	208	208
Model R^2	0.16	0.09	0.2	0.17	0.11	0.07
Smoking¶ pack-yrs	-2.8 (<0.001)	-1.1 (<0.01)	0.0	0.0	-3.4 (<0.001)	-1.9 (<0.01)
Current smoker+	-1.6	0.0	-1.9	3.2	-1.3	2.0
BMI kg·m^{-2}	-0.3 (<0.01)	-0.5 (<0.001)	-0.4	-1.0 (0.03)	-0.1	0.3(0.02)
Pre-PAPR mixer§	-6.1 (0.03)	0.5	-11.8 (0.02)	-3.7	3.9	-0.3
PAPR mixerƒ	-2.7	0.1	6.5	5.7	4.4	10.5
Intermittent pre-PAPR mixing##	3.2 (0.03)	2.9 (0.03)	5.0	5.6	-1.1	-3.5
Quality assurance¶¶	-0.8	0.2	NA	NA	-2.6	-3.1

Data other than subjects and model R^2 are presented as estimated change in % predicted (p-value). BMI: body mass index; PAPR: powered air-purifying respirator; NA: not applicable. #: p⩽0.10 reported; ¶: per 10 packs-yrs smoking history; +: current smokers compared with never- and former smokers; §: mixing room employees prior to April 2003 compared with employees with no mixing room or quality assurance employment; ƒ: mixing room employees beginning April 2003 with no pre-PAPR mixer experience compared with employees with no mixing room or quality assurance employment; ##: employees with >30 min·month^{-1} estimated time in mixing room pre-PAPR compared with employees with no mixing room or quality assurance employment; ¶¶: quality assurance employees compared with employees with no mixing room employment.

TABLE 4 Relationship between dichotomised cumulative diacetyl exposure and forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) in non-Asian males and females and Asian males#

Variable	Non-Asian males		Asian males		Non-Asian females	
	FEV_1 % pred	FVC % pred	FEV_1 % pred	FVC % pred	FEV_1 % pred	FVC % pred
Subjects n	400	400	52	52	208	208
Model R^2	0.16	0.08	0.18	0.14	0.11	0.06
Smoking¶ pack-yrs	-2.7 (<0.001)	-1.1 (0.01)	0.0	0.0	-3.3 (<0.001)	-1.9 (<0.01)
Current smoker+	-2.4	-0.5	-2.1	2.9	-1.5	1.6
BMI kg·m^{-2}	-0.3 (<0.01)	-0.5 (<0.001)	-0.3	-1.0 (0.04)	-0.2	-0.3 (0.01)
High exposure to diacetyl§	-10.3 (<0.01)	-5.4 (0.08)	-12.7 (<0.01)	-4.6	2.0	-1.4

Data other than subjects and model R^2 are presented as estimated change in % predicted (p-value). #: $p \leqslant 0.10$ reported; ¶: per 10 packs-yrs smoking history; +: current smokers compared with never- and former smokers; §: high cumulative exposure to diacetyl defined as $\geqslant$0.8 ppm-yrs and compared with <0.8 ppm-yrs. Of those with $\geqslant$0.8 ppm-yrs, 25 (81%) out of 28 were within the pre-powered air-purifying respirator mixer group.

In order to evaluate a potential short-term progressive decrease in lung function associated with current exposure levels of diacetyl, a sub-analysis was conducted on employees who participated in all three PFTs. Current personal exposure levels at the time of first PFT were dichotomised into <0.05 and $\geqslant$0.05 ppm. As there were no Asians with an exposure level $\geqslant$0.05 ppm, the analysis was confined to non-Asians only (n=343). Employees were divided into three categories: those with a progressive increase in FEV_1 of 330 mL or $\geqslant$8% from first to third PFT (n=14), those with a FEV_1 decrease of $\geqslant$8% or 330 mL (n=24) and the remainder (n=305) who were considered controls. A logistic regression model adjusted for packs-yrs and BMI found no significant association between current exposure level and a short-term persistent increase or decrease in FEV_1.

DISCUSSION

The present study of employees producing microwave popcorn at four plants demonstrated no statistically significant impact of diacetyl exposure on FEV_1 % pred values in nonmixing room employees. Also, no increase in an obstructive pattern by age or smoking category in the overall study cohort based on expected rates from NHANES III data was observed. A significant decline in FEV_1 % pred in males who historically worked as mixers within the mixing room was observed. The decline in FEV_1 % pred for non-Asian and Asian males with $\geqslant$0.8 ppm-yrs cumulative diacetyl exposure was -10.3 and -12.7% pred ($p<0.01$ for both), respectively. Similar findings were identified in non-Asian and Asian males within the pre-PAPR mixer exposure group. In addition, an eight-fold increased risk of an obstructive pattern involving the pre-PAPR mixer groups after adjusting for BMI, current smoking and packs-yrs was found. These changes were observed at a lower mean diacetyl exposure level than shown in the 2002 study at a Missouri microwave popcorn plant [7].

However, there are study limitations. The FVC and FEV_1 % pred values for Asians were calculated as 6% less than Caucasians and may underestimate the adjustment factor for Asians [24, 25]. As the present analysis studied current workers, the impact of past diacetyl exposure on workers who may have left employment prior to February 2005 cannot be determined. Furthermore, of the 725 employees, 93 (13%) left employment during the 1-yr testing period. These ''leavers'' could create a participation bias as they may have left for health reasons. This possibility was investigated. Of the 93 leavers, 44% were laid off or fired, 31% left for personal reasons that included another job, retirement, attending school or moving, 24% left for an unspecified personal reason and one died as a result of a nonrespiratory condition. Based on company exit interviews, none of the 93 reported leaving for health reasons. As only six leavers were Asian, a sub-analysis was conducted on the 87 non-Asian leavers, comparing them with the non-Asian nonleavers. On their first PFT, the leavers

TABLE 5 Expected and observed number of cases of obstructive pulmonary function test patterns among all employees categorised by smoking status and age

	Age		Total
	$\leqslant$39 yrs	$\geqslant$40 yrs	
Current or former smokers			
Subjects n	209	225	434
Expected	8.05	27	34.6
Observed	7	30	37
Prevalence ratio	0.87	1.11	1.07
Never-smokers			
Subjects n	144	179	323
Expected	3.33	4.82	7.46
Observed	6	3	9
Prevalence ratio	1.80	0.62	1.21
All			
Subjects n	353	404	757
Expected	10.87	34.06	41.8
Observed	13	33	46
Prevalence ratio	1.20	0.97	1.10

Expected values obtained from the third National Health and Nutrition Examination Survey and calculated by KREISS *et al.* [7]. Includes all employees with valid tests (n=757), including 11 office workers and 21 employees reporting asthma prior to employment who were excluded from the primary analyses. All prevalence ratios were nonsignificant ($p>0.05$).

TABLE 6 Cross-sectional logistic regression analysis of exposure groups and obstructive pulmonary function test (PFT) pattern in non-Asian males

Variable	Non-Asian males#	Non-Asian males without pre-employment asthma¶
Smoking history+ pack-yrs	1.61 (1.28–2.03)	1.61 (1.27–2.02)
Current smoker§	1.12 (0.39–3.21)	1.35 (0.46–4.00)
BMI kg·m^2	0.96 (0.89–1.05)	0.97 (0.89–1.05)
Pre-PAPR mixerƒ	8.12 (2.26–29.24)	8.22 (2.25–29.95)
PAPR mixer##	5.7 (1.23–26.24)	3.24 (0.56–18.71)
Intermittent pre-PAPR mixing¶¶	0.95 (0.29–3.15)	0.99 (0.30–3.31)

Data are presented as OR (95% CI). No employees in the quality assurance group had an obstructive PFT pattern. Therefore, for this analysis these employees were included in the nonmixing room employment group. BMI: body mass index; PAPR: powered air-purifying respirator. #: n=400, employees reporting pre-employment asthma and who were currently on asthma medication were removed (n=21); ¶: n=384, employees reporting any history of pre-employment asthma were removed (n=37); +: OR given for 10-yr increase in pack-yrs; §: current smokers compared with never and former smokers; ƒ: mixing room employees prior to April 2003 compared with employees with no mixing room employment; ##: mixing room employees after April 2003 with no pre-PAPR experience compared with employees with no mixing room employment; ¶¶: employees with >30 min·month^{-1} estimated time in mixing room pre-PAPR compared with employees with no mixing room employment.

versus nonleavers were only slightly different, with an adjusted FEV1 and FVC % pred of 96.8 and 99.5% pred compared with 97.1 and 100.2% pred, respectively. Hence, the findings are unlikely to be related to worker participation.

Butter flavourings, diacetyl and airway obstruction

At the Missouri plant, KREISS *et al.* [7] showed that in nonsmokers, airway obstruction was significantly increased. Furthermore, as cumulative diacetyl exposure increased, there was a corresponding decrease in FEV1 % pred and an increased prevalence of airway obstruction. Cumulative diacetyl exposure ranged from 0 to 126 ppm-yrs. At the time of the Missouri study, mixing room area diacetyl levels averaged 33.3 (range 1.3–97.9) ppm. A survey of workers by KANWAL *et al.* [26] at five additional microwave popcorn plants demonstrated a significant decrease in FEV1 to 90% pred in ever-mixers compared with 95% pred in never-mixers. The ever-mixers with >12 months of mixing exposure had a significantly reduced FEV1 of 80% pred compared with those with <12 months ever-mixing exposure at 95% pred. The mean and upper limit of area diacetyl exposures ranged from 0.2 to 1.2 ppm and 0.6 to 2.7 ppm, respectively. These levels were substantially lower than at the Missouri plant where liquid flavourings were heated before being combined with heated vegetable oil, but comparable to levels reported in the present study. A corresponding increased prevalence of airway obstruction was noted in packaging area workers in plants with inadequately isolated tanks at 11.5 *versus* 5.5% in plants with isolated tanks. No increase in airway obstruction was seen in quality control or maintenance employees.

Results from the current study are comparable with previous studies that demonstrate that employees involved with mixing butter flavourings including diacetyl are at risk for decrements in FEV1 % pred and airway obstruction. These findings were noted at sampled mean diacetyl exposures obtained through personal breathing zone monitoring ranging from 0.348 to 0.860 ppm. Removal of the pre-PAPR mixer group from plant 3 where the mean exposure was similar to the nonmixer exposure at 0.057 ppm increased the risk for airway obstruction from eight to 10-fold in the remaining three pre-PAPR mixer groups. The present results demonstrated no significant decrements in FEV1 % pred or a significant increased prevalence of airway obstruction in plant employees other than pre-PAPR mixers, including quality control employees where mean diacetyl exposure levels ranged from 0.014 to 0.074 ppm. In comparison, KREISS *et al.* [7] demonstrated airway obstruction in quality control workers at mean (range) area diacetyl exposure of 0.56 (0.33–0.89) ppm and KANWAL *et al.* [26] in packagaing workers at 0.3 (0.2–0.4) and 0.7 (0.4–1.2) ppm. Neither the current study nor previous referenced studies had available historical exposure monitoring data, thus providing the possibility for cumulative exposure misclassification. Recent work at NIOSH indicates that the methods used to collect and analyze the samples result in an underestimate of exposure when workplace humidity exceeds 30% [27]. Any reanalysis of our data including this humidity effect will likely result in slightly higher estimates of mean exposure by job group; therefore the values cited here may include a small safety factor.

Of those working in the mixing room prior to PAPR usage (n=39), an obstructive pattern was identified in two individuals at plant 1 (mean diacetyl level 0.678 ppm) and three individuals at plant 2 (mean diacetyl level 0.348 ppm). They all reported work-related respiratory symptoms, one individual had pre-existing asthma and another had respiratory symptoms prior to work. The individuals ranged in age from 25–54 yrs old; included never-, former and current smokers; demonstrated a decreased diffusing capacity below 75% pred in two of the three workers tested; and had a positive bronchodilator response in all three workers tested. Three individuals underwent HRCT (inspiratory and expiratory views) scans and air trapping was observed on expiratory views in two individuals. The contribution of exposure to butter flavouring with diacetyl to these clinical findings is uncertain.

Three individuals whose ages ranged from 28 to 61 yrs had an obstructive pattern in the PAPR group. One individual had pre-existing asthma and the other two had a 24 and 63 pack-yrs history of cigarette smoking. No pre-placement PFTs were available as of 2003 for comparison purposes. In the view of the mandatory use of PAPR respirators as a post April 2003 mixer, findings in these three individuals were likely due to a pre-existing health condition (asthma) and a long history of cigarette smoking. However, a contribution from potential unprotected short-term diacetyl exposure related to not following guidelines regarding PAPR respirator usage 100% of the time while in the mixing room cannot be excluded.

Butter flavourings, diacetyl and BO

Further investigation of the original cases of BO in the Missouri plant as reported by PARMET and VON ESSEN [6] showed that five of the nine cases worked in the mixing room and four in the microwave packaging area. Based on fixed airway obstruction, bronchiectasis and air trapping on HRCT, the findings were considered clinically consistent with BO. Measured FEV_1 ranged from 14.0 to 66.8% pred and lung function stabilised after leaving employment. Two of the three cases undergoing thoracoscopic lung biopsies had histological changes supporting the diagnosis of BO [28]. The study of the five additional microwave popcorn plants by KANWAL *et al.* [26] identified three mixers and three packaging line workers with fixed airway obstruction, normal diffusing capacity and evidence of air trapping on chest computed tomography scans. Three of these six had biopsy findings consistent with BO [26].

In the Netherlands, three cases of clinical BO in the high exposed process operators have been diagnosed in a diacetyl facility based on fixed airway obstruction and air trapping on HRCT [29]. Besides diacetyl, exposure to acetoin and acetaldehyde was listed as potential contributor to the study findings. In addition, three cases clinically consistent with BO were recently reported in California (USA) in the flavouring industry; area measurements for diacetyl ranged from 0.38 to 73.7 ppm and personal samples were as high as 83 ppm [30].

In regard to BO, short-term high peak exposure to various reactive chemicals represents a substantial risk [2]. In a flavouring manufacturer in California with reported cases of clinical BO, peak diacetyl levels exceeded 100 ppm at blending machines [30]. Within the Missouri plant studied in 2002, opening the lid on a tank of heated flavouring prior to being mixed with heated oil may have resulted in brief exposures as high as 1,230 ppm [30]. At another plant where a mixer was felt to have developed clinical BO, the mean area and personal time-weight average of diacetyl was 0.2 and 0.02 ppm, respectively [26, 30]. However, short-term peak exposures while open handling butter flavourings at the same plant exceeded 80 ppm [26].

As with other published studies, these findings rely on recent exposure monitoring. These results may under-estimate exposure prior to the sampling period as well as lifetime cumulative diacetyl exposure and do not consider the potential for short-term higher diacetyl exposure. The use of average diacetyl levels for job groups may also result in misclassification; this is likely, however, to be a nondifferential bias. In addition, butter flavouring is a complex mixture that when heated can result in the generation of over 150 volatile organic chemicals including acetoin and 2-nonanone and butanoic and hexanoic acids [31]. Diacetyl was used as a marker of butter flavouring exposures. Any conclusions pertaining to causation are based on the assumption that diacetyl is either causal or correlated with other chemical constituents of butter flavourings.

In conclusion, increased exposure to a reactive substance such as diacetyl by itself or in combination with other constituents of butter flavouring likely increases the risk of obstructive lung diseases including BO in susceptible individuals. Exposures, such as those that occurred in mixing rooms in the current study, result in a significantly higher prevalence of airway obstruction and decreased FEV_1 % pred. Similar findings were not seen in the lower exposed nonmixer group of employees. Control of exposure to butter flavouring including diacetyl and other reactive flavouring agents for primary prevention is warranted both in regard to an 8-h workday exposure and short-term peak exposure. In addition, pre-placement and ongoing medical surveillance that include spirometry should help with the early identification and reassignment of those individuals who develop accelerated loss in airflow and in the identification, replacement and control of potential offending flavouring agents.

SUPPORT STATEMENT

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STATEMENT OF INTEREST

Statements of interest for J.E. Lockey, T.J. Hilbert, K.L. White, E.K. Borton, R.T. McKay and G.K. LeMasters and the study itself can be found at www.erj.ersjournals.com/misc/statements.dtl

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