Pulmonary Impairment and Risk Assessment in a Diacetyl-Exposed Population

Microwave Popcorn Workers

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Objectives: The butter flavoring additive, diacetyl (DA), can cause bronchiolitis obliterans (BO) by inhalation. A risk assessment was performed using data from a microwave popcorn manufacturing plant. **Methods:** Current employees' medical history and pulmonary function tests together with air sampling over a 2.7-year period were used to analyze forced expiratory volume in 1 second (FEV_1) and FEV_1/forced vital capacity (FVC). The exposure responses for declining pulmonary function and for possible early onset of BO were estimated using multiple regression methods. Several exposure metrics were investigated; benchmark dose and excess lifetime risk of impairment were calculated. **Results:** Forty-six percent of the population had less than 6 months exposure to DA. Percent-of-predicted FEV_1 declined with cumulative exposure (0.40 per ppm-yr, $P < 10^{-7}$) as did percent FEV_1/FVC (0.13 per ppm-yr, $P = 0.0004$). Lifetime respiratory impairment prevalence of one per thousand resulted from 0.005 ppm DA and one per thousand lifetime incidence of impairment was predicted for 0.002 ppm DA. **Conclusion:** DA exposures, often exceeding 1 ppm in the past, place workers at high risk of pulmonary impairment.

Keywords: benchmark dose, bronchiolitis obliterans, flavorings, lifetime risk, susceptibility

When inhaled, the flavoring agent diacetyl can cause a disabling and potentially fatal disease of the small airways, bronchiolitis obliterans (BO).[1–4] Based on animal toxicology studies, the mechanism of action of this and other similarly behaving α-diketones appears to involve (1) protein modification; (2) DNA modification; and (3) cell injury by reactive oxygen species.[5–8] Diacetyl (DA) is widely used in manufactured foods like microwave popcorn, dairy products, confections, and in frying oil for retail food preparation. The National Institute for Occupational Safety and Health (NIOSH) has conducted numerous health hazard evaluations (HHEs) at workplaces with DA exposures.[9–15] Based on study population size, retrospective and longitudinal exposure assessments and repeated medical evaluations performed, one microwave popcorn plant was judged to have sufficient data to support quantitative risk assessment.[15] A recent mortality analysis for 511 workers in this same population observed 4 out of 15 deaths to be due to chronic obstructive pulmonary disease (COPD, 27%).[16] In healthy occupational populations COPD typically accounts for 4% to 6% of deaths which implies for this diacetyl-exposed population a relative risk of about five (four observed, 0.75 expected, Poisson one-tailed $P = 0.007$). Exposure levels over a working-lifetime corresponding to specified levels of risk were calculated based on an analysis of this plant. This work contributed to the publication of a NIOSH criteria document which specifies recommended exposure limits (REL) for both diacetyl and 2,3-pentanedione.[17] The analysis presented in this manuscript use an alternate job-exposure matrix to assess worker exposures, and used a different method to assess smoking in workers missing age at start of smoking data. The overall results of this analysis are very close to those reported in the criteria document. No assessment of 2,3-pentanedione exposure or health effects is provided here.

Learning Objectives

- Become familiar with previous evidence on the association between occupational diacetyl exposure and the risk of pulmonary impairment.
- Discuss the rationale for and methods of the new risk assessment among diacetyl-exposed workers at a microwave popcorn manufacturing plant.
- Summarize the findings on diacetyl exposure and risk of pulmonary impairment, including comparison with the previous NIOSH criteria document.

METHODS

Study Population and Work History

Eight cross-sectional surveys were conducted at a popcorn plant in Missouri from November 2000 to August 2003 in which pulmonary function was evaluated and medical and work history taken.[15] Environmental air-sampling was performed during those surveys and on one other occasion.[15,18] Work history at the plant was compiled by worker interview and consisted of specific department and job title assignments with corresponding dates. The current workforce varied between 135 and 165 workers and 368 employees (providing work histories) participated in at least one survey, for an average participation rate of about 80%. The workers studied were current employees at their first survey but could have terminated employment prior to subsequent evaluations. Information on workers terminating prior to the first survey was provided by the employer and former workers could participate in the surveys but exposure information going back several years prior to 2000 was lacking and greater selection bias was a concern for this group. For less than 2% of subjects missing age first smoking information, smoking pack-years were calculated assuming start at age 20. This differs from the criteria document which excluded those with missing data.

Environmental Assessment and Exposure Estimation

The environmental assessment for DA comprised full-shift personal breathing zone ($n = 314$) and area ($n = 269$) DA air

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samples following NIOSH Method 2557.[15,18] Air contaminants identified in addition to DA included acetoin and acetaldehyde. Problems in sampling with NIOSH Method 2557 related to humidity and time-to-extraction, specific to DA, were subsequently uncovered for which an appropriate correction was developed.[19] For DA determinations below the limit of detection (LOD), the sample value was set equal to LOD/2, a common procedure. Other methods of accounting for the LOD (set to LOD, 0, LOD/$\sqrt{2}$) were tested and the impact on the risk assessment was insignificant (data not shown). The mean estimated concentrations for non-detects were, respectively, a factor of 163 and 444 below the means for personal and area samples that were above the LOD. Over the course of nine exposure assessments at the plant a dramatic downward trend in DA air concentrations was observed, reflecting implementation of engineering and administrative controls recommended by NIOSH. It is not known what changes in environmental controls occurred prior to the first assessment but, based on interviews including plant management, these were determined to be minor. Other problems in the retrospective exposure assessment for DA included uncertainty over when DA was introduced (est.: July, 1986), the forms and extent of its use in different products over time, and seasonal variation in ventilation. In making exposure assignments across a worker's employment history three issues were addressed: (1) the relation between area and personal samples, (2) downward time trends over the 2.7-year period of air sampling, and (3) mapping sampling locations to work history categories (department, job). The following steps led to creation of a job-exposure matrix (JEM) for the plant.

(a) Air samples were classified in three process groups (PG): (1) mixing, (2) microwave line other than mixing, (3) all other; observed to have relatively high, medium, and low levels, respectively.
(b) In order to utilize area-samples, their values were converted to personal-equivalents within the three PGs by using personal-to-area ratios of mean concentrations within each group from all surveys where both personal and area samples were collected (surveys 2 to 9; roughly equal numbers of personal and area samples were taken during surveys 2 to 4, when exposures were highest; survey 1 collected only area samples).
(c) All personal-equivalent samples (all surveys) were modeled on time since first survey (November 13, 2000) in each of the three PGs. Best fit was obtained modeling log(PPM) with square-root dependence on time-since-first survey (t_1): $\ln(\mathrm{PPM}(t)) = a + b(\mathrm{sqrt}(t - t_1)$, $b < 0$ (in three PGs, $R^2 = 0.40$, 0.53, 0.19, respectively) (Fig. 1).
(d) A map from air sampling to work-history (department/job) locations was derived. When combining samples across sampling group locations, the average of all samples was used, that is, with weights reflected in the numbers of samples collected.
(e) For each department/job combination the predicted value of the corresponding PG mean at a specific time was multiplied by the ratio of the department/job personal-equivalent mean for all surveys to the PG personal-equivalent mean for all surveys. Thus, all department/job combinations in a given PG shared the same proportional change over time but their actual levels reflected their mean values across all surveys (Fig. 1).
(f) For times after the introduction of DA and before the first survey (November 13, 2000), exposures were fixed equal to those derived from the time-trend models at the time of first survey.

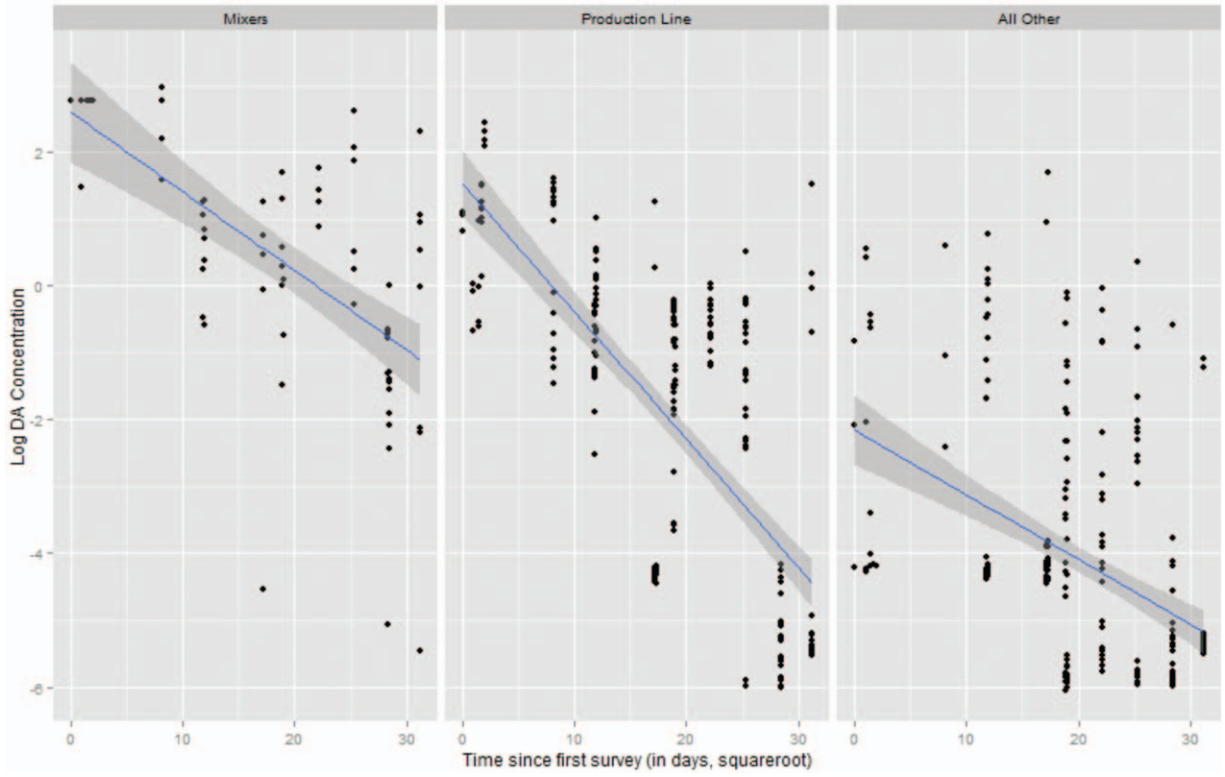

FIGURE 1. Diacetyl personal-equivalent airborne concentrations (ppm) during 2000 to 2003 in three groups: mixers, production line excluding mixers, and all others. Time scale: $(t - t_1)^{0.5}$, where t_1 is time at first survey (in days). Fitted models with 95% confidence intervals ($t > t_1$): Mixers: $\log(\mathrm{PPMP}(t)) = 2.59 - 0.119(\mathrm{sqrt}(t - t_1))$. Production line: $\log(\mathrm{PPMP}(t)) = 1.52 - 0.191(\mathrm{sqrt}(t - t_1))$. All other: $\log(\mathrm{PPMP}(t)) = -2.09 - 0.097(\mathrm{sqrt}(t - t_1))$.

In the criteria document, the JEM followed a similar protocol in creating the process groups, but then estimated average DA concentrations for grouped job categories in discrete time intervals reflecting known engineering and policy changes after Nov 2000. Using this approach,[17] models of exposure response exhibited explained variance and estimates of risk similar to those presented here.

Exposure Metrics

Cumulative exposure, cum(DA), defined as the summation of DA air concentrations over time (in ppm-years), was the primary exposure metric. Dose–rate effects were examined by calculating the time summation of the 0.5 and 2.0 powers of DA concentrations corresponding respectively to diminishing and increasing marginal responses to increasing exposure intensity. Transformed cumulative exposure as the square root, square, or logarithm were also evaluated as were duration of exposure and average exposure concentration (cumulative exposure divided by duration of exposure). Peak exposures were not directly available from the full shift (8-hour) time-weighted average sample concentrations or, for most jobs, by direct reading methods. To indirectly assess the impact of peak exposures, an analysis was conducted excluding the mixers.

Outcomes

Cases of bronchiolitis obliterans present a largely obstructive picture but with some restrictive spirometric pattern as well.[3,20–23] FEV_1 (forced expiratory volume in 1 second) is a commonly used spirometric (pulmonary function) measure for assessing impairment caused by hazardous agents, regardless of the specific nature (obstructive, restrictive, or combined). American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations are to use FEV_1 to assess the severity of any type of spirometric abnormality.[24] The health effects outcomes in this risk assessment therefore included (1) cross-sectional reductions in FEV_1, (2) reductions in FEV_1/FVC (expressed as a percent; FVC: forced vital capacity – total forced exhalation volume), and (3) longitudinal onset of two case conditions specified as: (a) FEV_1 below the lower limit of normal (LLN[25]; $n = 39$) and (b) both FEV_1 and FEV_1/FVC below their lower limits of normal ($n = 22$, a criterion more specific to airway obstruction). These outcomes plausibly would include cases of developing bronchiolitis obliterans (BO). Predicted values for pulmonary function tests and lower limits of normal were calculated based on age, height, sex, ethnicity, and race using prediction equations produced from the third National Health and Nutrition Examination Survey (NHANES).[25,26]

Exposure–Response Analysis

The spirometry determinations (1) percent of predicted FEV_1 ($ppFEV_1$), and, (2) the ratio, FEV_1/FVC, from a worker's last recorded spirometry, were analyzed as continuous outcomes in multiple linear regression models. Terms in the models included sex, ethnicity (Hispanic, African-American), ever-smoked, pack-years, and pack-years squared as of the date of testing. Pack-years squared permits some nonlinearity in the smoking response as might occur with survival or susceptibility effects. Models of FEV_1/FVC (as %) also included an age term (centered at 40). Models were assessed using the P value for exposure terms as well as the model multiple correlation coefficient (R^2). In the absence of exposure effects, the expected intercept for $ppFEV_1$ would be 100 and the expected intercept for FEV_1/FVC would be approximately 80%, and would depend on age.

For analyses of impairment incidence, date of onset for cases was defined as the average date a worker reported the start of continuing symptoms (cough, wheezing, shortness of breath, tightness of chest or phlegm), based on questionnaire items, provided those dates followed the first exposure to DA. Using the average date of symptom onset, rather than the first date, was intended to provide a more robust estimate of symptom onset attributable to DA exposure. If no qualifying symptom date existed (would include asymptomatic workers with new onset abnormal lung function), then date of onset was set to the date of first case-qualifying spirometry (<LLN; $n = 12$, case definition-1; $n = 4$, case definition-2) unless this was the worker's first survey in which case the worker was excluded from analysis of incidence because of unknown date of onset ($n = 42$, case definition-1; $n = 21$, case definition-2). These excluded workers may have had onset of impairment prior to exposure but could also have included early, asymptomatic BO cases arising prior to their first survey.

The incidence of new cases was modeled using Poisson regression[27] with both loglinear and linear relative rate (RR) specifications, which also estimated the background rate needed for a life-table-based calculation of excess lifetime risk. Observation time was compiled beginning with the date a worker was first exposed to DA. Models were fit using PROC COUNTREG in SAS 9.2 (SAS Institute Inc, Cary, NC)[28] and S-Plus software (Insightful Inc, Seattle WA),[29] and model fit assessed with the likelihood ratio test. This study design had potential bias leading to possibly underestimated rates arising from the selective removal of more susceptible or symptomatic workers from employment between the time of first exposure and the first survey or between surveys. Cases arising in those periods were available for analysis only if the individual remained in employment until, and chose to participate in, a spirometry-medical survey. In addition to exposure metrics and demographic covariates (age, sex, race, smoking), employment duration terms were included in some models to address survivor bias.

Assessment of Risk

Benchmark Dose

For continuous endpoints such as FEV_1, the benchmark dose approach permits estimation of excess impairment prevalence as a function of prior exposure.[30–32] From regression models and population data on the distribution of FEV_1 from NHANES III,[26] the proportions of the workforce impaired after working at specified exposure levels can be predicted. This calculation, implemented in S-Plus software,[29] requires specifying what deficit constitutes impairment and identifies the exposure concentration associated with a given increase in impairment prevalence, thereby defining a risk-based "benchmark dose" (BMD). For impairment defined in relation to the lower limit of normal, the BMD procedure is less direct because the distribution of FEV_1 in relation to LLN in a normal, healthy population is not easily described. LLN is specific to an individual's age and height. Therefore, an alternate approach was taken: in the NHANES population[26] the cumulative exposure that would reduce an individual's FEV_1 or (FEV_1/FVC) to their LLN was calculated using the exposure–response estimate from regression models. The excess proportion of individuals that would fall below their LLN as a function of exposure sustained over 45 years was then determined in the NHANES III population. From this could be derived an "empirical" benchmark dose; this procedure was implemented in SAS.[28]

Excess Lifetime Risk for Pulmonary Impairment

Using the life-table approach implemented in the BEIR IV report[33] together with the observed exposure–response relationship from models of incidence rate, one can estimate excess lifetime risk, the excess numbers of cases of DA-associated impairment that would occur over a working lifetime, with exposure at various concentrations. This method assumes irreversibility and removes

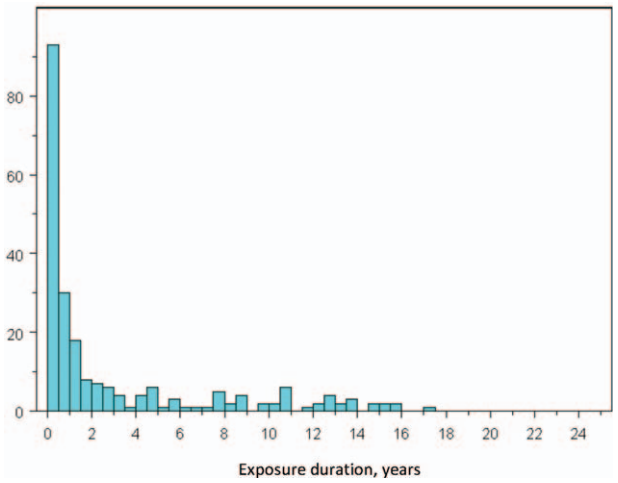

FIGURE 2. Distribution of diacetyl exposure duration in study population ($n = 368$, in 6 months intervals).

incident cases from the population at risk with increasing age along with deaths arising from the usual causes in the general population. A national life-table from Social Security data was used.[34] The surviving population (living but not yet a case) was calculated annually starting at age 20 and assuming exposure ceases at age 65. Excess lifetime risk was calculated with and without consideration of the effects of smoking. Excess lifetime risk was also calculated for exposure durations of 4 years, starting at age 20 and at age 40.

Attributable Mortality

Declining pulmonary function is a risk factor for mortality independent of age, sex, race, smoking, and body mass index (BMI). Five studies analyzed mortality and current FEV_1[35–39] three of which provide estimates of rate ratios that can be applied in a life-table analysis of excess lifetime mortality risk[35,37,38] resulting from pulmonary impairment.

RESULTS

Cross-Sectional Pulmonary Function Changes

The study population attributes have been described.[1,15] The mean duration of exposure to DA (equal to duration of employment unless hired before 1986) for the 368 subjects at the time of their last participation in a survey was 2.7 years (range: less than 1 to 17 years). Seventy-nine percent of the study population had less than 4.0-year duration and 46% had 6 months or less duration (Fig. 2). The mean cumulative exposure was 4.8 ppm-yr and the population time-averaged exposure was 1.87 ppm DA. At the time of the first plant survey the average DA exposure levels were estimated to be 13.3 ppm in mixers, 4.6 ppm on the production line, and 0.12 among support workers and the levels declined rapidly over the next 2.7 years (Fig. 1).

Multiple regression analyses for all subjects at the time of their last participation in a survey ($n = 368$) controlling for sex, ethnicity, and smoking, revealed statistically significant declining $ppFEV_1$ for all metrics, with Cum(DA) ($P = 5 \times 10^{-8}$) and $\{Cum(DA)\}^{0.5}$ ($P = 9 \times 10^{-9}$) performing considerably better than employment duration alone, and with Avg(DA) and $\{Cum(DA)\}^{2.0}$ performing less well (Table 1). The estimate for the exposure–response with Cum(DA) was a 0.40 reduction in $ppFEV_1$ for each ppm-year of cumulative exposure. (After 10 years at 5 ppm a worker's $ppFEV_1$, starting at 100, would be predicted to be $80 = 100 - 10 \times 5 \times 0.4$.) In the models with the better predicting metrics, sex and ethnicity (possible indicators of differential healthy worker selection) were unimportant predictors. Cumulative smoking, in pack-years, predicted a decline in $ppFEV_1$ but ever-smoking had a positive effect on $ppFEV_1$ (implying that, initially, beginning smokers may be healthier than those choosing not to smoke or that effect is not linear in pack-yrs); both effects were statistically significant. Regression models based on spirometry at a worker's first survey, rather than last, yielded similar estimates of DA exposure response (data not shown), suggesting that the pulmonary changes are irreversible (effects of earlier exposures not diminishing) and that the exposure assessment was consistent between the periods prior to first survey and after it. For FEV_1/FVC (as %) per ppm-yr a regression model with Cum(DA) predicted a decline of

TABLE 1. Multiple Regression Models for Percent of Predicted FEV_1 and for FEV_1/FVC (%) With Diacetyl Exposure Metrics

	Percentage of Predicted FEV_1					FEV_1/FVC				
Metric(DA)	R^2	**int.**	**est.**	t	P	R^2	**int.**	**est.**	t	P
Full population ($n = 368$)										
Avg(DA)	0.1313	94.92	−1.570	3.38	0.0007	0.3511	76.94	−0.990	4.45	8×10^{-6}
$(Cum(DA))^{2.0}$	0.1413	94.15	−0.0060	3.97	7×10^{-5}	0.3245	76.02	−0.0017	2.24	0.02
Duration	0.1501	96.94	−0.980	4.43	9×10^{-6}	0.3216	76.76	−0.235	1.87	0.06
$Cum(DA^{2.0})$	0.1547	94.70	−0.050	4.66	3×10^{-6}	0.3432	76.50	−0.021	3.92	9×10^{-5}
Cum(DA)	0.1720	95.44	−0.401	5.45	5×10^{-8}	0.3380	76.64	−0.134	3.52	0.0004
$Cum(DA^{0.5})$	0.1781	96.06	−0.913	5.71	10^{-8}	0.3363	76.80	−0.284	3.38	0.0007
$(Cum(DA))^{0.5}$	0.1792	96.83	−2.682	5.75	9×10^{-9}	0.3469	77.34	−1.007	4.17	3×10^{-5}
$\{Cum(DA^{0.5})\}^{0.5}$	0.1826	98.05	−4.125	5.90	3×10^{-9}	0.3439	77.73	−1.471	3.96	7×10^{-5}
Population with <4 years exposure duration ($n = 292$)										
Cum(DA)	0.0961	100.0	−0.765	2.65	0.0085	0.3242	77.17	−0.596	4.44	9×10^{-6}
$(Cum(DA))^{0.5}$	0.0873	100.3	−2.161	2.05	0.042	0.3194	77.59	−2.051	4.19	3×10^{-5}
Population with >4 years exposure duration ($n = 76$)										
Cum(DA)	0.1637	86.13	−0.265	2.01	0.048	0.2192	75.98	−0.110	1.46	0.15
$(Cum(DA))^{0.5}$	0.1735	89.08	−2.347	2.22	0.030	0.2271	77.46	−1.027	1.69	0.097

Partially missing smoking information imputed for seven subjects.

Separate model for each metric (mult. linear regression): $ppFEV_1 = \alpha + \beta sex + \gamma Hispanic + \delta race + \varepsilon smoker + \sigma packyrs + \theta(packyrs)^2 + \mu metric(DA)$.

Sex: male = 0, female = 1; Hispanic = 1, other = 0; race: black = 1, other = 0; smoker = ever smoked; (0,1); t—t-statistic for exposure metric effect estimate (est.); int.—model intercept; P—two-tailed P-value.

Avg(DA), average DA exposure, ppm; Cum(DA), cumulative DA exposure, ppm-yrs; DA, diacetyl; Duration, yrs of DA exposure.

TABLE 2. Models of Incidence Rate of Impairment by Poisson Regression With Loglinear Specification

	Case Definition-1, $n = 39$ ($FEV_1 <$ LLof N)			Case Definition-2, $n = 22$ (FEV_1 and $FEV_1/FVC <$ LLoN)		
Model	**est.**	***t***	***P***	**est.**	***t***	***P***
1						
Duration	−0.074	1.40	0.15	−0.075	1.14	0.26
Baseline rate		6.24×10^{-2}			2.24×10^{-2}	
-2ln(L)		679.816 (ref.)			409.318 (ref.)	
2						
Cum(DA)	0.015	1.28	0.20	0.016	1.23	0.22
Baseline rate		3.74×10^{-2}			2.50×10^{-2}	
-2ln(L)		680.455			409.344	
3						
Duration	−0.171	−2.30	0.022	−0.258	−2.25	0.024
Cum(DA)	0.039	2.54	0.011	0.055	2.57	0.010
Baseline rate		6.12×10^{-2}			2.26×10^{-2}	
−2ln(L)		673.881			402.396	
Δ(−2ln(L), 1df		5.94 ($P = 0.015$)			6.92 ($P = 0.008$)	
4						
Duration	−0.184	−2.45	0.014	−0.356	−2.78	0.005
$(Cum(DA))^{0.5}$	0.301	2.71	0.007	0.589	3.30	0.001
Baseline rate		4.83×10^{-2}			1.29×10^{-2}	
−2ln(L)		672.178			395.592	
Δ(−2ln(L), 1df		7.64 ($P = 0.006$)			13.72 ($P = 0.0002$)	
5						
Avg(DA)	0.170	2.73	0.011	0.325	3.93	0.00008
Baseline rate		3.04×10^{-2}			5.92×10^{-3}	
−2ln(L)		675.392			397.082	

Duration: yrs; Cum(DA), cumulative DA exposure, ppm-yrs; Avg(DA), average DA exposure, ppm.
Model (loglinear): $\text{rate} = \exp(\alpha + \beta\text{smoker} + \gamma\text{sex} + \delta(\text{age-40}) + \varepsilon(\text{age-40})^2 + \theta\text{packyrs} + \sigma(\text{packyrs})^2 + \eta\text{duration} + \mu\text{metric(DA)})$.
t, *t*-statistic for exposure metric estimate; *P*, two-tailed *P*-value from Wald statistic.
Δ(−2ln(L))—improvement in model fit with exposure term (and *P*-value for likelihood ratio test) compared with duration alone.

0.134 ($P = 0.0004$) and the model R^2 values were consistently larger compared with the $ppFEV_1$ regressions but the exposure effects were generally less significant with the exception of Avg(DA) which was the strongest predictor (Table 1).

Restricting the population on duration of exposure produced divergent results. The reduction in $ppFEV_1$ with less than 4 years duration ($n = 292$) was 0.77 per ppm-yr ($P = 0.009$), and with more than 4 years duration ($n = 76$) was 0.27 per ppm-yr ($P = 0.048$) (Table 1). With less than 4 years FEV_1/FVC declined by 0.60% per ppm-yr ($P = 10^{-5}$) compared with 0.11% at more than 4 years. With less than 4 years $\{Cum(DA)\}^{0.5}$ was no longer a stronger predictor than Cum(DA) for $ppFEV_1$ or FEV_1/FVC (Table 1).

The mixer job classification had intermittent high exposures. To assess whether those high exposures account for most of the DA effect in the population, analyses were repeated restricted to workers who had never been mixers ($n = 348$). The resulting DA effect estimate with the Cum(DA) metric was slightly larger in magnitude (−0.426 vs −0.401) and the effect remained highly statistically significant ($P = 8 \times 10^{-7}$, data not shown). A similar result obtained for the $\{Cum(DA)\}^{0.5}$ metric.

Using product terms for ever-smoking and smoking pack-years with the Cum(DA) and $\{Cum(DA)\}^{0.5}$ exposure metrics, there was some evidence of a DA-smoking interaction: a non statistically significant protective effect in smokers (data not shown). In smokers, the Cum(DA) effect estimate was 25% smaller, and 50% smaller with the $Cum(DA)^{0.5}$ metric. The reduced DA effect in the group with more than 4 years exposure was not accounted for by increased smoking; the proportion of ever-smokers was reduced in the more than 4 years group.

Acetoin, another flavoring component that is strongly associated with DA at this plant (corr = 0.85), was not subject to the humidity degradation problem in air sampling. When the procedure used for constructing the exposure matrix for DA was applied to acetoin, multiple linear regressions predicting $ppFEV_1$ produced the same pattern of results as observed with DA but with somewhat better model fit. For the metric square root of cumulative exposure, the R^2 observed for acetoin and DA were 0.183 and 0.179, respectively; the corresponding *t*-statistics for the exposure terms were 5.92 and 5.75, respectively. Current data indicate that acetoin is considerably less hazardous than diacetyl and it does not have the reactive α-dicarbonyl group, which has been implicated in the toxicity of diacetyl and 2,3-pentanedione.[40–42]

Incidence of Pulmonary Impairment

In Poisson regression analyses with a log-linear specification, for two case definitions, duration of DA exposure predicted a diminishing rate of onset while cumulative DA exposure predicted an increasing incidence rate but neither was statistically significant ($P \sim 0.2$, Table 2, models 1, 2). This is a surprising result given the expected colinearity of duration and measures of cumulative exposure. The metrics cumulative exposure and square root of cumulative exposure had significant effects only in the presence of a negative, statistically significant, duration term (Table 2, models 3, 4) and average exposure to DA, by itself, was a statistically highly significant predictor of increased onset (model 5). When the joint distribution of cases on exposure duration and cumulative exposure was examined, there was a cluster of cases with low duration and cumulative exposure. For example, there were three cases (definition-1) in the cell with second lowest duration and lowest exposure category (35 person-yr) and another three cases in a cell with highest exposure and 1 to 2 years duration (46 person-yr) (Table 3, part 1a). Thus there was a lower incidence rate in a cell with greater than 10-fold larger

TABLE 3. Cases of Impairment and Predicted Incidence Rate Ratios When Observation Time Is Classified by Duration and Cumulative Exposure

Case Definition-1 (FEV_1 Falling Below Lower Limit of Normal; 706.7 person-yrs at risk)						
	Cumulative Diacetyl Exposure (ppm-yrs)					
Duration of Exposure	**<0.5**	**0.5 < 2.0**	**2.0 < 3.0**	**3.0 < 5.0**	**≥5.0**	**All**
1a	Observed cases					
<0.5 yr	4	3	0	0	0	7
0.5 <1.0 yr	3	0	0	1	0	4
1.0 <2.0 yr	2	0	0	0	3	5
2.0 <4.0 yr	1	0	0	0	7	8
≥4.0 yr	2	0	0	1	12	15
All	12	3	0	2	22	39
1b	Rate ratio – relative to baseline: 0.022					
<0.5 yr	2.97	3.02	2.98	2.45	2.51	2.98
0.5 <1.0 yr	2.77	2.99	2.79	3.03	3.00	2.86
1.0 <2.0 yr	2.57	2.19	2.68	2.92	3.16	2.87
2.0 <4.0 yr	2.11	2.14	2.22	2.10	3.28	2.87
≥4.0 yr	1.00	1.33	1.07	1.25	2.61	2.01
All	2.30	2.08	2.72	2.53	2.89	2.55

Model (loglinear): rate = $\exp(\alpha + \beta\text{smoker} + \gamma\text{sex} + \delta(\text{age-40}) + \varepsilon(\text{age-40})^2 + \theta\text{packyrs} + \sigma(\text{packyrs})^2 + \eta\text{duration} + \mu\text{cum(DA)})$.
Rate ratio: predicted rate (adjusted for age, smoking, sex) divided by (baseline rate predicted for ≥4.0 yr and <0.5 ppm-yrs): 0.022 for case defn-1; 0.00663 for case defn-2.

cumulative exposure. The predicted baseline incidence (setting exposure = 0 in the Poisson regression model with duration and cum. exp.) is elevated in the early years of employment, falling from 0.061 (6.1% per year) in the first 6 months, to 0.022 (2.2% per year) after 4 years. Dividing the model-predicted total rate by 0.022 yields an incidence rate that declines with increasing duration within most cumulative exposure strata (Table 3, part 1b). Relatively early onset of BO cases has been reported in other DA investigations.[1,20,43,44] Examination of onset, graphically, confirmed that many cases arose after relatively short employment duration particularly among those of recent hire (not displayed to preserve confidentiality). This pattern of onset was consistent with DA exposures being lower at this plant in the earlier years of DA flavoring, prior to the introduction (c. 1994) of ''low-fat'' products which had higher DA content. A similar pattern was exhibited in the 46 cases (defn 1) identified among participating workers who were no longer employed at the time of their first survey (data not shown) and was also observed (case defn 1, $n = 25$) in the pooled population from two other popcorn enterprises with less well characterized exposures (data not shown).[13,14] In the present study plant, the second case definition produced a similar pattern but now with fewer cases ($n = 22$ vs 39) and now 11 of the 22 cases had less than 4 years duration (data not shown).

Recognizing that most hires have left employment within 4 years and that the remaining workforce may have had lower risk (lower susceptibility), a Poisson regression model was fit using a linear relative rate model specification that included a term intended to capture the possibly changing composition of the population with time. An exponential decline was assumed for the portion of the population that was initially in the majority and declining, with those remaining in employment having lower risk. Half-lives of 0.5, 1, and 2 years were evaluated. For case definition-2 a model with a term of the form:

$$(\text{Avg[DA]})^2 \times \exp\left(\frac{-0.693 \times \text{duration}}{2.0}\right)$$

having a half-life of 2 years and squared average exposure, produced the best fit among several choices (for the two DA terms, lrt = 13.54, 2df, $P = 0.001$; Table 4). The estimated baseline rate, in person-yrs,

TABLE 4. Linear Relative Rate Models of Incidence of Impairment

	Case Definition-1				Case Definition-2			
	est.	**RR**	**lrt**	***P***	**est.**	**RR**	**lrt**	***P***
Intercept	−9.218				−15.34			
smoke_ever	−0.721	0.49			−0.085	0.92		
ind:female	0.305	1.36			0.442	1.56		
age-40	0.0021	1.002			0.035	1.035		
$(\text{age-40})^2$	4×10^{-4}	1.0004			-3×10^{-4}	0.9997		
packyrs	0.078	1.08	2.95	0.043	8.57	9.57	3.12	0.039
cum(DA)	0.0081	1.0081	0.089	>0.5	10.7	11.7	2.49	0.057
shortdur(DA)	0.0637	1.0637	3.038	0.040	31.6	32.6	10.04	0.0008
Baseline rate		3.6×10^{-2}				8.0×10^{-5}		
Δ(−2ln(L), 2df)		4.218, $P = 0.12$				13.54, $P = 0.0011$		

Model (linear relative rate) rate = $\{\exp(\alpha + \beta\text{smoker} + \gamma\text{sex} + \delta(\text{age-40}) + \varepsilon(\text{age-40})^2\}\{1 + \theta\text{packyrs} + \sigma\text{shortdur(DA)} + \mu\text{cumDA}\}$.
shortdur(DA), short duration risk exposure term for half-life = 2.0 yr: shortdur(DA) = $[\text{DA}]^2 \exp(-0.693\text{duration}/2)$.
Person-time in days.
lrt, likelihood ratio test for removal of term from model; *P*, one-tailed *P*-values; RR, relative rate.

TABLE 5. Benchmark Dose for Impairment on Percent of Predicted FEV_1 ($ppFEV_1$) Based on 45 Years Exposure and cum(DA) Metric in Full Population and With <4 Year Duration

		Excess Prevalence of Impairment (Per Thousand)							
		Full Population				Population <4 years Duration			
DA (ppm)	Cum(DA) (ppm-yrs)	Pred.* $ppFEV_1$	<60% of pred.	$<5^{th}$%ile	<90% of pred.	Pred. $ppFEV_1$	<60% of pred.	$<5^{th}$%ile	<90% of pred.
1	45.0	84.07	76.8	259.0	431.0	65.58	356.5	654.4	675.3
0.5	22.5	92.04	19.3	92.2	211.8	82.79	69.0	240.6	412.4
0.2	9.00	96.81	5.0	28.6	79.5	93.12	12.4	63.8	158.4
0.1	4.50	98.41	2.1	13.1	38.6	96.56	4.7	27.0	75.7
0.05	2.25	99.20	1.0	6.2	19.0	98.28	2.0	12.4	36.8
0.02	0.90	99.68	0.4	2.4	7.5	99.31	0.8	4.7	14.4
0.01	0.45	99.84	0.2	1.2	3.7	99.66	0.4	2.3	7.2
0.005	0.225	99.92	0.1	0.6	1.9	99.83	0.2	1.1	3.6
0.002	0.090	99.97	0.0	0.2	0.7	99.93	0.1	0.4	1.5
0.001	0.045	99.98	0.0	0.1	0.4	99.97	0.0	0.2	0.7

Baseline prevalence for impairment defined as <60% of pred., $<5^{th}$%ile, and <90% of predicted is, respectively, 0.0057, 0.0507, 0.2635.
*Model-predicted $ppFEV_1$ assuming baseline = 100.

was very small: 0.008% per year ($365.25 \times \exp[-15.34] = 0.00008$; intercept in person-days), indicating that virtually all cases were attributable to either DA exposure or smoking. For smoking the estimated rate ratio increased by 8.6 for each additional pack-year and, for each additional ppm-yr of DA exposure in the long duration group, the rate ratio increased by 10.7. The initial (start of exposure) rate ratio for the entire population, most of whom would work for less than 4 years, DA was 32.6 at 1 ppm. The strong association with the term representing short duration of exposure supports the conjecture that the risk of most hires is substantially elevated above that of long-term employees. For the less selective case definition-1, the fit for the linear relative rate model was marginal (lrt = 4.22, 2df, $P = 0.12$; Table 4).

Benchmark Dose

With the linear regression results for percent-predicted FEV_1 with the metric Cum(DA), the excess prevalence of falling below (1) 60% of predicted (moderately severe impairment[23]), (2) the 5th percentile of normal (a common, traditional medical criterion for impairment corresponding to about 80% of predicted), or (3) 90% of predicted (10% loss of lung function), after 45 years of exposure, was calculated (Table 5). Thus, a 1/1000 excess prevalence after 45 years was found for these three pulmonary impairments at DA exposures of about 0.050, 0.008, and 0.003 ppm DA, respectively (BMDs, central tendency estimates). With the exposure–response estimate from the less than 4 years population (majority), the corresponding BMDs were 0.025, 0.005, and 0.0015 ppm (Table 5).

The ''empirical'' BMD procedure (using the empirical, nonparametric distribution of the NHANES population) yielded BMDs for both FEV_1 and FEV_1/FVC that were similar to those for $ppFEV_1$ in relation to impairment at the 5th percentile of normal (Table 6). The excess prevalence for FEV_1 below LLN after 45 years at 0.01 ppm DA was 1.8/1000 versus 1.2/1000 below the 5th percentile of normal (Tables 5 and 6). At DA concentrations below 0.01 ppm, the excess prevalence of FEV_1/FVC below the LLN was roughly comparable to that of FEV_1 for all employment duration (Table 6) but, for less than 4 years duration, the excess prevalence was higher for FEV_1/FVC.

Using the exposure metric, $\{Cum(DA)\}^{0.5}$, which better predicts $ppFEV_1$ in the full population, substantially lower BMDs result; 1/1000 excess risk for impairment at the 5th percentile after 45 years occurs with a DA exposure concentration of less than

TABLE 6. Empirical Benchmark Dose for FEV_1 and FEV_1/FVC Impairment Defined by Lower Limit of Normal Based on 45 Years Exposure and Cum(DA) Exposure Metric in Full Population and in Population With Exposure Duration <4 Years

	Excess Prevalence of Impairment (Per Thousand)			
	FEV_1		FEV_1/FVC	
DA (ppm)	All	<4 yr	All	<4 yr
1	399.8	783.0	217.4	888.2
0.5	148.4	375.7	81.9	707.3
0.2	45.1	103.5	27.2	182.4
0.1	19.9	42.5	12.4	69.7
0.05	9.2	18.6	6.7	30.3
0.02	4.0	7.5	3.2	11.2
0.01	1.8	3.7	2.2	6.4
0.005	1.1	1.6	1.0	3.5
0.002	0.4	0.6	0.4	1.5
0.001	0.2	0.4	0.3	0.9

Benchmark doses derived from BMD procedure with empirical distribution.
FEV_1, forced expiratory volume in 1 second; FEV_1/FVC, forced expiratory volume in 1 second/forced vital capacity.

TABLE 7. Excess Lifetime Risk Based on Life-Table (Per Thousand) Using an Incidence Rate Model (Case Definition-2) With Terms Distinguishing Short-Duration From Long-Term Employment

	45 yr Diacetyl Exposure			4 yr Diacetyl Exposure @ Age 20			4 yr Diacetyl @ Age 40
DA (ppm)	All*	Non-smokers†	Smokers†	All*	Non-smokers†	Smokers†	All*
1	328.3	827.9	118.9	73.7	230.5	29.7	63.4
0.5	230.0	639.9	82.2	38.3	123.0	15.5	32.3
0.2	116.8	355.5	41.8	15.7	51.2	6.4	13.0
0.1	63.7	200.9	22.9	7.9	25.9	3.2	6.5
0.05	33.3	107.0	12.0	4.0	13.1	1.6	3.3
0.02	13.7	44.5	4.9	1.6	5.2	0.6	1.3
0.01	6.9	22.6	2.5	0.80	2.6	0.32	0.66
0.005	3.5	11.3	1.2	0.40	1.3	0.16	0.33
0.002	1.4	4.6	0.50	0.16	0.53	0.06	0.13
0.001	0.70	2.3	0.25	0.08	0.26	0.03	0.07

Case definition-2: $FEV_1 <$ LLof N and $FEV_1/FVC <$ LLof N.
*Model of case incidence with no smoking terms (Table 4, case defn-2).
†Model of case incidence with smoking terms and excess lifetime risk calculated separately for non-smokers and smokers.

0.0005 ppm (data not shown) versus 0.01 ppm with the Cum(DA) metric. Although this metric accounts for reduced risk with long duration, the increasing (negative) slope of the exposure response with smaller values of the exposure metric may represent an inappropriate extrapolation.

Excess Lifetime Risk

Because smoking information was used in modeling, several variants for excess lifetime risk were calculable (Table 7). For example, at 0.01 ppm DA, using an incidence model (case definition-2) that ignores smoking, the excess lifetime risk was 6.9/1000. Using a model that includes smoking, the excess lifetime risk at 0.01 ppm DA for nonsmokers was 22.7/1000, while for smokers (one pack/d) it was 2.5/1000. Excess lifetime risk was also calculated assuming a 4-year duration of employment starting at age 20 and at age 40 (Table 7). The contribution of the exposures in the first 4 years would be about the same but the effects of cumulative exposure following employment termination would impact a shorter period (by 20 years) for those hired at age 40.

Excess Mortality

Published estimates of mortality relative risk associated with declining FEV_1, range from 1.010 to 1.019 per percent decline in FEV_1 in men, and from 1.010 to 1.025 in women,[34,36,37] after controlling for smoking and other risk factors. Assuming a relative rate of 1.015 per percent decline in FEV_1, and using the estimate of FEV_1 decline from the cross-sectional analysis using Cum(DA) (Table 1), a life-table analysis produced estimates of excess lifetime mortality risk. These estimates happen to be comparable to those based on the incidence of pulmonary impairment, for example, FEV_1 falling below LLN and the benchmark dose estimates, (Table 8) however, they are the result of a generic effect of declining FEV_1 on mortality not specific to BO. It is plausible that this mortality effect is in addition to mortality proceeding from advancing BO disease itself at high DA exposures (ie, >0.5 ppm). Using the estimate of FEV_1 decline restricted to those exposed less than 4 years produced higher excess mortality estimates by a factor of about 2.0 (Table 8).

Summary of Risk Assessments

Excess prevalence and lifetime risk estimates variously derived, for 45 years of DA exposure were similar (Table 8). Excess risk of 1/1000 corresponds to approximately 0.002 to 0.005 ppm DA (7.0 to 17.5 $\mu g/m^3$).

DISCUSSION

The results observed in this analysis were consistent with the findings in the NIOSH criteria document. Using a JEM in which exposures were modeled over time, representing the changes in engineering controls at each survey, yielded very similar results to the analyses presented here.

Interpretation of Modeling Results

The observation that considerably more cases met the first case definition than the second (39 vs 22), due to the added requirement: FEV_1/FVC is less than LLN, suggests that there is both obstructive and restrictive (or air-trapping[20]) lung impairment (that FVC is also diminished, sometimes resulting in a ''normal'' FEV_1/FVC). The relative fit of various model specifications for incidence rate (case definition-2) indicated that, for a single metric, average exposure fit best in both loglinear and linear relative rate models, but there was some improvement using other exposure metrics along with a duration term or a term distinguishing low employment duration (linear relative rate design). In the loglinear models with a (negative) duration term, the excess cases at short duration are actually being treated as part of a declining background rate, that is, not attributable to DA exposure.

The metric cumulative square root of DA concentration—$Cum(DA^{0.5})$—was a somewhat stronger predictor of spirometry changes than simple cumulative exposure, and $Cum(DA^{2.0})$ was weaker (Table 1), implying that if there is any dose–rate effect it is probably negative—lower exposures make a larger than proportional contribution to decreasing lung function. This argues against only high DA exposures conferring risk and against the apparent survivor effect being an artifact of a positive dose–rate effect. With no survivor effect, lower exposures (and longer durations) would have greater than predicted effects not less (as observed in Table 3).

The slightly stronger prediction of spirometry changes with square root of cumulative DA concentration—$[Cum(DA)]^{0.5}$—suggests that with accumulating dose, there is attenuation of increasing risk. This too is consistent with declining susceptibility associated with high workforce turnover and a surviving low risk population. Within the traditional occupational risk assessment paradigm, the existence of a transient workforce or variable susceptibility poses a challenge because the composition of the population with respect to the factor modifying risk is changing in an unknown manner over time. The lower excess lifetime risk from DA for smokers can be

TABLE 8. Risk Assessment Synthesis: Excess Prevalence or Lifetime Risk for 45 Years Exposure to Diacetyl

	Method						
	BMD: Excess Prevalence (Per Thousand)				Life-Table: Excess Lifetime Risk (Per Thousand)		
	Impairment*				Case Onset	Mortality‡	
	FEV_1 (<LLN)		FEV_1/FVC (<LLN)		Case defn.-2†	All Cause	
DA (ppm)	All	<4 yr	All	<4 yr		All	<4 yr
0.10	19.9	42.5	12.4	69.7	63.7	21.0	39.5
0.05	9.2	18.6	6.7	30.3	33.3	10.6	20.0
0.02	4.0	7.5	3.2	11.2	13.7	4.3	8.1
0.01	1.8	3.7	2.2	6.4	6.9	2.1	4.1
0.005	1.1	1.6	1.0	3.5	3.5	1.1	2.0
0.002	0.4	0.6	0.4	1.5	1.4	0.4	0.8
0.001	0.2	0.4	0.3	0.9	0.7	0.2	0.4

FEV_1, forced expiratory volume in 1 second; FEV_1/FVC, forced expiratory volume in 1 second /forced vital capacity.

*Based on multiple regression predicting fall in percent-predicted FEV_1 with DA exposure (0.40% per ppm-yr DA in all, and 0.765 per ppm-yr DA in <4 yr population).

†Case definition-2: FEV_1 < LLof N and FEV_1/FVC < LLof N; assumes worker exposed 45 yr.

‡Based on (1) estimate of all-cause mortality dependence on FEV_1 after controlling for age, sex, BMI, smoking, and various cardiovascular risk factors (1.5% increase in mortality rate per 1% decline in FEV_1) and (2) regression coefficients for declining FEV_1 in all and <4 yr workers; smokers: one pack/d.

explained by smoking being a strong competing cause for becoming a case and because smoking appears to be slightly protective for the DA effect based on the observed smoking–DA interaction that was observed here.

Risk Assessment

This risk assessment pertains to the development of pulmonary impairment believed to be a precursor of a disabling and potentially fatal disease. The natural history of BO with continuing DA exposure, or after termination of exposure, is not known except to the extent of extrapolating from studied populations where exposures generally were for less than 10 years.[19] This 45-year risk assessment thus extrapolates considerably beyond the existing data. Variable susceptibility, suggested by these analyses, implies that for some individuals, the onset of impairment comes more slowly than for most or, alternatively, that average susceptibility declines with continuing DA exposure.

The HHE investigation utilized here included extensive and repeated exposure and spirometric measurements. It also included an invitation to former employees to participate in the surveys. However, former employees were excluded from this analysis because of the reasons stated previously. In the present analysis there are several sources of bias expected to result in underestimation of DA effects: (a) the plant population studied represents a survivor cohort (symptomatic incident cases leaving employment prior to the first survey were excluded or missed), (b) some asymptomatic cases were excluded in the incidence analysis, (c) exposures prior to 1994 may have been overestimated, and (d) the correction required for DA air sample determinations probably contributed to nondifferential exposure misclassification (independent of outcome status). Observing similar results using a JEM based on discrete time intervals[17] rather than models of continuous exposure levels over time reveals the robustness of both approaches. The only other methodological deviation with the previously published report[17] was the assumption of age beginning smoking at 20 years when missing, in less than 2% of the population.

Acting against sources of underestimation bias is the possibility that study participants may have included a more than representative proportion of cases. However, the high participation rate (~80%) limits the potential bias arising from selective participation. The variability in apparent susceptibility to DA effects could be related to host factors like differences in diacetyl metabolism and respiratory fitness itself. The short-duration cases did not differ from others on BMI. Healthy worker effect bias from population-based prediction equations was minimized by analyses using internal exposure comparisons.

Two other popcorn plant HHEs considered for risk assessment purposes had much lower exposures than the plant described here, based on many fewer air samples taken in a single survey[13,14]; the exposures prior to those surveys were unknown but probably higher based on plant histories obtained. Estimated parameters for the exposure–response relationship from analyses of these two HHEs were larger than that for the current study plant and would have generated lower estimated exposures to achieve the range of life-time risks considered. It is also possible that materials and process conditions at the two other plants were more typical of the industry than those of the plant evaluated in this assessment, in which case the current risk assessment could be an underestimate of the risks of DA exposure.

The exposure metric, average exposure (cumulative exposure divided by duration) was a strong predictor of pulmonary impairment in some analyses. It is implausible that average exposure, in a homogeneous population, would predict impairment without consideration of duration unless duration was very uniform, which was not at all the case in this study. Rather, it seems likely that the association of impairment with average exposure reflects not only a cumulative exposure response but also the changing composition of the population with employment duration. More responsive individuals (which appear to be in the majority) leaving the population sooner than some others would diminish the apparent importance of cumulative exposure. Thus average exposure might predict impairment, but could be population-specific depending on how the particular plant population changed over time, and would not permit a generalizable exposure response.

All of the risk assessments developed here assume some degree of low-dose linearity, with effects diminishing proportionally with decreasing exposure levels held constant over 45 years. Over periods of less than 10 years, this linearity assumption is consistent with the observed effects at exposures within the range of most of the observed data (career-average exposures to DA were less than 0.01 ppm in 17% of workers), particularly when restricted to workers with less than 4 years exposure (Table 1). Below 0.01 ppm, there could be some significant departure from linearity, although

observing a negative dose–rate effect argues against a threshold in the observable range, and diversity in response would tend to favor linearity to lower levels.[45,46]

The health significance of small spirometry changes, such as a 1% decline in FEV_1 after 2 years at 1 ppm DA, depends in part on whether such changes are early indications of lung pathology that eventually would manifest as BO. In studies of BO arising from lung transplantation, unrelenting irreversible FEV_1 decrements are observed that ultimately lead to the diagnosis of BO and fatal disease,[47] but this is a pathophysiologically distinct disease from DA-related BO. Incomplete knowledge of the natural history of BO development with DA exposure is a limitation in the present risk assessment. For individuals already below their LLN for other reasons, further decrements such as from DA exposure take on increasing importance. Moreover, small changes, even if their progression is arrested by reduction or elimination of exposure, are risk factors for future adversity. Not only is risk for mortality increased, as estimated in this risk assessment, quality of life is degraded[48] and risk is increased for other respiratory and cardiovascular disease.[49–54]

Findings from Other Studies

At four plants of another popcorn manufacturer, comparing high versus low DA exposed worker groups, Lockey et al[21] observed significant losses of FEV_1 and FVC in high-exposed groups (DA >0.8 ppm year) but observed no significant association between percent predicted FEV_1 and duration of DA exposure, suggesting a possible survivor effect as seen here. These investigators reported no significant associations of FEV_1 or FVC with a continuous cumulative DA exposure metric but the DA determinations were not corrected for humidity. In the present study where exposures were higher than in Lockey et al, there was a significant decline in $ppFEV_1$ (increasing impairment) with duration of exposure but the incidence of new cases of impairment also decreased with increasing duration (Table 2). In a cross-sectional study of diacetyl manufacturing workers in the Netherlands, van Rooy et al[55] observed clearly increased respiratory symptoms but pulmonary function appeared to improve with duration of exposure, which the authors interpret possibly due to a strong healthy worker survivor bias as was observed in the present study, or to exposure misclassification. In a longitudinal study of flavoring workers, Hines et al[56] also failed to observe an association between cumulative DA exposure and lung function overall, but among workers with less than 2 years exposures, they observed a significant increasing rate of FEV_1 decline across three levels of increasing DA exposure. This observation of early changes parallels observation in the present study which we interpret to represent diminishing susceptibility in the population being followed, that is, a survivor effect.

Maier et al[57] reviewed the animal and human-data options for a quantitative risk assessment for diacetyl concluding that there is insufficient human epidemiology on which to base this effort. Their concerns with HHEs centered on the adequacy of retrospective exposure assessment, and their evaluation of the Akpinar-Elci et al[20] findings did not consider possible selection or susceptibility effects that now have been observed in several studies including the present one. Egilman et al[58] challenged this dismissal of the available human epidemiology and reported positive findings in data presented in Akpinar-Elci et al[20] and in the HHE[15] on which the present assessment was based. Using the available data and a simple extrapolation, they derive a "proposed safe exposure level" for DA of approximately 1 ppb (0.001 ppm).

Ronk et al[59] reanalyzed pulmonary function data from a NIOSH HHE at a facility manufacturing flavorings, including diacetyl,[60] and found no decrease in lung function in these workers. However, as with typical cross-sectional studies, there is evidence of survivor bias in their analysis. Over the 10-year period of observation it was reported that only 2 out of 112 employees terminated employment, a highly suspect accounting (0.2% turnover per year), and inconsistent with their reported average employment duration of 16 years (in a steady-state population with 16 years average duration and a 32 years career duration there would be greater than 3% turnover per year and 27% turnover over 10 years). The estimates of pulmonary impairment showed increasingly negative associations (ie, less risk, but nonsignificant) with tenure in jobs having higher exposure potential. No diacetyl air concentrations were used in the analysis and the manufacturing processes may have involved much lower exposures than occur, for example, in downstream applications such as mixing and injecting hot flavoring fluids in the packaging lines for microwave popcorn production.

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