CRITERIA FOR A RECOMMENDED STANDARD

# Occupational Exposure to Diacetyl and 2,3-Pentanedione


**DEPARTMENT OF HEALTH AND HUMAN SERVICES**
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

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**August 12, 2011**
**External Review Draft**

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**DHHS (NIOSH) Publication No. 20XX-XXX**

## Executive Summary

Diacetyl is used extensively in the flavoring and food production industry, and occupational exposure to this substance has been associated with severe obstructive lung disease, bronchiolitis obliterans, and decrease in lung function. Bronchiolitis obliterans is a life threatening disease and decreased pulmonary function has been associated with degraded quality of life and increased mortality [Cullen et al. 1983; Ebi-Kryston et al. 1989; Heng et al. 1998; Mannino and Davis 2006; Mannino et al. 2006]. 2,3-Pentanedione has been used as a substitute for diacetyl and is of concern because of structural similarities with diacetyl and because of animal studies showing similar pathology as seen with diacetyl in exposed animals and workers [Hubbs et al. 2010; Morgan et al. 2010].

In 1985, two workers with fixed obstructive lung disease suggestive of bronchiolitis obliterans were observed in a facility where flavorings with diacetyl were made for the baking industry [NIOSH 1985]. The link between exposure to diacetyl and the risk of bronchiolitis obliterans was identified in the early 2000s when research confirmed a relationship exists between diacetyl exposures and lower pulmonary function [Kreiss et al. 2002]. Occupational exposures to diacetyl have been assessed in various food production and flavoring facilities [Kanwal et al. 2006; Martyny et al. 2008; NIOSH 2003a, b, 2004a, b, 2006, 2007a, 2008a, b, 2009d, 2011b]. Mean diacetyl air concentrations measured at the index microwave popcorn facility were the highest in the mixing room (57.2 ppm), followed by the packaging area for machine operators (2.8 ppm) [Kanwal et al. 2011]. Mean diacetyl air concentrations at five other microwave popcorn plants were lower: 0.02 to 0.83 ppm in the packaging areas and 0.63 to 1.54 ppm in the mixing rooms/areas.

Using cross-sectional pulmonary function data from diacetyl exposed workers, NIOSH conducted analyses to determine the exposure-response relationship and identify risk of pulmonary function decrease at various levels of diacetyl exposure. NIOSH found that a relationship exists between diacetyl exposures and lower pulmonary function.

Utilizing this quantitative risk analysis, NIOSH recommends that exposure to diacetyl be kept below a concentration of 5 parts per billion (ppb) as a time-weighted average (TWA) during a 40-hour work week. NIOSH has determined that workers exposed to diacetyl at this concentration should have no more than a 1 in 1000 chance of suffering reduced lung function associated with diacetyl exposure and less chance for developing bronchiolitis obliterans. To further protect against effects of short-term exposures, NIOSH recommends a short-term exposure limit (STEL) for diacetyl of 25 ppb for a 15-minute time period.

In many operations, 2,3-pentanedione and other substances are being used to substitute for diacetyl. There is little health effect data on these substances but it is appropriate to consider some of them as potentially as hazardous as diacetyl. Specifically, 2,3-pentanedione is structurally very similar to diacetyl because it is a 5-carbon alpha-diketone, and diacetyl is a 4-carbon alpha-diketone. Published reports on the toxicity of 2,3-pentanedione suggest that in rats 2,3-pentanedione causes airway epithelial damage similar to that produced by diacetyl [Hubbs et al. 2010; Morgan et al. 2010]. The toxic potency of the two substances appears to be roughly comparable. Therefore, NIOSH recommends keeping occupational exposure to 2,3-pentanedione below a level comparable to that recommended for diacetyl. However, analytical limitations of the recommended method indicate that 2,3-pentanedione can only be reliably quantified to 9.3 ppb. This is slightly higher than what is recommended for diacetyl. NIOSH recommends that exposure to 2,3-pentanedione be kept below a concentration of 9.3 ppb in a TWA during a 40-hour work week. NIOSH also recommends a STEL for 2,3-pentanedione of 31 ppb during a 15-minute period.

Engineering and work practices are available to control diacetyl and 2,3-pentanedione exposures below the RELs [Eastern Research Group 2009c; NIOSH 2008a, d]. Validated analytic methods are available that allow measurements at the RELs [Eide 2008, 2010; Simmons and Hendricks 2008]. For diacetyl, NIOSH recommends an action level of approximately one half the REL (2.6

ppb). For 2,3-pentanedione, because the REL is established at the reliable quantification limit, no action level is recommended.

NIOSH recommends that employers develop and implement comprehensive occupational safety and health programs to protect workers with potential exposure to diacetyl or 2,3-pentanedione and other potentially hazardous flavoring chemicals. This program should include exposure and medical monitoring, and implementation and assessment of exposure controls. The exposure and control assessments should (1) determine worker exposure to diacetyl and 2,3-pentanedione and other flavoring chemicals used in the workplace, (2) evaluate the effectiveness of work practices and engineering controls, and (3) facilitate the selection of appropriate protective equipment.

NIOSH recommends that medical monitoring and surveillance be implemented for workers with occupational exposure to diacetyl or 2,3-pentanedione. This involves developing a medical monitoring program that includes spirometry testing for pulmonary function, medical evaluation for workers found with abnormality on spirometry, removal from exposure pending this evaluation, and analysis of medical surveillance and spirometry data on a group basis (epidemiologic surveillance) to assess work-related risk factors based on job, task, area, and other exposure indices. The purpose of epidemiologic surveillance is to assist monitoring physicians in prioritizing and evaluating the effectiveness of interventions, if indicated. Identifying excessive declines in spirometry, even within the normal range of spirometry, offers the best opportunity to intervene to prevent the development of impairing occupational lung disease.

While the focus of this document is on diacetyl and 2,3-pentanedione, NIOSH has concern about other flavoring substitutes with structural similarities to diacetyl or moieties that are biologically active and capable of producing similar toxic effects as diacetyl. Therefore, NIOSH recommends that such exposures also be considered and controlled to as low as reasonably achievable.

# ABBREVIATIONS

| | |
|---|---|
| ACGIH | American Conference of Governmental Industrial Hygienists |
| ACOEM | American College of Occupational and Environmental Medicine |
| AH | absolute humidity |
| AIC | Akaike Information Criterion |
| AIHA | American Industrial Hygiene Association |
| AL | action level |
| ANSI | American National Standards Institute |
| APF | assigned protection factor |
| ATS | American Thoracic Society |
| BLS | Bureau of Labor Statistics |
| BMD50 | benchmark dose modeling conducted for a response rate of 50% |
| BMD | benchmark dose |
| BMDL | lower bound on benchmark dose |
| BOOP | bronchiolitis obliterans organizing pneumonia |
| BOS | bronchiolitis obliterans syndrome |
| Cal/OSHA | California Occupational Safety and Health Administration |
| CAS | Chemical Abstract Service |
| CDC | Centers for Disease Control and Prevention |
| CDPH | California Department of Public Health |
| CFD | computational fluid dynamics |
| CFD-PBPK | computational fluid dynamic-physiologically based pharmacokinetic model |
| cfm | cubic feet per minute |
| CI | confidence interval |
| COP | cryptogenic organizing pneumonitis |
| COPD | chronic obstructive pulmonary disease |
| CT | computed tomography |
| cumDA | cumulative exposure to diacetyl |
| DA | diacetyl |
| DCXR | dicarbonyl/L-xylulose reductase |
| DLco | diffusing capacity for carbon monoxide |
| ECRHS | European Community Respiratory Health Survey |
| EPA | Environmental Protection Agency |
| ERS | European Respiratory Society |
| ESD | encapsulated starter distillate |
| ET | extrathoracic |
| °F | degrees Fahrenheit |
| FASEB | Federation of American Societies for Experimental Biology |

| | |
|---|---|
| FDA | Food and Drug Administration |
| FEMA | Flavor and Extract Manufacturers Association |
| $FEV_1$ | forced expiratory volume in one second |
| FISHEP | Flavor Industry Safety and Health Evaluation Program |
| fpm | feet per minute |
| FTIR | Fourier transform infrared |
| FVC | forced vital capacity |
| g/kg | grams per kilogram |
| GC | gas chromatography |
| GC-ECD | gas chromatography using an electron capture detector |
| GC-FID | gas chromatography using a flame ionization detector |
| GC-MS | gas chromatography-mass spectrometry |
| GC-NPD | gas chromatography-nitrogen/phosphorus detection |
| GM | geometric mean |
| GHS | Globally Harmonized System of Classification and Labeling of Chemicals |
| GSD | geometric standard deviation |
| HEC | human equivalent concentrations |
| HEPA | high efficiency particulate air |
| HHE | health hazard evaluation |
| HPLC | high performance liquid chromatography |
| HRCT | high resolution computerized tomography |
| HSE | Health and Safety Executive |
| HVAC | heating, ventilating, and air-conditioning |
| IDLH | immediately dangerous to life or health |
| IR | infrared |
| JEM | job exposure matrix |
| kg | kilogram |
| LD50 | median lethal dose |
| LEV | local exhaust ventilation |
| LLD | limit of longitudinal decline |
| LLofN | lower limit of normal |
| L/min | liters per minute |
| LOD | limit of detection |
| LOQ | limit of quantification |
| LRT | likelihood ratio test |
| m/s | meters per second |
| $mg/m^3$ | milligrams per cubic meter of air |
| mg/mL | milligrams per milliliter |
| mg/L | milligrams per liter of air |
| mL/min | milliliters per minute |
| mM | millimole |
| MSDS | material safety data sheet |

| | |
|---|---|
| NAICS | North American Industry Classification System |
| NHANES III | Third National Health and Nutrition Examination Survey |
| NLM | National Library of Medicine |
| NIOSH | National Institute for Occupational Safety and Health |
| NMAM | NIOSH Manual of Analytical Methods |
| NTP | National Toxicology Program |
| OEL | occupational exposure limit |
| OR | odds ratio |
| OSHA | Occupational Safety and Health Administration |
| OV | organic vapor |
| PAPR | powered air-purifying respirator |
| PBZ | personal breathing zone |
| PEL | permissible exposure limit |
| PFBHA | O-(2, 3, 4, 5, 6-pentafluorobenzyl) hydroxylamine |
| PFT | pulmonary function test |
| PID | photoionization detector |
| ppb | parts per billion |
| PPE | personal protective equipment |
| ppm | parts per million |
| QC | quality control |
| Rt | transepithelial resistance |
| REL | recommended exposure limit |
| RH | relative humidity |
| SAR | supplied air respirator |
| SPIROLA | Spirometry Longitudinal Data Analysis |
| SPME | solid phase microextraction |
| STD | standard deviation |
| STEL | short-term exposure limit |
| TB | tracheobronchial |
| TLV | threshold limit value |
| TWA | time-weighted average |
| VE | minute volume |
| VOC | volatile organic compound |

## Glossary

***2,3-pentanedione:*** a diketone (Chemical Abstracts Service No. 600-14-6) used as a synthetic flavoring agent and aroma carrier. It has a buttery taste and smell. It may be used as either a solid (powder) or as a liquid. It is structurally very similar to diacetyl.

***Acetoin:*** a hydroxy ketone (Chemical Abstracts Service No. 513-86-0) found in butter flavoring. Acetoin may be converted to diacetyl through oxidation.

***American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV):*** voluntary exposure guidelines recommended by ACGIH, a professional organization, for use by industrial hygienists and others trained in this discipline to assist in the control of health hazards

***Asthma:*** a chronic inflammatory airway disease that causes episodic wheezing, shortness of breath, chest tightness, and coughing.

***Bronchiolitis obliterans:*** a potentially fatal irreversible lung disease characterized by fixed airway obstruction. The bronchioles are compressed and narrowed by either fibrosis or inflammation. Symptoms include a dry cough and shortness of breath, and may progress gradually or occur suddenly.

***Diacetyl:*** an alpha-diketone (Chemical Abstracts Service No. 431-03-8) used as a synthetic flavoring agent and aroma carrier. It has a buttery taste and smell. It may be used as either a solid (powder) or as a liquid.

***Emphysema:*** an irreversible progressive disease of the lungs that destroys the alveolar tissues of the lungs.

***Encapsulated powder:*** ingredients such as diacetyl or other flavor enclosed within a material to decrease volatility and allow a subsequent release or flavor burst.

***Fibrosis:*** a condition in which lung tissue is replaced over time with scar tissue. This process restricts the lungs and reduces total lung capacity.

***Fixed airways obstruction:*** a respiratory problem marked by reduced airflow out of the lungs that, unlike asthma, is not reversible with a bronchodilator medication.

***Gas chromatography/mass spectrometry:*** a method of analyzing mixtures of chemicals qualitatively and quantitatively

***Material safety data sheet****:* a listing of a hazardous chemical's health and physical hazards, exposure limits, and precautions.

***Mid-expiratory flow rate:*** the maximum rate of airflow measured between exhaled volumes of 25% and 75% of the forced vital capacity as measured during a forced exhalation.

***Occupational exposure limit:*** levels of exposure that most employees may be exposed to for up to 10 hours per day, 40 hours per week, for a working lifetime, without experiencing adverse health effects.

***N-95 filtering facepiece respirator:*** a term that describes the class of respirators that use N95 filters to remove particles from the air that is breathed through them. An N95 filter removes at least 95% of airborne particles during "worst case" testing using a "most-penetrating" sized particle during NIOSH testing.

***NIOSH recommended exposure limit (REL):*** An 8- or 10-hour time-weighted average or ceiling exposure concentration recommended by NIOSH that is based on an evaluation of the health effects data.

***OSHA permissible exposure limit (PEL):*** regulatory limits on the amount or concentration of a substance in the air. OSHA PELs are based on an 8-hour time weighted average exposure.

***Organic vapor cartridge:*** devices used in respirators to remove organic vapors from the air

***Personal protective equipment:*** respirators, work gloves, work boots, and other equipment that reduce or eliminate worker exposure to hazards.

***Prevalence:*** the number of cases of a disease or condition present in a particular population at a given time.

***Respiratory rate:*** the number of breaths taken within a certain amount of time, commonly measured in breaths per minute.

***Silicosis:*** a respiratory disease caused by inhaling silica dust.

***Slurry:*** a mixture of liquid and powder ingredients.

***Spirometer/Spirometry:*** an instrument and method for performing a pulmonary function test (PFT) that measures the volume or flow of air that can be inhaled or exhaled to assess lung function.

***Starter distillate:*** a steam distillate of the culture of bacteria grown on a medium consisting of skim milk usually fortified with about 0.1 percent citric acid. It contains mostly water, and the remainder is a mixture of butter-like flavor compounds. The major flavoring ingredient is diacetyl, but starter distillate also contains minor amounts of acetaldehyde, ethyl formate, ethyl acetate, acetone, ethyl alcohol, 2-butanone, acetic acid, and acetoin.

***Supplied-air respirator system:*** an atmosphere-supplying respirator for which the source of breathing air is not carried by the user.

***Tidal volume:*** the volume of air inhaled or exhaled during a single breath at rest.

***Time-weighted average:*** the average exposure during a normal 8- to 10-hour workday.

***Volatile organic compound (VOC):*** an organic chemical compounds with high vapor pressure and low boiling point.

# ACKNOWLEDGMENTS

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# DIACETYL AND 2,3-PENTANEDIONE

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# Chapter 1: Introduction

## 1.1 Purpose

This document presents the criteria and components of a recommended standard necessary to reduce or eliminate significant risk of health impairment from exposure to diacetyl and 2,3-pentanedione and prevent flavorings-related lung disease. This document was developed in accordance with the Occupational Safety and Health Act of 1970 [29 U.S.C. 669(a)(3) ; 29 U.S.C. 671(c)(1)]. This Act charges NIOSH with recommending occupational safety and health standards and developing criteria for toxic materials. These criteria are to describe exposures that are safe for various periods of employment, including but not limited to the exposures at which no worker will suffer diminished health, functional capacity, or life expectancy as a result of his or her work experience.

The purpose of the criteria document is to evaluate and analyze the scientific literature concerning potential health effects, toxicology, risk assessment, engineering controls, work practices, personal protective equipment, and recommendations pertaining to diacetyl and 2,3-pentanedione. The criteria document provides the basis for the recommended exposure limit (REL) for diacetyl and 2,3-pentanedione, although compliance with this recommended standard is not the sole objective. The intended outcome of the document is to reduce occupational exposures to diacetyl and 2,3-pentanedione and thereby prevent flavoring-related lung disease through hazard guidance implementation. In its entirety, the RELs and the guidance are intended to help employers develop a more healthful work environment. The REL and guidance will also provide useful information to help employees actively participate in their own protection.

### 1.2 Scope

This criteria document contains a review of relevant scientific information related to diacetyl and 2,3-pentanedione, and provides the rationale and criteria for establishing appropriate risk management recommendations. The basis for developing a criteria document on diacetyl and 2,3-pentanedione is described in this chapter. Chapter 2 provides an overview of studies conducted to characterize occupational exposure to diacetyl and 2,3-pentanedione. Chapter 3 describes the health effects observed in workers exposed to diacetyl and other flavoring ingredients. Chapter 4 describes toxicology research from diacetyl and 2,3-pentanedione while Chapters 5 and 6 describe the assessment of risk based on available human and animal data. Chapter 7 provides the basis for a REL for diacetyl and 2,3-pentanedione. Chapter 8 describes procedures for informing workers about the safety of diacetyl and substitutes as well as engineering interventions that could significantly reduce exposures when appropriately applied and fully operational. Also included in Chapter 8 are recommendations for establishing an effective respiratory protection program. Chapter 9 provides medical surveillance guidelines for the ongoing evaluation of the health status of workers. Chapter 10 describes the components of an effective exposure monitoring program and work practices that when implemented correctly can reduce occupational exposures. Chapter 11 presents key research needs.

### 1.3 Background

Diacetyl is one of the main components in butter flavoring that imparts the buttery taste and has been identified as a prominent volatile organic compound (VOC) in air samples from microwave

popcorn plants and flavoring manufacturing plants [Akpinar-Elci et al. 2004; Ashley et al. 2008b; Kanwal 2003; Kanwal et al. 2006; Kanwal and Martin 2003; Martyny et al. 2008; NIOSH 2004a; Parmet and Von Essen 2002]. Diacetyl is used as a synthetic flavoring ingredient and aroma carrier in butter, caramel, vinegar, dairy products, and coffee and is also naturally found in foods. A number of flavor formulations use diacetyl to create other flavors including but not limited to strawberry, caramel, hazelnut, and butterscotch. Occupational exposures in the flavoring and food production industry have been associated with respiratory disease, including bronchiolitis obliterans, an uncommon lung disease characterized by fixed airways obstruction. Bronchiolitis obliterans refers to disease processes that show some degree of inflammation, narrowing, or obliteration of small airways (bronchioles) in the lung and is discussed in more detail in Chapter 3, specifically section 3.1.1. Although a causative relationship between diacetyl and respiratory disease has been observed, diacetyl may not be the only flavoring ingredient related to health impairment. Other flavoring ingredients such as acetaldehyde, butyric acid, and acetoin, have been associated with adverse health effects [Lockey et al. 1998; van Rooy et al. 2007]. In addition, new diacetyl substitutes with little or no toxicological information related to occupational safety and health are being used in production.

Given its related chemical structure and flavor properties similar to diacetyl, 2,3-pentanedione has been used as a diacetyl substitute in many flavor manufacturing facilities. Day et al. [Day et al. 2011] has observed 2,3-pentanedione in food production facilities. Published reports on the toxicity of 2,3-pentanedione from experimental inhalation studies with rats indicate that exposure causes airway epithelial damage similar to that produced by diacetyl [Hubbs et al. 2010; Morgan et al. 2010].

No state or national registries are available to identify potential bronchiolitis obliterans cases among workers. In 1985, two workers with fixed obstructive lung disease suggestive of bronchiolitis obliterans were observed in a facility where flavorings with diacetyl were made for the baking industry [Kreiss et al. 2002; NIOSH 1985]. Catastrophic fixed airways disease suggestive of bronchiolitis obliterans was observed in two former mixing workers who were young nonsmokers with job tasks that involved blending corn starch and flour with various flavorings. Two additional current workers who formerly had mixing responsibilities also had otherwise unexplained obstruction, whereas two current mixers were unaffected. A review of common ingredients listed diacetyl among other flavoring ingredients.

In the microwave popcorn industry, the first occurrences of bronchiolitis obliterans were observed in 2000 when eight workers formerly employed in a microwave popcorn facility were diagnosed with the disease [Kreiss et al. 2002]. The observation of this case series led to the identification of another case of bronchiolitis obliterans in a separate facility [Parmet 2002]. Since then, numerous cases of bronchiolitis obliterans have been observed in the microwave popcorn industry [Akpinar-Elci et al. 2004; CDC 2002; Ezrailson 2002; Kanwal et al. 2006; NIOSH 2003a, 2004a, b, 2006; Parmet 2002; Schachter 2002]. In addition, a retrospective epidemiologic study found cases of bronchiolitis obliterans in workers who were employed in a chemical plant with exposures to diacetyl, acetoin, acetic acid, and acetaldehyde [van Rooy et al. 2007].

In 2004 and 2006, two cases of bronchiolitis obliterans among workers who made food flavorings were reported to the California Department of Public Health (CDPH). An industry-wide public

health investigation performed by CDPH, the California Occupational Safety and Health Administration (Cal/OSHA) and NIOSH initially found an additional five workers with severe, fixed obstructive lung disease [CDC 2007]. Outreach to the industry on the diacetyl hazard, including Cal/OSHA consultation site visits, prompted quick implementation of exposure controls and medical surveillance programs. A longer-term effort focused on companies' installation of effective engineering controls and further assessment of medical surveillance findings over time by CDPH and NIOSH. A cross-sectional analysis of medical surveillance data from 16 companies confirmed the risk of lung disease among workers at companies using diacetyl [Kim et al. 2010]. In 2010, California passed the first occupational standard for diacetyl [California Code of Regulations. Title 8, §5197].

Employees within the flavoring production industry have complex exposures in terms of the physical form of the agents (solid, liquid, and gas) and the number of different chemicals used. Although thousands of flavoring ingredients are in use, few have occupational exposure limits. The Flavor and Extract Manufacturing Association reports that of the more than 1,000 flavoring ingredients considered to be potential respiratory irritants or hazards, only 46 have established Occupational Safety and Health Administration (OSHA) permissible exposure limits (PELs) [FEMA 2004]. Given the lack of occupational exposure limits for most flavoring ingredients, assessing workplace exposures and developing exposure control guidance are critical to help reduce the risk of flavoring-related lung disease.

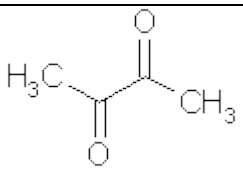
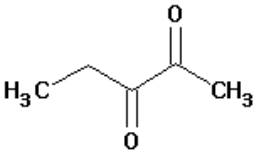
### 1.4 Chemical and Physical Properties

The chemical diacetyl is an alpha-diketone with Chemical Abstract Service (CAS) number 431-03-8. Diacetyl has several synonyms including 2,3-butanedione (IUPAC nomenclature), 2,3-butadione, 2,3-diketobutane, biacetyl, dimethyl diketone, dimethylglyoxal and glyoxal, dimethyl. The chemical 2,3-pentanedione is another alpha-diketone, with CAS number 600-14-6. It is also referred to by the name acetylpropionyl. The diacetyl and 2,3-pentanedione molecules contain two carbonyl groups, oxygen molecules attached to carbon by a double bond. These carbonyl groups are ketones, meaning that the carbonyl carbons are attached only to carbon molecules. In these molecules, the two carbonyl groups are adjacent, which puts diacetyl and 2,3-pentanedione into a group of compounds known as alpha-dicarbonyl compounds; more specifically, diacetyl and 2,3-pentanedione are alpha-diketones. A listing of physical and chemical properties of diacetyl and 2,3-pentanedione is presented in Table 1.1.

Human and animal toxicological mechanisms are discussed in Chapters 3 and 4, respectively.

Table 1.1 Chemical and physical properties

| Property | Diacetyl | 2,3-Pentanedione |
|---|---|---|
| CAS # | 431-03-8 | 600-14-6 |
| Synonyms | 2,3-butanedione; biacetyl; dimethyl diketone; dimethylglyoxal; 2,3-diketobutane [Merck and Co. Inc. 2006] | Acetylpropionyl [Lide 2008]; |
| Molecular formula | $C_4H_6O_2$ | $C_5H_8O_2$ |
| Molecular weight | 86.090 [Lide 2008] | 100.117 [Lide 2008] |
| Molecular structure | | |

| |  |  |
|---|---|---|
| Density | 0.9808 g/mL (18ºC) [Lide 2008] | 0.9565 g/mL (19ºC) [Lide 2008] |
| Refractive index | 1.3951 (20ºC) [Lide 2008] | 1.4014 (19ºC) [Lide 2008] |
| Melting point | −1.2ºC [Lide 2008]; −2.4ºC [IPCS 2009]; −4 ºC [Fischer Scientific 2007] | −52ºC [Merck Chemicals International 2010] |
| Boiling point | 88°C [Lide 2008]; 89°C–90°C [Illovo Sugar Limited 2009] | 108°C [Lide 2008]; 110ºC – 112ºC [Merck Chemicals International 2010]; 112ºC [Chem Service Inc. 1988] |
| Vapor density | 3 [IPCS 2009] | 3.45 [Illovo Sugar Limited 2010] |
| Vapor pressure | 52.2 mm Hg (20°C) [Sigma Aldrich 2010] | 21.4 mm Hg (20ºC) [Merck Chemicals International 2010] |
| Saturated vapor concentration [Perez 1991] | 184 g/m$^3$ (20ºC); 246 g/m$^3$ (20ºC) | 117 g/m$^3$ (20ºC) |
| Water solubility | 200 g/L (25ºC) [IPCS 2009]; | 60 g/L (15ºC) [Merck Chemicals International 2010] |
| Flash point, closed cup | 6°C [IPCS 2009]; 7°C [Sigma Aldrich 2010] | 18ºC [Merck Chemicals International 2010] |
| Autoignition temperature | 365°C [IPCS 2009]; 345°C [Sigma Aldrich 2010] | 265ºC [Merck Chemicals International 2010] |
| Explosive limits in air | 2.4% (V) – 13% (V) [IPCS 2009] | 1.8% (V) – 10.9% (V) [Merck Chemicals International 2010] |
| Odor | Quinine odor, vapors have chlorine-like odor [Merck and Co. Inc. 2006]; strong rancid, chlorine-like, butter-like [Fischer Scientific 2007]; sweet-butter [Diaz et al. 2004]; buttery [Illovo Sugar Limited 2010] | Fruity/pleasant [Chem Service Inc. 1988]; buttery [Illovo Sugar Limited 2010] |
| Odor threshold<br><br>concentration in water | 0.05 µg/L [Diaz et al. 2004]; 4 ug/L [Schlichtherle-Cerny et al. 2008] *<br><br>*recognition threshold | 30 ug/L [Schlichtherle-Cerny et al. 2008] *<br><br>*recognition threshold |

| | | |
|---|---|---|
| Odor threshold<br><br>concentration in air | 0.09 ppb [Illovo Sugar Limited 2009]; 0.01-0.02 ppb [Blank et al. 1992] | 0.01-0.02 ppb [Blank et al. 1992] |
| Octanol/water partition<br><br>coefficient, Log $P_{ow}$ | −1.34 [IPCS 2009] | −0.85 [Illovo Sugar Limited 2010] |
| Henry's Law Constant | $1.97 \times 10^{-7}$ atm-$m^3$/mol at 25°C [Meylan and Howard 1991] *; $1.35 \times 10^{-5}$ atm-$m^3$/mol at 25°C [Betterton 1991]; $1.75 \times 10^{-5}$ atm-$m^3$/mol at 25°C [Snider and Dawson 1985]<br><br>* estimated using bond contribution method | $2.62 \times 10^{-7}$ atm-$m^3$/mol at 25°C [Meylan and Howard 1991] *<br><br>* estimated using bond contribution method |
| Appearance | Yellowish green liquid [Merck and Co. Inc. 2006]; green to yellow liquid [IPCS 2009] | Dark yellow liquid [Lide 2008]; colorless to yellow [Chem Service Inc. 1988] |
| Electron Impact Mass<br><br>Spectrum, m/z (%) | 43 (100%), 15 (34%), 86 (11%), 14 (10%), 42 (7%), 13 (3%), 26 (2%), 29 (2%) [Nottingham University 1983] | 43 (100%), 29 (69%), 57 (35%), 27 (30%), 15 (26%), 100 (10%), 26 (9%), 14 (9%) [Nottingham University 1983] |
| Infrared Spectrum | 1715.6 $cm^{-1}$, 1420.7 $cm^{-1}$, 1353.2 $cm^{-1}$, 1115.5 $cm^{-1}$, 537.2 $cm^{-1}$, [Pouchert 1985] | 2982.5 $cm^{-1}$, 1715.1 $cm^{-1}$, 1408.0 $cm^{-1}$, 1349.6 $cm^{-1}$, 1094.2 $cm^{-1}$, 908.7 $cm^{-1}$, 581.4 $cm^{-1}$ [Pouchert 1985] |

### *1.4.1 How Diacetyl and 2,3-Pentanedione are Prepared*

Diacetyl can be synthesized chemically from four starting materials: (1) from methyl ethyl ketone either by converting it to an isonitroso compound and then hydrolyzing with hydrochloric acid or by partial oxidation of methyl ethyl ketone over a copper or vanadium oxide catalyst [Aquila et al. 2001; National Toxicology Program 2007], (2) from 2,3-butanediol by oxidative dehydrogenation of 2,3-butanediol over a copper or silver catalyst [National Toxicology Program 2007], (3) from acetoin (obtained by electrochemical oxidation of methyl ethyl ketone) by reacting acetoin with molecular oxygen in the presence of copper oxide catalyst [Aquila et al. 2001], or (4) from 1-

hydroxyacetone (obtained by dehydrogenation of 1,2-propanediol) by the acid-catalyzed condensation of 1-hydroxyacetone with formaldehyde [National Toxicology Program 2007].

Diacetyl is also a byproduct of fermentation. Natural diacetyl is used in the form of starter distillate, a concentrated flavor distillate, which may contain different concentrations of diacetyl depending on production conditions [Burdock 1997].

The compound 2,3-pentanedione is also naturally produced by fermentation and is recovered from dairy waste to be used as a flavoring ingredient [Miller et al. 1998]. The chemical synthesis of 2,3-pentanedione is achieved in the following ways: (1) the condensation of lactic acid and an alkali metal lactate [Miller et al. 1998], (2) the acid-catalyzed condensation of 1-hydroxyacetone with paraldehyde [Lambrecht et al. 2004], or (3) the oxidation of 2-pentanone with excess sodium nitrite and diluted hydrochloric acid in the presence of hydroxylamine hydrochloride [Burdock 1997].

### 1.5 Production Uses and Applications

The flavor manufacturing industry commonly uses diacetyl and 2,3-pentanedione during flavor formulation production. Flavor formulations are then sold to downstream users for the production of flavored food products. Flavored food production is the process of manufacturing food and beverage products that contain added flavor formulations or flavorings to enhance or modify the taste of the product. Examples of flavored food products include cake mixes, flour, beer, wine, margarines and soft spreads, cheese, candy, bakery products, crackers, cookies, ice cream, frozen foods, and many other food and beverage products. The addition of concentrated flavorings

including diacetyl is a cost effective way to impart the desired properties to manufactured food items.

In flavor formulations, diacetyl and 2,3-pentanedione are typically found as components in liquid solutions but can also be added to powders in dry mixtures to create a particulate formulation. Many volatile compounds are also encapsulated in an amorphous carbohydrate, producing more stable products with more manageable properties. Encapsulated diacetyl powder is often created with a spray dryer, which converts a slurry mixture into a powder in which the diacetyl and/or 2,3-pentanedione is surrounded by the powder, instead of simply coating the particle. When the encapsulated powder comes into contact with water, or saliva, a "flavor burst" occurs where the release of the flavor from the encapsulation is generally fast and complete upon contact with moisture [Ubbink and Schoonman 2002].

The percentage of diacetyl or 2,3-pentanedione in a particular flavor formulation varies widely depending upon the product and its use. In past years, microwave popcorn contained the highest proportion of diacetyl ranging from 1% to 25% diacetyl [Hallagan 2007]. The diacetyl content in flavor formulations has declined rapidly as many manufacturers have reduced or substituted diacetyl with other flavoring ingredients with similar characteristics. Most confectionary flavors contain up to 1% diacetyl while marshmallow production uses up to 5% [Hallagan 2007].

Starter distillate, produced by fermenting milk with starter cultures, contains diacetyl in the range of 1% to 5% and is often used in the dairy industry. Diacetyl is the major flavor component of starter distillate, constituting as much as 80% to 90% of the mixture of organic flavor compounds

[FDA 2009]. A NIOSH health hazard evaluation (HHE) at a modified dairy products company found concentrations of airborne diacetyl ranging to 2.14 ppm on a full-shift TWA basis. [NIOSH 2009d].

Diacetyl is also used as a chemical modifier of arginine residues in proteins in studying glycation (the nonenzymatic browning of foods or the nonenzymatic binding of sugar and protein molecules in the body) [Saraiva et al. 2006]. Other uses for diacetyl include reactant/starting material in chemical or biochemical reactions, analytical reagent, antimicrobial/preservative, electron stabilizing compound and modifier of radiation response for chemical and biological systems, and photoinitiator/photosensitizer in polymerizations [National Toxicology Program 1994].

### 1.6 Potential for Exposures

It is difficult to quantify the number of employees directly involved with flavor manufacturing and more specifically having diacetyl or diacetyl substitute exposure in the United States. According to the Environmental Protection Agency (EPA) Non-Confidential Inventory Updating Report, diacetyl had an aggregate production volume between 10,000 and 500,000 pounds in 2002 [EPA 2011]. The North American Industry Classification System (NAICS) category 311, the most relevant category, indicates nearly 1.5 million workers are employed in food manufacturing. Bureau of Labor and Department of Commerce data provide a breakdown of a portion of that number into categories shown in Table 1.2. According to the Flavor Extract Manufacturers Association, whose members account for approximately 95% of all flavors

produced in the United States, a total of 6,520 employees work directly in the flavor manufacturing or laboratory activities in membership companies [Hallagan 2010].

Table 1.2. Breakdown of workers in various categories of the food manufacturing industry

| Category description | No. of workers | NAICS code | Ref. |
|---|---|---|---|
| Bakeries and Tortilla Manufacturing | 280,900 | 3118 | [BLS 2008] |
| Other Food Manufacturing | 164,100 | 3119 | [BLS 2008] |
| Dairy Product Manufacturing | 129,100 | 3115 | [BLS 2008] |
| Sugar and Confectionery Product Manufacturing | 70,800 | 3113 | [BLS 2008] |
| Beverage industry | 177,000 | 3121 | [BLS 2008] |

Initial research concerning occupational exposure to diacetyl has focused on workers who directly produce flavorings or use them in the microwave popcorn industry. The employment figures for the food production industry suggest that some other workers have potential exposure to diacetyl and other food flavorings. For example, respiratory issues have been anecdotally reported for cheese production (Wisconsin), yogurt production (Ohio), and potato chip manufacturing [Alleman 2002].

Employers in the food manufacturing sector are generally small business owners with 89% in establishments employing fewer than 100 workers and nearly 53% in establishments employing fewer than 10 workers [United States Census Bureau 2004]. Industries that comprise food manufacturing can be found in every state in the United States. However, concentrations of specific industries are found in general geographic locations. For example, in 2004, Wisconsin had 33% of the cheese manufacturing workers employed in the United States, and California had

20% of the fruit and vegetable preservation industry workers employed in the United States [BLS 2007].

There is increasing likelihood that various substances will be used to substitute for diacetyl or 2,3-pentanedione. The potential for both workers' exposure and disease from exposure to these substitutes still remains largely unstudied.

# Chapter 2: Assessing Occupational Exposure in Workers

## 2.1 Introduction

Measurement of diacetyl and 2,3-pentanedione exposures is helpful in preventing flavorings-related lung disease, even though flavorings exposures are often more complex. Diacetyl and 2,3-pentanedione can be monitored using personal and environmental (area) air samples since the predominant route of exposure is inhalation. Results from air sampling can be compared with established criteria such as the NIOSH RELs. Measuring diacetyl and other alpha-diketone exposures may help to identify hazards, guide corrective actions such as engineering controls, identify improved work practices, and select appropriate respiratory protection to reduce or eliminate exposures.

This chapter discusses (1) available sampling and analytical techniques for monitoring diacetyl and 2,3-pentanedione vapor in the workplace, (2) techniques for measuring diacetyl and 2,3-pentanedione in airborne particulate and bulk materials, (3) real-time techniques for measuring relevant airborne analytes, (4) sampling and analytical techniques for other flavoring ingredients, and (5) results of some occupational exposure assessments by NIOSH and others of facilities that use diacetyl and 2,3-pentanedione.

Many work environments have mixed exposures, with multiple chemical agents present. Although the primary focus of this criteria document is diacetyl and 2,3-pentanedione, other compounds can also be of concern. Depending upon the processes employed in a workplace, sampling should be conducted for agents of concern to maintain safe work environments. Common sampling and analytical methods to determine concentrations of diacetyl and 2,3-pentanedione are presented in Appendix 1.

## 2.2 Time-integrated Air Sampling and Analytical Methods for Diacetyl and 2,3-Pentanedione Vapor

Personal breathing zone sampling is the preferred approach for estimating the exposure characteristics of a worker performing specific tasks. For personal sampling, a worker wears the air sampling equipment and the collection medium positioned within the worker's breathing zone. Area sampling is performed to evaluate exposure characteristics associated with an area or process, and frequently to determine the efficiency of control systems. While the same sampling equipment may be used in some cases for both personal and area sampling, area sampling is stationary, in contrast to personal sampling which allows for mobility by accompanying the worker throughout the sampling period.

### *2.2.1 OSHA Methods 1012 and 1013*

In response for the need to sample for longer sampling time periods with a lower detection limit or reliable quantitation limit, OSHA validated two sampling and analytical methods for diacetyl and acetoin in 2008 [Eide 2008; Simmons and Hendricks 2008]. As of the publication of this document, there are the recommended methods for diacetyl. After the introduction of diacetyl substitutes in flavorings, OSHA also used Methods 1012 and 1013 for 2,3-pentanedione [OSHA 2011]. These methods can be used simultaneously for diacetyl, and acetoin. OSHA Method 1012 pulls air through two 600 mg sorbent tubes in series containing specially cleaned and dried silica gel (SKC Inc., Eighty Four, PA, Catalog no. 226-183). A flow rate of 50 mL/min for 180 minutes is recommended for the determination of time-weighted average (TWA) concentrations, and a flow rate of 200 mL/min for 15 minutes is recommended for short term concentration measurements. A target concentration of 0.05 parts per million (ppm) (0.18 mg/m$^3$) TWA diacetyl is provided. An opaque sampling tube protective cover should be used in conjunction with the sampler. It prevents the sampled employee from being injured from the sharp ends of the glass sampling tubes in addition to preventing the sampler, glass shards from the sampling tubes, or its contents from contaminating flavorings or food products. The cover also protects the sample from light which can decompose diacetyl and acetoin. After sampling, the two tubes should be separated, capped, and protected from light with aluminum foil or other opaque material. Samples are extracted and derivatized with a solution of 95:5 ethyl alcohol:water containing 2 mg/mL of

O-(2, 3, 4, 5, 6-pentafluorobenzyl) hydroxylamine hydrochloride and analyzed by gas chromatography using an electron capture detector (GC-ECD).

OSHA Method 1013 [Simmons and Hendricks 2008] is similar to Method 1012 in that it recommends the same sampling media, flow rate and sample collection procedures. A higher target concentration of 0.5 ppm (1.8 mg/m$^3$) TWA diacetyl is provided. Samples are extracted with a solution of 95:5 ethyl alcohol:water and analyzed by gas chromatography using a flame ionization detector (GC-FID). This method also requires samples be protected from light during and after sampling. An advantage of Method 1013 is that sample preparation can be performed in one hour, whereas the derivatization step of Method 1012 requires 36 hours. After samples have been extracted and analyzed using Method 1013, if needed (e.g., if sample concentration is not detectable), they can be derivatized and analyzed using Method 1012 to benefit from its lower detection capability.

#### *2.2.2 OSHA Method 1016*

OSHA Method 1016 [Eide 2010] can be used to measure 2,3-pentanedione concentrations. Samples are collected through two 600 mg tubes containing specially cleaned and dried silica gel (SKC Inc., Eighty Four, PA, Catalog no. 226-183) in series at 0.05 L/min for 200 minutes for a TWA concentration or 0.2 L/min for 15 minutes for short term concentration. Samples are extracted with ethyl alcohol:water solution and analyzed by GC-FID following refrigerated overnight shipment.

#### *2.2.3 OSHA Method PV2118*

Superseded by methods 1012 and 1013, OSHA Method PV2118 [Shah 2003] is an air sampling method that uses two 150/75 mg silica gel sorbent tubes in series (SKC Cat. No. 226-10) at a recommended flow rate of 50 milliliters per minute (mL/min) for one hour. Unlike some other sampling and analytical methods for diacetyl, there is no requirement that samples be kept cold during shipping or storage. For analysis, each section of these tubes is placed in separate vials for desorption with a 95:5 ethyl alcohol:water solution and analyzed by GC-FID.

After issues with NIOSH Method 2557 were identified, a "modified" OSHA Method PV2118 was used by some practitioners in the field. This modified OSHA method used larger collection tubes (400/200 mg, SKC Cat. No. 226-10-03) with specially cleaned silica gel sorbent material which provided greater capacity to minimize breakthrough of contaminant to the backup tube. Sample analysis remained unchanged.

### *2.2.4 NIOSH Method 2557*

While no longer recommended for use, NIOSH initially developed Method 2557 [NIOSH 1994] for measuring diacetyl in air. It called for the collection of sample on a 150/75 mg carbon molecular sieve solid sorbent tube (Cat. No. 226-121, SKC Inc., Eighty Four, PA) at a calibrated air flow rate between 10 and 200 mL/min for a sample volume between 1 and 10 liters (L). The method specifies extraction with acetone:methanol (99:1) and analysis by gas chromatography using flame ionization detection (GC-FID) within 7 days of sampling.

Until 2007, this was the predominant air sampling and analytical method for diacetyl used in the field, but it is no longer recommended for use [Ashley et al. 2008a]. In 2007, field and chamber investigations indicated the method was adversely affected by humidity resulting in an underestimation of true diacetyl concentrations. To aid in the evaluation of sampling and analytical methods for diacetyl, a field comparison study between new and existing sampling collection methods was conducted [Ashley et al. 2008a]. Side-by-side field samples were collected and analyzed according to NIOSH Method 2557, OSHA Method PV2118, and a modified version of the OSHA method in flavoring manufacturing facilities (these other methods are discussed below). The results of this field work confirmed the tendency of the NIOSH method to underestimate the true concentration of diacetyl. However, no mathematical correlation was found in this data set which would produce an adjustment factor to allow for correction of results.

As a result, NIOSH researchers collaborated with scientists at the OSHA Salt Lake Technical Center laboratory to study the effects of humidity on measured diacetyl air concentrations using NIOSH Method 2557. This laboratory has chamber facilities for the generation of known diacetyl

air concentrations with the ability to control both temperature and relative humidity (RH). During each of the tests, they sampled air of a known diacetyl concentration in the chamber through an array of sampling tubes at calibrated flow rates. The test results indicated that diacetyl recoveries for NIOSH Method 2557 were affected by absolute humidity (AH), days to sample extraction, and diacetyl air concentration. The study resulted in the development of a correction procedure to adjust diacetyl concentrations previously measured using NIOSH Method 2557. The procedure is presented in Appendix 2 and is also published elsewhere [Cox-Ganser et al. 2011].

### *2.2.5 Other Air Sampling Method(s) in Development*

Because of current interest in exposure to flavoring compounds, new methods continue to be developed for their measurement. At this time, however, none of these methods are validated to any degree.

A method is being developed by NIOSH to measure alpha-dicarbonyl compounds (such as diacetyl, 2,3-pentanedione, etc.) in air via derivatization with 1,2-phenylenediamine. 1,2-Phenylenediamine is known to react with alpha-dicarbonyl compounds to form stable quinoxaline derivatives [Rodrigues et al. 1999]. In this method, air is sampled through a sorbent tube containing silica gel coated with 1,2-phenylenediamine at 0.1% by weight. In the lab, the tube is desorbed with ethanol and the solution analyzed by gas chromatography-nitrogen/phosphorus detection (GC-NPD). A potential advantage of this method is greater sampling volume and sampling time without the breakthrough experienced with bare silica gel tubes. Experiments to date indicate no breakthrough of diacetyl, 2,3-pentanedione, or 2,3-hexanedione after passage of 24L air at 80% RH. This suggests the ability to sample 8 hours without changing out sampling tubes. Another advantage is the high sensitivity of the NPD detection, which will enable measurement of alpha-dicarbonyl compounds below the proposed REL of 5 ppb.

A method for priority flavoring compounds [FEMA 2004] is being investigated that involves replacement of FID detection in OSHA Method 1013 with mass spectrometry operated in the selected ion monitoring mode. The advantage of using mass spectrometry detection instead of

FID detection is that it provides greater confidence in correct identification and accurate quantification in complex environments. Carbon-13-labelled diacetyl is being investigated as an internal standard. As OSHA Method 1013 has been validated only for diacetyl and acetoin, the method using mass spectrometry detection should validate the desorption procedure for additional analytes. Also, limits of detection and quantification should be determined.

Finally, a method for priority flavoring compounds [FEMA 2004] is being investigated that utilizes a novel sampler – the helium diffusion sampler [Entech Instruments Incorporated 2011]. The helium diffusion sampler collects a whole air sample for either short-term or full-shift sampling. The advantages of helium diffusion sampling are that no air sampling pump is required, there is no concern about incompatibility of the analyte with the sorbent, there is no concern about breakthrough of the sample components, and there is minimal sample handling in the laboratory. A portion of the collected air sample is analyzed by gas chromatography-mass spectrometry (GC-MS) in the selected ion monitoring mode. Although helium diffusion sampling will not support limits of detection achieved by thermal desorption – GC-MS because of the relatively small air volume sampled (20 mL), it may have adequate sensitivity to measure diacetyl at the proposed REL.

### *2.2.6 NIOSH Method 2549–Qualitative Determination of Volatile Organic Compounds*

To sample for diacetyl, 2,3-pentanedione, as well as a wide range of other flavoring VOCs, thermal desorption tubes provide a high degree of sensitivity and diversity. This technique is primarily qualitative, but specific compounds can be quantified if corresponding standards are analyzed along with the samples. It uses a multi-bed thermal desorption tube to maximize collection of VOCs. The thermal desorption tube is usually a stainless steel tube (configured for a specific thermal desorber) filled with various adsorbent beds of graphitized carbons, carbon molecular sieves, and/or Tenax. The adsorbents can be heated to high temperatures without breakdown or artifacts, so they can be cleaned and reused multiple times. The tubes are analyzed with a thermal desorption system interfaced to a gas chromatograph with mass selective detector [NIOSH 1994].

In addition to diacetyl, acetoin, 2,3-pentanedione, acetic acid, furfural, benzaldehyde, and numerous other flavoring compounds, this technique has also detected $C_5$-$C_{16}$ aliphatic hydrocarbons, benzene, and substituted benzenes, alcohols, some aldehydes, ketones, acetates, and fatty acids.

## 2.3 Sampling for Diacetyl and 2,3-Pentanedione in Airborne Dust and in Bulk Materials

### *2.3.1 Size-selective Air Sampling for Dust*

Although diacetyl is normally found in liquid form it can also be contained in a powder, either by encapsulation or adherence to a substrate. Air sampling for diacetyl–containing dust that may be generated during handling of powders can be achieved by active sampling methods. During the sample collection, some of the diacetyl may volatize, i.e., release from the dust particles (due to impaction, contact with moisture, diffusion, etc). The remaining dust can be analyzed via gravimetric means which will provide the mass of the dust as well as any adsorbed substances such as diacetyl. Measurement of the particles according to their size (e.g., total, inhalable, thoracic, or respirable) can help to understand where they may deposit in the respiratory tract. Several types of sampling devices are available (e.g., sampling cassettes, inhalable dust samplers, cyclones, impactors) to provide measurements of different size fractions of airborne dust. Sampling media and collection should be hydrophobic in nature (e.g., polyvinyl chloride). Filters should be measured gravimetrically before being subjected to analysis for diacetyl content. Validated methods such as NIOSH Method 0500 for total dust and Method 0600 for respirable dust [NIOSH 1994] are available for the collection and gravimetric analysis of airborne dust.

### *2.3.2 Sampling for Diacetyl and 2,3-Pentanedione in Airborne Dust*

A sampling and analytical method is being developed by NIOSH for quantitation of diacetyl, 2,3-pentanedione, and potentially other flavoring ingredients in dust. The method involves collecting airborne dust onto filters, extracting the filters with water, and taking a headspace sample above the aqueous solutions with a solid-phase microextraction (SPME) sampler. This sample will then be analyzed by gas chromatography-mass spectrometry, using the internal standard 4,4,4-$d_3$-2-

butanone, to quantitatively measure diacetyl or 2,3-pentanedione in the particulate air sample. This same procedure can be used to measure diacetyl, 2,3-pentanedione, and potentially other flavoring ingredients in samples of bulk powders.

Dissolution of a dust particle in water not only extracts diacetyl and/or 2,3-pentanedione adsorbed onto the particle surface, but breaks down the particle structure, releasing any of the chemical that is encapsulated within the dust particle.

Headspace SPME involves an equilibrium process in which the volatile analytes establish equilibria among the sample solution, the headspace above the solution, and the polymer-coated fused SPME fiber. The mechanism by which the analytes are extracted from the headspace is based on adsorption of the analytes onto the fiber. The fiber is then inserted directly into a GC injection port where the analytes are desorbed from the fiber and concentrated onto an analytical column. Because the entire sample collected on the fiber is introduced into the GC-MS instrument for analysis (as opposed to an aliquot of the sample for methods in which a solvent extract is used), lower detection limits can be achieved. In addition, mass spectrometric detection provides high sensitivity as well as high selectivity, and with the use of an internal standard, the method will also provide reliable reproducibility. Upon completion, this analytical method will be published in the NIOSH Manual of Analytical Methods and will be accessible via the NIOSH website.

### *2.3.3 Bulk Liquids and Solids*

#### 2.3.3.1 Sample collection

Although the review of material safety data sheets (MSDSs) may be helpful to identify flavor ingredients and potential exposures; they are not always comprehensive or specific. Collection and analysis of bulk flavoring materials can be useful to identify chemical ingredients and physical characteristics, establish flavoring ingredient concentrations in raw material or final products, and guide personal exposure assessment strategies. Prior to collecting bulk samples, sampling objectives should be clear, i.e., the purpose and uses of data from bulk sampling. Other important considerations include the flavoring materials to be sampled, the physical state of these

materials (powder, liquid, paste, or other), the need to sample opened or unopened containers, the sampling locations, the number of samples to collect, and the amount of bulk sample to collect (often determined by requirements of the laboratory analysis). When collecting bulk samples, they should be representative, derived from a variety of sampling locations, and obtained from multiple batches to capture batch-to-batch variability.

When sampling, it is important to collect and transport the sample in a manner that does not contaminate or cross-contaminate the bulk materials. Sampling technique should involve disposable gloves and only clean or unused sample containers that are compatible with the bulk material sampled. In general, leak-proof glass containers are ideal because they will not react with most chemicals, but polyethylene or polypropylene containers may also be appropriate. A convenient container is a 20-mL glass scintillation vial with polytetrafluoroethylene-lined cap. Each container should be clearly labeled with information about the bulk sample including: sample number, material sampled, company or product number, site of sampling, date of sample collection, laboratory or analytical sequence number and any hazards or precautions to be taken regarding the bulk sample.

After sampling, consideration should be given to the storage (refrigeration, exposure to light) and shipping of these bulk samples. Care should be taken to preserve the integrity of the samples and to follow established Department of Transportation shipping regulations. The containers should be labeled as required by Department of Transportation under their regulations, 49CFR Part 171-177. For most materials classified as "Flammable" or "Poisonous," amounts up to 1 quart can be shipped by any carrier. Most bulk dusts are not covered by Department of Transportation regulations. Specific restrictions and labeling requirements should be checked prior to shipping any samples. In the case of volatile bulk samples (and some air samples), samples should be shipped with “blue ice” or dry ice depending upon the advice from the accredited laboratory. Bulk materials should not be shipped together with air samples. The carrier should be contacted as there may be restrictions on the amount of dry ice they will accept in a package (usually 5 pounds or less is acceptable). Specific shipping box labels are usually required when dry ice is used.

#### 2.3.3.2 Measurement of Diacetyl or 2,3-Pentanedione Content of Bulk Powders

The analytical procedure being developed for airborne dust samples described in Section 2.3.2 will also be used for analysis of bulk powder samples.

## 2.4 Real-time Techniques for Diacetyl and Other Flavoring Ingredients

There are several analytical methods that provide real-time or near real-time measurements of volatile compounds in the air such as diacetyl and 2,3-pentanedione. These methods have the unique advantage of providing continuous exposure information over very short averaging periods that can be viewed as it is being generated during sampling or later if the instrument has data-logging capabilities. The abundance of measurement information provides valuable insight into variations in concentrations throughout the sampling period as well as the short-term concentration peaks that can possibly be associated with their sources. While real-time monitoring instruments generally lack sufficient sensitivity and specificity for monitoring REL levels of diacetyl and 2,3-pentanedione, they can be useful for screening, identifying appropriate work practices, and to find leaks and "hotspots." This information can be very useful in the development of exposure controls.

### *2.4.1 Photoionization Detectors*

Photoionization detectors (PIDs) have been used to monitor VOC air concentrations in various industrial work environments, including flavoring manufacturing facilities, and have become favored instruments for on-site monitoring because of ease of operation, reliability, versatility, cost, and response to a wide variety of substances. PID instruments measure the relative concentration of VOCs by passing the molecules of those compounds past an ultraviolet lamp that emits radiation over a narrow wavelength range in the ultraviolet region of the electromagnetic spectrum. Photons of ultraviolet radiation will form a molecular ion by removing an electron from orbit around that molecule, allowing for electronic detection of that ion, hence the name.

The energy of the radiation emitted by the lamp is inversely proportional to its wavelength, and common PID lamps produce energy in the range from approximately 8 to 12 eV. The amount of

work required to form a molecular ion by removing an electron from orbit, a property known as ionization potential, varies by compound but for many hydrocarbons is in the range from 7 to 11 eV. Since nitrogen, oxygen, and many of the minor components of air (i.e., water vapor, carbon monoxide, carbon dioxide, argon) have ionization potentials significantly higher than 12 eV, they are not ionized by the photons emitted from a PID. This property allows for the continuous monitoring of air to obtain an estimate of total hydrocarbon concentration.

PIDs respond to a broad range of VOCs and do not provide concentrations specific to any particular compound. They are often calibrated for isobutylene and can commonly detect total VOC concentrations from 1 to 2000 ppm. Modern PIDs can be programmed to measure the concentration of VOCs at fixed time intervals and store these data for subsequent download to a computer.

### *2.4.2 Infrared Analyzers*

The absorption of infrared (IR) radiation, while more commonly used as a qualitative tool, can also be used to quantify many substances by determination of response relative to known concentrations of that substance. Absorption of electromagnetic radiation in the IR region of the spectrum will produce transitions among vibrational and rotational states of the molecules absorbing that rotation. This absorption can only occur at wavelengths exactly matching the vibrational frequency of a chemical bond, and by selecting the proper analytical wavelength it is possible to obtain reasonable specificity in the compound being quantified.

Diacetyl can be detected and measured by using an IR gas analyzer such as the Thermo Electron MIRAN® "SapphIRe" (Thermo Fisher Scientific Inc., Waltham, MA), which is a portable direct-reading instrument that has the advantage of displaying real-time concentrations. The SapphIRe is a single beam IR spectrophotometer with a pathlength of 0.5 or 12.5 meters. It has a sample cell volume of 2.23 liters and a built-in pump that runs at approximately 14 liters per minute. Single sample analyses are updated every 0.5 seconds. The detector is available with pre-loaded factory calibrations for over 100 gases, but since diacetyl is not in this standard library it should be set up

for this application by the factory. The concentration range that can be measured is dependent on the compound in question. The high and low settings for the pathlength extend this range considerably.

The predecessor model, the Foxboro/Wilks MIRAN 1A, has adjustable wavelength and pathlength controls and can be calibrated for gases or vapors using the closed loop system available. There are many MIRAN 1A models still in use in the field. The best wavelength for measuring diacetyl is about 9 micrometers. Neither water nor carbon dioxide should interfere significantly at that wavelength. The minimum detectable concentration should be less than 0.5 ppm at the highest pathlength.

Fourier transform infrared (FTIR) spectroscopy can be used to analyze a sample of gaseous molecules for both chemical composition and for the concentration of individual chemical constituents. In this analysis, chemical functional groups absorb IR radiation at specific, unique frequencies producing a characteristic spectrum of absorbed versus transmitted radiation. From this spectrum, identification and quantitation of the gas is possible. FTIR analysis can produce real-time quantitation of flavoring chemicals in air providing chemical specific full-shift, partial-shift, and peak concentration measures although interferences can pose analytical difficulties in quantifying specific flavoring ingredients in complex environments with multiple organic chemicals present.

### *2.4.3 Photoacoustic Spectroscopy (Infrared Absorbance) Techniques*

Because the absorption of infrared radiation produces transitions among vibrational states of molecules, the application of rapid pulses of IR photons at the proper wavelength can be used to produce pressure variations in the air surrounding the molecules absorbing that radiation. Those pressure variations can be detected as sound waves, the amplitude of which is proportional to the concentration of the analyte of interest. Using IR radiation and measuring this resultant amplitude to quantify an analyte is the technique of photoacoustic spectroscopy.

Diacetyl has been detected by using the Innova photoacoustic infrared gas analyzers, which are direct-reading instruments that have the advantage of displaying real-time concentrations. Both personal and area concentrations were measured during tasks involving exposure to diacetyl in liquid and powder form and then 8-hour TWA exposures were calculated. The powder exposures only measured vapor released and did not include diacetyl adsorbed on the powder [Martyny et al. 2008].

Current available models of the photoacoustic analyzer are the 1314 and 1412, available from California Analytical Instruments, Inc., Orange, CA. The measurement system is based on photoacoustic infrared detection and provides the capability of measuring virtually any gas that absorbs in the infrared spectrum. Gas selectivity is achieved through the use of optical filters which provide both a means of detecting the gas of interest and compensating for interfering gases and water. Specifications on the unit indicate a dynamic range of 4 orders of magnitude and a repeatability of 1% of the measured value. The analyzer displays updated concentrations approximately every 30 seconds. The analyzer can be calibrated using diacetyl standards and can analyze diacetyl concentrations from the parts per billion range to hundreds or thousands of parts per million.

### 2.5 Industrial Hygiene Surveys and Exposure Assessments

Several investigations have been completed by NIOSH and others within the flavoring and food production industries. Exposure conditions vary widely, depending upon site-specific parameters and the processes employed. Many diacetyl samples have been collected to evaluate occupational exposures in the workplace and are described below. When pertinent data on absolute humidity and time to sample extraction were available, measurements obtained using NIOSH Method 2557 were subsequently corrected for the method's tendency to underestimate [Cox-Ganser et al. 2011]. An overview of diacetyl samples collected during multiple investigations is presented in Table 1.

**Table 1: Multiple Investigations of flavoring and food production industries**

| Study | Method | Location | Diacetyl concentration in ppm (Sample Type) | | |
|---|---|---|---|---|---|
| | | | **Arithmetic Mean** | **Geometric Mean** | **Range** |
| **Microwave Popcorn Plants** | | | | | |
| Company G (NIOSH 2006) | NMAM 2557 (corrected) | Mixing room<br>Packaging area<br>QC lab<br>Maintenance<br>Other areas | 57.2 (full shift TWA)<br>2.8 (full shift TWA)<br>0.8 (full shift TWA)<br>0.9 (full shift TWA)<br>>0.15 (full shift TWA) | | |
| Six companies (Kanwal 2006) | NMAM 2557 (corrected) | Packaging areas (area samples)<br>Packaging areas (personal samples)<br>Mixing rooms/areas (area samples)<br>Mixing rooms/areas (personal samples) | 0.019 – 3.0<br>0.023 – 1.16<br>0.63 – 57.2<br>0.035 – 1.33 | | |
| (White et al. 2010) | NMAM 2557 | Mixers<br><br>Non-mixers | 0.057 – 0.860 (full shift)<br>0.014 – 0.074 (full shift) | 0.029 – 0.231 (full shift)<br>0.001 – 0.018 (full shift) | 0.004 – 3.900 (full shift)<br>0.004 – 1.000 (full shift) |
| **Flavoring Production Plants** | | | | | |
| Company B, (NIOSH 2007) | NMAM 2557 (corrected) | Powdered flavoring production area | 2.73 (full shift TWA)<br>25.9 (partial shift) | | 204 (real-time peak) |
| Company C (NIOSH 2008) | NMAM 2557, OSHA PV2118 | Liquid flavoring production area<br>Powdered flavoring production area<br>Task-based (pouring diacetyl) | 0.46 (full shift TWA)<br>0.34 (full shift TWA) | | 11 (10 minute peak) |
| Company H (NIOSH 2008) | OSHA PV2118 | Liquid production room<br>Powder production | 0.26 (full shift TWA)<br>0.07 (full shift TWA) | | |
| | NMAM 2557 | Liquid production (personal samples)<br>Powder production (personal samples) | 0.10<br>0.05 | | |

| | | | | | |
|---|---|---|---|---|---|
| Company D<br>(NIOSH 2009) | OSHA<br>PV2118 | Starter distillate room<br>Starter distillate room (personal samples)<br>Spray dry room<br>Spray dry room (personal samples)<br>Flavors room<br>Flavors room (personal samples)<br>Spray dry room, task-based (moving diacetyl between containers)<br>Spray dry room, task-based (cleaning barrel with hose) | 1.06 (full shift TWA)<br>1.78 (full shift TWA)<br>1.07 (full shift TWA)<br>0.756 (full shift TWA)<br>0.171 (full shift TWA)<br>0.329 (full shift TWA) | | 90<br>(real-time peak)<br>18<br>(real-time peak) |
| Company I<br>(NIOSH 2011) | NMAM<br>2557 | Spray drying<br>Spray drying (personal samples)<br>Other production areas<br>Other production areas (personal samples) | | 0.169 (full shift TWA)<br>0.123 (full shift TWA)<br>0.375 (full shift TWA)<br>0.762 (full shift TWA) | |
| | OSHA<br>PV2118 | Spray drying<br>Spray drying (personal samples)<br>Coffee and tea area<br>Liquid compounding area (personal samples) | | 0.167 (full shift TWA)<br>0.182 (full shift TWA)<br>0.076 (full shift TWA)<br>1.90 (full shift TWA) | |
| (Martyny et al. 2008) | NMAM<br>2557 | All areas (personal samples) | 2.48 (1–3 hours) | | 0.01 – 60<br>(1–3 hours) |
| **Diacetyl Production** | | | | | |
| (van Rooy 2007) | | Task specific | | | 0.6 – 83<br>(real-time peaks) |
| **Food Production** | | | | | |
| Company M<br>(NIOSH 2007) | NMAM<br>2557<br>NMAM<br>2549 | All areas<br>Directly above heated popping oil | No diacetyl detected | | 0.14<br>(real-time peak) |
| Company E<br>(NIOSH 2009) | OSHA<br>PV2118,<br>OSHA<br>1013 | All areas | No diacetyl detected | | |
| Company F<br>(NIOSH 2008) | OSHA<br>PV2118 | All areas | Below limit of detection (>0.02) | | |

### *2.5.1 NIOSH Microwave Popcorn Production Exposure Assessments*

NIOSH conducted health hazard evaluations at six microwave popcorn plants from 2000 to 2003 [Kanwal et al. 2006]. In these facilities diacetyl-containing butter flavorings (liquids, pastes, or powders) were mixed with heated soybean oil in large heated mixing tanks. Salt and coloring were added to the flavoring mixture which was transferred to packaging lines and combined with kernel popcorn in microwaveable bags. Diacetyl concentrations were measured with NIOSH Method 2557 in multiple production locations using personal and area samples.

In the plants, 29 area and 17 personal samples were collected in mixing areas, and 67 area and 65 personal samples were collected in packaging areas. Humidity-corrected mean diacetyl air concentrations ranged from 0.63 to 57.2 ppm for area samples and from 0.035 to 1.33 ppm for personal samples in the mixing areas. In the packaging areas, mean concentrations ranged from 0.019 to 3.0 ppm for area samples and from 0.023 to 1.16 ppm for personal samples. In general, diacetyl concentrations were higher in the mixing rooms when the diacetyl-containing butter flavorings were heated.

### *2.5.2 Other Microwave Popcorn Production Exposure Assessments*

White et al. [White et al. 2010] conducted a comprehensive, repeated exposure monitoring campaign at four microwave popcorn plants. A total of 639 full shift diacetyl samples were collected during both the day and night shifts in multiple production areas including all employees who worked in the slurry (mixing) room. In that study 49% of 639 samples were below their limit of detection with the maximum measurement of 11.72 ppm after correction for humidity [White et al. 2010]. Overall, exposures were higher for mixers compared to non-mixers and were consistent with diacetyl concentrations observed during previous NIOSH investigations. Diacetyl exposures declined substantially for mixers after the installation of engineering controls.

### *2.5.3 NIOSH Flavoring Manufacturing Exposure Assessments*

In 1985, NIOSH conducted a health hazard evaluation at a plant in Indiana that produced flavorings for the baking industry [NIOSH 1985]. Case histories showed severe fixed obstructive lung disease among workers in a mixing room. Data from previous air monitoring indicated a high dust concentration in the personal breathing zone of a worker during a mixing operation. Diacetyl was on a list of ingredients commonly used at this facility but airborne measurements of diacetyl or other flavoring compounds were not made. Although the investigators were unable to identify specific etiology at that time, they concluded that workers' disease was most likely caused by some agent in the mixing room at the plant.

NIOSH conducted evaluations at three California flavoring manufacturing facilities where they measured exposures to diacetyl and other related compounds [NIOSH 2007a, 2008a, b, c]. The objectives of these surveys included identifying common work tasks, plant processes, and procedures, as well as characterizing potential occupational exposures within the flavoring industry. Most of the data collected were from the liquid and powder production areas, with some information also coming from spray drying, preproduction, quality assurance, administration, and research and development locations.

At one plant [NIOSH 2007a], the mean TWA diacetyl exposure, after NIOSH Method 2557 humidity-based correction, from full-shift air sampling in the powdered flavoring production area was 2.73 ppm. Measurements made with partial-shift air sampling during the production of butter and vanilla powdered flavorings showed a diacetyl exposure of 25.9 ppm. Workers' real-time diacetyl exposures measured with an FTIR monitor during the packaging of these powders were as high as 204 ppm. At another plant [NIOSH 2008b], mean TWA diacetyl air concentrations from full-shift air sampling using modified OSHA Method PV2118 in November 2006 (area and personal samples combined) were 0.46 ppm in liquid flavoring production and 0.34 ppm in powdered flavoring production. A task-based personal air sample measured a diacetyl air concentration of 11 ppm when a worker poured diacetyl from a 55-gallon drum into multiple 5-gallon containers over a 10-minute period. Using modified OSHA Method PV2118 for area air sampling at the other plant [NIOSH 2008a], the mean full-shift concentration of diacetyl in the

liquid production room was 0.26 ppm, while in the powder production room it was 0.07 ppm. For personal samples that were collected with NIOSH Method 2557 and not corrected for humidity and time to extraction, the mean concentrations in liquid production and powder production rooms were 0.10 ppm and 0.05 ppm. This work also indicated high variability in concentrations of volatile organic compounds (as measured with a photoionization detector) and dust (as measured with personal dust monitors) with time.

A health hazard evaluation was conducted at a facility in Wisconsin [NIOSH 2009b] that manufactured flavorings, modified dairy products, and bacterial additives. One of the flavoring products made at this plant was liquid starter distillate, a product of distillation of fermented milk stock, which contains about 4.5% diacetyl. Starter distillate and liquid diacetyl were used to make a variety of powdered (via spray drying processes) and liquid flavorings. NIOSH staff obtained 21 personal and 29 area air samples using modified OSHA Method PV2118 for diacetyl throughout the facility. They found the highest full-shift TWA concentrations in the starter distillate room (geometric mean of 1.78 ppm for personal and 1.06 ppm for area samples), followed by the spray dry room (0.756 and 1.07 ppm) and the flavors room (0.329 and 0.171 ppm). In the spray dry room, FTIR real-time measurements indicated peak diacetyl concentrations up to 90 ppm in the worker's breathing zone while dumping diacetyl from buckets to mixing tanks and while pumping diacetyl from a barrel into buckets. A peak exposure of about 18 ppm was measured in the breathing zone of a worker in the same room while cleaning a barrel with a water hose.

Company air sampling data were obtained during a health hazard evaluation at an Indiana flavorings plant that used many ingredients, including diacetyl and starter distillate, in the batch production of a variety of liquid and powdered flavorings [NIOSH 2011b]. Using NIOSH Method 2557 prior to the HHE request to measure diacetyl, they collected 22 samples. The geometric mean full-shift TWA diacetyl concentration in spray drying operations was 0.123 ppm for personal samples and 0.169 ppm for area samples, while in the other production areas, mean concentrations up to 0.762 ppm and 0.375 ppm were measured for personal and area samples, respectively. Because of the problems with NIOSH Method 2557, these results were likely underestimations of the true concentrations. No data on humidity or time from collection to

analysis was available, so no correction could be estimated. Subsequent measurements (45 personal and 71 area samples) by the company, after some control intervention, were collected using validated OSHA sampling Methods PV2118 and 1012 for diacetyl. In the spray drying operations, the geometric mean for full-shift diacetyl personal samples was 0.182 ppm, and for area samples it was 0.167 ppm. The highest mean concentration in the other production areas was 1.900 ppm for personal samples (liquid compounding area) and 0.076 ppm for area samples (coffee and tea area).

### *2.5.4 Other Flavoring Manufacturing Exposure Assessments*

In a study evaluating diacetyl exposures in 16 flavor manufacturing companies, Martyny et al. [Martyny et al. 2008] measured levels of that compound from the limit of detection (0.01 to 0.18 ppm depending on sample duration) to as high as 60 ppm. Using a protocol designed to obtain measurements during worst-case exposures by collecting samples only during processes in which diacetyl was being used, 181 personal and area samples were collected generally for 1 to 3 hours. Samples for diacetyl were collected and analyzed using NIOSH Method 2557 [NIOSH 1994] which was subsequently found to underestimate actual diacetyl concentrations. Without sampling environment absolute humidity information to make corrections, the results of this study likely underestimate true values.

Results indicated personal exposures during the selected work processes ranged from <0.01 to 60 ppm, with a mean of 2.48 ppm. Eight-hour TWA concentrations were calculated with the assumption that there was no exposure to diacetyl during the un-sampled 5 to 7 hours of a work shift. However real-time monitoring of airborne diacetyl vapor concentrations, made using a photoacoustic IR analyzer, indicated a background of approximately 2 ppm diacetyl according to Figure 1 of that paper.

Data indicated that concentrations varied by process, with powder compounding having the highest mean and median diacetyl exposures. Martyny also concluded, "Compared with the

microwave popcorn industry, there is wide variability in frequency and duration of use of diacetyl among flavor companies."

### *2.5.5 NIOSH Flavored Food Production Exposure Assessments*

NIOSH researchers conducted health hazard evaluations at food production facilities including a bakery mix production plant [NIOSH 2009b], a popcorn popping plant [NIOSH 2007b], and three office building cafeterias [NIOSH 2009c].

At the bakery mix production facility, workers combined liquid and powdered flavorings with flour, sugar, salt and other solid ingredients to produce baking mixes. For about a year up to July 2008, the company used a buttermilk flavoring that contained 15% to 20% diacetyl and then began using a reformulated buttermilk flavoring that contained less than 1% diacetyl. The reformulated flavoring also contained the diacetyl substitute 2,3-pentanedione. Diacetyl was detected in qualitative screening air samples using NIOSH Method 2549 during industrial hygiene air sampling by NIOSH investigators in late September 2008, but the concentrations were too low to be detected in any of the 9 personal or 10 area samples collected with the modified OSHA Method PV2118. Diacetyl was again not detectable in a second industrial hygiene survey in May 2009 when NIOSH investigators collected 13 personal and 11 area air samples using OSHA Method 1013; however, one personal sample showed an air concentration of 2,3-pentanedione of 91 ppb (parts per billion parts air), and a corresponding area sample showed an air concentration of 78 ppb. Nearly half of the samples detected 2,3-pentanedione in the air. Area air sampling using a method under development (see section 2.2.5 above) did not detect diacetyl in any of the 11 samples, but it measured 2,3-pentanedione in 7 samples, at concentrations ranging from 48 to 95 ppb. The sample that showed an air concentration of 95 ppb was obtained in the same area where a sample obtained with OSHA Method 1013 showed an air concentration of 78 ppb.

At the popcorn popping plant, neither the two personal nor the twelve area air samples found diacetyl concentrations above the minimum detectable concentration of 0.01 ppm using NIOSH Method 2557 during popcorn popping operations with butter-flavored oil. Diacetyl was detected

in all three thermal desorption tube samples from the room with semiquantitative analyses (NIOSH Method 2549) but with very low abundances. A one-minute real-time concentration of 0.14 ppm diacetyl was measured with an FTIR monitor directly above the heated popping oil.

At the three cafeterias, two of seven cooking oil products being used contained diacetyl. Neither diacetyl nor acetoin was found at or above the minimum detectable concentration (0.02 ppm) using the modified OSHA Method PV2118 to collect 20 personal and area air samples during grilling operations.

### *2.5.6 OSHA Site Visits Related to Diacetyl and Flavorings that Contain Diacetyl*

Between January 2008 and January 2010, an OSHA contractor measured diacetyl exposure to employees in a series of twelve industrial hygiene surveys at various facilities that use (11 facilities) or manufacture (1 facility) flavorings, including diacetyl [Eastern Research Group 2008a, b, c, d, 2009a, b, c, d, e, 2010a, b, c]. In the first 2 surveys, conducted in January, 2008 diacetyl was measured using OSHA Method PV2118. In the subsequent 10 surveys, OSHA Methods 1012 and 1013 were used. At all facilities, visual observation was made of engineering controls in place at the various operations evaluated.

The measured range of diacetyl concentrations are presented in Table 2 below, along with the type of facility and synopsis of controls. ERG returned to Company G in 2010 to remeasure airborne diacetyl concentrations following the installation of engineering controls and work-practice changes at that facility. In this follow-up study measurements were also made for 2,3-pentanedione in samples which contained diacetyl. 2,3-pentanedione was not detected.

**Table 2: Investigations of facilities using or producing diacetyl**

| FACILITY | PRODUCT | CONTROLS IN PLACE | MEASUREMENT RANGE |
|---|---|---|---|
| OSHA A<br>[ERG 2008a] | Coffee | dilution ventilation | ND – 54 ppb (TWA)<br>8 ppb (STEL) |
| OSHA B<br>[ERG 2008b] | Commercial bakery | dilution ventilation,<br>LEV, process containment | ND – 2703 ppb (TWA)<br>ND – 1012 ppb (area) |
| OSHA C<br>[ERG 2008c] | Seasoned snack product | ND* | |
| OSHA D<br>[ERG 2008d] | Baked snack food | Secondary heat ventilation for<br>heating and powder dumping | ND – 164 ppb (TWA)<br>ND – 139 ppb (STEL)<br>ND – 111 ppb (area) |
| OSHA E<br>[ERG 2009a] | Sauce production | Engineering controls for other<br>purposes, heat removal, etc. | ND – 5.3 ppb (TWA)<br>ND – 10.5 ppb (STEL)<br>ND – 2.4 ppb (area) |
| OSHA F<br>[ERG 2009b] | Low-calorie cracker | Canopy hoods in heated<br>Production process #1, dilution ventilation | ND – 195.7 ppb (TWA)<br>ND – 701.1 ppb (STEL)<br>ND – 13.1 ppb (area) |
| OSHA G<br>[ERG 2009c] | Buttered popcorn<br>Production | Before:<br>Heat Extraction Hoods<br>Dilution Ventilation<br><br>After:<br>Dilution ventilation in production area;<br>slot hood over tumbler<br>QC after work practice change | <br>24.8 – 71.2 ppb (TWA)<br>466.8 – 2298.7 ppb (STEL)<br>9.1 – 8660.2 ppb (area)<br><br><2.7 - <9.8 ppb (TWA)<br><10.4 – 98.8 ppb (STEL)<br>2.7 – 5.4 ppb (area) |
| OSHA H<br>[ERG 2009d] | Sour cream production | Exhaust ventilation for dust | ND – 32.4 ppb (TWA)<br>ND – 138.6 ppb (STEL)<br>ND – 4.6 ppb (area) |
| OSHA I<br>[ERG 2009e] | Ice cream | Controls for other mechanisms,<br>submerging water bath,<br>dilution ventilation | ND to 1.6 ppb (TWA)<br>ND (STEL)<br>ND (area) |

| | | | |
|---|---|---|---|
| OSHA J [ERG 2010a] | Cottage cheese | Dilution ventilation | ND – 55.3 ppb (TWA)<br>32.3 – 317 ppb (STEL)<br>1.3 – 32.4 ppb (area) |
| OSHA K [ERG 2010b] | Food flavor production | Dilution ventilation | ND – 2,990 ppb (TWA)<br>ND – 12,373.1 ppb (STEL)<br>29.1 – 381.5 (area) |
| OSHA L [ERG 2010c] | Retail bakery | Dilution with oven ventilation | ND – 50.4 ppb (TWA)<br>ND – 118.5 ppb (STEL)<br>ND – 30.9 ppb (area) |

*ND No limit of detection was reported.

# Chapter 3: Effects of Exposure in Workers

Information on the effects on workers' health of exposures to diacetyl and other flavoring chemicals comes from case reports and case series and from cross-sectional and longitudinal medical and environmental surveys conducted at several flavoring and food manufacturing plants (Table 3.1). NIOSH has conducted cross-sectional surveys as part of HHEs at six microwave popcorn plants where diacetyl-containing butter flavorings were used, at five flavoring manufacturing plants that used diacetyl and other flavoring chemicals to produce different flavors for use in food products such as microwave popcorn, at a plant that used flavorings (including buttermilk flavoring) to produce baking mixes, and at three restaurants where grill cooks used butter-flavored oil. Academic researchers have also conducted studies at other food and flavoring manufacturing plants and at a chemical plant in the Netherlands that produced diacetyl. Surveillance with a longitudinal component has been conducted by NIOSH in two HHEs, by the California Department of Public Health, and by academic researchers.

## 3.1 Obstructive Lung Disease Consistent with Constrictive Bronchiolitis Obliterans

The most significant health consideration for flavoring-exposed workers is the development of lung airways obstruction. Airways obstruction is characterized by a decreased forced expiratory volume in one second ($FEV_1$) and a decreased $FEV_1$ to forced vital capacity (FVC) ratio on spirometry testing and is associated with varying degrees of shortness of breath. The magnitude of decline in $FEV_1$ determines the severity of the disorder.

Table 3.1. Literature pertinent to flavoring health effects

| Reference | Study Type(s) and Industry | Contribution |
|---|---|---|
| Akpinar-Elci et al. [2004] | Case report, MICROWAVE POPCORN MANUFACTURING | Nine former workers at the index plant exhibited moderate to very severe fixed airways obstruction; 5 of the cases were on lung transplant lists. |
| CDC [2002] | Public health investigation, MICROWAVE POPCORN MANUFACTURING | Eight cases of suspected bronchiolitis obliterans among former workers at the index plant resulted in identification of excess risk for mixers compared to packaging workers, with no cases in workers outside of microwave production. |
| CDC [2007] | Case report and public health investigation, FLAVORING MANUFACTURING | Reports of bronchiolitis obliterans case-patients from two different companies resulted in a public health surveillance effort identifying 5 additional cases of fixed obstruction in young non-smoking workers who worked in flavor compounding or packaging. |
| Kanwal et al. [2006] | Summary of six plant surveys, MICROWAVE POPCORN MANUFACTURING | Synthesis of cross-sectional NIOSH surveys (four described below) identified an industry-wide risk of fixed airways obstruction in five plants, one of which had mixing-area diacetyl exposures as low as 0.02 ppm. Mixers with longer work histories and packaging workers near nonisolated tanks of oil and flavorings had higher prevalences of respiratory symptoms and airways obstruction. |
| Kanwal et al. [2011] | Intervention study, MICROWAVE POPCORN MANUFACTURING | Ventilation and isolation of flavor mixing resulted in one to three orders of magnitude reduction in diacetyl air concentrations in different areas. Workers with high past exposures had stable chest symptoms, decreased mucous membrane and skin symptoms, and higher prevalence of rapid declines in lung function than workers hired after interventions began. These new workers had lower symptom prevalences and higher lung function, demonstrating that intervention resulted in improved health for new workers. |
| Kim et al. [2010] | Cross-sectional industry-wide public health investigation, FLAVORING MANUFACTURING | California flavoring workers had 2.7 times more severe airways obstruction than the general population. Risk factors for the 18 cases with obstruction among 467 workers were younger age, Hispanic ethnicity, liquid and powder production work, greater company diacetyl usage, and having a coworker with obstruction. Severity of obstruction was related to tenure. At least 12 workers had probable occupational fixed airways obstruction. |
| Kreiss et al. [2002] | Cross-sectional survey, MICROWAVE POPCORN MANUFACTURING | The 117 current workers at the index plant had 2.6 times the expected rates of respiratory symptoms and 3.3 times the expected rate of airways obstruction, with never-smokers having 10.8 times the expected rate. Quartile of cumulative exposure to diacetyl was related to the frequency and extent of airways obstruction. |
| Lockey et al. [2009] | Longitudinal survey, MICROWAVE POPCORN MANUFACTURING | Study of 765 workers at four plants at two 6-month intervals showed significant $FEV_1$ declines in mixers, who also had an 8-fold risk of obstructive abnormality. Cumulative diacetyl exposure of 0.8 ppm-yr or more conferred an odds ratio of 9.2 for obstruction. |
| NIOSH [1985] | Cross-sectional survey, FLAVORING MANUFACTURING | Two young workers with no known risk factors developed severe, fixed obstructive lung disease suggestive of bronchiolitis obliterans within one year of employment. |
| NIOSH [2003] | Cross-sectional survey, MICROWAVE POPCORN MANUFACTURING | Elevated prevalence of airways obstruction when compared to national rates; all observed obstruction was fixed. |
| NIOSH [2004a] | Cross-sectional survey, MICROWAVE POPCORN MANUFACTURING | A study of 157 workers in a plant having one mixing room worker previously diagnosed with fixed obstructive lung disease consistent with bronchiolitis obliterans found abnormal lung function in six of 13 mixers—three had fixed airways obstruction and three had spirometric restriction. |

Table 3.1. Literature pertinent to flavoring health effects

| Reference | Study Type(s) and Industry | Contribution |
|---|---|---|
| NIOSH [2004b] | Cross-sectional survey, MICROWAVE POPCORN MANUFACTURING | A survey of 205 workers at a plant with a mixer previously diagnosed with severe fixed obstructive lung disease consistent with bronchiolitis obliterans, identified 3 of 12 mixers and 5 of 110 packaging workers with fixed airways obstruction and normal diffusing capacity. |
| NIOSH [2006] | Eight cross-sectional surveys, MICROWAVE POPCORN MANUFACTURING | Studies of 373 current workers over 2.75 years at the index plant determined that inhalation of butter flavoring chemicals is a risk for occupational disease. Comparisons of workers hired before and after implementation of exposure controls document declines in the prevalence of eye, nose, and throat irritation, although the prevalences of chest symptoms and spirometric abnormalities did not decline. Large drops in $FEV_1$ (> 300 ml or 10%) were observed in 4 of 9 mixers; one young mixer lost 2800 ml over 2.75 years. |
| NIOSH [2007a] | Two cross-sectional surveys, FLAVORING MANUFACTURING | Two former workers and one current worker who made powdered flavorings (of 18 with current or previous production experience) had severe fixed obstructive lung disease consistent with bronchiolitis obliterans. |
| NIOSH [2007b] | Cross-sectional survey, FOOD PRODUCTION | All 3 workers in a popcorn popping business developed symptoms of airways disease during their tenure; all were lifetime non-smokers. One of the workers had significant reversible airways obstruction with clinical evidence suggesting possible bronchiolitis obliterans in addition to asthma. |
| NIOSH [2008] | Two cross-sectional surveys, FLAVORING MANUFACTURING | One of 14 workers with production experience had severe fixed airways obstruction (subsequently confirmed as bronchiolitis obliterans), and an additional production worker developed mild fixed obstruction following the loss of 1 liter in $FEV_1$ during a 4.5 months screening interval. |
| NIOSH [2009b] | Cross-sectional survey, FOOD PRODUCTION | At a plant using a newly reformulated flavoring that included 2,3-pentanedione, no obstruction was identified in the 22 workers tested. Participants had higher than expected rates of shortness of breath, physician-diagnosed asthma, and a restrictive pattern on spirometry (4 cases ranging from mild to moderately severe), compared to U.S. adults. Some participants reported symptoms with a work-related pattern. |
| NIOSH [2009c] | Three cross-sectional surveys, FOOD PREPARATION | Studies of workers at three sites found higher prevalences of spirometric restriction, wheeze, dyspnea on exertion, nasal and eye irritation, and nasal allergies when compared to national rates. Cooks were 3-4 times more likely to report work-related respiratory symptoms. Fixed airways obstruction identified in two workers did not appear to be work-related. |
| NIOSH [2009d] | Cross-sectional survey, FLAVORING MANUFACTURING | This study of 34 workers found that bacterial products workers had higher prevalences of work-related eye symptoms and post-hire skin problems than flavoring workers; both groups reported lower respiratory symptoms related to the substances they handled at work. One worker was identified with fixed airways obstruction and 2 workers with restriction on spirometry. |
| van Rooy [2007] | Case reports from cross-sectional survey, DIACETYL MANUFACTURING | Four cases of bronchiolitis obliterans (previously attributed to COPD or asthma) were identified in screening of 103 process operators in a retrospective cohort of production plant workers. |
| van Rooy [2009] | Cross-sectional survey, DIACETYL MANUFACTURING | Excess respiratory symptoms and asthma indices occurred among 175 production plant workers compared to an internal reference group and a general population sample, with evidence of diacetyl exposure-response for $FEV_1$. |

Airways obstruction can occur in diseases such as smoking-related emphysema and chronic obstructive pulmonary disease (COPD) and in asthma. In emphysema, the airways obstruction is usually fixed (i.e., does not respond to bronchodilator medications), whereas in asthma, the airways obstruction is at least partially responsive to bronchodilators (reversible airways obstruction). Most workers who have developed obstructive lung disease while exposed to diacetyl and other flavoring chemicals have had fixed airways obstruction. Additional medical tests in severely affected workers have generally revealed findings consistent with the irreversible obstructive lung disease constrictive bronchiolitis obliterans (discussed in detail in section 3.1.2, Evidence from field studies). Serial lung function testing with spirometry indicates that affected workers can experience very rapid lung function declines.

Obstructive lung disease in workers exposed to diacetyl and other flavoring chemicals was first reported in workers in the microwave popcorn industry. Scientific publications that have reported on the occurrence and natural history of the illness have used different diagnostic terms including fixed obstructive lung disease [CDC 2002], popcorn worker's lung [Schachter 2002], flavorings-related lung disease [Kanwal et al. 2006; NIOSH 2009a], clinical bronchiolitis obliterans [Kreiss et al. 2002], bronchiolitis obliterans syndrome [Akpinar-Elci et al. 2004], and flavoring-related bronchiolitis obliterans [Kreiss 2007]. Of the few surgical lung biopsies that have been performed in affected workers, some have been interpreted as showing evidence of "constrictive bronchiolitis" or "obliterative bronchiolitis" [Akpinar-Elci et al. 2004; Kanwal 2008]. The term *fixed obstructive lung disease* is the least specific of the terms. The term *popcorn worker's lung* refers to the population of workers in which the disease was first identified. The term *flavorings-related lung disease* refers to the full spectrum of lung diseases that may be related to flavorings exposure and is not necessarily limited to obstructive conditions. The terms *flavoring-related bronchiolitis obliterans, constrictive bronchiolitis,* and *obliterative bronchiolitis* refer to pathologic findings of inflammation and fibrosis primarily involving the bronchioles, leading to irreversible airflow limitation. Terminology is complicated by the fact that, historically, researchers have applied the

term "bronchiolitis obliterans" to different distinct disorders that involve the bronchioles [King 2003; King and Kinder 2008]. The terms clinical bronchiolitis obliterans and bronchiolitis obliterans syndrome refer to those who are felt to suffer from this pathologic condition on clinical grounds, but have not undergone lung biopsy for pathological confirmation. Additional discussion regarding diagnostic terminology in relation to the different recognized forms of bronchiolitis is included in section 3.1.1.

### *3.1.1 Bronchiolar Disease and Terminology*

Bronchiolitis obliterans refers to disease processes that show some degree of inflammation, narrowing, or obliteration of small airways (bronchioles) in the lung [King 2003; King and Kinder 2008]. Historically, bronchiolitis obliterans has been classified into two groups: *proliferative* bronchiolitis obliterans and *constrictive* bronchiolitis obliterans [King 2003; King and Kinder 2008]. The disorder known as BOOP (bronchiolitis obliterans organizing pneumonia) is included in the proliferative group. BOOP is characterized pathologically by intraluminal polyps in the respiratory bronchioles, alveolar ducts, and alveolar spaces, accompanied by organizing pneumonia in the more distal parenchyma. Clinically it is usually associated with diffuse alveolar opacities on chest x-ray and computed tomography scan; pulmonary function testing may show a restrictive defect [King 2003; King and Kinder 2008]. BOOP was first described in 1985. Prior to this, many cases that matched the description for BOOP were classified as idiopathic bronchiolitis obliterans [King 2003; King and Kinder 2008]. The American Thoracic Society (ATS) and the European Respiratory Society (ERS) have recommended the use of the term cryptogenic organizing pneumonitis (COP) instead of BOOP to avoid confusion with the disease constrictive bronchiolitis obliterans [ATS and ERS 2002]. While proliferative bronchiolitis can be idiopathic (e.g., COP), known associations include collagen vascular diseases (e.g., systemic lupus erythematosus), acute infections (e.g., influenza, mycoplasma), organ transplantation (rare), and aspiration pneumonitis. Proliferative bronchiolitis is generally responsive to corticosteroid medications and is usually reversible [King and Kinder 2008].

Constrictive bronchiolitis (also referred to as constrictive bronchiolitis obliterans [ATS and ERS 2002], obliterative bronchiolitis [Schlesinger et al. 1998; Visscher and Myers 2006], and bronchiolitis obliterans [King 2003; King and Kinder 2008]) is a rare disorder characterized by alterations in the walls of respiratory and membranous bronchioles that cause concentric narrowing or complete obliteration of the airway lumen, without involvement of the distal lung parenchyma by inflammation or organizing pneumonia [King 2003; King and Kinder 2008]. In affected individuals, pulmonary function tests show airways obstruction and hyperinflation [King and Kinder 2008]; chest x-rays may be normal or show hyperinflation, peripheral attenuation of the vascular markings, and nodular or reticular opacities [King 2003]. The predominant finding of constrictive bronchiolitis on high resolution computed tomography (HRCT) scan is heterogeneity of lung density due to mosaic perfusion and air trapping [King 2003; King and Kinder 2008]. Other findings of bronchiolitis on HRCT scan include centrilobular thickening, bronchial wall thickening, bronchiolar dilatation, and the tree-in-bud pattern. Cylindrical bronchiectasis is frequently associated with bronchiolitis obliterans; scans with both inspiratory and expiratory views are helpful because expiratory views are important in assessing air trapping [King 2003]. Identification of the constrictive bronchiolitis lesion on lung biopsy may be difficult due to its patchy distribution [Estenne et al. 2002; Schlesinger et al. 1998; Visscher and Myers 2006], often requiring step-sectioning and special staining to identify airway walls [King 2003; King and Kinder 2008]. In comparison to proliferative bronchiolitis, constrictive bronchiolitis is generally unresponsive to corticosteroid medications and usually progresses to more severe disease [King and Kinder 2008].

As mentioned previously and discussed in detail in the next section (3.1.2, Evidence from Field Studies), the medical evaluations of workers who have developed lung disease during exposure to diacetyl and other flavoring chemicals have generally revealed findings consistent with constrictive bronchiolitis obliterans. Because of concerns for patient welfare and the invasive nature and low sensitivity of lung biopsy for diagnosing constrictive bronchiolitis obliterans, most patients have been diagnosed based upon

clinical findings [King 2003; King and Kinder 2008; Schlesinger et al. 1998; Visscher and Myers 2006]. Despite the small number of lung biopsies conducted, findings consistent with constrictive bronchiolitis obliterans have been identified in multiple flavorings-exposed patients [Akpinar-Elci et al. 2004; NIOSH 2007a]. Patients exposed to sulfur mustard gas are another patient population where constrictive bronchiolitis has been diagnosed in a small subfraction of the patients while other patients are diagnosed using contemporary clinical criteria, including HRCT scans [Ghanei et al. 2004a; Ghanei et al. 2004b; Ghanei et al. 2008; Rowell et al. 2009]. Other known causes of constrictive bronchiolitis obliterans include uncontrolled inhalation exposures to ammonia, chlorine, phosgene, nitrogen dioxide and sulfur dioxide, collagen vascular diseases (especially rheumatoid arthritis), infections, and organ transplantation (bone marrow, heart-lung, lung) [King and Kinder 2008].

Because of the difficulty of identifying the lesions of constrictive bronchiolitis obliterans on lung biopsy, and because the disease occurs commonly after heart-lung and lung transplants, in 1993 a committee sponsored by the International Society for Heart and Lung Transplantation proposed a clinical description for the disease termed bronchiolitis obliterans syndrome (BOS). The syndrome refers to graft deterioration secondary to persistent airflow obstruction as defined by pulmonary function changes with or without histolopathology confirmation. Probable risk factors for BOS include acute graft rejection and cytomegalovirus pneumonitis [Estenne et al. 2002]. The term BOS has also been used in cases of constrictive bronchiolitis obliterans resulting from chemical injury and diagnosed using clinical criteria with or without biopsy [Akpinar-Elci et al. 2004; Ghanei et al. 2004a].

Because the terminology used in the peer-reviewed literature of flavorings-exposed workers has included several different accepted and frequently interchanged diagnostic terms, and indeed may have been influenced by the peer-review process itself, this criteria document provides the terms used in the cited papers and includes the criteria used in the patient evaluations.

### *3.1.2 Evidence from Field Studies*

NIOSH first learned of the potential risk of constrictive bronchiolitis obliterans in microwave popcorn workers exposed to butter flavorings in August 2000 when they were asked by the Missouri Department of Health and Senior Services for technical assistance in investigating the occurrence of this illness in eight former workers (index cases) of a microwave popcorn plant (index plant). NIOSH reviewed medical records for these workers and in November 2000 conducted a medical survey of current and former workers of this plant [CDC 2002]. Survey results and medical records review for the eight index cases and a current worker with lung disease showed several findings consistent with constrictive bronchiolitis obliterans: all cases had moderate to very severe airways obstruction ($FEV_1$s between 14.9% and 58.4% predicted), fixed in most cases; six of seven cases tested had increased residual volume consistent with air trapping; diffusing capacity for carbon monoxide ($DL_{CO}$) was normal initially in five of seven cases tested; all cases had chest x-rays that were normal or showed hyperinflation; all eight cases that had HRCT scans showed marked bronchial wall thickening and mosaic attenuation with air trapping (five cases also showed mild cylindrical bronchiectasis); and in two of three cases that underwent lung biopsy the reviewing pathologist reported findings that supported or were consistent with a diagnosis of bronchiolitis obliterans [Akpinar-Elci et al. 2004]. These nine workers had developed a dry persistent cough, shortness of breath on exertion, and wheezing after a median of 1.5 years of employment. At the time of symptom onset, five of the workers had been working in the room where butter flavorings, salt, and colorings are combined with heated soybean oil; the other four workers had been working in the adjacent room where the oil and flavoring mixture is combined with kernel popcorn in microwavable bags (packaging area). None of these workers was initially diagnosed by their personal physicians as having bronchiolitis obliterans. Initial diagnoses received by these workers included pneumonia, asthma, emphysema, bronchitis, COPD, hay fever, and sinusitis. Five of the workers had minimal smoking history. All nine workers had been prescribed oral corticosteroids, but none had improvement in lung function. Five of the workers had been placed on lung transplant waiting lists by their personal physicians [Akpinar-Elci et al. 2004].

### 3.1.2.1 Index plant lung function testing

The NIOSH medical survey at the index microwave popcorn plant in November 2000 included lung function testing with spirometry and $DL_{CO}$, chest x-rays, and a questionnaire [Kreiss et al. 2002; NIOSH 2006]. NIOSH compared the prevalences of respiratory symptoms, self-reported physician-diagnosed asthma and chronic bronchitis, and airways obstruction on spirometry to data from the Third National Health and Nutrition Examination Survey (NHANES III) [CDC 1996]. Of 135 current workers, 117 (87%) completed the questionnaire; 97 (83%) of the survey participants worked in the microwave popcorn production areas of the plant; the remaining 20 survey participants worked in areas where butter flavorings were not used (i.e., plain kernel popcorn packaging, offices, warehouse, outside receiving). The prevalences of respiratory and systemic symptoms, mucous membrane irritation, and skin irritation were higher among workers in microwave popcorn production areas than in other areas. Among all survey participants, the prevalences of chronic cough and shortness of breath when hurrying on level ground or walking up a slight hill were 2.6 times higher than expected; the prevalence of wheezing was three times higher than expected. The prevalences of self-reported physician-diagnosed asthma and chronic bronchitis were 1.8 and 2.1 times higher than expected, respectively. Of the 116 workers who underwent spirometry, 21 had airways obstruction, 3.3 times higher than expected; airways obstruction in nonsmokers was 10.8 times higher than expected; only two workers with airways obstruction had a significant response to administered bronchodilator. Five of six workers in the quality control (QC) laboratory had airways obstruction; these workers popped up to 100 bags of microwave popcorn in microwave ovens per worker per 8-hour work shift. Of the 115 survey participants who had an x-ray, 111 had no abnormalities, two had evidence of emphysema, one had saber-sheath tracheal narrowing attributable to COPD or tracheal stenosis, and one had focal upper-zone scarring and atelectasis at the left lung base. $DL_{CO}$ was normal in 96 of 103 workers tested, including all but one of those with airways obstruction.

#### 3.1.2.2 Index plant environmental survey

In addition to the cross-sectional medical survey, NIOSH conducted a detailed environmental survey at the index microwave popcorn plant in November 2000 [Kanwal et al. 2011; NIOSH 2006]. The predominant VOC in the air of the plant was the butter flavoring chemical diacetyl. All measurements above detectable limits (except where noted otherwise below) were subsequently corrected for underestimation inherent to NIOSH Method 2557 related to absolute humidity and days to extraction [Cox-Ganser et al. 2011]. The mixing room had the highest mean air concentration of diacetyl (57.2 ppm); the next-highest mean air concentration of diacetyl was in the packaging area for machine operators (2.8 ppm). The mean air concentration of diacetyl in the QC laboratory was 0.8 ppm, and for maintenance was 0.9 ppm. Mean diacetyl air concentrations in other plant areas were less than 0.15 ppm. These area-specific diacetyl concentrations and work history data provided by workers on the medical survey questionnaire were used to calculate estimated cumulative exposure to diacetyl for each survey participant. When survey participants were grouped into quartiles of increasing estimated cumulative exposure to diacetyl (uncorrected for underestimation by NIOSH Method 2557), the prevalence of airways obstruction on spirometry was 10.3% in the lowest two exposure quartiles and 24.1% and 27.6% in the highest two exposure quartiles (statistically significant; *P* for trend = 0.03). Average lung function as indicated by the $FEV_1$ on spirometry was 4.5%, 8.9%, and 12.5% lower in the second, third, and fourth (highest) exposure quartiles, respectively, compared to the first (lowest) exposure quartile [Kreiss et al. 2002].

#### 3.1.2.3 Findings of index plant follow-up surveys

NIOSH conducted seven follow-up medical and environmental surveys at the index microwave popcorn plant from 2001 to 2003 [Kanwal et al. 2011; NIOSH 2006]. These surveys were conducted to follow worker symptoms and lung function over time as exposures decreased with the implementation of engineering controls. NIOSH recommended a respiratory protection program for mixing room workers to minimize their exposures while engineering controls were being implemented; this program was initiated at the time of the November 2000 NIOSH survey. Starting in February 2001, the

company began implementing several controls to decrease air concentrations of flavoring chemicals in the mixing room, the main source of air contaminants in the plant. An exhaust fan was installed in an outer wall of the mixing room to move contaminated air from this room to the outdoors and to maintain this room under negative air pressure relative to the rest of the plant. An air lock was installed at the entrance to the mixing room to further isolate the room from the rest of the plant. Local exhaust ventilation of the air space (headspace) above the contents of the heated flavoring tanks and the mixing tank (where flavorings are mixed into heated soybean oil) was accomplished via ducts connecting the tank lids to the wall exhaust fan. A pump was installed to facilitate closed transfer of heated butter flavorings into the mixing tank. In 2002, the company constructed and began using a new mixing room that was more isolated from the packaging area than the original mixing room. In the packaging area, additional general dilution ventilation was implemented in 2001 along with local exhaust ventilation for seven heated holding tanks (located on a mezzanine above the packaging lines) that contained soybean oil and butter flavoring mixtures (transferred via pipes from the mixing room). The entire mezzanine was walled off from the packaging area in 2003. Additional general dilution ventilation was also implemented in the QC laboratory in 2001. In 2003, all microwave ovens were eventually moved into a separate "popping room" adjacent to the QC laboratory with additional exhaust ventilation.

Compared to the mean diacetyl air concentrations NIOSH measured at the index microwave popcorn plant in November 2000, concentrations measured in November 2001 were approximately 96% lower in the mixing room, 85% lower in the microwave popcorn packaging machine operator area, and 51% lower in the QC laboratory. After the implementation of a new, more isolated mixing room in fall 2002, mean diacetyl air concentrations in the microwave popcorn packaging machine operator area further declined to less than quantifiable limits (~0.004 ppm) in January 2003 [Kanwal et al. 2011].

In their analyses of data from the eight NIOSH medical surveys at the index microwave popcorn plant from November 2000 to August 2003, NIOSH compared health outcomes in microwave popcorn production workers hired after the implementation of exposure controls to health outcomes in workers who had been working at the plant prior to the implementation of controls [Kanwal et al. 2011]. For these analyses, investigators classified workers according to their hire date as follows: "Group 1" consisted of workers who were already working at the plant at the time of the November 2000 survey (i.e., before exposure controls were implemented), and "Group 2" consisted of workers who started work at the plant after the November 2000 survey (i.e., after the company started implementing exposure controls and exposures had declined). Because of a high turnover rate among workers hired after the November 2000 survey, participation in more than one medical survey was much higher in Group 1 (100 of 146 [68%] Group 1 survey participants) than in Group 2 (86 of 227 [38%] Group 2 survey participants). Mean length of employment for Group 1 survey participants was approximately 6 years, compared to 6 months for Group 2 survey participants. For all Group 1 microwave popcorn production workers who participated in one of the last two surveys in February 2003 and August 2003 and in an earlier survey, NIOSH compared symptoms and lung function on their first survey to their last survey results. Most Group 2 workers who participated in more than one survey worked in the packaging area. Therefore, for all Group 2 packaging area workers who participated in more than one survey, investigators compared symptoms and lung function on their first survey to their last survey results. In Group 1, the only statistically significant change in symptom prevalence over time was a decline in reported eye, nose, or throat irritation. There were no statistically significant changes in the prevalence of airways obstruction or in mean percent predicted $FEV_1$. Based on data from workers' first surveys, packaging area workers in Group 2 had lower prevalences of respiratory symptoms and airways obstruction on spirometry, and mean percent predicted $FEV_1$ was significantly higher, compared to packaging area workers in Group 1 (all differences were statistically significant except for usual cough). There were no statistically significant changes in the prevalences of symptoms, airways obstruction, or

mean percent predicted $FEV_1$ from first to last survey in Group 2 packaging area workers [Kanwal et al. 2011].

#### 3.1.2.4 Other NIOSH HHEs

NIOSH conducted HHEs that included cross-sectional medical and environmental surveys at five other microwave popcorn plants from 2001 to 2003 [Kanwal 2003; Kanwal and Martin 2003; NIOSH 2003a, 2004a, b]. These plants and the index plant were similar with regard to some production and exposure characteristics; however, there were some important differences as well [Kanwal et al. 2006]. The similarities in production and exposure characteristics at the six microwave popcorn plants (including the index plant) evaluated by NIOSH were as follows:

(1) At each plant, one to three workers per work shift (i.e., mixers) measured butter flavorings (liquids, pastes, and powders) in open containers such as 5-gallon buckets and poured the flavoring into heated soybean oil in large (e.g., 500-gallon) heated mixing tanks, most of which had loose-fitting lids.
(2) Most mixers did not use respirators (only one mixer at one plant reported consistent use of a respirator with organic vapor cartridges during mixing tasks).
(3) Mixers added salt and coloring to the oil and flavoring mixture, which was then transferred by pipes to nearby packaging lines to be combined with kernel popcorn in microwaveable bags.
(4) Workers on the packaging lines operated the packaging machines and facilitated the placement of the finished product into cartons and boxes.

In most plants, QC workers popped product in microwave ovens that were usually located in a separate QC laboratory. Other workers were located in warehouse and office areas. In separate areas of some plants, workers also packaged plain kernel popcorn in plastic bags without oil or flavorings. The six microwave popcorn plants differed in size as follows:

(1) Two small plants had fewer than 15 workers, one or two mixing tanks, and one packaging line.

(2) One medium-sized plant had approximately 50 workers, one mixing tank, three holding tanks for heated oil and butter flavoring mixtures, and three packaging lines.
(3) The three largest plants had over 100 workers, five or more tanks, and seven or more packaging lines.

In some plants, flavoring-mixing activities and tanks were in a separate room adjacent to the packaging area. In other plants, some or all tanks of heated oil and flavoring were adjacent to or were inadequately isolated from the packaging lines [Kanwal et al. 2006].

In addition to the workers with bronchiolitis obliterans at the index microwave popcorn plant, workers with fixed airways obstruction and air trapping on HRCT scans consistent with constrictive bronchiolitis obliterans were identified at four of the other five microwave popcorn plants where NIOSH conducted HHEs [Kanwal et al. 2006]. Including the index plant, the three largest plants and one of the small plants had affected mixers [Akpinar-Elci et al. 2004; Kanwal and Martin 2003; NIOSH 2004a, b]. Like the index plant, the medium-sized plant had affected packaging area workers. At both of these plants, packaging area workers worked near tanks of heated oil and butter flavorings [NIOSH 2003a, 2006]. The biopsies of three of the six workers who underwent lung biopsy at the medium-sized plant were reported by the reviewing pathologists as having findings consistent with bronchiolitis obliterans [Kanwal et al. 2006; NIOSH 2003a]. Compared to mean diacetyl air concentrations measured at the index microwave popcorn plant, mean corrected diacetyl air concentrations at the other five microwave popcorn plants were lower: 0.02 to 0.83 ppm in the packaging areas and 0.63 to 1.54 ppm in the mixing rooms/areas [Kanwal et al. 2006].

NIOSH conducted analyses of aggregated data from the medical surveys conducted at the six microwave popcorn plants [Kanwal et al. 2006]. (Only the data from the first survey at the index microwave popcorn plant was aggregated with the data from the surveys at the other plants.) Compared to workers who had never worked as mixers, workers who had at least one day of experience mixing butter flavorings into heated soybean oil had statistically significant ($P < 0.05$) higher prevalences of respiratory symptoms and a

statistically significant lower mean percent predicted $FEV_1$. Compared to mixers with 12 months or less experience, mixers with more than 12 months experience had higher prevalences of respiratory symptoms (shortness of breath was statistically significant) and airways obstruction on spirometry. Mean percent predicted $FEV_1$ was 82% in mixers with more than 12 months experience compared to 95% in mixers with 12 months or less experience ($P = 0.004$). The same pattern of higher prevalences of respiratory symptoms and worse lung function in ever mixers (who had ever worked at least one day mixing flavorings in oil) and in mixers with more than 12 months experience was still evident after index plant data were excluded from the analyses [Kanwal et al. 2006]. Compared to packaging area workers at plants where tanks of heated oil and butter flavorings were isolated from the packaging lines, packaging area workers at plants where tanks were adjacent to or inadequately isolated from the packaging lines had higher prevalences of respiratory symptoms and airways obstruction on spirometry and lower mean percent predicted $FEV_1$ (29% vs. 10% for wheezing, $P = 0.001$; 14% vs. 5% for airways obstruction, $P = 0.06$; $P > 0.05$ for all other comparisons). Of 27 packaging area workers with airways obstruction at plants where tanks were adjacent to or inadequately isolated from the packaging lines, 21 of 23 who were administered a bronchodilator had fixed airways obstruction. After excluding index plant data from the analyses, packaging area workers in plants where tanks were adjacent to or inadequately isolated from the packaging lines still had higher prevalences of airways obstruction (11.5% vs. 5.5%; not statistically significant) and wheezing (25% vs. 10.7%, $P = 0.01$) compared to packaging area workers at plants where tanks were isolated. The prevalences of other respiratory symptoms were similar in both groups [Kanwal et al. 2006].

#### 3.1.2.5 Results of private surveys

A large food company hired private consultants to conduct medical and environmental surveys at the company's four microwave popcorn plants [Lockey et al. 2009; White et al. 2010]. (One of the company's plants was among the six microwave popcorn plants evaluated by NIOSH. A mixer at this plant had developed severe airways obstruction and other findings consistent with constrictive bronchiolitis obliterans.) The investigators

conducted spirometry tests three times at each plant from February 2005 through January 2006. During this time, 765 full-time employees worked at the four plants. Four workers were not tested because of significant cardiovascular disease or pneumonia, and four had unusable tests. The investigators excluded from subsequent analyses the test results of 11 office workers and 21 workers with a history of asthma that began prior to employment and who were taking asthma medications. The investigators classified workers into five groups for data analyses: (1) non-mixers (i.e., workers in the packaging line area, warehouse, or shipping/receiving areas), (2) mixers with mixing experience before the company implemented mandatory use of powered air-purifying respirators (PAPRs) for mixers in April 2003, (3) mixers who only had mixing experience after implementation of mandatory use of PAPRs, (4) mechanics and supervisors who spent more than 30 minutes per month in the mixing room, and (5) quality assurance workers who popped approximately 50 bags of microwave popcorn per day. The investigators identified the following statistically significant associations from their data analyses:

(1) Work as a mixer before the implementation of mandatory PAPR use was associated with a decrease in the $FEV_1$ percent of predicted of 6.1% for non-Asian males and 11.8% for Asian males, in comparison to employees with no mixing room or quality assurance employment ($P$=0.03 and $P$=0.02, respectively).

(2) Having a cumulative diacetyl exposure greater than or equal to 0.8 ppm-years was associated with a decrease in the $FEV_1$ percent of predicted of 10.3% for non-Asian and 12.7% for Asian males, compared to having a cumulative diacetyl exposure less than 0.8 ppm-years.

(3) Among non-Asian males, work as mixer before the implementation of mandatory PAPR use was associated with an eight-fold increased risk of airways obstruction (95% confidence interval [CI] 2.26–29.24), and work as a mixer after the implementation of mandatory PAPR use was associated with a 5.7-fold increased risk of airways obstruction (95% CI 1.23–26.24).

(4) Having a cumulative diacetyl exposure greater than or equal to 0.8 ppm-years was associated with airways obstruction (odds ratio 9.2, 95% CI 2.29–36.75).

To assess for evidence of rapid lung function decline, the investigators identified workers with a progressive increase or decrease in $FEV_1$ of greater than 8% or 330 milliliters (mL) over 12 months among workers who participated in all three spirometry tests [Lockey et al. 2009]. They found no association between current diacetyl exposure (less than 0.05 ppm or greater than/equal to 0.05 ppm) and a short-term persistent increase or decrease in $FEV_1$, adjusted for pack-years of smoking and body mass index. Of 39 mixers with mixing experience before the implementation of mandatory PAPR use, five had airways obstruction. Three of the five had bronchodilator administered, and all three had a bronchodilator response. Three of the five had HRCT scans; two of the scans showed air trapping on the expiratory view. The investigators concluded that, "The contribution of exposure to butter flavouring with diacetyl to these clinical findings is uncertain." Three mixers who began mixing after the implementation of mandatory PAPR use were found to have airways obstruction. Preplacement spirometry was not available for these individuals. One of the three workers had pre-existing asthma, and the other two had long smoking histories (24 and 63 pack-years, respectively). The investigators concluded that the airways obstruction in these three individuals was likely due to asthma and smoking but could not rule out the possibility that short-term exposure to diacetyl contributed to the airways obstruction when respirators had not been used 100% of the time.

#### 3.1.2.6 Field studies at flavoring manufacturing plants

Workers at several flavoring manufacturing plants have developed severe fixed airways obstruction and other findings consistent with constrictive bronchiolitis obliterans [Kanwal 2008]. The first known publically available report of bronchiolitis obliterans in flavoring manufacturing workers is a 1986 report of a NIOSH HHE at a plant that manufactured flavors for the baking industry [NIOSH 1985]. At this plant, two young previously healthy male employees (28 and 30 years old; nonsmokers) who prepared batches of flavorings developed severe fixed obstructive lung disease within 7 months of employment. Each worker developed progressive shortness of breath on exertion and nonproductive cough 4 to 5 months after starting work. Pulmonary function testing

within 1 to 2 months of symptom onset revealed an $FEV_1$ of 1.2 and 0.7 liters, respectively, in the two workers. The NIOSH reported that one worker had a "mild" response to bronchodilators and the other had a "minimal" response. Neither worker showed significant improvement in lung function within 1 to 2 years after they stopped working at the plant. Diffusing capacity was initially normal in both workers, and chest x-rays were normal or showed hyperinflation. The NIOSH concluded that, even without pathological confirmation, the clinical picture was more compatible with bronchiolitis obliterans than with emphysema. One of the two workers was relocated to work in the loading dock but eventually left the job 11 months after starting work at the plant because of shortness of breath. The other worker left the job when he was identified with severe fixed airways obstruction 5 months after starting work at the plant. Two current mixers with 5 to 6 years experience were asymptomatic and had normal lung function on spirometry. Two other former mixers (36 and 38 years old) had asymptomatic airways obstruction on spirometry. One had moderately severe airways obstruction and a normal chest x-ray; the other had mild airways obstruction, normal $DL_{CO}$, and a normal chest x-ray. Both were former smokers.

At the time of the NIOSH HHE, mixers produced flavors by mixing liquid flavor ingredients into dextrose and corn starch powder in large blenders (two "Day mixers (ribbon blenders)" with capacities of 300 and 500 pounds, and a 1,500-pound capacity Littleford Mixer) [NIOSH 1985]. Workers used approximately 200 FDA-approved flavor ingredients to produce different flavors. A list of commonly used ingredients at this plant included diacetyl. A supplied-air respirator system had been installed several months before the first worker to develop severe fixed airways obstruction had started work. Management had required workers to wear respirators when weighing or adding the flavors or base ingredients to the mixers. However, workers did not always wear respirators during clean-up activities where exposure to powdered flavors was possible. NIOSH concluded that it was probable that some agent in the mixing room produced severe fixed obstructive lung disease in two employees. They did not identify a specific etiologic agent, but suspected an airborne agent because the lung was the only affected

organ and because air sampling by the Indiana Division of Labor had revealed high dust exposures. (The Indiana Division of Labor collected 20-minute air samples that showed dust air concentrations of 20 $mg/m^3$ in an employee's breathing zone and 2.5 $mg/m^3$ inside the hood of an employee's supplied-air respirator.) NIOSH analyzed bulk ingredient samples for levels of proteolytic enzymes and endotoxin. They did not identify proteolytic activity in any of the samples; endotoxin levels were "below levels seen in other workplaces where endotoxin has been associated with large decrements in $FEV_1$" [NIOSH 1985]. Air sampling for specific flavoring chemicals was not conducted.

In 2007, the California Department of Public Health reported that seven flavoring manufacturing workers from four California plants had severe fixed airways obstruction [CDC 2007]. NIOSH conducted HHEs that included cross-sectional medical and environmental surveys at two of these plants [NIOSH 2007a, 2008b]. One of these HHEs was conducted at a company that produces liquid and powdered flavorings; powdered flavorings are produced by combining liquid flavoring ingredients such as diacetyl with powder ingredients in ribbon blenders. Out of a workforce of 36 at the time of the NIOSH HHE, 12 worked in the flavoring production room. Before July 2006, management provided production workers with 3M® N-95 filtering-facepiece respirators for voluntary use. In 2005, a 42-year-old production worker (who had worked for 7 years primarily making powdered flavorings) developed cough and progressive shortness of breath. Medical tests conducted by this worker's personal physicians revealed the following: fixed airways obstruction with an $FEV_1$ of 0.55 liters (18% of predicted) on spirometry; an HRCT scan of the chest that showed small areas of patchy ground-glass opacities in the lungs; a follow-up CT scan that revealed a small amount of scarring in the right lower lobe and lingula (part of the left lung) and resolution of the ground-glass opacities; and an open lung biopsy that was interpreted as showing peribronchial fibrosis and some granulomas. An occupational pulmonary medicine physician who evaluated this worker favored a diagnosis of bronchiolitis obliterans over hypersensitivity pneumonitis. This worker stopped working at the plant in December 2005 because of severe cough and shortness of breath on exertion. In the July 2006 NIOSH medical

survey, spirometry testing in this worker again showed severe fixed airways obstruction ($FEV_1$ of 0.54 liters; 21% of predicted). Another former worker and a current worker who had worked in powdered flavoring production also had severe fixed airways obstruction on NIOSH spirometry tests. The $FEV_1$ was 1.11 liters (32% of predicted) for the former worker and 0.78 liters (23% of predicted) for the current worker. The current worker with severe airways obstruction reported a past history of asthma, but he said that he was asymptomatic when he began working at the plant. He reported the onset of difficulty breathing within 2 weeks of starting work in powdered flavoring production. He had been relocated to the warehouse just before the NIOSH HHE because of severe shortness of breath on exertion. An open lung biopsy was interpreted by the reviewing pathologist as showing bronchiolitis obliterans. An additional current production worker was found to have mild restriction on spirometry; the rest of the medical survey participants (31 of 36 current workers and three former workers) had normal spirometry tests [NIOSH 2007a].

NIOSH conducted an HHE at a second flavoring manufacturer over several visits to the plant from October 2006 to July 2007 [NIOSH 2008b]. This company produces liquid and powdered flavorings (encapsulated and nonencapsulated powders) and colors. Nonencapsulated powdered flavorings are produced by combining liquid flavoring ingredients such as diacetyl with powder ingredients in ribbon blenders. Encapsulated powdered flavorings are produced by drying a slurry (a mixture of powdered and liquid ingredients) in a spray dryer. With encapsulated powder flavors, volatile flavor ingredients such as diacetyl are enclosed within an encapsulant material to decrease volatility. Out of a workforce of 47 at the time of the NIOSH HHE, 12 were production workers. Forty-one workers participated in the first NIOSH medical survey conducted from October 30 to November 1, 2006. Of 41 workers tested, 3 had abnormal spirometry: a laboratory/QC worker had mild restriction, a flavoring production worker had borderline obstruction, and a worker in the warehouse with several years of experience in flavoring production had severe fixed airways obstruction. This last worker had started working at the plant in powdered flavoring production in 1995 at age 26. He used an N95 filtering facepiece respirator from 1995 to 1999 and then started using a full-face,

negative-pressure, air-purifying respirator; he was not fit-tested for either respirator. Because of respiratory symptoms, he was reassigned to liquid flavoring production in 2000. In April 2006, he was reassigned to the warehouse. His personal physician diagnosed chronic rhinitis in 2003 and acute bronchitis in 2004. A spirometry test in March 2005 showed severe fixed airways obstruction ($FEV_1$ 20% of predicted). In May 2005, a pulmonologist diagnosed bronchiectasis of unknown etiology based on HRCT scan of the chest. The worker was hospitalized twice for his lung condition. NIOSH spirometry testing in October 2006 showed severe fixed airways obstruction ($FEV_1$ 17.9% of predicted). On follow-up spirometry testing by NIOSH at the plant in March 2007 his $FEV_{1,}$ was 20.7% of predicted. The flavoring worker who had borderline airways obstruction on NIOSH testing in October 2006 was found to have mild fixed airways obstruction in March 2007; his $FEV_1$ had dropped approximately one liter (percent predicted $FEV_1$ declined from 86% to 64%).

#### 3.1.2.7 Lung disease in flavoring manufacturing workers

The California Department of Public Health provided information on a flavoring production worker who developed bronchiolitis obliterans while working at another California flavoring plant [California Department of Public Health 2007a; CDC 2007]. This worker primarily prepared powdered flavorings by pouring "diacetyl and other liquid ingredients through a hole on the blender lid." [California Department of Public Health 2007a]. He started working at the plant in October 2001 at the age of 27. Two years later he developed progressive shortness of breath on exertion, decreased exercise tolerance, intermittent wheezing, left-sided chest pain, and a productive cough. In November 2003, his physician prescribed antibiotics and bronchodilators for suspected bronchitis and allergic rhinitis. He stopped working in January 2004, but his shortness of breath continued to worsen. An HRCT scan of his chest showed cylindrical bronchiectasis in the lower lobes, with scattered peribronchial ground-glass opacities. Spirometry in April 2004 showed severe fixed airways obstruction ($FEV_1$ 28% of predicted). Lung volume measurements showed severe air trapping. Diffusing capacity was normal. A follow-up HRCT with inspiratory and expiratory views in October 2004

showed central peribronchiolar thickening with central airway dilatation and subtle areas of mosaic attenuation scattered throughout the lungs, predominantly in the right lower lobe [CDC 2007].

In 2006, the California Department of Industrial Relations, Division of Occupational Safety and Health (Cal/OSHA) and the California Department of Public Health (CDPH) developed a lung disease prevention program for workers of California flavoring manufacturing plants. In analyses of aggregated medical surveillance data (questionnaire and spirometry results) from 467 workers at 16 companies who had usable questionnaires and acceptable spirometry tests, 18 workers (3.9%) from six companies (with 315 participating workers) had airways obstruction [Kim et al. 2010]. This prevalence was similar to that expected in comparison to national data from NHANES III. However, the distribution by severity of obstruction was highly skewed, with six mild cases, seven moderate, one severe, and the remaining four very severe. The prevalence of severe and very severe obstruction combined was 2.7 times higher than expected overall (95% CI 1.2–6.4) and 15 times higher than expected in workers less than 40 years old (CI 5.1, 44.1). Sixteen obstructed cases worked in four companies using $\geq$ 800 pounds of diacetyl annually compared to two obstructed cases in companies using less diacetyl (prevalence of 5.3% versus 1.2%), for an OR of 4.5 (95% CI 1.03–19.9). The prevalence of obstruction in workers currently doing any production task was 4.5% compared to 2.0% in production support workers (laboratory technicians/scientists, quality control technicians, maintenance/repair workers, warehouse workers, and truck drivers) and 2.3% in office workers. Of the 18 workers with obstruction, 14 currently worked in production, two worked in production support (one had just moved from production because of dyspnea), one with previous production experience currently worked in the office, and one could not be classified. Tenure was statistically significantly higher in workers with moderate or worse obstruction than in workers with mild obstruction (1.5 versus 9.0 years; $P$=0.02). Half of the 18 workers with obstruction reported no chest symptoms (five of six workers with mild obstruction and four of seven with moderate obstruction). Of the 13 with documented postbronchodilator spirometry, 12 had fixed

obstruction (including four of four with severe or very severe obstruction). Of the 12 of 18 with obstruction who had medical evaluation results submitted to CDPH, eight were diagnosed by their physicians to have either bronchiolitis obliterans or fixed obstruction related to flavorings; all eight had moderate to very severe disease [Kim et al. 2010]. (Some of the cases included in this analysis of California flavoring worker surveillance data were presented above in the descriptions of two NIOSH HHEs at California flavoring plants.)

A cluster of cases of bronchiolitis obliterans among production workers at a flavoring manufacturing company was reported by Dr. James Lockey at the 2002 American Thoracic Society International Conference [Lockey et al. 2002]. After identification of an index case of bronchiolitis obliterans at this plant, a survey of the workforce identified an additional four workers with clinical findings consistent with bronchiolitis obliterans. All five workers with bronchiolitis obliterans had normal spirometry tests at the start of employment. These workers went on to develop moderate to severe fixed airways obstruction. For 4 to 5 years after cessation of exposure to flavoring chemicals, the affected workers had no further declines in their lung function.

A 2007 NIOSH HHE included a medical and environmental survey at a flavoring manufacturer in Wisconsin [NIOSH 2009d]. At the time of the HHE, this plant manufactured flavors, colors, and bacterial blends (used as silage inoculants and probiotics). One of the flavor products produced at this plant is starter distillate, a diacetyl-containing distillate of a milk stock produced from fermented dairy cultures. The diacetyl concentration in this distillate was 4.5%. Other flavor products made at this plant included powdered encapsulated starter distillates and other butter flavors produced by spray drying, and other liquid flavors. The NIOSH medical survey included a questionnaire, spirometry testing, and methacholine challenge testing (to identify airways hyperresponsiveness as occurs in asthma). Of 40 workers in production areas, the quality control laboratory, the warehouse, and in maintenance who were invited to participate in the medical survey, 34 agreed to participate. Of these 34 workers, 15 worked in jobs

where they could potentially be exposed to flavoring-related chemicals including diacetyl. Of 10 former workers who had worked in flavoring production areas and were invited to participate in the medical survey, three agreed to participate. Of the 15 current workers with jobs in which they could potentially be exposed to flavoring-related chemicals including diacetyl, one worker with a pre-employment history of asthma was found to have mild fixed airways obstruction mixed with restriction. NIOSH recommended that this worker pursue additional medical evaluation to look for further evidence of bronchiolitis obliterans or another illness; follow-up results were not available to NIOSH. In addition to the worker with mild fixed airways obstruction (mixed with restriction), two workers had restrictive abnormalities. Five of the 15 workers with potential exposures to flavoring-related chemicals reported having currently active physician-diagnosed asthma. All five were diagnosed with asthma before starting work at the plant; no workers reported post-hire recurrence of pre-existing asthma that had been inactive for 2 or more years prior to hire. Two of 11 workers with normal spirometry who underwent methacholine challenge testing were found to have airways hyperreactivity. Both of these workers had physician-diagnosed asthma before coming to work at the plant.

#### 3.1.2.8 Lung disease in diacetyl production workers

Lung disease consistent with bronchiolitis obliterans has been reported among workers of a plant in the Netherlands that produced diacetyl [van Rooy et al. 2007]. From 1960 through 2003 (when diacetyl production ceased), 206 employees had potentially been exposed to diacetyl at this plant. Of 196 workers still alive, 175 consented to participate in a medical survey conducted by Dutch investigators. The survey included a questionnaire, spirometry, and review of medical files of the Occupational Health Service. Workers with possible airways obstruction on screening spirometry were referred for additional medical evaluation including an HRCT scan with inspiratory and expiratory views. Of the 175 survey participants, 102 worked as "process operators." The other participants worked in other jobs such as the quality control laboratory, "technical service," management, research and development, and logistics. Four workers were found

to have fixed airways obstruction. One of these four workers (with a predicted $FEV_1$ of 72%) refused further evaluation. $FEV_1$ percent predicted in the other three workers (all process operators) ranged from 35% to 42%. All three workers had evidence of air trapping on HRCT scan expiratory views. One of these three workers underwent a thoracoscopic lung biopsy that did not show evidence of constrictive bronchiolitis. Two of these three workers were nonsmokers who had initially been diagnosed with COPD; the third worker (with a 14 pack-year smoking history) had initially been diagnosed with COPD and asthma. Two of these three workers developed shortness of breath on exertion within a year or two of starting work at the plant (at ages 45 and 39 years). The other worker developed shortness of breath at age 52, 14 years after starting work. A fourth worker (process operator; nonsmoker) with severe fixed airways obstruction and findings compatible with bronchiolitis obliterans on HRCT scan was identified among survey nonparticipants after the survey. During production of diacetyl, workers were also potentially exposed to acetoin, acetaldehyde, and acetic acid. The diacetyl plant was one of several in operation at the production site; all process workers also worked at other chemical plants at the production site. The investigators noted that "Among the gaseous chemicals identified in the plants, only ammonia and chlorine were of potential concern for bronchiolitis obliterans, but none of the cases reported having had significant exposure to these agents" [van Rooy et al. 2007].

The investigators who evaluated the workforce of the diacetyl-producing plant in the Netherlands compared respiratory symptom and asthma prevalence among male workers to data from the Dutch section of the European Community Respiratory Health Survey [van Rooy et al. 2009]. Compared to the Dutch European Community Respiratory Health Survey population, the diacetyl plant workforce had significantly higher prevalences of continuous trouble with breathing, daily cough, and asthma attacks. Compared to a minimally exposed internal comparison group, process operators (including the three with severe fixed airways obstruction and evidence of air trapping on HRCT scan expiratory views who were identified in the medical survey [van Rooy et al. 2007]) and quality control laboratory workers reported ever trouble with breathing significantly more

often. Operators also reported significantly more shortness of breath in the last year. Spirometry test results for the 149 Caucasian male diacetyl plant workers did not differ significantly from the Dutch European Community Respiratory Health Survey population after adjusting for smoking history. The investigators were not able to demonstrate an exposure-response relationship between relative cumulative exposure to diacetyl and $FEV_1$. However, they had previously demonstrated an average 292 mL decrement in $FEV_1$ in process operators in comparison to a minimally exposed internal reference group [van Rooy et al. 2007].

Available information on TWA and peak exposures to diacetyl in flavoring and diacetyl manufacturing plants where workers have developed bronchiolitis obliterans indicates that workers' exposures in these plants may have been similar to workers' exposures at microwave popcorn plants. At one flavoring plant [NIOSH 2007a], the mean TWA diacetyl exposure from full-shift air sampling in the powdered flavoring production area was 2.73 ppm. Measurements made with partial-shift air sampling during the production of butter and vanilla powdered flavorings showed a diacetyl exposure of 25.9 ppm. Workers' real-time diacetyl exposures during the packaging of these powders were as high as 204 ppm. At a second flavoring plant [NIOSH 2008b], mean TWA diacetyl air concentrations from full-shift air sampling in November 2006 (area and personal samples combined) were 0.46 ppm in liquid flavoring production and 0.34 ppm in powdered flavoring production. A task-based personal air sample measured a diacetyl air concentration of 11 ppm when a worker poured diacetyl from a 55-gallon drum into multiple 5-gallon containers over a 10-minute period. At the diacetyl production plant in the Netherlands where Dutch investigators identified four former workers with severe fixed airways obstruction and evidence of air trapping on HRCT scan expiratory views, task-specific diacetyl exposures ranged from 3 to 396 $mg/m^3$ (0.6 ppm to 83 ppm) during discharge of diacetyl from a reactor vessel into containers [van Rooy et al. 2007]. The measured diacetyl exposures at these three plants are comparable to exposures (adjusted for absolute humidity and days to extraction) measured at the six microwave popcorn plants evaluated by NIOSH. In the mixing room at the index microwave popcorn plant,

the mean TWA diacetyl air concentration from area samples in November 2000 was 57.2 ppm. At the three other microwave popcorn plants where mixers developed bronchiolitis obliterans, TWA diacetyl exposures from personal samples were 0.31 ppm, 0.69 ppm, and 1.33 ppm [Kanwal and Martin 2003; NIOSH 2004a, b]. Real-time measurements at one of these plants showed that a mixer's diacetyl exposures increased up to 80 ppm to 120 ppm when he added liquid butter flavorings to a mixing tank [NIOSH 2004a].

### 3.2 Rapid Lung Function Decline

Indirect and direct evidence shows that workers exposed to flavoring-related chemicals can experience rapid lung function decline. Indirect evidence comes from reviews of medical records and work histories of flavoring-exposed workers who developed bronchiolitis obliterans. In a case series summarizing the eight affected former workers and one additional current worker at the index microwave popcorn plant, the median length of employment prior to symptom onset was 1.5 years; the median duration of employment was 2 years [Akpinar-Elci et al. 2004]. At a company that manufactures flavors for the baking industry, two flavoring production workers developed respiratory symptoms and severe fixed airways obstruction within 7 months of starting work at the plant [NIOSH 1985]. Although these workers did not have baseline spirometry tests before they began working with flavorings, it is unlikely that their lung function was already significantly decreased when they started work. Production jobs such as preparing the oil and flavoring mixture for microwave popcorn production and mixing liquid and powder flavor ingredients in flavoring manufacture often require the worker to lift 50- to 100-pound containers. It is unlikely that workers could have performed such tasks if their lung function was already severely compromised when they started work. Some affected workers stopped working when they could no longer do the job due to severe shortness of breath on exertion, while others were relocated to less strenuous jobs [NIOSH 1985, 2007a, 2008b]. Severe airways obstruction as seen in constrictive bronchiolitis obliterans is rare in the general population. Data from NHANES III show that, among individuals less than 50 years old (including both smokers and never-

smokers), the prevalence of obstruction with an $FEV_1$ less than 40% of predicted is 0.1% (1 in 1000 people) [CDC 1996].

Direct evidence that workers exposed to flavoring-related chemicals can experience rapid lung function decline comes from exposed workers who have had serial spirometry tests. Normal average $FEV_1$ decline is about 30 ml/year, and percent predicted $FEV_1$ does not usually change in the absence of disease because the predicted value is age-corrected. Three of the affected former workers from the index microwave popcorn plant had declines in their $FEV_1$ percent of predicted of approximately 20% to 30% over approximately 2 years [Akpinar-Elci et al. 2004]. NIOSH evaluated data from the eight NIOSH medical surveys at the index microwave popcorn plant for evidence of rapid lung function decline [Kanwal et al. 2011]. They chose as the criterion for rapid decline a decrease in $FEV_1$ of 300 mL and/or 10% from a worker's initial (baseline) spirometry test to the worker's last spirometry test. This criterion was similar to a later study of spirometry surveillance data in coal miners in which the researchers concluded that "when healthy working males perform spirometry according to American Thoracic Society standards, a yearly decline in $FEV_1$ greater than 8% or 330 mL should not be considered normal…" [Wang and Petsonk 2004]. The more stringent criterion used by the investigators, who did not annualize declines, was chosen because of the potential severity of the irreversible health outcome and the high quality of the pulmonary function tests, which allows for a sensitive cutpoint. For their analysis of the data from the surveys at the index microwave popcorn plant, investigators excluded survey participants with fewer than three interpretable spirometry tests because interpretation of change over time based on only two tests is less reliable [Pellegrino et al. 2005].

Of the 88 survey participants who participated in three or more NIOSH medical surveys at the index microwave popcorn plant and had started working there prior to the implementation of exposure controls ("Group 1"), 19 (22%) had $FEV_1$ declines of greater than 300 mL and/or 10% from their first to their last spirometry test. Four of these 19 workers had worked at some point in the mixing room, including one worker who

experienced a 1300-milliliter decline from the first test in November 2000 to the next test 5 months later; the next spirometry test 4 months later showed an additional decline in $FEV_1$ of 600 mL, prompting the worker to stop working at the plant. This worker's $FEV_1$ continued to fall after leaving employment, with a total fall of 2800 mL over 2.75 years, representing a decline from 96% of predicted $FEV_1$ to 39% of predicted $FEV_1$. In comparison to survey participants who began working at the plant before the company started implementing exposure controls, only 3 (7%) of 41 survey participants with three or more spirometry tests who were hired after the company began implementing controls ("Group 2") had $FEV_1$ declines of greater than 300 mL and/or 10% from their first to their last spirometry test [Kanwal et al. 2011]. Of the 27 Group 1 workers who participated in all eight medical surveys, mean annualized decline in $FEV_1$ in the first year of follow-up was 144 mL per year. Annualized decline in the second year of follow-up fell to 40 mL per year as exposures were controlled, and the annualized decline fell to 22 mL per year in the third year of follow-up, a rate of decline consistent with normal aging-related lung function decline [Kreiss 2007].

NIOSH identified rapid lung function decline at a flavoring plant where a production worker had developed severe fixed airways obstruction [NIOSH 2008b]. Another flavoring production worker at this plant had borderline airways obstruction on his first spirometry test (defined as a normal $FEV_1$ with a $FEV_1$/FVC ratio below the lower limit of normal). This worker was found to have mild fixed airways obstruction on his second test 5 months later; his $FEV_1$ had declined approximately one liter in the 5 months between tests.

NIOSH found evidence of rapid lung function decline among flavoring production workers at a flavoring manufacturing company in Indiana [NIOSH 2011b]. Diacetyl was used nearly daily in the plant and was measured in many areas of the plant. In the course of an HHE at this facility, NIOSH reviewed results of spirometry tests obtained by the company on 112 production workers. Interpretable spirometry results were available for

106 current and former production workers. NIOSH compared the results of each worker's spirometry test to reference values based on U.S. population data on healthy nonsmokers from NHANES III [Hankinson et al. 1999]. The investigators calculated changes in $FEV_1$ over time for 70 workers with more than one spirometry test result. To assess abnormal excessive declines in $FEV_1$, they determined the average within-person variation in $FEV_1$ to be 5%. Using SPIROLA, a NIOSH freeware program that adjusts for data quality (within-person variation) and length of follow up [NIOSH 2010b], NIOSH found that 19% (13) of workers with serial spirometry had excessive decline in $FEV_1$ based on a 12.4% longitudinal decline supplemented by a reference decline of 30 ml/year. Five of the 13 still had spirometry values within the normal range despite their excessive declines. Workers currently working in areas with higher potential of flavorings exposure had a 7.0-fold odds of having excessive $FEV_1$ decline (95% CI 1.3–38.2) in comparison to workers who were not currently working in areas with higher potential for exposure. The areas with higher potential for exposure included dry blend, extract and distillation, liquid compounding, process flavors and spray dry areas. The workers in these areas had 2.8 times greater average annual declines in $FEV_1$ than workers in other areas. Historical measurements of diacetyl and other flavoring chemicals were insufficient to evaluate quantitative exposure-response relations. NIOSH also found a high prevalence of a restrictive pattern on spirometry tests in this workforce (see section 3.2).

The California Department of Public Health received serial spirometry test data for 416 flavor manufacturing workers administered from 2004 until early 2009, of whom 9.6% (40) had abnormal $FEV_1$ decline [Kreiss et al. 2011]. Abnormal $FEV_1$ decline rates (per person-month of follow up) were greater at companies using $\geq$800 lbs/year diacetyl than at companies using lesser amounts (7.3 versus 3.0 per 1000 person-months, $P$=0.01) and greater in companies previously shown to have 4-person clusters of spirometric obstruction than at companies with no or only one worker with obstruction. Using only high quality serial spirometry data on a subset of 289 workers, 21 (7.3%) had abnormal decline using the 4% within-individual variation that characterized this subset [NIOSH

2010]. Only one of the 21 had airways obstruction; this worker lost 23.9% (−980 mL) of his baseline $FEV_1$ over 25 months. The greatest annualized $FEV_1$ decline in the group with good quality data was −2534 mL/year (−1700 mL in 8 months), and the average annualized $FEV_1$ loss in this group was −85 mL/year. The mean $FEV_1$ change for workers in companies using ≥ 800 lbs/yr of diacetyl was −113.6 mL/yr compared to −51.6 mL/yr in companies using less diacetyl (*P*=0.06).

Other investigators have reported rapid declines in flavoring-exposed or diacetyl-exposed workers. The bronchiolitis obliterans syndrome cases identified in the Dutch diacetyl manufacturing plants had accelerated declines in $FEV_1$, with one case having an annualized decline of 175 mL/year from 1995 to 2003 [van Rooy et al. 2007]. In a microwave popcorn manufacturing cohort studied over 12 months, no relationship was demonstrated between current exposure level (dichotomized at 0.05 ppm) and an abnormal decrease in $FEV_1$ (found in 7% of workers), adjusted for pack-years of smoking and body mass index [Lockey et al. 2009].

### 3.3 Pulmonary Restriction

Among the former workers who developed bronchiolitis obliterans while working at the index microwave popcorn plant, lung function tests in one worker showed a reduced total lung capacity and reduced residual volume in addition to airways obstruction. These reduced lung volumes indicate that this worker had restrictive lung disease as well as airways obstruction [Akpinar-Elci et al. 2004].

NIOSH found a high prevalence of a restrictive pattern among production workers at a flavoring manufacturing plant in Indiana [NIOSH 2011b]. Among the 106 workers with interpretable spirometry test results obtained by the company, 30 (28%) had a restrictive pattern (22 with a mild abnormality, six with a moderate abnormality, one with a moderately severe abnormality, and one with a severe abnormality). In addition, three workers had obstructive abnormalities and one had a very severe mixed abnormality.

Combining all spirometric abnormalities with those with only excessive decline in $FEV_1$ in the subset of workers with serial abnormalities, 39 (37%) workers had abnormal findings. In comparison to the U.S. general population, the worker prevalence of restrictive spirometric abnormalities was 3.8 times higher than expected, after adjustment for race, ethnicity, gender, age, smoking status, and body mass index. NIOSH also found evidence of rapid lung function decline in this workforce (see section 3.2). As in other flavoring plants, chemical exposures were diverse, although diacetyl was used nearly daily. Personal samples of diacetyl using NIOSH Method 2557 (unadjusted for absolute humidity and days to extraction) ranged to 0.76 ppm and area measurements to 10.2 ppm. Company samples in 2008–2009 using OSHA methods (not requiring adjustment) ranged to 1.9 ppm for personal and 2.9 for area.

NIOSH also found a high prevalence of a restrictive pattern on spirometry among workers at a plant where production workers combined liquid and powdered flavorings with flour, sugar, salt and other solid ingredients to produce baking mixes [NIOSH 2009b]. Of 41 workers, 23 (including 18 of 27 production workers) participated in a NIOSH medical survey that included spirometry testing. Of 22 workers with interpretable spirometry results, four (18%) had a restrictive pattern. All other spirometry tests were normal. The prevalence of restriction was approximately three times greater than expected compared to U.S. general population data from NHANES III [CDC 1996]. From June 2007 through May 2008, the company had used a buttermilk flavoring that contained 15% to 20% diacetyl. The company began using a reformulated buttermilk flavoring that contained less than 1% diacetyl in July 2008. The reformulated buttermilk flavoring contained 2,3-pentanedione, a diacetyl substitute that contains an additional methyl group. Use of the buttermilk flavoring was reported to be infrequent. In an industrial hygiene survey conducted by NIOSH from September 30, 2008, to October 2, 2008, diacetyl was detected qualitatively in screening air samples obtained with thermal desorption tubes and analyzed with gas chromatography/mass spectrometry according to NIOSH Method 2549. However, the diacetyl air concentrations were too low to be quantified or detected with the modified OSHA Method PV2118. In a second industrial

hygiene survey conducted by NIOSH in May 2009, air sampling with OSHA Method 1013 again did not reveal detectable or quantifiable concentrations of diacetyl; however, one personal sample showed an air concentration of 2,3-pentanedione of 91 ppb, and a corresponding area sample showed an air concentration of 78 ppb. Area air sampling with an additional NIOSH method (SMP2) did not detect diacetyl but did show 2,3-pentanedione in several areas, at concentrations ranging from 48 to 95 ppb. The sample that showed an air concentration of 95 ppb was obtained in the same area where a sample obtained with OSHA Method 1013 showed an air concentration of 78 ppb.

In 2008 NIOSH conducted an HHE of three cafeterias located at three different office buildings in New York City [NIOSH 2009c]. The HHE request was motivated by concern about diacetyl in butter-flavored cooking oils used on grill surfaces. Laboratory analyses of bulk samples of butter and two samples of one brand of cooking oil used at the three facilities did reveal diacetyl. Air samples obtained by NIOSH at the three facilities showed that air concentrations of diacetyl were below the limit of detection (0.02 ppm). NIOSH conducted a medical survey that included a questionnaire and spirometry tests. Approximately 80% of the workforce at the three facilities participated in the medical survey (116 of 141 workers completed the questionnaire; 104 of 111 workers who underwent spirometry testing had a valid test). Five workers (5%) had airways obstruction, and two workers had fixed obstruction. Both workers with fixed obstruction had started work at their current facility after butter-flavored cooking products were no longer in use. All five workers with obstruction denied having ever worked as professional cooks. Fifteen workers (14%) had restriction on spirometry, for a prevalence that was twice as high as expected compared to general population data from NHANES III [CDC 1996]. Only three of the 15 reported cooking experience, and 13 reported cleaning experience. Compared to workers who did not cook at work, workers who reported cooking among their job duties were twice as likely to report asthma-like symptoms; more than three times as likely to report shortness of breath after exercise, cough, and work-related wheezing; approximately five times more likely to report work-related shortness of breath following exercise; and more than twice as likely to report

work-related nasal symptoms. Workers who reported cleaning among their job duties were three times more likely to report asthma-like symptoms or shortness of breath while hurrying on level ground or walking up a slight hill than workers who did not clean at work. Workers who reported cleaning hot surfaces at work were almost four times more likely to report shortness of breath following exercise than those who had not cleaned hot surfaces at work.

### 3.4 Asthma

At the index microwave popcorn plant and at one of the other five microwave popcorn plants that NIOSH evaluated, the prevalence of self-reported physician-diagnosed asthma was approximately two times higher than expected [NIOSH 2004b, 2006]. This suggests the possibility that some workers exposed to diacetyl and other flavoring chemicals may be at increased risk for asthma (reversible airways obstruction) while others might be at risk for bronchiolitis obliterans (fixed airways obstruction). However, few of the survey participants with airways obstruction at these two plants who were administered a bronchodilator medication had a significant response (i.e., their airways obstruction was fixed); therefore, it is possible that some of these individuals had a different lung disease, not asthma. Some workers at microwave popcorn plants and flavoring plants who were initially diagnosed with asthma were ultimately found to have fixed airways obstruction and other findings consistent with constrictive bronchiolitis obliterans [Akpinar-Elci et al. 2004; van Rooy et al. 2007].

It is possible that individuals with pre-existing asthma may experience an exacerbation of their asthma due to the irritant properties of diacetyl or similar vapors. Diacetyl has also been reported to be a potential sensitizer [Anderson et al. 2007]. If sensitization to diacetyl were to occur in a susceptible individual, that individual might develop allergic-type asthma, with diacetyl exposure triggering airways obstruction and respiratory symptoms.

NIOSH conducted an HHE at a small plant where workers popped popcorn in heated oil and applied flavorings (including butter flavorings) prior to packaging [Sahakian et al. 2008]. Before 2002, they had used diacetyl-containing salt, and they used butter-flavored oil at the time of the survey. All three workers (lifelong nonsmokers) who had ever worked at the company developed respiratory disease while working there. One former worker had a mixed pattern of airways obstruction and restriction on spirometry; the airways obstruction was responsive to administered bronchodilator. This worker eventually died as a result of his respiratory disease. "Status asthmaticus with acute cardiopulmonary arrest" was listed as the primary diagnosis on the hospital discharge summary. Of the two other workers who had symptoms of asthma, one had an $FEV_1$ that improved by 480 ml (11%) and an FVC by 510 (8%) within the normal ranges after bronchodilator administration. The other worker had abnormal airways resistance of 322% of predicted; 19% improvement of the mid maximal forced expiratory flow after bronchodilator; and modest improvement in $FEV_1$ (6%) after bronchodilator. While employed at the plant, all three workers experienced worsening of their respiratory symptoms on the days they worked. HRCT scans of the chest showed findings suggesting possible bronchiolitis obliterans in the worker who died and in one of the other two workers. Air sampling results indicated that aldehydes were the predominant type of VOC in the plant air during production processes. Air samples obtained with thermal desorption tubes and analyzed with gas chromatography/mass spectrometry according to NIOSH Method 2549 showed that diacetyl was present in the plant air. However, the 2-hour and 4-hour diacetyl concentrations were less than the minimal detectable concentrations of 0.02 and 0.01 ppm, respectively with NIOSH Method 2557 [NIOSH 2007b].

### 3.5 Mucous Membrane Irritation (Eye, Upper Respiratory)

Eye, nose, and throat irritation has been frequently reported by workers in NIOSH medical surveys at microwave popcorn plants and flavoring manufacturing plants. At the index microwave popcorn plant, among workers who started work in microwave popcorn

production prior to the implementation of exposure controls, approximately 65% reported eye, nose, or throat irritation on their first medical survey. Only 33% of these workers reported eye, nose, or throat irritation on their last survey after exposures had declined. Microwave popcorn packaging area workers who started work after exposures had declined had a similar lower prevalence of irritant symptoms (25%) [NIOSH 2006]. At the two small microwave popcorn plants NIOSH evaluated, most workers reported eye and/or nasal irritation [Kanwal 2003; Kanwal and Martin 2003]. At one of these two plants, several workers developed severe eye irritation and blurred vision when the company started using a new butter flavoring [Kanwal and Martin 2003]. After the company stopped using the new flavoring and halted production for several days, the workers' eye problems resolved. At one of the large microwave popcorn plants NIOSH evaluated, management implemented use of full-facepiece respirators for mixing room workers soon after the company began producing microwave popcorn (before the respiratory hazard from butter flavoring vapors had been recognized), because these workers experienced severe eye irritation from butter flavoring vapors [NIOSH 2004a]. However, workers did not wear respirators consistently at all times during which they might be exposed [NIOSH 2004a]. At another microwave popcorn plant evaluated by NIOSH, 83% of workers in the mixing room reported nasal irritation [NIOSH 2004b]. All laboratory and warehouse workers who participated in the NIOSH medical survey at a flavor manufacturer reported post-hire nasal irritant symptoms; 80% of workers in the production room and the laboratory reported post-hire eye irritation [NIOSH 2007a]. 93% of workers who had ever worked in production at another flavor manufacturer reported post-hire eye irritation [NIOSH 2008b]. One worker reported eye burning from exposure to diacetyl and starter distillate during a NIOSH survey at a third flavoring producer [NIOSH 2009d].

### 3.6 Dermatologic Effects

Of the former workers who developed bronchiolitis obliterans while working at the index microwave popcorn plant, one worker also developed a severe skin rash [Akpinar-Elci et

al. 2004]. The worker developed thick keratotic plaques and fissures of the palms and soles, associated with dystrophic fingernails. Skin punch biopsy revealed mild acanthosis and spongiosis with focal superficial epidermal necrosis and an associated subepidermal dense lymphohistiocytic infiltrate. Patch testing showed early and late reactions to two butter flavorings and late reactions to six other butter flavorings, all used in the plant. This worker's dermatitis improved when he stopped work.

Prevalences of reported post-hire skin problems at microwave popcorn plants and flavoring plants have ranged from 12% at one of the six microwave popcorn plants NIOSH evaluated [NIOSH 2003a] to 36% among production workers at a flavoring plant. Post-hire skin problems were reported by 60% of workers who primarily made liquid flavorings at this plant [NIOSH 2007a].

### 3.7 Discussion

Medical evaluations of workers who have developed progressive shortness of breath while working at several microwave popcorn plants and flavoring plants have shown findings consistent with the severe irreversible lung disease constrictive bronchiolitis obliterans. Some affected workers have experienced extremely rapid declines in lung function, with severe airways obstruction occurring within several months of the start of exposure to flavoring chemicals in some cases [Akpinar-Elci et al. 2004; NIOSH 1985]. Workers as young as 22 years old have been affected. Some affected workers have been placed on lung transplant waiting lists by their physicians because of the severity of their disease [Akpinar-Elci et al. 2004]. The findings from investigations and studies conducted at multiple plants have revealed a link between exposure to diacetyl and risk for severe occupational lung disease. These findings meet the criteria which are often used to determine if the results of multiple studies indicate that an exposure is the likely cause of specific health effects [Gordis 1996; Hill 1965].

The first of these criteria is *temporality*: the exposure precedes disease development. Evidence of this comes from the many instances where initially asymptomatic diacetyl-exposed workers developed progressive shortness of breath within months of starting work and then were found to have severe fixed airways obstruction [van Rooy et al. 2007]. Additionally, NIOSH documented rapid falls in lung function in exposed workers with initially normal spirometry at three plants [NIOSH 2006, 2007a, 2011b]. Lockey et al. reported that five flavoring workers who developed moderate or severe fixed airways obstruction had normal spirometry at the start of employment [Lockey et al. 2002]. California public health surveillance showed that excessive $FEV_1$ decline occurred in workers in flavor manufacturing plants that participated in a preventive program attempting to lower flavoring exposures [Kreiss et al. 2011].

Temporality requires the exposure to precede disease development, and the inverse is that disease should decline in a population with *cessation of exposure*. Follow-up medical and environmental surveys at the index microwave popcorn plant revealed evidence of decreased lung disease risk with control of exposures. In workers hired before exposures were controlled, the prevalences of respiratory symptoms and airways obstruction and mean percent predicted $FEV_1$ did not change significantly over time (consistent with an irreversible disease). However, workers hired after exposures were controlled had lower prevalences of respiratory symptoms and airways obstruction and higher mean percent predicted $FEV_1$ on their first medical survey than workers hired before exposures were controlled, and these findings did not change significantly over time [Kanwal et al. 2011; NIOSH 2006]. Additionally, among 27 workers who participated in all eight NIOSH medical surveys from 2000 to 2003, annualized declines in $FEV_1$ improved from 144 mL per year to 40 mL per year to 22 mL per year, the last being consistent with normal aging-related lung function decline [Kreiss 2007]. Similarly, the former worker index cases with clinical bronchiolitis obliterans had stable $FEV_1$ within about 2 years of exposure cessation [Akpinar-Elci et al. 2004].

Another criterion is *strength of the association*: the magnitude of the apparent health risk due to the exposure. In analyses of data from the initial NIOSH medical survey at the index microwave popcorn plant, the prevalence of airways obstruction among nonsmoking current workers was approximately 11 times higher than expected in comparison to national data from NHANES III. It was approximately three times higher than expected in older smokers [Kreiss et al. 2002]. In analyses of California flavoring worker surveillance data, the prevalence of severe airways obstruction was approximately three times higher than expected among all workers compared to national data. The prevalence in workers less than 40 years old was 15 times higher than expected [Kim et al. 2010].

The *strength of the association* between diacetyl exposure and development of severe occupational lung disease is also apparent in the number of plants where workers have been affected and the number of production workers in these plants. The six microwave popcorn plants NIOSH evaluated represent a large segment of the microwave popcorn industry in the United States. Workers who developed bronchiolitis obliterans at these plants prepared the mixture of butter flavorings and soybean oil ("mixers") or worked nearby in the packaging area. Four of the six microwave popcorn plants NIOSH evaluated had affected mixers [Kanwal et al. 2006]. Each of these plants had one to three mixers per work shift at the time of the NIOSH HHEs. The occurrence of multiple cases of severe airways obstruction in such a small job category (approximately 20 mixers across the six plants) is far greater than expected when compared to the U.S. population prevalence of severe airways obstruction from NHANES III data (0.1%, or 1 in 1000, in individuals less than 50 years old, including smokers and never-smokers) [CDC 1996]. A similar magnitude of risk exists in some flavoring companies. At least six flavoring production workers developed bronchiolitis obliterans at three flavoring plants where NIOSH conducted medical surveys. There were approximately 30 production workers across these three plants at the time of the NIOSH HHEs [NIOSH 1985, 2007a, 2008b].

Additional criteria to support a causal link between diacetyl exposure and severe lung disease include *replication of findings (consistency)*, *biologic plausibility*, *dose-response relationship*, and *consideration of alternate explanations*. *Replication of findings (consistency)* is supported by the occurrence of lung disease consistent with constrictive bronchiolitis obliterans in diacetyl-exposed workers in at least five microwave popcorn plants evaluated by NIOSH, at least eight flavoring manufacturing plants, and a diacetyl production plant [Akpinar-Elci et al. 2004; CDC 2007; Kanwal et al. 2006; Kim et al. 2010; NIOSH 1985, 2007a, 2008b; van Rooy et al. 2007]. Private consultants who conducted medical and environmental surveys at four microwave popcorn plants owned by one large food company also found in their data analyses that a history of working as a mixer and higher cumulative exposure to diacetyl were associated with decreased lung function [Lockey et al. 2009]. *Biologic plausibility* is supported by the evidence of diacetyl toxicity identified in several animal exposure studies and other nonhuman research (see Chapter 4).

NIOSH found evidence of a *dose-response relationship* (i.e., worse lung disease or more workers affected with higher diacetyl exposure) in analyses of medical survey data from the index microwave popcorn plant and in analyses of aggregated data from medical surveys at the index plant and five additional microwave popcorn plants. The analyses of data from the initial survey at the index plant showed an increasing prevalence of abnormal spirometry with increasing quartiles of estimated cumulative diacetyl exposure [Kreiss et al. 2002]. Analyses of aggregated data from surveys at the six microwave popcorn plants showed higher prevalences of respiratory symptoms and worse lung function in mixers with more than 12 months experience and in packaging area workers at plants where heated tanks of oil and flavorings were not adequately isolated, compared to less exposed comparison groups [Kanwal et al. 2006]. Additional evidence of a *dose-response relationship* was found in analyses of California flavoring worker surveillance data. An analysis of obstruction by amount of plant diacetyl use showed that there were sixteen workers with obstruction in four companies that used more than 800 pounds of diacetyl annually compared to two workers with obstruction in companies that used less

diacetyl (prevalence of 5.3% versus 1.2%), for an OR of 4.5 (95% CI 1.03 – 19.9) [Kim et al. 2010].

In diacetyl-exposed workers with severe fixed airways obstruction and other findings of constrictive bronchiolitis obliterans, a *consideration of alternate explanations* should take into account the fact that while obstructive lung diseases such as asthma and smoking-related emphysema are common in the general population, severe airways obstruction is rare, especially in young individuals. Asthma is characterized by episodes of reversible airways obstruction–some individuals with severe or inadequately treated asthma can develop fixed airways obstruction. However, asthma does not appear to be a possible explanation for cases of severe lung disease among diacetyl-exposed workers for the following reasons:

(1) Most affected workers denied having any pre-existing lung disease or symptoms at the start of exposure.
(2) Once shortness of breath developed, it did not improve when workers were away from the workplace as would be expected in workers with occupational asthma (either new onset asthma or exacerbation of pre-existing asthma).
(3) Workers' illnesses did not improve when they took medications for asthma such as bronchodilators and corticosteroids.
(4) Most workers did not have a significant response to administration of bronchodilators in any of their spirometry tests (i.e., airways obstruction was fixed).

While some diacetyl-exposed workers who developed severe lung disease were smokers, the natural history of smoking-related disease and the results of medical evaluations of affected workers make it unlikely that any of the cases of severe fixed airways obstruction among diacetyl-exposed workers are smoking-related. Compared to the normal decline in lung function that occurs with aging ($FEV_1$ declines approximately 30 mL/year), in a subset of smokers lung function declines more rapidly ($FEV_1$ declines on average approximately 45–70 mL/year). An estimated 10%–15% of all smokers develop clinically important airflow obstruction [Ryu and Scanlon 2001]. Smokers who

experience rapid lung function decline will start to become short of breath once their $FEV_1$ falls below 60% of predicted; this usually occurs around age 50. Severe airways obstruction (e.g., $FEV_1$ less than 40% predicted) typically doesn't occur before 55–60 years of age [Wise 2008]. Several diacetyl-exposed workers developed severe fixed airways obstruction while still in their 20s and 30s. Any smoking history among these affected workers (as well as in affected workers younger than 50) would not explain their severe fixed airways obstruction. Additional evidence against smoking as a cause of severe lung disease in these workers is the fact that most workers' $DL_{CO}$ measurements were normal. In airways obstruction due to smoking-related emphysema, $DL_{CO}$ is reduced.

Constrictive bronchiolitis obliterans is known to occur as a result of a variety of exposures or in association with other nonpulmonary diseases (e.g., overexposure to highly irritating gases or vapors such as chlorine, ammonia, and nitrogen oxides or in association with connective tissue diseases such as systemic lupus erythematosus and rheumatoid arthritis). The diacetyl-exposed workers who developed severe fixed airways obstruction did not have histories or medical evaluation findings to suggest that they had developed constrictive bronchiolitis obliterans due to another exposure or medical condition. Airways obstruction can also occur due to diseases that affect other airways besides the bronchioles (e.g., bronchiectasis; upper airway lesions) [Ryu and Scanlon 2001]. However, individuals with airways obstruction from such other causes typically have characteristic history, physical exam, and medical test findings that usually serve to reveal the nature of the illness (e.g., copious sputum in someone with bronchiectasis or evidence of upper airway obstruction on spirometry). Such findings were not apparent in diacetyl-exposed workers who developed severe fixed airways obstruction.

Investigations of severe lung disease consistent with constrictive bronchiolitis obliterans among diacetyl-exposed workers have provided substantial evidence of a causal relationship between diacetyl exposure and development of this disease. Exposure preceded disease development and lung disease risk decreased with control of exposures.

Analyses of data from workplace medical and environmental surveys revealed a strong, consistent association of the disease with diacetyl manufacture, use of diacetyl in flavoring production, and use of diacetyl-containing butter flavorings in microwave popcorn production. The investigations have also shown evidence of a dose-response effect, and animal and other laboratory studies have provided evidence of biologic plausibility. Medical evaluations of affected workers did not identify alternative explanations for their illness besides their workplace exposure to diacetyl and other flavoring chemicals.

# Chapter 4: Toxicology of Diacetyl and 2,3-Pentanedione

## 4.1. Diacetyl Chemistry and Metabolism

Chemical and physical properties of diacetyl and 2,3-pentanedione are presented in Section 1.4, above. As mentioned there, diacetyl is an alpha-dicarbonyl. Endogenous alpha-dicarbonyl compounds are among the reactive carbonyl species implicated in the formation of advanced glycation end products [Nakagawa et al. 2002a; Wondrak et al. 2002]. Like other alpha-dicarbonyl compounds, diacetyl is reactive, with a tendency to cause protein cross-links [Miller and Gerrard 2005]. The reactivity of the alpha-dicarbonyl compounds is enhanced by electron attracting groups and decreased by electron donors [Roberts et al. 1999]. Thus, diacetyl is a reactive compound, but its alkyl components are electron donors that may somewhat decrease the reactivity of the adjacent carbonyl groups [Roberts et al. 1999].

Diacetyl and related alpha-dicarbonyl compounds can inactivate proteins, principally through reactions with the amino acid, arginine [Epperly and Dekker 1989; Saraiva et al. 2006]. The related alpha-dicarbonyl flavoring, 2,3-pentanedione, has been reported to be even more reactive with arginine groups than diacetyl [Epperly and Dekker 1989].

### *4.1.1 Diacetyl in Food*

Diacetyl has a long history as a component of food, suggesting that exposures can occur in diverse workplaces. Diacetyl occurs as a natural product in many foods. It imparts the flavor and aroma of butter to many common foods and drinks, including butter, cheese,

yogurt, beer and wine. Diacetyl in food plays an important role in food preference [Liggett et al. 2008]. Bacteria and yeast produce diacetyl during fermentation [Chuang and Collins 1968]. It can be produced by metabolism of an acetaldehyde-thiamine pyrophosphate complex in the presence of acetyl-coenzyme A [Speckman and Collins 1968]. Microbes can also produce diacetyl from pyruvate in the presence of acetyl-coenzyme A [Chuang and Collins 1968]. Microbial culture conditions, such as pH, can influence the relative amount of diacetyl produced during fermentation [Garcia-Quintans et al. 2008; St-Gelais et al. 2009]. In addition, the steam distillate of several bacterial cultures grown on skim milk is known as "starter distillate" and is also considered a flavoring [FASEB 1980; FDA 1983]. Major components of starter distillate include diacetyl and acetic acid [FASEB 1980]. Thus, diacetyl can occur naturally in food, may be added to food as diacetyl per se, and is an anticipated component when starter distillate is added as a flavoring.

#### *4.1.2 Diacetyl Metabolism in Mammalian Cells*

In the rat and hamster liver, diacetyl is metabolized principally by reduction to acetoin in an enzymatic reaction catalyzed by dicarbonyl/L-xylulose reductase (DCXR) with either NADH or NADPH as coenzymes [Nakagawa et al. 2002a; Otsuka et al. 1996; Sawada et al. 1985]. Acetoin can be further reduced to 2,3-butanediol in an NADH-dependent manner [Otsuka et al. 1996]. This diacetyl reductase activity is higher in the liver than in the kidney, and kidney activity is higher than in the brain. However, after oral administration of diacetyl, the levels of acetoin are much higher in the brain than in the

kidney or liver. 2,3-Butanediol accumulates in liver, kidney, and brain after the administration of diacetyl, acetoin or 2,3-butanediol. Oral administration of acetoin and, to a lesser extent, 2,3-butanediol, in rats also causes diacetyl accumulation in the liver and brain in vivo [Otsuka et al. 1996]. However, liver homogenates do not produce significant diacetyl from acetoin or 2,3-butanediol [Otsuka et al. 1996]. Thus, the metabolic interconversion of the 4-carbon compounds, acetoin, diacetyl, and 2,3-butanediol, occurs in mammalian systems in vivo and in vitro but the full spectrum of metabolic pathways remains incompletely investigated.

As mentioned above, in mammalian cells, the predominant metabolic pathway for diacetyl is catalyzed by DCXR, a tetrameric protein also known as diacetyl reductase, that is comprised of four subunits, each 244 amino acid long [El-Kabbani et al. 2005; Ishikura et al. 2001; Nakagawa et al. 2002a; Sawada et al. 1985]. In addition to the reductive metabolism of diacetyl, DCXR catalyzes the metabolism of several other dicarbonyl compounds, including 2,3-pentanedione, 2,3-hexanedione. 2,3-heptanedione and 3,4-hexanedione [Nakagawa et al. 2002a]. In addition, DCXR catalyzes the reductive metabolism of a number of monosaccharides, including L-xylulose, and plays a role in the glucuronic acid/uronate cycle of glucose metabolism as well as the metabolism of carbonyl compounds [Carbone et al. 2005; El-Kabbani et al. 2005; Nakagawa et al. 2002a]. Inhibitors of DCXR are well described and include *n*-butyric acid, 2-furoic acid, benzoic acid, and nicotinic acid [Carbone et al. 2005; Nakagawa et al. 2002a]. At least one of these DCXR inhibitors, n-butyric acid, is well absorbed in the nose, and its presence in vapor mixtures causes small but significant decreases in diacetyl absorption

in the nasal mucosa and, thereby, leaves more diacetyl in the vapor stream of the nasal airways for delivery to the lung [Morris and Hubbs 2009]. In the rat kidney, DCXR is localized in the distal tubules and collecting ducts and colocalizes with carboxylmethyllysine, an advanced glycation end product [Nakagawa et al. 2002a]. In the mouse kidney, DCXR is localized to the brush border of the proximal renal tubules [Nakagawa et al. 2002a]. In the human prostate epithelial cells and in normal human skin, DCXR is associated with the cell membrane [Cho-Vega et al. 2007a; Cho-Vega et al. 2007b]. In human skin, DCXR is located near the adhesion molecules, e-cadherin and β-catenin [Cho-Vega et al. 2007b]. Similarly, in the vascular endothelium in the dermis, DCXR localizes near intercellular junctions, suggesting a potential role for DCXR in cell adhesion [Cho-Vega et al. 2007b]. In addition, DCXR activity is present in the respiratory mucosa of the rat, with the highest activity in the olfactory epithelium [Morris and Hubbs 2009]. DCXR knockout mice are not well characterized phenotypically but are reported to be fertile [Nakagawa et al. 2002b]. People without DCXR excrete pentose in their urine but are otherwise believed to be healthy, indicating that DCXR and the major diacetyl metabolic pathway are not essential for life [Flynn 1955; Lane and Jenkins 1985].

Importantly, the metabolism of diacetyl is not exclusively by DCXR. For example, AKR1C15 is a newly described aldo-keto reductase expressed in rat lung that can metabolize alpha-diketones [Endo et al. 2007]. Recently, a low affinity, high capacity and a high affinity, low capacity pathway for diacetyl metabolism has been described in the respiratory tract of the rat [Gloede et al. 2011]. The high affinity pathway was

inhibited by sodium benzoate indicating that it is DCXR. The low affinity pathway is not believed to play a major role at diacetyl concentrations associated with most exposures.

Table 4.1. Experimental immunotoxicology and respiratory toxicology studies

| Reference | Test Subject | Diacetyl exposure | Effects of exposure |
|---|---|---|---|
| Anderson et al. [2007] | Female BALB/c mice | Repeated dermal exposure, 1.25-24% diacetyl in 25 µL 4:1 acetone/olive oil (AOO) | Quantitative structure-activity relationship (QSAR) programs were used in tandem with local lymph node assays (LLNA); both predicted diacetyl as a sensitizer. Diacetyl was shown to likely be a T-cell-mediated sensitizer. |
| Fedan et al. [2006] | In vitro preparations of guinea pig trachea | 1 mM<br>3 mM<br>10 mM<br>30 mM | Direct effect of diacetyl:<br>Very weak tracheal contractions with threshold at 1 mM, relaxation at exposures above 3 mM.<br>Diacetyl effect on response to intraluminal (mucosal) methacholine:<br>4 hour perfusion with 3 mM diacetyl increased methacholine reactivity 10x; 10 mM diacetyl completely inhibited methacholine response.<br>Depolarization of transepithelial potential difference at 3 and 10 mM.<br>Decrease in transepithelial potential difference at 10 mM. |
| Fedan et al. [2010](abstract) | Male Sprague-Dawley rats | 100 ppm<br>200 ppm<br>300 ppm<br>360 ppm<br>n = 6-9 | Decrease in reactivity to inhaled methacholine at 360 ppm |
| Fedan et al. [2011](abstract) | In vitro preparations of guinea pig trachea | 1 mM<br>3 mM<br>10 mM<br>30 mM | Contraction and relaxation responses to diacetyl are not mediated by the airway epithelium |
| Gloede et al, 2011 | F344 rats | Respiratory uptake of diacetyl in F344 rats was used to validate a model of respiratory tract uptake of diacetyl | At a given inhaled diacetyl dose, the predicted dose to the bronchiolar epithelium of a lightly exercising worker is predicted to be more than 40x the dose to the bronchiole of a rat.<br><br>Describes a low affinity, high capacity and a high affinity, low capacity pathway for diacetyl metabolism in the rat respiratory epithelium. The high affinity pathway is inhibited by sodium benzoate, indicating that it is DCXR. |

Table 4.1. Experimental immunotoxicology and respiratory toxicology studies

| Reference | Test Subject | Diacetyl exposure | Effects of exposure |
|---|---|---|---|
| Hubbs [2002] | Male Sprague-Dawley rats | Control (n=16)<br>0 ppm | |
| | | Diacetyl exposures were to vapors of a complex mixture of diacetyl-containing butter flavoring | |
| | | Exposure for 6 hours: | |
| | | Low constant exposure (n=6)<br>203 ppm of the diacetyl component | Airway epithelial damage with necrosis and inflammation in nose |
| | | Middle constant exposure (n=3)<br>285 ppm of the diacetyl component | Airway epithelial damage with necrosis and inflammation in nose and lungs (bronchi) |
| | | High constant exposure (n=3)<br>352 ppm of the diacetyl component | Airway epithelial damage with necrosis and inflammation in nose and lungs (bronchi) |
| | | High pulsed exposure (n=3)<br>371 ppm (range 72–940) of the diacetyl component | Airway epithelial damage with necrosis and inflammation in nose and lungs (bronchi and bronchioles) |

Table 4.1. Experimental immunotoxicology and respiratory toxicology studies

| Reference | Test Subject | Diacetyl exposure | Effects of exposure |
|---|---|---|---|
| Hubbs [2008] | Male Sprague-Dawley rats | Control (n=18) 0 ppm | |
| | | Exposure for 6 hours: | |
| | | 99.3 ppm (n=6) | Airway epithelial damage with necrosis and inflammation in the nose (1/6) |
| | | 198.4 ppm (n=6) | Airway epithelial damage with necrosis and inflammation in nose (6/6) |
| | | 294.6 ppm (n=6) | Airway epithelial damage with necrosis and inflammation in nose (6/6) and bronchi (2/6) |
| | | Four ~15 minute pulses in 6 hours (TWA): | |
| | | 122 ppm (n=6) | Airway epithelial damage with necrosis and inflammation in nose (2/6) |
| | | 225 ppm (n=6) | Airway epithelial damage with necrosis and inflammation in nose (6/6) and trachea (2/6) |
| | | 365 ppm (n=6) | Airway epithelial damage with necrosis and inflammation in the nose (6/6), trachea (6/6) and bronchi (6/6) |
| | | Continuous exposure for 6 hours to match pulse TWA: | |
| | | 120 ppm (n=6) | Airway epithelial damage with necrosis and inflammation in nose (5/6) and bronchus (1/6) |
| | | 224 ppm (n=6) | Airway epithelial damage with necrosis and inflammation in nose (6/6), trachea (5/6) and bronchus (1/6) |
| | | 356 ppm (n=6) | Airway epithelial damage with necrosis and inflammation in nose (6/6), trachea (6/6) and bronchi |
| | | Single ~15 minute pulse exposure | |
| | | 1949 ppm (92.9 TWA) (n=6) | Airway epithelial damage with necrosis and inflammation in nose (3/6) |

Table 4.1. Experimental immunotoxicology and respiratory toxicology studies

| Reference | Test Subject | Diacetyl exposure | Effects of exposure |
|---|---|---|---|
| Larsen et al. [2009] | Male BALB/cJ mice | 191 ppm<br>334 ppm<br>790 ppm<br>1154 ppm<br>Challenge after acute exposure<br>555 ppm | Pulmonary irritation at 790 and 1154 ppm, decrease in respiratory rate, increase in "time of break"<br><br>Higher diacetyl concentrations at the acute exposure reduced sensitivity to challenge exposure, lower acute exposure increased sensitivity; challenge exposure did not alter lung inflammation |
| Morgan et al. [2008] | Male C57BI/6 mice | Subacute inhalation, whole body 6 h/day, 5 days<br><br>0<br><br>200<br><br>400 ppm<br><br>Intermittent inhalation, nose only<br><br>0, 100, 200, or 400 ppm 1 h/day, 5 days/week, 2 weeks<br><br>0, 100, 200, or 400 ppm 1 h/day, 5 days/week, 4 weeks | <br><br><br>Necrosis and inflammation in mucosa of the nose and larynx<br><br>Necrosis and inflammation in the mucosa of the nose, larynx and bronchi<br><br><br>Chronic-active inflammation in the nose of all diacetyl exposed mice (100, 200 and 400 ppm) , squamous metaplasia of respiratory epithelium of the nose(1/5 at both 100 and 200 ppm; 4/4 at 400 ppm; necrosis and ulceration of respiratory epithelium of the nose at 400 ppm (3/4); atrophy of olfactory epithelium of the nose (1/4 at 100 ppm and 2/2 at 400 ppm; lymphocyte infiltrates around bronchi in the lung (4/5 at 100 ppm; 5/5 at 200 and 400 ppm)<br><br>Chronic-active inflammation in the nose (2/5 at 100 ppm; 5/5 at 200 and 400 ppm); squamous metaplasia of the respiratory epithelium of the nose (1/5 at 100 ppm; 5/5 at 200 and 400 ppm). Atrophy of olfactory epithelium (1/5 at 200 ppm; 5/5 at 400 ppm) Lymphocytic infiltrates around bronchi (2/3 at 100 ppm; 5/5 at 200 and 400 ppm). |

Table 4.1. Experimental immunotoxicology and respiratory toxicology studies

| Reference | Test Subject | Diacetyl exposure | Effects of exposure |
|---|---|---|---|
| | | 0 or 1200 ppm<br>15 min twice a day, 5 days/week, 4 weeks | Chronic-active inflammation in the nose (5/5); necrosis and ulceration of the respiratory epithelium of the nose (3/5); squamous metaplasia of the respiratory epithelium of the nose (5/5); atrophy of the olfactory epithelium (4/5); both peribronchial and peribronchiolar lymphocytic infiltrates (5/5) |
| | | Subchronic inhalation, whole body | |
| | | 0, 25, 50, or 100 ppm<br>6 h/day, 5 days/week, 6 weeks | Necrosis and ulceration of the respiratory epithelium of the nose (2/5 at 50 ppm and 5/5 at 100 ppm); squamous metaplasia of the respiratory epithelium of the nose (1/4 at 25 ppm; 3/5 at 50 ppm; 5/5 at 100 ppm); atrophy of the olfactory epithelium (3/5 at 50 and 1/5 at 100 ppm); peribronchial lymphocytic inflammation (3/5 at 25 ppm; 5/5 at 50 ppm; 5/5 at 100 ppm); denudation and atrophy of bronchial epithelium (5/5 at 100 ppm). |
| | | 0, 25, 50, or 100 ppm<br>6 h/day, 5 days/week, 12 weeks | Necrosis and ulceration of the respiratory epithelium of the nose (1/5 at 50 ppm and 5/5 at 100 ppm); squamous metaplasia of the respiratory epithelium of the nose (2/5 at 25 ppm; 4/5 at 50 ppm; 5/5 at 100 ppm); atrophy of the olfactory epithelium (5/5 at 100 ppm); Peribronchial lymphocytic infiltrates (2/5 at 25 ppm; 4/5 at 50 ppm; 5/5 at 100 ppm); denudation and attenuation of bronchial epithelium (5/5 at 100 ppm)) |
| | | Oropharyngeal aspiration<br>0, 100, 200, or 400 mg/kg body weight, single dose<br>4-days postaspiration | Foci of fibrohistiocytic proliferation with little or no inflammation present at the junction of the terminal bronchioles and alveolar ducts (400 mg/kg) |
| Morris and Hubbs [2009] | Male Sprague-Dawley rats | 100 or 300 ppm in airstream flows of 100-400 mL/min | A hybrid computational fluid dynamic-physiologically based pharmacokinetic model (CFD-PBPK) was used to extrapolate diacetyl and butyric acid uptake in rat airways epithelium to human airways epithelium. The CFD-PBPK suggests that nasal injury in rats can predict a risk to deep lung tissue in man. Diacetyl damages airway epithelium when it reaches a critical concentration in the target cells; the CFD-PBPK indicates that more diacetyl will be absorbed in the nose of rats than in |

Table 4.1. Experimental immunotoxicology and respiratory toxicology studies

| Reference | Test Subject | Diacetyl exposure | Effects of exposure |
|---|---|---|---|
| | | | man. Butyric acid in butter flavoring vapors (BFV) is shown to shift diacetyl absorption from the nose and trachea into deeper lung tissue. |
| Roberts et al. [1999] | Mice | Repeated dermal exposure | The results of murine local lymph node assays (LLNA) of a series of alpha,beta-diketones were correlated with reactive alkylation index (RAI) values, showing a structure-activity relationship (SAR) for alpha,beta-diketones as skin sensitizers. |
| Zaccone et al. [2010](abstract) | In vitro preparation of rat trachea | 60 ppm<br>100 ppm<br>200 ppm<br>300 ppm<br>360 ppm<br>n = 6-11 | Increase in reactivity to methacholine at 360 ppm |

### 4.2 In Vivo and In Vitro Diacetyl Toxicology Studies

Diacetyl may be consumed in food, its vapors may be inhaled, and it may come in contact with skin. In vivo studies have modeled these routes of human exposure.

Table 4.1 summarizes key immunotoxicology and respiratory toxicology findings for diacetyl.

#### *4.2.1. In Vivo Toxicology of Orally-Administered Diacetyl*

Studies of acute oral toxicity have used gavage exposures in rats. Based upon gavage administration of a 20% diacetyl solution in water, the $LD_{50}$ for a single oral dose of diacetyl is estimated to be 3 g/kg in female rats and 3.4 g/kg in male rats [Colley et al. 1969].

Subchronic (90 day) gavage administration of 540 mg diacetyl/kg/day caused multiple changes in exposed rats, including decreased body weight, increased water consumption, increased adrenal weight, increased relative kidney and liver weights (in females absolute kidney and liver weights were also increased), decreased blood hemoglobin concentration and gastric ulceration [Colley et al. 1969]. No adverse effects were noted at the next highest dose level, which was 90 mg/kg/day. On a mg/kg basis, the 90 mg/kg/level was estimated to be roughly 500-fold greater than the estimated human maximum daily intake of diacetyl from foods consumed at that time, with 50 ppm diacetyl being the highest estimated concentration in any food [Colley et al. 1969].

### *4.2.2. Sensitization by Topically-Applied Diacetyl In Vivo*

Topical application of diacetyl causes sensitization based on the results of a murine local lymph node assay [Anderson et al. 2007; Roberts et al. 1999]. Significant increases in ear swelling observed after 3 days of topical applications at concentrations of 2.5% (v/v) and higher also support a role for diacetyl as a skin irritant. On the basis on the results of the local lymph node assay and immune cell phenotyping, it is suggested that diacetyl functions as a T-cell mediated chemical sensitizer [Anderson et al. 2007]. Cutaneous sensitization by diacetyl may be initiated through haptenation of diacetyl with proteins containing the amino acids lysine and arginine [Roberts et al. 1999].

### *4.2.3. Toxicology of Inhaled Diacetyl In Vivo*

In rats, acute exposures to diacetyl or diacetyl-containing butter flavoring vapors cause necrosis in the epithelial lining of nasal and pulmonary airways. Rats inhaling vapors of butter flavoring that contained diacetyl developed multifocal necrotizing bronchitis one day after a 6-hour exposure. The mainstem bronchus was the most affected intrapulmonary airway. However, nasal airways were more affected than intrapulmonary airways. Necrosuppurative rhinitis was seen in rats inhaling butter flavoring vapors at concentrations of butter flavoring that did not cause damage in intrapulmonary airways [Hubbs et al. 2002]. As a single agent acute exposure in rats, diacetyl caused epithelial necrosis and inflammation in bronchi at concentrations of $\geq$ 294.6 ppm and caused epithelial necrosis and inflammation in the trachea and larynx at concentrations of $\geq$224 ppm [Hubbs et al. 2008]. In a pattern reminiscent of airway damage from butter

flavoring vapors, diacetyl causes greater damage to nasal airways than to intrapulmonary airways [Hubbs et al. 2008].

Eighteen hours after a 6-hour exposure to inhaled diacetyl (100, 200, 300 or 360 ppm), in anesthetized rats 360 ppm elevated slightly basal airway resistance ($R_L$) and dynamic lung compliance ($C_{dyn}$) [Fedan et al. 2010]. Subsequent inhalation of methacholine aerosol (0.3 – 10 mg/ml) revealed that airway reactivity was decreased after exposure to diacetyl at 360 ppm. It had been predicted, based on extensive epithelial damage noted after diacetyl inhalation, that reactivity to inhaled methacholine would be increased.

In mice, inhaling diacetyl at concentrations of 200 or 400 ppm for 6 hours/day for up to 5 days causes respiratory tract changes similar to those seen in rats inhaling diacetyl or butter flavoring vapors [Morgan et al. 2008]. At both 200 and 400 ppm, diacetyl caused necrotizing rhinitis in mice that was most prominent in the front portion of the nose. At 400, but not at 200 ppm, the olfactory epithelium demonstrated vacuolar degeneration and apoptosis. Necrotizing laryngitis was consistently observed in all mice inhaling 400 ppm diacetyl, while only one mouse inhaling 200 ppm diacetyl had comparable necrotizing laryngitis, but erosive laryngitis was present in 9 of 10 mice inhaling the 200 ppm concentration.

Exposing mice to diacetyl for 1 hour/day at 100, 200, or 400 ppm diacetyl, 5 days per week, for 2 to 4 weeks eliminated epithelial necrosis in mice inhaling 200 ppm diacetyl and decreased the severity of epithelial necrosis in mice inhaling 400 ppm diacetyl as compared with the 6-hour/day exposures. Lymphocytic inflammation was seen around the bronchi in some mice

inhaling 100 ppm and in all mice inhaling 200 or 400 ppm diacetyl [Morgan et al. 2008]. Exposing mice to diacetyl for 15 minutes per day at 1200 ppm diacetyl, 5 days/week for 2 weeks also caused lymphocytic infiltrates around bronchi and lymphocytic infiltrates extended deeper into the lung, reaching the level of the preterminal bronchioles [Morgan et al. 2008].

Subchronic, 12-week, diacetyl inhalation for 6 hours/day, 5 days/week caused significant histopathologic changes in mice at all concentrations studied. Peribronchial lymphocytic infiltrates were seen at terminal sacrifice at 12 weeks in all subchronically-exposed mice inhaling 100 ppm diacetyl and in some mice inhaling 25 or 50 ppm diacetyl. In mice inhaling 100 ppm diacetyl, bronchial epithelial changes included denudation, attenuation, and hyperplasia [Morgan et al. 2008]. Chronic active nasal inflammation was seen in all mice inhaling 50 or 100 ppm and in four of five mice inhaling 25 ppm diacetyl for 12 weeks, an exposure that also caused minimal to mild lymphocytic bronchitis in two of five mice. This suggests that the no observable adverse effect level in mice for subchronic inhalation may be less than 25 ppm diacetyl.

Butyric acid caused a small but significant reduction in nasal uptake of diacetyl in the rat nose, and, thereby, increased the diacetyl exposure to the lung due to a reduced "scrubbing" effect [Morris and Hubbs 2009].

Oropharyngeal aspiration permits exposures that bypass the rodent nose and, hence, scrubbing at that site [Foster et al. 2001; Rao et al. 2003]. A single aspiration exposure to 400 mg/kg diacetyl produced a fibrohistiocytic response at the bronchioloalveolar junction of mice after 4 days. While oropharyngeal aspiration of diacetyl delivers a high bolus dose of diacetyl, the unusual

fibrohistiocytic response could suggest that the smallest airways may be particularly susceptible to diacetyl-induced epithelial injury and fibrosis [Morgan et al. 2008]. A subsequent study demonstrates development of bronchiolitis obliterans in rats after intratracheal instillation of diacetyl [Palmer et al. 2011]. In this model, diacetyl-induced bronchiolitis obliterans was associated with abnormal repair of the injured bronchiolar epithelium. However, as noted in the study, the very large single dose used in these studies may have limitations for the use of single exposure intratracheal instillations for risk assessment purposes [Palmer et al. 2011]. Additional investigation is required.

Pulmonary function changes have been investigated in mice after acute or subchronic diacetyl exposure. In mice, acute 2-hour diacetyl inhalation at concentrations from 191 to 1154 ppm caused a decrease in respiratory rate and an increase in the "time of break" between inhalation and exhalation, an indicator of sensory irritation [Larsen et al. 2009]. In addition, acute diacetyl inhalation in mice caused decreases in tidal volume and mid-expiratory flow rate. Mice previously exposed to high diacetyl concentrations were *less* sensitive to the sensory irritation effects of a diacetyl challenge exposure, while mice previously exposed to low diacetyl concentrations were *more* sensitive to a diacetyl challenge exposure. Extrapolation of the mouse dose-response relationship to humans suggested no sensory irritation to warn workers during acute diacetyl exposures at concentrations less than 20 ppm [Larsen et al. 2009]. Conversely, a recent study suggests that acute diacetyl inhalation exposures can actually increase the number of substance P positive neurons in the jugular ganglia of exposed rats [Goravanahally et al. 2010]. As a group, these studies suggest dysregulation of airway sensory innervation and responses. Additional studies support the potential for diacetyl to alter pulmonary function in exposed

rodents. Mice inhaling 100 ppm diacetyl for 12 weeks had concentration-dependent decreases in respiratory rate and minute volume after 3 and 6 weeks of exposure; mice inhaling 50 ppm diacetyl had decreased respiratory rates after 6 weeks exposure, but pulmonary function improved with time with continued exposure at these exposure concentrations [Morgan et al. 2008]. However, after 18 weeks of exposure respiratory rates in mice inhaling 25 ppm diacetyl were significantly lower than in controls [Morgan et al. 2008].

The effects of diacetyl inhalation may not be limited to the respiratory tract. Inhaling 2500 ppm diacetyl for 45 minutes increased 2-deoxyglucose uptake in foci in the posterior portion of the rat brain olfactory bulb [Johnson et al. 2007]. While this finding has generally been interpreted as being related to olfaction [Johnson et al. 2007], the potential exists for toxicity to olfactory neurons that radiate into the olfactory bulb.

#### *4.2.4. In Vitro Toxicology of Diacetyl*

Diacetyl is mutagenic in the *Salmonella typhimurium* tester strains TA100 and TA104 [Kim et al. 1987; Marnett et al. 1985]. However, diacetyl also reacts with mutagenic heterocyclic amines and suppresses the mutagenicity of heterocyclic amines in *Salmonella typhimurium* tester strain TA98. Diacetyl also enhances chromosome loss by proprionitrile in *Saccharomyces cerevisiae.* Recently, diacetyl in the presence of human S9 demonstrated a high degree of mutagenicity in a mouse lymphoma mutation assay [Whittaker et al. 2008]. Additional studies on the genotoxicity of diacetyl have been reviewed in the background documents available online as part of the National Toxicology Program [National Toxicology Program 2007].

In isolated mitochondria, diacetyl closes the mitochondrial permeability transition pore and renders it insensitive to $Ca^{2+}$ [Eriksson et al. 1998]. This effect of diacetyl occurs at concentrations that could occur in tissues of diacetyl-exposed individuals, with half-maximal inhibition of the mitochondrial transition reported to be at a diacetyl concentration of 1 mM [Eriksson et al. 1998]. The effect of diacetyl on the mitochondrial permeability transition pore appears to be caused by arginine modification (see above) [Eriksson et al. 1998]. In addition, diacetyl can be metabolized by pig heart mitochondrial pyruvate kinase to form acetate and acetyl-CoA with a $K_m$ value of 0.46 mM. Diacetyl is also a competitive inhibitor of pyruvate metabolism by pyruvate dehydrogenase with a $K_i$ of 0.43 mM.

The isolated, perfused trachea system employing tracheas from unexposed guinea pigs has been used to investigate the effects of diacetyl in vitro [Fedan et al. 2006]. In this model, the direct, potentially toxic effects of the agent on epithelium may be examined. Agents such as diacetyl may be applied to the epithelium (mucosal surface) or separately to the smooth muscle (serosal surface) of the trachea while measuring contractile or relaxant responses of the airway smooth muscle. An advantage of this model is that the effects of the diketone do not involve an inflammatory response, inasmuch as the trachea has been removed from the animal and there is no source for the recruitment of inflammatory cells into the wall of the airway.

In unstimulated tracheas, diacetyl applied to the mucosal surface in concentrations between $10^{-7}$ to 1 mM dissolved in a physiological salt solution elicited small contractions; in concentrations higher than 3 mM (i.e., 10 and 30 mM), contractions to diacetyl were followed by relaxations.

The relaxation responses were larger than the contractile responses. Exploring these phenomena further, adding diacetyl to the mucosa of tracheas that were first contracted with methacholine, a bronchoconstrictor agonist, resulted in full relaxation of the smooth muscle over the same range of diacetyl concentrations. These findings indicate that diacetyl is a weak contractile agent when applied to the epithelial surface, but that it is capable of eliciting strong relaxant responses. Thus, a diversity of responses in the airway that depend on the diacetyl concentration are produced.

Investigation of inhaled diacetyl effects (60, 100, 200, 300 and 360 ppm) on reactivity of tracheas removed from exposed rats and studied in the isolated, pefused trachea model showed that reactivity to methacholine applied to the mucosal surface was increased slightly after inhalation of 360 ppm. Based on epithelial damage in airways after exposure to diacetyl, a larger increase in airway reactivity had been anticipated [Fedan et al. 2010].

Diacetyl inhalation elicits substantial histopathologic changes to airway epithelium, including denudation and necrosis (see section 3.3.3.). Commonly, damage to respiratory epithelium leads to airway hyperreactivity. For example, after ozone inhalation, airway reactivity of guinea pigs to inhaled methacholine is increased; likewise, reactivity to methacholine applied to the mucosa of isolated, perfused trachea is also increased [Fedan et al. 2000]. Incubation of perfused trachea with diacetyl dissolved in a physiological salt solution and applied to the mucosal surface led to no effect (1 mM diacetyl), an approximately 10-fold increase in reactivity to methacholine (3 mM), or full suppression of contraction to methacholine (10 mM) [Fedan et al. 2006]. The effects of diacetyl in isolated airways from naïve animals does not involve the airway epithelium [Fedan et al. 2011].

Damage to the epithelium after diacetyl inhalation suggests that epithelial ion transport and electrical resistance could be affected by the diketone. In rat tracheal segments investigated in vitro with Ussing chambers, diacetyl dissolved in physiological salt solution at 3 mM decreased transepithelial potential difference ($V_t$, mV), indicative of a decrease in electrogenic ion transport and/or an effect on paracellular ion transport involving tight junctions, whereas 10 mM diacetyl reduced $V_t$ further and decreased transepithelial resistance ($R_t$, $\Omega \cdot cm^2$). $R_t$ is an index of tight junction permeability. Thus, ion transport and epithelial integrity are affected directly by diacetyl.

The diacetyl concentrations observed to affect tracheal diameter and elicit bioelectric responses, i.e., 1 to 3 mM, are within the range estimated to occur in the rat tracheal mucosa after diacetyl inhalation. Using a computational fluid dynamic-physiologically based pharmacokinetic model, Morris and Hubbs [Morris and Hubbs 2009] calculated that inhalation levels of 100, 200, and 300 ppm diacetyl could yield concentrations in the mucosa of 1.1 to 1.2, 2.3 to 2.5, and 3.7 to 3.8 mM diacetyl, respectively. This suggests that some or all of the observed in vitro effects may occur in the airways during vapor inhalation.

The mechanism(s) of the effects of diacetyl on trachea in vitro are not known at present. However, a related structure, 2,3-butanedione monixime, has been reported to inhibit contraction of smooth muscle, perhaps as a result of inhibiting phosphorylation of myosin light chains [Lizarraga et al. 1998; Siegman et al. 1994; Stowe et al. 1997; Waurick et al. 1999]. Bioelectric responses of neurons also have been reported to be inhibited by 2,3-butanedione monixime [Lizarraga et al. 1998].

### *4.2.5. Toxicology of Inhaled Diacetyl Substitutes In Vivo*

Diacetyl is the compound largely responsible for the flavor of butter in butter [FASEB 1980; FDA 1983]. However, exposures in workplaces that make or use butter flavoring and emissions from heated butter flavoring involve multiple volatile compounds [Boylstein et al. 2006; Kullman et al. 2005]. Among the potential replacements for diacetyl, starter mix contains high concentrations of diacetyl [FASEB 1980; FDA 1983]. Another chemical that adds the flavor of butter to food is acetoin, but its toxicity is incompletely investigated, and it was present along with diacetyl in many of the workplaces where bronchiolitis obliterans occurred in workers who make or use diacetyl [Kullman et al. 2005; van Rooy et al. 2007]. As well, the butter flavoring, 2,3-pentanedione, is structurally very similar to diacetyl because it is a 5-carbon alpha-diketone, and diacetyl is a 4-carbon alpha-diketone. The available evidence suggests that 2,3-pentanedione causes in rats airway epithelial damage similar to that produced by diacetyl [Hubbs et al. 2010; Morgan et al. 2010].

Eighteen hours after a six-hour inhalation exposure to 2,3-pentanedione 120, 240, 320 and 360 ppm), $R_L$ and $C_{dyn}$ in anesthetized rats were decreased slightly at the 360 ppm exposure [Fedan et al. 2010]. Subsequently, airway reactivity to inhaled methacholine aerosol was decreased after inhalation of 120, 240 and 320 ppm 2,3-pentanedione. 2,3-Pentanedione produced a greater effect on reactivity to methacholine than diacetyl's. Airway hyperreactivity to methacholine had been anticipated, in view of the epithelium damage caused by the flavoring.

Following inhalation exposure of rats to these same concentrations of 2,3-pentanedione and perfusion of the trachea in vitro, reactivity to mucosally-applied methacholine was increased by 240, 320 and 360 ppm 2,3-pentanedione [Fedan et al. 2010]. The magnitude of this effect surpassed that caused by diacetyl inhalation.

Application of 2,3-pentanedione to the mucosal surface of the guinea-pig isolated, perfused trachea from naïve animals evoked a contraction (>3 mM) followed by relaxation [Fedan et al. 2011]. 2,3-Pentanedione was more efficacious than diacetyl in causing contraction. As in the case of diacetyl, the epithelium did not mediate responses to 2,3-pentanedione.

#### *4.2.6 Relevance of Diacetyl Animal Studies to Humans*

Animal exposure studies have revealed that the upper airways of rodents are sensitive to flavoring-induced toxicity, whereas the lower airways of humans are most affected by these agents. As detailed below, computational fluid dynamic-physiologically based pharmacokinetic models indicate that these differences in site of injury reflect interspecies differences in diacetyl dosimetry within the respiratory tract [Gloede et al. 2011; Morris and Hubbs 2009]. Nevertheless, the toxicological effects observed in rodents are consistent with the epithelium as the initial cell target in the airways [Hubbs et al. 2008; Morgan et al. 2008].

Rodents are obligate nasal breathers while humans are oronasal breathers. Inhaled air may bypass the human nose, particularly during exertion [Conolly et al. 2004]. In addition, the rodent nasal passageways and the rodent trachea are much narrower than the corresponding human

nasal passageways and trachea. Small airway diameter increases the percentage of the airstream which is in contact with the mucous layer, increases resistance, and thereby increases mucosal absorption of water soluble vapors [Frederick et al. 1998; Morris 1997]. Thus, the dimensions of the rodent nose predict much greater absorption of diacetyl vapors in the rodent than in the human nose. However, the surface area of the airways within the human lung is 100 times greater than the surface area of airways in the rat lung [Mercer et al. 1994]. These anatomic differences predict that, at a given exposure concentration, the mucosa in the rodent nose receives a much higher diacetyl dose than does the human nose and that the human lung receives a much higher dose than the rodent lung.

To investigate these dosimetry predictions, a CFD-PBPK model of diacetyl uptake was developed [Morris and Hubbs 2009]. The CFD-PBPK model also predicted greater intrapulmonary diacetyl concentrations in the lung of humans than in rats at a given exposure concentration, especially during mouth breathing [Morris and Hubbs 2009]. Under resting conditions at an exposure concentration of 100 ppm, the rat nose and trachea are predicted to remove 39% of the inhaled diacetyl, while the trachea of a mouth breathing human would remove 4% of the inhaled diacetyl. This same study suggests the potential importance of nasal lesions in rats for predicting pulmonary toxicity in humans [Morris and Hubbs 2009]. Because the respiratory epithelium of the terminal bronchiole of humans is believed to be the target tissue for the development of bronchiolitis obliterans [King 1989], and because diacetyl doses reaching the respiratory epithelium in the nose of rodents can be similar to diacetyl doses reaching the respiratory epithelium of the deep lung in man, it may be appropriate to consider toxicity to the respiratory epithelium lining the nose of rodents in evaluating the risk of diacetyl to mouth-

breathing workers. In addition, butyric acid, which is a component of some butter flavorings, caused a small but statistically significant reduction in nasal uptake of diacetyl in the rat nose, and thereby increased the diacetyl exposure to the lung due to a reduced "scrubbing effect" [Morris and Hubbs 2009]. A subsequent computational fluid dynamic – physiologically based pharmacokinetic model indicates that with low levels of exercise that could occur in the workplace, diacetyl dose to the bronchiolar epithelium of man may be more than 40-fold greater than the dose received by the bronchiolar epithelium of experimentally exposed rodents[Gloede et al. 2011].

### 4.3 Conclusions

Inhalation toxicity studies in rats and mice, and in vitro studies in guinea pig tracheal preparations indicate that diacetyl-containing butter flavoring vapors can damage airway epithelium and cause inflammation in the respiratory tract after acute or subchronic exposure. In addition, in vivo local lymph node assays indicate that diacetyl is a sensitizer and in vitro studies indicate that diacetyl is mutagenic. Diacetyl can react with arginine residues causing structural changes in proteins that influence the function of the altered proteins. These functional changes in proteins include changes in enzyme activity and the mitochondrial permeability pore. Pharmacologic studies in vitro indicate that diacetyl can alter ion transport and reduce epithelial integrity. CFD-PBPK modeling indicates that diacetyl concentrations in the deep airways of humans may be higher than those in laboratory rodents, explaining the tendency for diacetyl-induced airway damage to be more anterior in the respiratory tract of rodents than in humans.

Most recently, studies of the related alpha-diketone, 2,3-pentanedione, suggest that the airway toxicity of diacetyl may be shared with other structurally related, alpha-diketones.

# Chapter 5: Quantitative Risk Assessment Based on Worker Data

The goal of this chapter is first to present a numerical estimate of the risk of developing respiratory disease due to occupational exposure to diacetyl using standard epidemiological methods. This estimate is based on mathematical models that describe the relationship between exposure to diacetyl and the development of impaired lung function in a known population of exposed workers. Using these models, a further goal is to estimate an exposure level below which there would be a relatively low risk. Exposure-response modeling requires making assumptions about the exposures of the persons studied over the course of their working lifetime, and about the precise mathematical form of the exposure-response relationship. One approach that is used (benchmark dose) is to estimate how many additional people would have abnormally low lung function after a lifetime of working at various exposure levels. Another approach estimates how many new cases of abnormal lung function would develop over a working lifetime (excess lifetime risk). Finally, the various methods are used to develop a range of plausible risk estimates for various levels of occupational exposure to diacetyl.

Although diacetyl causes bronchiolitis obliterans, a debilitating and potentially fatal condition, it may be associated with a spectrum of disorders. Clinical observations present a picture of largely obstructive disease with a combination of reduced $FEV_1$ and $FEV_1$/FVC ratio ($FEV_1$: forced expiratory volume in one second; FVC: forced vital capacity). However, it may also cause restrictive ventilatory impairment, characterized by reduced $FEV_1$ and normal $FEV_1$/FVC ratio [Akpinar-Elci et al. 2004; Kreiss 2007; Lockey et al. 2009]. $FEV_1$ is the most commonly used outcome variable to assess lung function impairment caused by hazardous agents, regardless of the specific nature (obstructive or restrictive or combined) of impairment. American Thoracic Society/ European Respiratory Society (ATS/ERS) recommendations are to use $FEV_1$ to assess the severity of any type of spirometric abnormality [http://www.thoracic.org/statements/resources/ pfet/pft5.pdf ]. The health effects outcomes of diacetyl exposure that we used in risk assessment therefore included (1) reductions in $FEV_1$ (which would be seen in either obstruction or restriction), (2) reductions in $FEV_1$/FVC (a measure more specific to obstruction), and (3) onset of cases defined by symptoms in workers

whose $FEV_1$ and $FEV_1$/FVC are below their lower limits of normal, conditions that plausibly would include cases of developing bronchiolitis obliterans.

## 5.1 Methods: Study Population, Exposure Assessment, and Outcomes

### *5.1.1 Study Population*

Six NIOSH health hazard evaluations (HHEs) conducted at workplaces producing microwave popcorn with diacetyl exposures were reviewed for possible use in risk assessment [NIOSH 2001, 2003a, b, 2004a, b, 2006]. Four were determined to have the potential to provide sufficient work history, environmental assessment, and outcome information (pulmonary function) to support modeling of exposure response: Company G [NIOSH 2006], Company L [NIOSH 2004b], Company K [NIOSH 2004a] and Company N [NIOSH 2003a] (Table 5.1). In three of these HHEs a single episode of environmental and health outcomes assessment was conducted, but for Company G [NIOSH 2006] nine different surveys (eight with spirometry assessments) were performed, providing the possibility of a longitudinal analysis. With estimates of the diacetyl exposure response, standard risk assessment procedures can be applied.

### *5.1.2 Environmental Assessment and Exposure Estimation*

For workplace environmental assessments, the HHE surveys generally collected full-shift personal breathing zone (PBZ) and area diacetyl air samples using NIOSH Method 2557. This sampling identified a number of air contaminants in addition to diacetyl, including acetoin and acetaldehyde (Table 5.2). Problems in diacetyl sample determination with NIOSH Method 2557, related to humidity at the time of sampling and the elapsed time to sample extraction, were subsequently uncovered. Extensive work was performed by NIOSH researchers to understand this problem and derive an appropriate correction for estimating diacetyl levels [Cox-Ganser et al. 2011]. This adjustment procedure, based on absolute humidity and time to extraction, was applied to the diacetyl exposure levels reported in the selected HHEs. For other chemical exposures in microwave popcorn production, such as acetoin, no humidity/extraction correction was needed.

For laboratory diacetyl determinations below the limit of detection (LOD), the sample value was set equal to LOD/2. For determinations above the LOD but below the limit of quantification (LOQ), the actual diacetyl determination was used. All diacetyl determinations above detection limits were then adjusted for absolute humidity and time–to-extraction in the same manner as other samples. At Company K, 44 out of 60 samples (personal and area) were < LOD. At Company L, 4 out of 125 samples were < LOD and at Company G, 105 out of 262 personal and 146 out of 346 area samples were < LOD.

The characterization of historical exposures was limited by the absence of air sampling prior to the NIOSH HHEs. In the case of Company L, engineering modifications had been implemented prior to the NIOSH exposure assessment, including adjustment of factory air pressures to reduce migration of diacetyl from the mixing areas. In contrast, the situation at Company N at the time of the assessment was perceived not to have changed over time. Over the course of the nine evaluations at Company G a dramatic downward trend in diacetyl air concentrations was observed, reflecting implementation of NIOSH recommendations and consultations for controlling exposures. However, it is not known what changes, if any, may have occurred prior to the first assessment. The NIOSH exposure assessment for Company K found diacetyl airborne concentrations to be quite low and similar to Company L airborne concentrations. Company K had taken exposure control steps, including provision of powered, air-purifying respirators (PAPRs) for diacetyl mixers, soon after the introduction of microwave production following an outbreak of eye irritation. NIOSH-measured diacetyl exposure levels for key process locations showed considerable variation across the HHE sites with higher levels at Company G and Company N (Table 5.3). The generally lower airborne concentrations at Company K and Company L may have occurred because the mixing operations at those two plants were isolated from the production areas unlike the situation at Company G and Company N. Mean diacetyl exposures for the Company K, L and N populations were calculated classifying by department and job (Appendix 3, Tables A3.1–A3.3) based on the absolute humidity and extraction delay-adjusted measured air concentrations of diacetyl.

The most extensive and representative diacetyl exposure data and the largest body of respiratory outcomes data were available from the HHE at the Company G microwave popcorn plant [Kullman et al. 2005; NIOSH 2006]. This population had the largest number of air samples, over nine surveys, and it was assumed that there were no significant exposure control changes prior to the first survey. Therefore, the risk assessment largely relied on the Company G findings, comprising repeated environmental assessments between November 2000 and July 2003 (2.7 yr). The estimation of diacetyl exposure levels at Company G over time within department/job combinations was based on a job exposure matrix (JEM) that was constructed through collaboration between NIOSH and OSHA (Appendix 4). Plant job titles were aggregated into eight exposure categories based on work and environmental similarities (Table 5.4) [Corn and Esmen 1979]. Starting with the humidity- and time-to-extraction-adjusted personal breathing zone sample concentrations (in parts per million), means were calculated for the cells in the JEM (Appendix 3, Table A3.4). Arithmetic means of personal samples are the preferred measure of central tendency for estimating cumulative exposure in chronic disease investigations [Smith 1992]. However, for the first industrial hygiene survey (November 2000), only area samples were collected, and thus personal sample-equivalent exposures were estimated (Appendix 4). Unique exposure time periods were developed for each of the eight exposure categories to reflect impact of the exposure control changes implemented at the plant from November 2000 to July 2003. Within the time periods for each JEM exposure category, exposures were assumed to be constant. The exposure estimates in the JEM were assigned to workers based on their work histories using information on the jobs performed, their duration, and the calendar time period. For work history prior to the first industrial hygiene survey, exposure estimates from the first time period were used. For some workers such as those in the mixers exposure category, the measured personal diacetyl exposure was adjusted for the use of respirators for selected exposure periods.

Problems in the retrospective exposure assessment for diacetyl include (1) uncertainty over when diacetyl was introduced and on the extent of its use as a flavoring component over time (and therefore on employee exposure levels), (2) variation in diacetyl content across different product lines over time, (3) the relative presence of diacetyl as a vapor vs. adsorbed to powders or

encapsulated, and (4) seasonal variation in the role of natural ventilation. Cumulative exposure and other exposure metrics were calculated starting at the dates when diacetyl was believed to be first used in regular production at the four plants: Company K (July 1, 1988), Company L (January 1, 1994), Company G (July 1, 1986), and Company N (July 1, 1986). These dates are uncertain, particularly for Company N.

### *5.1.3 Work History*

The workers studied were current employees at time of survey except at Company G where some former employees were also examined. All results presented are for current workers except at Company G where, due to repeated pulmonary testing over months or years, initially current workers could become former workers at a subsequent survey. Participation was voluntary and generally quite high among current employees (66%–91%) (Table 5.1). Work history was routinely collected in HHEs by worker interview and consisted of successive periods of employment in specific department and job title assignments with corresponding beginning and ending dates. Gaps in employment were treated as unexposed and not included in duration-of-exposure measures.

### *5.1.4 Outcomes*

Reported symptoms and pulmonary function test (PFT) – spirometry – results defined the HHE outcomes. A medical questionnaire was administered that included standard American Thoracic Society (ATS) items on respiratory health [Ferris 1978b] as well as dermal symptoms, allergies, detailed smoking history, and questions on other exposures and protective equipment used. Sustained-symptom onset dates were also collected. Spirometry testing was performed following ATS guidelines [Ferris 1978a]. The *predicted* and *lower limit of normal* (LLofN) values for $FEV_1$, FVC and $FEV_1$/FVC were calculated using prediction equations produced from the third National Health and Nutrition Examination Survey (NHANES) [Hankinson et al. 1999]. The lower limit of normal has been defined by ATS as approximately the lower $5^{th}$ percentile of ventilatory function within the general population classified by age, gender, race and height.

For risk assessment purposes workers' percent of predicted values for $FEV_1$ and actual $FEV_1$/FVC ratios were the outcomes modeled as continuous variables. In identifying possible developing bronchiolitis obliterans cases, a classification of pulmonary impairment was defined based on $FEV_1$ or $FEV_1$/FVC being less than their respective LLofN. This discrete outcome, onset of impairment, was analyzed by modeling incidence rates. Bronchiolitis obliterans is thought of as largely irreversible obstruction; reversibility of obstructive changes was assessed in these HHEs using bronchodilator medication for individuals with $FEV_1$/FVC and $FEV_1$ less than their respective LLofNs. However, fifty seven percent of the cases defined using $FEV_1$ were not tested for reversibility, and only one of the cases tested was reversible (increases in $FEV_1$ of at least 200 ml and 12%). Frequently there was a substantial residual deficit after bronchodilation. Therefore cases were defined without regard to reversibility. The classification of cases was not based on clinical diagnoses because the systematic medical data collected in the HHEs were limited to the questionnaire and spirometry tests. A complete diagnostic work-up of probable cases is not routinely performed in NIOSH HHEs though full disclosure of individual test results and recommendations for referral are provided to participating workers.

## 5.2 Methods: Analysis of Exposure Response

### *5.2.1 Exposure Metrics*

The most appropriate measure of past exposure to diacetyl for predicting health consequences is not known and hence should be determined by assessment of the statistical fit of various models using different exposure terms. Cumulative exposure (cum(DA)) was the starting choice for exposure metric but dose-rate effects were examined by calculating the time summation of the square root or square of diacetyl concentration corresponding respectively to diminishing and increasing marginal responses to increasing exposure intensity (dose-rate effects) as follows:

$\text{cum(DA)} = \Sigma_i\,(\text{DA})$, $\text{cum}(\text{DA}^{0.5}) = \Sigma_i\,(\text{DA}^{0.5})$ and $\text{cum}(\text{DA}^{2.0}) = \Sigma_i\,(\text{DA}^{2.0})$ where the summation was over calendar days.

Transformed cumulative exposures as the square root, square, or logarithm were evaluated as were duration of exposure and average exposure concentration (*cumulative exposure*/*duration of exposure*). Peak exposures were not directly available from full shift (8-hour) TWA concentrations although selected jobs were analyzed using a real-time method (Fourier Transform Infrared gas analyzer) to assess time-variability.

#### *5.2.2 Models of Percent Predicted $FEV_1$ and $FEV_1$/FVC*

The spirometry determinations, (1) percent of predicted $FEV_1$ ($ppFEV_1$), and, (2) $FEV_1$ /FVC, were analyzed as continuous outcomes in multiple linear regression models. Terms in the models included gender, ethnicity (Hispanic/Non-Hispanic), race (African American/Other), 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. Known potential confounders were retained in models regardless of statistical significance according to good epidemiologic practice. Models of $FEV_1$ /FVC included age (centered at 40). Models were assessed using over-all model $R^2$ as well as the *P* value for exposure metric terms. In the case of Company G with repeated survey outcomes, the last recorded spirometry was used for analyses unless stated otherwise. In models of $ppFEV_1$, the expected intercept in the absence of exposure effects would be 100. Models were fit using PROC REG in SAS 9.2 [SAS Institute Inc. 2008].

To make full use of the serial spirometry determinations at Company G, a longitudinal analysis of $ppFEV_1$ was performed in which exposure metrics were calculated from time of first diacetyl exposure up to the time of each successive spirometry determination. This analysis included workers with one or more spirometry results. All workers were active at their first survey but could have left employment prior to a subsequent survey. These models were fit using PROC MIXED in SAS 9.2 [SAS Institute Inc. 2008] with random effects permitted for individual workers' intercepts and exposure responses. A second set of metrics was calculated with exposure cumulation starting at the time of a worker's first survey and used in a subsidiary

longitudinal analysis. This analysis permitted a test of homogeneity, i.e., (1) for exposure effects before and after the first survey and (2) for possible survivor bias.

Pooled analyses were conducted for two plant populations (Company K, L) with similar reported average exposures and estimated exposure responses. Plant effects were introduced to allow for systematic differences between the two sites, and there was a test of heterogeneity in the exposure effects.

### *5.2.2 Models of the Incidence of Pulmonary Obstruction*

For analyses of onset of discrete adverse effect outcomes, conducted for the Company G population (n=361), two case-definitions of pulmonary impairment were applied:

1) $FEV_1 <$ LLofN; n=36

2) $FEV_1 <$ LLofN and $FEV_1/FVC <$ LLofN; n=19

with definition 2 representing a measure more specific to obstruction. For the combined Company L and Company K populations, the case definition for determining onset of pulmonary impairment was: $FEV_1 <$ LLofN (n=25). The more restrictive definition produced too few cases for meaningful analysis.

In identifying cases, a date of onset for a condition resulting in impairment and possibly representing bronchiolitis obliterans was estimated as the average of the dates on which the worker reported the start of one or more continuing symptoms (cough, wheezing, shortness of breath, tightness of chest or phlegm, based on questionnaire items), provided those symptom dates were after the date of first exposure to diacetyl. The average date was chosen over the first date to be more robust for recalled dates. If no qualifying symptom date existed, then date of onset was set to the date of first case-qualifying spirometry result (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 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, according to the participating HHE clinicians, also may have included asymptomatic cases caused by diacetyl exposure.

The incidence of new cases was modeled using Poisson regression [Checkoway et al. 2004]. This method produces an estimate of the background rate needed for a life-table-based calculation of excess lifetime risk. Observation time was compiled beginning with date first exposed to diacetyl. Models were fit using PROC COUNTREG in SAS 9.2 [SAS Institute Inc. 2008] and model fit assessed with the likelihood ratio test. Employment duration and the other covariates (age, gender, smoking) were included in these models. This design has potential bias leading to underestimated rates arising from the departure of affected workers from employment between the times of a worker's first exposure and first survey. Cases arising in that period are available for analysis only if the individual remains in employment until, and chooses to participate in, a spirometry-medical survey.

### 5.3 Results: Exposure Response

#### *5.3.1 Cross-sectional Pulmonary Function Changes*

Multiple regression analyses for the Company G population (the largest group, n=361, with the most extensive exposure assessment) controlling for gender, ethnicity, and smoking, revealed statistically significant declining $ppFEV_1$ for all diacetyl exposure metrics, with Cum(DA) ($p=10^{-6}$) and $(Cum(DA))^{0.5}$ ($p=4\times10^{-7}$) performing considerably better than exposure duration alone, and with Avg(DA) and $Cum(DA^{2.0})$ performing less well than duration (Table 5.5). The estimate for the exposure-response with Cum(DA) was a 0.5 reduction in $ppFEV_1$ for each ppm-year of cumulative exposure (Table 5.6). (After 1 year at 1 ppm a worker's $ppFEV_1$, starting at 100, would be predicted to be 99.5.) In the models with the better predicting metrics, gender and ethnicity (possible indicators of differential healthy worker selection) were unimportant predictors. Ever-smoking was associated with an increase in $ppFEV_1$ but cumulative smoking, in pack-years, predicted a decline in $ppFEV_1$ (implying that, initially, smokers may be healthier

than nonsmokers); both effects were statistically significant (Table 5.6). Regression models based on the first Company G spirometry determination rather than the last yielded similar estimates of diacetyl effects (data not shown). The metric *cumulative square root of diacetyl concentration* was a slightly stronger predictor of spirometry changes than simple cumulative exposure (Table 5.6), implying that if there is any dose-rate effect it is probably negative—lower exposures have larger than proportional association with decreases in spirometry. Results for Company N, based on a small number (n=35) of workers, are not presented but were generally comparable to Company G results.

The best-predicting exposure metric depended on the HHE population analyzed (Tables 5.7, 5.8). In predicting $FEV_1$/FVC the R-square values were consistently larger compared with the $ppFEV_1$ regressions but the exposure effects were sometimes less significant. For Company G, Avg(DA) and $(Cum(DA))^{0.5}$ were the better predictors of $FEV_1$/FVC; for Company K, Cum(DA) was best while for Company L, Cum(DA), $(Cum(DA))^{0.5}$ and $Cum(DA^{2.0})$ were all equivalent better predictors. For $ppFEV_1$, model fit at Company K was strongest for $(Cum(DA))^{2.0}$, however, Cum(DA) provided a similar fit. For Company L, average exposure was the strongest predictor of $ppFEV_1$. In the pooled analysis of the Company K and Company L plants with the better predicting metrics Cum(DA) and $(Cum(DA)^2$, the differences in exposure response between the plants for $ppFEV_1$ and $FEV_1$/FVC were highly significant (Tables 5.9, 5.10). The pooled estimate for the Cum(DA) metric was a fall in $ppFEV_1$ of 4.22 per ppm-yr of cumulative exposure (Table 5.9), almost an order of magnitude higher than the Company G estimate (Table 5.6). At these two plants, many of the environmental samples collected were below the limit of detection for diacetyl, and the HHE environmental assessments were cross-sectional and not necessarily reflective of exposures prior to the survey date. This may explain the divergence in optimum exposure metrics compared with the Company G results. For example, if jobs with the highest exposures were given priority for control interventions, then the subsequently measured levels would be underestimated most for the jobs originally having the highest levels. Therefore, an exposure metric like Cum $(DA^{2.0})$, which gives greater weight to high values, might better predict spirometry changes than Cum(DA), as was observed at Company K for $ppFEV_1$ and $FEV_1$/FVC, and at Company L for $FEV_1$/FVC (Table 5.5). Because of the inconsistencies

between them and less certain exposure histories, the results for Company K and Company L were not the basis for the NIOSH risk assessment for diacetyl which, instead, relied on the Company G findings.

Acetoin is another exposure in the popcorn flavoring environment (typically present with diacetyl in flavoring additive packages) and its presence is strongly associated with diacetyl (corr = 0.85) but it is not subject to the humidity degradation problem in air sampling. In response to concerns that the corrected historical exposure measurements for diacetyl were imprecise, we repeated models of exposure-response relationships using acetoin measures. Applying to acetoin the procedure used for constructing the exposure matrix for diacetyl resulted in estimated acetoin concentrations over workers' work histories. Multiple linear regressions predicting percent of predicted $FEV_1$ based on acetoin exposure metrics produced the same pattern of results as observed with diacetyl and with almost identical model fit. For the metric square root of cumulative exposure, the $R^2$ observed was .1743 and .1737, respectively, for acetoin and diacetyl; the t-statistics for the exposure terms were 5.09 and 5.06 respectively. Because there is little support for acetoin itself playing a role other than as surrogate for diacetyl in pulmonary toxicity, based on animal or other human studies, this result supports the validity of the diacetyl exposure assessment and subsequent findings but also suggests that the diacetyl effects are being underestimated as a result of misclassification.

#### *5.3.2 Longitudinal Analyses of ppFEV$_1$ at Company G*

Longitudinal mixed effect models of $ppFEV_1$ (where individual intercepts and responses are treated as random effects) show smaller effects for both Cum(DA) and $Cum(DA)^{0.5}$ exposure metrics compared to the analyses based on the $FEV_1$ at last survey (Tables 5.7, 5.8); the effects remain statistically significant (Table 5.11, models 1 and 2). Differences in effects of exposures accruing in workers following their initial evaluation, compared to exposures prior to their first survey, using either the metric Cum(DA) or $Cum(DA)^{0.5}$, were small and not statistically significant ($p > .7$) (Table 5.11, models 3 and 4). This supports the inference that the likely

exclusion of workers terminating prior to the surveys has not biased exposure-response estimation for the decline in ppFEV$_1$.

#### *5.3.3 Incidence of Pulmonary Impairment at Company G*

Poisson regression analysis with the log-linear specification was applied to model incidence rates adjusted for gender, age, and smoking (race and ethnicity were not important predictors). Excluding candidate cases for which no qualifying date of onset was available (after start of a worker's exposure) and those missing smoking data left 314 subjects for analysis. The original sentinel cases of bronchiolitis obliterans reported from this plant were not participants in this analysis. For the first definition of case (FEV$_1$< LLofN, n=36), both the duration of exposure and diacetyl cumulative exposure (Cum(DA)) predicted diminishing onset with increasing duration or exposure metric (Table 5.12, models 1,2). Model fit improved with both terms in the model but the duration effect remained negative (Tables 5.12, 5.13). Other diacetyl metrics performed similarly (Table 5.12) with avg(DA) and cum(DA) providing the best fit (largest Δ-2lnL, smallest LRT p). A negative duration term implies diminishing background rate with increasing duration. With the more stringent case definition (FEV$_1$< LLofN *and* FEV$_1$/FVC< LLofN, n=19), the negative duration effects were more significant and cumulative exposure was now statistically significant (*P*=.016); three other metrics yielded stronger associations, particularly the square-root of cumulative exposure ((Cum(DA))$^{0.5}$) and average exposure (Avg(DA)) (both *P*=.003) although with average exposure, duration was no longer significant (Tables 5.14, 5.15). In these models smoking effects were not statistically significant, and age and sex were marginally significant (Tables 5.13, 5.15).

#### *5.3.4 Evidence of Variable Susceptibility to Diacetyl Effects*

When the joint distribution of cases by exposure duration and cumulative exposure was examined (case definition 1; all jobs had exposures > 0.0), the pattern suggested the possible presence of a high-risk subpopulation or variable susceptibility. For example, there were five cases in the cell with lowest duration and lowest exposure and another five cases in a different

cell with comparable person-years of observation (89 yrs) in the highest exposure category and 2 to 4 years duration (Tables 5.16, 5.17). Thus similar rates were observed despite the greater than 10-fold difference in cumulative exposure. Sixteen of the 36 cases occurred in the first 2 years of exposed-employment. Cases of rapid onset of this disease have been reported [Akpinar-Elci et al. 2004; CDC 2007; Israel et al. 2009; Kreiss et al. 2002; NIOSH 2006, 2008b]. Examination of onset graphically (data not shown) also suggested that many cases arose after relatively short employment duration. A similar pattern was exhibited in the 46 cases (defn 1) identified among former employees (no longer employed at their first survey) (data not shown). The predicted baseline incidence (from the model with diacetyl exposure set = 0) in the same array (Table 5.18) has an elevated level 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 a rate ratio that appears to be systematically elevated at low durations of exposure (employment after 1986) and at high cumulative exposures (Table 5.19). The same situation was observed in the pooled Company L and Company K populations using the first case definition. Ten out of 25 cases occur in the low duration-low cumulative exposure strata (Table 5.20), with elevated rates predicted for low durations and high exposures (Table 5.21). With the second case definition in the Company G population, the same pattern is observed but now with fewer cases (n=19 vs. 36) and only *4* in the apparent high risk group (< 2yr duration and < 2 ppm-yr cum. exposure; Table 5.22). The predicted rate ratios relative to the long-duration baseline rate are again elevated at both low duration and high cumulative exposures (Table 5.23).

In the loglinear models using a (negative) duration term, the excess cases at short duration are actually being treated as part of the background rate, i.e., not attributable to diacetyl exposure. On the suspicion that a high risk subpopulation was present and declining with duration, either because it is being depleted (becoming cases) or leaving employment, a different Poisson regression model was fit using a linear relative rate specification that included a term intended to capture excess risk arising from diacetyl exposures in an unknown subpopulation diminishing with time. An exponential decline was assumed and half-lives of 0.5, 1 and 2 years were evaluated. A model with a term of the form $[\text{Avg(DA)}]^2 \times \exp(-0.693 * \text{Duration})$, i.e., half-life of 1 year and squared average exposure, produced a significant fit (LRT=7.97, 2df, p=.0186; Table

5.24, model 3) with the two exposure terms being considerably stronger predictors than in models with either one alone (Table 5.24, models 1, 2). Of the choices examined for parameters in the high-risk term, the best fit occurred with a half-life of 2.0 years and squared average exposure (LRT=9.52, 2df, p=.0086; Table 5.24, model 4; Table 5.25). In this model, the estimated rate ratio for 1.0 pack year of smoking was 17.7 and, for 1.0 ppm-yr of diacetyl exposure in the "low-risk" group, it was 12.3; the initial (start of exposure) rate ratio for the high-risk group at 1 ppm diacetyl was 69.8.

The relative fit of various incidence-rate model specifications (case definition 2) indicates that, for a single metric, the *average prior exposure* metric fits best, but considerable improvement comes with an added duration term (Table 5.26). The best fit was for a) cumulative exposure with duration squared terms or b) square root of cumulative exposure with duration term (loglinear design), and with c) cumulative exposure and the term for a high-risk subpopulation (linear relative rate design).

### *5.3.5 Interpretation of Modeling Results*

Multiple linear regression models of continuous spirometry outcomes at the Company G plant reveal that both cum(DA) and $(\text{cum(DA)})^{0.5}$ are preferred predictors of $FEV_1$ decline based on model fit. Average exposure was the weakest predictor of $ppFEV_1$. Subsidiary analyses indicate that a) a dose-rate effect, if present, is small and negative; b) bias arising from possible removal of earlier cases was small, and c) the bias introduced by the correction procedure addressing degradation of diacetyl air samples is also small although possibly causing underestimation of the diacetyl effect. Evidence for non uniform susceptibility suggests that the somewhat superior prediction by $(\text{cum(DA)})^{0.5}$ compared to cum(DA) may be a reflection of a heightened response in the population at short durations of exposure. Similarly, the relatively strong but implausible prediction of $FEV_1/FVC$ by average diacetyl exposure is interpreted as a likely consequence of varying susceptibility.

In the modeling of incidence, fewer cases met the second case definition than the first (19 vs. 36) due to the added requirement: $FEV_1/FVC < LLofN$. This was consistent with some restriction as was observed in regression models of forced vital capacity (FVC). Using the second case definition, the estimated baseline rate is very small (Table 5.24, models 3,4); baseline annual rate = 0.007% per year ($365.25 \times \exp(-15.48)=0.00007$), indicating that virtually all cases were attributable to either diacetyl exposure or smoking. The estimated rate ratio for 1.0 pack year of smoking was 17.7 and, for 1.0 ppm-yr of diacetyl exposure in the entire group, it was 12.3; the initial (start of exposure) rate ratio for the high-risk group at 1 ppm diacetyl was 69.8. The strong association with the term representing short duration of exposure supports the conjecture that a high risk subpopulation was present, declining by about half with each 2 years of exposure duration. Although average diacetyl exposure by itself is a strong predictor of incidence, as with prediction of $FEV_1/FVC$, this appears to be an artifact of changing population susceptibility and has little biological plausibility as a risk factor itself.

The existence of a transient high risk group poses a challenge for predicting excess cases over a 45-year working lifetime because the composition of the population with respect to the factor(s) conveying high risk cannot be anticipated with certainty due to workforce turnover and the continual introduction of a high-risk segment to employment.

In a population with relatively uniform response to diacetyl exposure (uniform susceptibility), the early new cases resulting from diacetyl exposure would in general constitute individuals who were already very close to their LLofN. For a given age and height, this subpopulation is proportional to the height of the $FEV_1$ distribution at the LLofN (Figure 1). With increasing cumulative exposure the $FEV_1$ distribution is shifted toward lower values so that the segment at immediate risk of falling below the LLofN would be increasing as long as the mean of the shifted distribution remains above LLofN. This is not what was observed; initially the rate of new cases is actually larger and declines with increasing cumulative exposure, implying variable susceptibility, i.e., some individuals in the exposed population are losing $FEV_1$ much faster than others.

## 5.4 Human Data-based Assessment of Risks

Using the impairment findings from the Company G plant NIOSH employed two approaches for assessing risk of diacetyl exposures. The first was the widely used benchmark dose procedure, which is appropriate for cross-sectional population surveys with continuous health outcomes, and the second was calculation of excess lifetime risk, a life-table procedure which accounts for competing risks using a model for the rate of onset of a discrete outcome. In these calculations, two exposure estimates were derived: for a life-time exposure (45 yr) and also for a 10 yr exposure (a more typical employment duration and implying a larger workforce ever exposed). The nominal standard for acceptable risk used was one per thousand excess risk, a choice often used in OSHA regulation.

### *5.4.1 Benchmark Dose*

#### 5.4.1.1 Methods

For continuously distributed respiratory endpoints such as $FEV_1$, the benchmark dose approach permits estimation of increased prevalence of impairment as a function of prior exposure history [Bailer et al. 1997; Clewell et al. 2003; Crump 1995; Park et al. 2006]. On the basis of linear regression models and population data on the distribution of $FEV_1$ from NHANES III [CDC and NCHS 2011], the proportions of the workforce predicted to be impaired after working at specified exposure levels can be calculated. This method requires specification of what degree of deficit constitutes impairment and the maximum increase in impairment prevalence that is considered acceptable, which are policy choices. The exposure resulting in a maximum allowable increase in impairment is called the "benchmark dose" (BMD).

#### 5.4.1.2 Risk assessment with percent predicted $FEV_1$

With the conventional benchmark dose procedure, the excess prevalence of an adverse condition is calculated using an exposure-response relationship derived from modeling. With the linear regression result for percent predicted $FEV_1$ and Cum(DA) (coef.=0.5, Table 5.6), the excess prevalence after 10 or 45 years of exposure for falling below (1) 60% of predicted, or (2) the 5th percentile of normal, was calculated as a function of exposure level (Table 5.27). Given these two pulmonary impairments, a 1/1000 excess prevalence after 45 years was found for diacetyl

exposures (BMDs, central tendency estimates) of about 0.04, and 0.007 ppm diacetyl, respectively. Using the exposure metric, $(Cum(DA))^{0.5}$, which better predicts $ppFEV_1$, substantially lower BMDs result (Table 5.28); 1/1000 excess risk for impairment at the 5th percentile after 45 years occurs with a diacetyl exposure concentration of less than 0.0001 ppm vs. 0.007 with the Cum(DA) metric (Table 5.27). These lower BMDs result from the increasing (negative) slope of the exposure response with diminishing exposure metric. With a high-risk subpopulation present that is diminishing with time, the calculated excess prevalence with decreasing cumulative exposure would increasingly pertain to that subpopulation, and therefore not be representative of the aggregate population. For this reason we chose Cum(DA) over $(Cum(DA))^{0.5}$ as the basis for risk assessment using the BMD procedure.

For impairment defined in relation to the lower limit of normal, the BMD procedure is less direct. LLofN is specific to age, height, gender and race so that the distribution of various functions of $FEV_1$ and LLofN, such as $FEV_1$/LLofN or ($FEV_1$- LLofN)/(pp $FEV_1$ - LLofN) are not readily specifiable. An alternate approach was taken: in the NHANES population [CDC, NCHS [2011] the cumulative exposure that would reduce individuals' $FEV_1$ to their LLofN was calculated using the exposure-response estimate from the preferred regression model of $ppFEV_1$. The prevalence of individuals predicted to be below their LLofN was then calculated in the NHANES III population as a function of exposure over 10 or 45 years. This "empirical" BMD procedure (using the empirical, nonparametric distribution of the NHANES population) yielded BMDs for both $FEV_1$ and $FEV_1$/FVC (Tables 5.29, 5.30). For $FEV_1$ below the LLofN ($FEV_1$) the BMD values were somewhat similar to those calculated the traditional way for $ppFEV_1$ in relation to impairment at the 5th percentile of normal; the excess prevalence after 45 years at 0.01 ppm diacetyl was 2.5/1000 and 1.5/1000, respectively (Tables 5.27, 5.29). BMDs for $FEV_1$/FVC below the LLofN ($FEV_1$/FVC) were comparable to those for $FEV_1$ (Tables 5.30, 5.29). In the pooled Company K and Company L population, where reported exposures were lower than at Company G, the estimated BMDs were much lower: for $FEV_1$, 0.0005 ppm and $FEV_1$/FVC, 0.0004 ppm (Table 5.31). Using the less satisfactory, average exposure, avg(DA), as the predicting metric in the Company G population, the excess prevalence was estimated to be

considerably lower (Table 5.32); the 1/1000 BMD for $FEV_1$, was correspondingly higher: 0.1 ppm diacetyl.

#### 5.4.1.3 Analysis for potential susceptible subgroup

These BMD results pertain to the risk predicted by the linear regression models using cumulative exposure and interpreted to best reflect the long-term risk experienced by workers not in the high-risk group. To encompass both the high-risk and regular population, the traditional BMD procedure was modified to include cases thought to arise from a high-risk group, as follows. From the 314 workers followed, 13 cases (case definition 1) were observed in the low duration/low exposure levels, of which 8.2 were estimated to be excess cases (Table 5.33) corresponding to 26.1 per 1000. The mean average exposure of the 13 total cases was 0.79 ppm diacetyl corresponding to this risk of 26.1/1000.Then, by linear extrapolation, 0.030 ppm corresponds to 1/1000, and 1 ppm ~ 33.0/1000. Adding the predicted high-risk cases for different levels of the exposure metric to the empirical BMD table provides composite BMDs including the high-risk contribution (Table 5.34). For the 10-year BMD calculations, corresponding to an average work tenure of 10 years, the high-risk contribution occurred one time, making the general assumption that one high-risk group per 1000 hires has been observed. For the 45-year calculation, the high-risk contribution was increased by a factor of 4.5 because with an average tenure of 10 years, 4.5 groups would be hired over the course of 45 years of equivalent employment. Alternatively, the 10-year composite excess prevalence estimates were multiplied by 4.5 to compare with the 45-year estimates (Table 5.34). At 0.01 ppm diacetyl, the excess prevalence after 45 years was 4.0/1000, compared with 2.5/1000 obtained without consideration of the high-risk group. The 10-year estimate, multiplied by 4.5, yielded 3.2/1000 excess prevalence.

### *5.4.2 Excess Lifetime Risk for Pulmonary Impairment*

#### 5.4.2.1 Methods

Using the life-table approach first implemented in the BEIR IV report [Committee on the Biological Effects of Ionizing Radiation 1988] together with the observed exposure-response relationship from models of incidence rate, one can estimate the excess numbers of cases of diacetyl-associated impairment that would occur as a result of lifetime exposures at various concentrations. This method assumes irreversibility and removes incident cases from the population at risk with increasing age along with deaths arising from the usual causes found in the general population. Although typical applications for the excess lifetime risk calculation are for fatalities arising from chronic diseases, the method can be applied to incidence of an irreversible condition provided a baseline incidence rate for the condition is known and an estimate of the exposure-related incidence rate ratio is available. In this analysis, Poisson regression models formed the basis of the calculation, with the model intercept describing the baseline risk.

#### 5.4.2.2 Risk Assessment: Excess Lifetime risk

A national life-table constructed from Social Security data [SSA 2005] was used. The surviving population (living but not yet a case) was calculated annually assuming exposure starts at age 20 and ceases at age 66, for a 45 yr exposure. For 10 years of exposure the life-table exposures covered ages 20–30. Because smoking information was used in modeling, several variants for lifetime risk are presented (Table 5.35). For example, at 0.01 ppm diacetyl, using an incidence model (case definition 2) that ignores smoking determinants, the excess lifetime risk (analogous to excess prevalence in the BMD approach) was 3.2/1000. On the basis of a model that includes the smoking determinants, the excess lifetime risk at 0.01 ppm diacetyl for nonsmokers was 11.2/1000, while for smokers (one pack/day) it was 2.2/1000. Smokers have a smaller lifetime risk because smoking is a strong competing cause for becoming a case.

A number of investigations have observed that declining pulmonary function is a risk factor for mortality independent of other possibly associated risk factors such as age, gender, smoking, and BMI. Three such studies investigated rate of decline in pulmonary function as a predictor of mortality [Mannino and Davis 2006; Mannino et al. 2006; Rodriguez et al. 1994] and five others predicted mortality using current $FEV_1$ [Bang et al. 1993; Hole et al. 1996; Ryan et al. 1999; Sabia et al. 2010; Schunemann et al. 2000; Sin et al. 2005]. Three studies provide estimates of rate ratios that can be applied to a life-table analysis of excess lifetime risk [Bang et al. 1993; Ryan et al. 1999; Schunemann et al. 2000]. The estimates range from 1.010 to 1.019 per percent decline in $FEV_1$ in men, and from 1.01 to 1.025 in women. Assuming a RR of 1.015 per percent decline in $FEV_1$, a life-table analysis produced estimates of excess lifetime risk (Table 5.36) that were comparable to those based on the incidence of pulmonary impairment, e.g., $FEV_1$ falling below LLofN (Table 5.35). These estimates of excess mortality are the result of a generic effect of declining $FEV_1$ on mortality not specific to bronchiolitis obliterans. This generic effect may not adequately predict mortality proceeding from advancing bronchiolitis obliterans disease itself with high exposures to diacetyl.

### 5.5 Sensitivity Analyses and Alternate Hypotheses

NIOSH evaluated many different statistical models and procedures using continuous and discrete outcomes based on different definitions of impairment, different exposure metrics, and data from different plants. For Company G, the risk estimates are surprisingly similar for the different modeling approaches and the diacetyl levels estimated for a given level of life-time prevalence or risk are generally pretty close, within an order of magnitude.

Models where percent predicted $FEV_1$ or $FEV_1$/FVC were used as the response to occupational diacetyl exposure showed declines in pulmonary function with increasing exposure, no matter which exposure metric was used. Similarly, when models looking at the rate of pulmonary impairment defined in two different ways were compared, the same pattern was observed indicating that there was an unexpected elevated effect in the low exposure, low duration group.

Alternate formulations such as for dose-rate, comparing exposure effects pre- and post-first survey, comparing prediction based on diacetyl vs. acetoin, a surrogate for diacetyl, all supported the final choices utilized in the risk assessment. On the question of unknown exposures prior to the NIOSH surveys, particularly the date when widespread diacetyl exposure were present at Company G, analyses specifying different years for the start of exposures suggested that the optimum starting year was about 1994 instead of 1986, but this assumption had only a small impact on the estimated exposure response.

Several alternate explanations were investigated for the apparent variability in susceptibility:
(a) The proportion of Hispanic workers was higher among the short duration cases but Hispanics also comprised a higher proportion among recent hires and the cross-sectional surveys tended to reflect more recent employees due to high turnover.
(b) For candidate cases lacking a symptom onset date, using the date of their first qualifying spirometry would increase rather than decrease the estimate of duration of exposure until onset and thus would not account for the short-duration cases.
(c) Recall bias on symptom onset: workers with fast onset probably estimated symptom onset in relation to hire date, not in relation to survey date. For example, a worker with three years employment probably would recall that symptoms began after about 6 months on the job, not two and a half years ago.
(d) Jobs with peak exposures (dose-rate effect) would not favor an early onset unless the cumulative exposure metric was underestimating the relevant exposure. The study could not address peak exposure effects directly because the exposure data didn't exist although a general pattern of a positive dose-rate effect (non linear concentration effect) was not evident in analyses using cumulative square root or squared concentrations. Serious exposure misclassification could cause a pattern indistinguishable from variable susceptibility; workers whose exposures were consistently underestimated would appear to respond more strongly (faster) with adverse health effects and conversely for workers whose exposures are overestimated. However, the "high risk" cases were not largely associated with specific job groups such as mixers or quality control; many came from the general production line. Undoubtedly misclassification was present but a

systematic discrepancy by a factor of 10, as observed between the "high risk" group and others seems implausible.

In summary, these sensitivity analyses substantiated the parameters, variables, and assumptions used in the final risk assessment and provide confidence in the risk estimates.

### 5.6 Discussion

The NIOSH HHE investigations in popcorn manufacturing were not specifically designed for quantitative risk assessment and have limitations in terms of unknown selection of study subjects and limited historical exposure information. Nonetheless, these observations of workers exposed to diacetyl are useful for risk assessment. The likelihood that the Company G population represents a survivor cohort together with the relatively high participation rate implies that underestimation of effects has probably resulted. Further underestimation has resulted from exclusion of asymptomatic cases in the analyses of incidence. Acting against bias from selection of a surviving population and missing cases is the possibility that participants may have included a more than representative proportion of cases. However, the high participation rate (~80 %) limits the potential bias arising from participation. Evidence has been observed for substantial population variability on susceptibility to diacetyl effects. This variability could arise from differing responses related to smoking status, other host factors like BMI, differences in diacetyl metabolism, and respiratory fitness itself.

The exposure metric, *average exposure*, which is simply the cumulative exposure divided by duration of exposure (employment duration since start of diacetyl use) 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. 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 over the time since exposures began. The more responsive individuals leaving the population sooner than

others would diminish the apparent importance of cumulative exposure. Thus average exposure might predict impairment, but it could be very population-specific depending on how the particular plant population changed over time, and would not be a generalizable exposure response. For this reason average exposure was not utilized in the risk assessment procedures.

Critical in the risk assessment and development of the REL for diacetyl is consideration that the health effects should be viewed in the complementary contexts of an individual worker's risk of impairment which is the clinician's measure of impact, and the risk incurred by the population of workers with diacetyl exposure. The American Thoracic Society, in a statement on the effects of air pollution, concluded that shifts in the respiratory health of a population resulting from some exposure, that diminish individual reserve function, are adverse "even in the absence of the immediate occurrence of frank illness." [ATS 2000] In the clinical context, if a worker's $FEV_1/FVC$ is less than 0.7 (or $FEV_1$ less than or equal to 80%), that can be considered mild COPD [GOLD 2011]. However, if diacetyl exposure decreases the mean pulmonary function of the exposed population this could be considered an adverse event [ATS 2000]. The case definition used in the NIOSH risk assessment ($FEV_1$ and $FEV_1/FVC$ ratio below the lower limits of normal) determined an adverse risk profile corresponding to a lifetime risk of one per 1000 workers in an exposed population.

The health significance of small spirometry changes, such as a 1% decline in $FEV_1$ after 2 years at 1 ppm diacetyl, depends partly on whether such changes are early indications of lung pathology that eventually would manifest as bronchiolitis obliterans. In studies of bronchiolitis obliterans arising from lung transplantation, unrelenting irreversible $FEV_1$ decrements are observed that ultimately lead to the diagnosis of bronchiolitis obliterans and fatal disease [Heng et al. 1998]. However, incomplete knowledge concerning the natural history of bronchiolitis obliterans development with diacetyl exposure is a limitation in the present risk assessment. Nonetheless, small changes, even if their progression is arrested by sufficient reductions in exposure, can still constitute impairment of current function and are risk factors for future serious adversity. Not only is risk for mortality increased, as estimated in this risk assessment, quality of life is degraded [Ferrer et al. 2002] and risk is increased for cardiovascular disease and

progressive respiratory disease [Cullen et al. 1983; Ebi-Kryston et al. 1989; Knuiman et al. 1999; Kuller et al. 1989; Schroeder et al. 2003; Wise 2006]. The decrease in $FEV_1$ predicted after working for 10 years in diacetyl exposures of 0.2 ppm is comparable to changes observed in children exposed to levels of air pollution that lead to clinical impairment in later life [Gauderman et al. 2004].

The presence of susceptibility variation poses issues for risk assessment. If most susceptible individuals are being removed from the work force and replaced by new hires, then the dynamic nature of the employment process ends up inducing disease among a larger proportion of the population than would be expected by hypothetically following 1000 workers over a theoretical 45 year work life. The results presented here suggest that a removal of likely cases of impairment takes place. If the turnover of the workforce corresponds to an average work duration of 10 years, then at a minimum, the impact of diacetyl-induced disease for the more susceptible workers would be 4.5 times higher than estimated by the usual 10-year risk assessment scenario. At Company G, the average employment duration among active employees when first surveyed was 1.8 yr implying an estimated mean Company G career employment of 2×1.8=3.6 yr. If exposure is playing a role in termination of employment, durations would be longer at exposures near the proposed REL.

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 10 or 45 years. At exposures within the range of most of the observed data (career-average exposures to diacetyl fell below 0.01 ppm in 17% of workers), this assumption is consistent with the observed effects. The proposed REL is only a factor of two below this range. Below 0.01 ppm, there can be some significant departure from linearity although diversity in response would tend to favor linearity to lower levels [Clewell and Crump 2005; National Research Council 2009].

### 5.7 Conclusion

Excess prevalence (BMD) and lifetime risk estimates variously derived for 45 years of diacetyl exposure were similar, based on Company G analyses (Table 5.37). Impairment has been defined here as pulmonary function falling below the lower limit of normal. The composite BMD estimate including high-risk cases is within a factor of 2.0 of the life-table estimates ignoring smoking. Excess risk of 1/1000 corresponds to approximately 0.003–0.005 ppm diacetyl (10.5-17.5 μg/m$^3$) in the general population and 0.0009 ppm (3.15 μg/m$^3$) for non-smokers. The robustness of the risk estimates was examined throughout the risk assessment process. NIOSH has selected Company G risk estimates for the basis for a recommended REL because Company G had the most extensive and representative diacetyl exposure data and largest body of respiratory outcomes data. In the pooled Company K/L population, believed by NIOSH to be a less adequate basis for risk assessment, the benchmark dose analysis for 1/1000 excess risk corresponds to approximately 0.0004-0.0005 ppm diacetyl. Diacetyl exposures predicted to result in various levels of risk are displayed in Table 5.38.

Although this risk assessment is based on pulmonary impairment, not clinical diagnoses, these results pertain to the development of a respiratory condition (bronchiolitis obliterans) that can ultimately be disabling and fatal. The natural history of the disease with continuing levels of diacetyl 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 yrs. Variable susceptibility, which is apparent in these analyses, indicates that for some individuals, the onset of impairment comes much faster than others.

Table 5.1. Study populations from NIOSH health hazard evaluations

| Name | G | K | N | L |
|---|---|---|---|---|
| Number of surveys | 9* | 1 | 1 | 1 |
| Total workforce at survey | 135–165 | 193 | 48 | 313 |
| Workforce evaluated (%) | 363† (73–91) | 157 (81) | 35 (73) | 206 (66) |
| Date of survey | Nov 2000–Jul 2003 | Jul 2002 | Nov 2002 | Mar 2003 |
| Start date for diacetyl use | 1-Jul-1986 | 1-Jul-1988 | 1-Jul-1986 | 1-Jan-1994 |

* Nine exposure assessments and eight medical evaluations were performed.
† Number of unique employees evaluated one or more times
Source: NIOSH health hazard evaluations

Table 5.2. Numbers of air samples by analyte from health hazard evaluation environmental assessments

| | Diacetyl | | Acetoin | |
|---|---|---|---|---|
| Company | Personal | Area | Personal | Area |
| N | 20 | 12 | 20 | 12 |
| K | 30 | 30 | 30 | 31 |
| L | 76 | 49 | 76 | 49 |
| G | 314 | 269 | 314 | 270 |

— indcates lack of data

Table 5.3. Mean air concentrations of diacetyl in major processes at four sites

| | Mixing | | Production | | Quality Control | | Maintenance | |
|---|---|---|---|---|---|---|---|---|
| **Company** | **n** | **Mean (SD)** | **n** | **Mean (SD)** | **n** | **Mean (SD)** | **n** | **Mean (SD)** |
| | | | | **Personal Samples** | | | | |
| N | 1 | 0.79 (0.00) | 7 | 0.740 (0.640) | 2 | 0.250 (0.014) | 2 | 0.160 (0.066) |
| K | 5 | 0.31 (0.41) | 7 | 0.040 (0.079) | 3 | 0.003 (0.001) | 3 | 0.020 (0.030) |
| L | 10 | 1.15 (0.74) | 36 | 0.028 (0.016) | 5 | 0.034 (0.019) | 6 | 0.014 (0.008) |
| G | 25 | 2.36 (3.92) | 112 | 0.490 (0.900) | 20 | 0.370 (0.390) | 17 | 0.080 (0.130) |
| | | | | **Area Samples** | | | | |
| N | 2 | 1.03 (0.45) | 2 | 0.620 (0.140) | 2 | 0.320 (0.140) | — | — |
| K | 2 | 2.42 (3.42) | 7 | 0.032 (0.052) | 3 | 0.002 (0.000) | 2 | 0.037 (0.048) |
| L | 6 | 1.54 (0.91) | 23 | 0.028 (0.015) | 6 | 0.018 (0.011) | 3 | 0.019 (0.014) |
| G | 47 | 16.80 (31.60) | 123 | 1.050 (1.870) | 24 | 0.250 (0.370) | 17 | 0.120 (0.330) |

n: number of samples, SD: standard deviation, — indicates lack of data
Note: Means are given in parts per million (ppm).

Table 5.4. Exposure categories used for constructing job exposure matrix at Company G

| Exposure Category | Jobs Included in Exposure Category |
|---|---|
| Warehouse | Warehouse |
| Maintenance | Maintenance |
| Outside Processing/Office | Outside Processing and Office |
| Polyethylene Line | Polyethylene Packer and Polyethylene Stacker |
| Microwave Mixing | Microwave Mixer |
| Microwave Packaging Line | Machine Operator, Packer, Stacker, Supervisor, and Inventory Control |
| Bag Print | Bag Print |
| Quality Control | Quality Control |

Table 5.5. Multiple regression models for percent predicted $FEV_1$ and various diacetyl exposure metrics for Company G

| | $R^2$ | Intercept | *t*-statistic (1df) for Exposure Metric | *P* value |
|---|---|---|---|---|
| Avg(DA) | 0.128 | 94.99 | 2.41 | 0.0167 |
| $Cum(DA^{2.0})$ | 0.142 | 94.62 | 3.41 | 0.0007 |
| $(Cum(DA))^{2.0}$ | 0.148 | 94.76 | 3.76 | 0.0002 |
| Duration | 0.161 | 97.17 | 4.43 | $9\times10^{-6}$ |
| Cum(DA) | 0.169 | 95.95 | 4.83 | $10^{-6}$ |
| $Cum(DA^{0.5})$ | 0.172 | 96.38 | 4.95 | $7\times10^{-7}$ |
| $(Cum(DA))^{0.5}$ | 0.174 | 97.34 | 5.04 | $4\times10^{-7}$ |
| $(Cum(DA^{0.5}))^{0.5}$ | 0.176 | 98.25 | 5.16 | $2\times10^{-7}$ |

Avg(DA) – time-weighted average exposure, = cum(DA)/duration
Cum(DA) – cumulative exposure = $\Sigma_i$ (DA)
$Cum(DA^{0.5}) = \Sigma_i (DA^{0.5})$
$Cum(DA^{2.0}) = \Sigma_i (DA^{2.0})$

Table 5.6. Multiple regression models for percent predicted $FEV_1$ and best-fitting diacetyl exposure metrics for Company G

| | **Cum(DA)** $R^2$ = 0.169 | | **(Cum(DA))$^{0.5}$** $R^2$ = 0.174 | | **Cum(DA$^{0.5}$)** $R^2$ = 0.172 | | **(Cum(DA$^{0.5}$))$^{0.5}$** $R^2$ = 0.176 | |
|---|---|---|---|---|---|---|---|---|
| | ***β*** | ***P*** | ***β*** | ***P*** | ***β*** | ***P*** | ***β*** | ***P*** |
| intercept | 95.95 | — | 97.34 | — | 96.38 | — | 98.25 | — |
| female | −0.386 | 0.82 | 0.092 | 0.96 | −0.306 | 0.86 | 0.078 | 0.96 |
| hispanic | 1.99 | 0.40 | 1.42 | 0.55 | 1.70 | 0.47 | 1.18 | 0.62 |
| black | 8.58 | 0.45 | 7.78 | 0.49 | 8.30 | 0.46 | 7.15 | 0.52 |
| smoke_ever | 7.29 | 0.0020 | 6.86 | 0.0038 | 6.88 | 0.004 | 6.49 | 0.0063 |
| packyrs | −0.571 | 0.0008 | −0.562 | 0.0009 | −0.560 | 0.0009 | −0.558 | 0.0009 |
| packyr2 | 0.0024 | 0.36 | 0.0024 | 0.34 | 0.0025 | 0.32 | 0.0025 | 0.31 |
| exposure metric | −0.500 | $10^{-6}$ | −2.77 | $4\times10^{-7}$ | −0.843 | $7\times10^{-7}$ | −3.70 | $2\times10^{-7}$ |

Table 5.7. Preliminary regression model results for percent predicted $FEV_1$ and $FEV_1$/FVC at Companies K, L, and G

| | | Duration | | | Avg(DA) | | | Cum($DA^{0.5}$) | | | Cum(DA) | | | $(Cum(DA))^{0.5}$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | % pred. $FEV_1$ | | | | | | | | | | | | | | |
| | n | $\beta$ | $R^2$ | *P* | $\beta$ | $R^2$ | *P* | $\beta$ | $R^2$ | *P* | $\beta$ | $R^2$ | *P* | $\beta$ | $R^2$ | *P* |
| K | 161 | −0.31 | 0.189 | 0.20 | −21.7 | 0.217 | 0.01 | −5.26 | 0.306 | $< 10^{-4}$ | −7.77 | 0.322 | $< 10^{-7}$ | −14.3 | 0.286 | $10^{-6}$ |
| L | 215 | −0.35 | 0.098 | 0.31 | −18.6 | 0.157 | 0.0001 | −3.57 | 0.135 | 0.0018 | −3.56 | 0.138 | 0.0012 | −9.15 | 0.146 | 0.0004 |
| G | 361 | −0.96 | 0.161 | $< 10^{-5}$ | −1.77 | 0.128 | 0.02 | −0.84 | 0.172 | $< 10^{-6}$ | −0.50 | 0.169 | $10^{-6}$ | −2.77 | 0.174 | $< 10^{-6}$ |
| | | $FEV_1$/FVC | | | | | | | | | | | | | | |
| | n | $\beta$ | $R^2$ | *P* | $\beta$ | $R^2$ | *P* | $\beta$ | $R^2$ | *P* | $\beta$ | $R^2$ | *P* | $\beta$ | $R^2$ | *P* |
| K | 161 | −0.28 | 0.323 | 0.068 | −13.8 | 0.357 | 0.0009 | −2.99 | 0.430 | $< 10^{-7}$ | −4.30 | 0.449 | $< 10^{-7}$ | −8.24 | 0.420 | $< 10^{-7}$ |
| L | 215 | −0.069 | 0.139 | 0.70 | −9.92 | 0.222 | $< 10^{-5}$ | −2.14 | 0.197 | $< 10^{4}$ | −2.16 | 0.213 | $< 10^{-5}$ | −5.26 | 0.212 | $< 10^{-5}$ |
| G | 358 | −0.26 | 0.333 | 0.035 | −1.26 | 0.348 | 0.0004 | −0.26 | 0.339 | 0.0044 | −0.16 | 0.342 | 0.0024 | −0.98 | 0.346 | 0.0007 |

$\beta$ – parameter estimate for diacetyl exposure metric

$R^2$ – R-squared measure of multiple regression model fit

*P* – P-value for exposure metric effect

Table 5.8. Preliminary regression model results for percent predicted $FEV_1$ and $FEV_1$/FVC with quadratic exposure metrics at Companies K, L, and G

| | | **Cum($DA^{2.0}$)** | | | **$(Cum(DA))^{2.0}$** | | |
|---|---|---|---|---|---|---|---|
| | | **% pred. $FEV_1$** | | | | | |
| | **n** | ***β*** | **$R^2$** | ***P*** | ***β*** | **$R^2$** | ***P*** |
| K | 161 | −8.79 | 0.300 | $< 10^{-6}$ | −1.36 | 0.325 | $< 10^{-7}$ |
| L | 215 | −2.64 | 0.136 | 0.0017 | −0.40 | 0.122 | 0.010 |
| G | 361 | −0.08 | 0.142 | 0.0007 | −0.01 | 0.148 | 0.0002 |
| | | **$FEV_1$/FVC** | | | | | |
| | **n** | ***β*** | **$R^2$** | ***P*** | ***β*** | **$R^2$** | ***P*** |
| K | 161 | −4.93 | 0.432 | $< 10^{-7}$ | −0.79 | 0.415 | $< 10^{-4}$ |
| L | 215 | −1.54 | 0.215 | $< 10^{-5}$ | −0.25 | 0.195 | $< 10^{-4}$ |
| G | 358 | −0.032 | 0.338 | 0.0059 | −0.004 | 0.334 | 0.021 |

L Company L

*β* – parameter estimate for diacetyl exposure metric

$R^2$ – R-squared measure of multiple regression model fit

*P* – *P* value for exposure metric effect

Table 5.9. Pooled analyses with Company L and Company K populations: % pred. $FEV_1$

| | **Duration** $R^2 = 0.129$ | | **Avg(DA)** $R^2 = 0.171$ | | **(Cum(DA))$^{0.5}$** $R^2 = 0.191$ | | **Cum(DA)** $R^2 = 0.188$ | | **(Cum(DA))$^{2.0}$** $R^2 = 0.165$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ***β*** | ***P*** | ***β*** | ***P*** | ***β*** | ***P*** | ***β*** | ***P*** | ***β*** | ***P*** |
| Intercept: K | 99.07 | — | 97.92 | — | 99.82 | — | 97.95 | — | 97.24 | — |
| Intercept: deviation* | −2.80 | 0.1240 | −1.19 | 0.47 | −1.11 | 0.49 | −1.51 | 0.35 | −1.50 | 0.36 |
| Exposure: pooled | −0.309 | 0.1092 | −17.6 | $< 10^{-5}$ | −10.4 | $< 10^{-7}$ | −4.22 | $< 10^{-7}$ | −0.47 | $< 10^{-5}$ |
| | **$R^2 = 0.129$** | | **$R^2 = 0.172$** | | **$R^2 = 0.197$** | | **$R^2 = 0.208$** | | **$R^2 = 0.202$** | |
| Intercept: K | 99.07 | — | 98.14 | — | 101.00 | — | 98.95 | — | 98.19 | — |
| Intercept: deviation | −2.81 | 0.28 | −1.51 | 0.38 | −3.05 | 0.13 | −2.83 | 0.090 | −2.52 | 0.12 |
| Exposure: K | −0.309 | 0.18 | −21.8 | 0.0061 | −14.31 | $< 10^{-5}$ | −7.83 | $< 10^{-7}$ | −1.37 | $< 10^{-7}$ |
| Exposure: deviation* | 0.001 | 0.99 | 5.35 | 0.55 | 6.36 | 0.10 | 5.13 | 0.0025 | 1.11 | $< 10^{-4}$ |

*Deviation from Company K estimate by Company L

*β* – parameter estimate for diacetyl exposure metric

*P* – *P* value for exposure metric effect

Table 5.10. Pooled analyses with Company L and Company K populations: $FEV_1$/FVC

| | **Duration** $R^2 = 0.209$ | | **Avg(DA)** $R^2 = 0.226$ | | **$(Cum(DA))^{0.5}$** $R^2 = 0.289$ | | **Cum(DA)** $R^2 = 0.292$ | | **$(Cum(DA))^{2.0}$** $R^2 = 0.268$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ***β*** | ***P*** | ***β*** | ***P*** | ***β*** | ***P*** | ***β*** | ***P*** | ***β*** | ***P*** |
| Intercept: K | 81.19 | — | 80.42 | — | 81.37 | — | 80.28 | — | 79.88 | — |
| Intercept: deviation* | −1.37 | 0.14 | 0.795 | 0.37 | 0.264 | 0.76 | 0.541 | 0.53 | 0.588 | 0.51 |
| Exposure: pooled | −0.227 | 0.044 | −10.8 | $< 10^{-7}$ | −6.27 | $< 10^{-7}$ | −2.65 | $< 10^{-7}$ | −0.315 | $< 10^{-7}$ |
| | **$R^2 = 0.213$** | | **$R^2 = 0.267$** | | **$R^2 = 0.295$** | | **$R^2 = 0.307$** | | **$R^2 = 0.299$** | |
| Intercept: K | 81.83 | — | 80.53 | — | 81.98 | — | 80.73 | — | 80.33 | — |
| Intercept: deviation | −2.62 | 0.048 | 0.963 | 0.30 | −1.24 | 0.23 | −1.11 | 0.21 | −1.07 | 0.22 |
| Exposure: K | −0.316 | 0.016 | −12.8 | .0011 | −8.35 | $< 10^{-7}$ | −4.29 | $< 10^{-7}$ | −0.749 | $< 10^{-7}$ |
| Exposure: deviation* | 0.275 | 0.18 | 2.64 | 0.55 | 3.32 | 0.081 | 2.33 | .0051 | 0.534 | $< 10^{-4}$ |

* Deviation from Company K estimate by Company L

*β* – parameter estimate for diacetyl exposure metric

*P* – *P* value for exposure metric effect

Table 5.11. Longitudinal analyses of percent predicted $FEV_1$ at Company G using random effects models

| Model No. | | Random: ID | | | Random: ID, DA-effect | | |
|---|---|---|---|---|---|---|---|
| | | ***β*** | ***t*** | ***P*** | ***β*** | ***t*** | ***P*** |
| 1 | Cum(DA) | −0.438 | −4.49 | 0.0001 | −0.427 | −2.90 | 0.016 |
| 2 | $Cum(DA)^{0.5}$ | −1.82 | −3.35 | 0.007 | −1.99 | −3.07 | 0.012 |
| 3 | Cum(DA) | −0.436 | −4.23 | 0.002 | −0.437 | −4.25 | 0.002 |
| | Cum(DA) since first survey | −0.0309 | −0.07 | 0.95 | −0.110 | −0.18 | 0.86 |
| 4 | $Cum(DA)^{0.5}$ | −2.59 | −4.82 | 0.0007 | −2.60 | −4.83 | 0.0007 |
| | $Cum(DA)^{0.5}$ since first survey | 0.718 | 0.37 | 0.71 | 0.766 | 0.34 | 0.74 |

*Note:* Cum(DA) is calculated up to each survey of a worker (two or more are in the analysis). $Cum(DA)^{0.5}$ *since first survey* is calculated from a worker's first survey up to each subsequent survey.

*β* – parameter estimate for diacetyl exposure metric

*t* – t-statistic for exposure metric effect

*P* – *P* value for exposure metric effect

Table 5.12. Company G incidence rate models: alternate exposure metrics (case defn 1)

| Model No. | Metric | Intercept Baseline Rate | Effect Estimate | RR 10yr @ 1 ppm | RR 5yr @ 2 ppm | Δ-2lnL | Wald *P* | LRT *P* |
|---|---|---|---|---|---|---|---|---|
| 1 | Duration | -8.61 | -0.081 | | | 0.0 | 0.14 | - |
| 2 | Cum(DA) | -8.96 | -0.0002 | - | - | - | 0.84 | - |
| 3 | Duration | −8.59 | −0.162 | | | | 0.063 | |
| | Cum(DA) | | 0.040 | 1.49 | 1.49 | 1.80 | 0.17 | 0.18 |
| 4 | Duration | −8.57 | −0.166 | | | | 0.17 | |
| | Cum($DA^{0.5}$) | | 0.067 | 1.95 | 1.60 | 0.74 | 0.41 | 0.39 |
| 5 | Duration | −8.76 | −0.016 | | | | 0.071 | |
| | $(Cum(DA))^{0.5}$ | | 0.220 | 2.01 | 2.01 | 1.64 | 0.21 | 0.20 |
| 6 | Duration | −8.87 | −0.086 | | | | 0.13 | |
| | Avg(DA) | | 0.161 | 1.17 | 1.38 | 1.94 | 0.14 | 0.16 |

LRT: likelihood ratio test

Rate = $\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2)+\theta packyrs + \eta cum(dur) + \mu cum(DA))$

RR – relative rate

Δ-2lnL – change in -2 × ln(likelihood)

Table 5.13. Company G incidence rate model (case defn 1, n = 36)

| Parameter | Estimate | SE | *t* | Wald *P* |
|---|---|---|---|---|
| Intercept | −8.59 | 0.417 | −20.62 | < 0.0001 |
| Ind:female | 0.357 | 0.347 | 1.03 | 0.30 |
| age-40 | 0.007 | 0.018 | 0.39 | 0.70 |
| $(age\text{-}40)^2$ | −0.0001 | 0.0014 | −0.08 | 0.94 |
| smoke_ever | −0.945 | 0.645 | −1.47 | 0.14 |
| Packyrs | 0.051 | 0.054 | 0.93 | 0.35 |
| $packyrs^2$ | −0.0003 | 0.0010 | −0.29 | 0.77 |
| Duration | −0.162 | 0.087 | −1.86 | 0.063 |
| cum(DA) | 0.040 | 0.029 | 1.38 | 0.19* |

Model likelihood ratio test, LRT = 1.80, $p = 0.18$

t – t-statistic for exposure metric effect

Table 5.14. Company G incidence rate models: alternate exposure metrics (case defn 2)

| Model No. | Metric | Intercept Baseline Rate | Effect Estimate | RR 10yr @ 1 ppm | RR 5yr @ 2 ppm | Δ-2lnL | Wald *P* | LRT *P* |
|---|---|---|---|---|---|---|---|---|
| 1 | Duration | -9.60 | -0.085 | - | - | 0.0 | 0.23 | - |
| 2 | Cum(DA) | -10.2 | 0.0124 | - | - | - | 0.60 | - |
| 3 | Duration | −9.61 | −0.300 | | | | 0.023 | |
| | Cum(DA) | | 0.090 | 2.46 | 2.46 | 5.31 | 0.16 | 0.021 |
| 4 | Duration | −9.51 | −0.555 | | | | 0.036 | |
| | $Cum(DA^{0.5})$ | | 0.316 | 23.7 | 9.37 | 5.50 | 0.041 | 0.020 |
| 5 | Duration | −10.3 | −0.411 | | | | 0.0085 | |
| | $(Cum(DA))^{0.5}$ | | 0.804 | 12.7 | 12.7 | 8.76 | 0.005 | 0.003 |
| 6 | Duration | −10.6 | −0.088 | | | | 0.24 | |
| | Avg(DA) | | 0.468 | 1.60 | 2.55 | 8.75 | 0.001 | 0.003 |

LRT: likelihood ratio test

Rate = $\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2)+\theta packyrs + \eta cum(dur) + \mu cum(DA))$

Table 5.15. Company G incidence rate models (case defn 2, n = 19)

| Parameter | Estimate | SE | *t* | Wald *P* |
|---|---|---|---|---|
| intercept | −9.61 | 0.647 | −14.85 | < 0.0001 |
| Ind:female | 0.845 | 0.518 | 1.63 | 0.10 |
| age-40 | 0.051 | 0.028 | 1.82 | 0.068 |
| $(age\text{-}40)^2$ | −0.0019 | 0.0022 | −0.86 | 0.39 |
| smoke_ever | −0.232 | 0.913 | −0.25 | 0.80 |
| packyrs | 0.012 | 0.068 | 0.17 | 0.86 |
| $packyrs^2$ | 0.0003 | 0.0011 | 0.29 | 0.77 |
| duration | −0.300 | 0.132 | −2.27 | 0.023 |
| cum(DA) | 0.090 | 0.037 | 2.41 | 0.016* |

Model likelihood ratio test, LRT = 5.306, $p = 0.021$

t – t-statistic for exposure metric effect

Table 5.16. Company G: observed cases (case defn 1) by duration and cumulative diacetyl

| | Observed Cases | | | | | |
|---|---|---|---|---|---|---|
| **Duration** | **Cumulative Diacetyl Exposure (ppm-yrs)** | | | | | |
| **(yrs)** | **< 0.5** | **0.5 < 2.0** | **2.0 < 3.0** | **3.0 < 5.0** | **≥ 5.0** | **All** |
| < 0.5 | 5 | 2 | 0 | 0 | 0 | 7 |
| 0.5 < 1.0 | 3 | 0 | 1 | 0 | 0 | 4 |
| 1.0 < 2.0 | 2 | 0 | 0 | 2 | 1 | 5 |
| 2.0 < 4.0 | 1 | 0 | 0 | 0 | 5 | 6 |
| ≥ 4.0 | 1 | 0 | 0 | 1 | 12 | 14 |
| All | 12 | 2 | 1 | 3 | 18 | 36 |

Table 5.17. Company G: person-yrs (case defn 1) by duration and cumulative diacetyl

| | Person-Yrs | | | | | |
|---|---|---|---|---|---|---|
| **Duration** | **Cumulative diacetyl Exposure (ppm-yrs)** | | | | | |
| **(yrs)** | **< 0.5** | **0.5 < 2.0** | **2.0 < 3.0** | **3.0 < 5.0** | **≥ 5.0** | **All** |
| < 0.5 | 89.0 | 29.6 | 0.3 | 0.1 | 0.0 | 119.0 |
| 0.5 < 1.0 | 27.2 | 26.7 | 16.9 | 0.7 | 1.2 | 72.7 |
| 1.0 < 2.0 | 23.5 | 10.2 | 12.4 | 39.0 | 10.7 | 95.8 |
| 2.0 < 4.0 | 14.9 | 4.7 | 7.1 | 14.2 | 88.8 | 129.7 |
| ≥ 4.0 | 25.2 | 16.0 | 1.7 | 9.4 | 222.1 | 274.5 |
| All | 179.8 | 87.2 | 38.5 | 63.5 | 322.9 | 691.8 |

Table 5.18. Company G: baseline rate (case defn 1) by duration and cumulative diacetyl

| Duration (yrs) | Predicted Baseline Rate (cum. exp. = 0) Cumulative Diacetyl Exposure (ppm-yrs) | | | | | |
|---|---|---|---|---|---|---|
| | **< 0.5** | **0.5 < 2.0** | **2.0 < 3.0** | **3.0 < 5.0** | **≥ 5.0** | **All** |
| < 0.5 | 0.061 | 0.063 | 0.039 | 0.032 | — | 0.061 |
| 0.5 <1.0 | 0.057 | 0.059 | 0.056 | 0.053 | 0.043 | 0.057 |
| 1.0 < 2.0 | 0.054 | 0.045 | 0.059 | 0.057 | 0.046 | 0.054 |
| 2.0 < 4.0 | 0.044 | 0.034 | 0.046 | 0.048 | 0.045 | 0.045 |
| ≥ 4.0 | 0.024 | 0.011 | 0.032 | 0.024 | 0.022 | 0.022 |
| All | 0.053 | 0.049 | 0.054 | 0.050 | 0.029 | 0.041 |

— indicates no person-time in stratum

Table 5.19. Company G: rate ratio (case defn 1) by duration and cumulative diacetyl

| Duration (yrs) | Rate Ratio (relative to baseline: 0.022) Cumulative Diacetyl Exposure (ppm-yrs) | | | | | |
|---|---|---|---|---|---|---|
| | **< 0.5** | **0.5 < 2.0** | **2.0 < 3.0** | **3.0 < 5.0** | **≥ 5.0** | **All** |
| < 0.5 | 2.77 | 3.00 | 1.96 | 1.64 | — | 2.82 |
| 0.5 <1.0 | 2.59 | 2.82 | 2.77 | 2.86 | 2.50 | 2.73 |
| 1.0 < 2.0 | 2.50 | 2.18 | 2.96 | 3.00 | 2.82 | 2.77 |
| 2.0 < 4.0 | 2.00 | 1.64 | 2.32 | 2.55 | 2.86 | 2.68 |
| ≥ 4.0 | 1.09 | 0.55 | 1.59 | 1.27 | 2.00 | 1.77 |
| All | 2.41 | 2.32 | 2.68 | 2.64 | 2.27 | 2.36 |

— indicates no person-time in stratum

Table 5.20. Company K and Company L plants pooled: cases (case defn 1) by duration and cumulative diacetyl

| | Observed Cases | | | | | |
|---|---|---|---|---|---|---|
| **Duration** | **Cumulative Diacetyl Exposure (ppm-yrs)** | | | | | |
| **(yrs)** | **< 0.5** | **0.5 < 2.0** | **2.0 < 3.0** | **3.0 < 5.0** | **≥ 5.0** | **All** |
| < 0.5 | 4 | 0 | 1 | 0 | 0 | 5 |
| 0.5 <1.0 | 2 | 1 | 0 | 0 | 0 | 3 |
| 1.0 < 2.0 | 0 | 3 | 0 | 1 | 0 | 4 |
| 2.0 < 4.0 | 0 | 0 | 3 | 4 | 1 | 8 |
| ≥ 4.0 | 0 | 0 | 2 | 0 | 3 | 5 |
| All | 6 | 4 | 6 | 5 | 4 | 25 |

Table 5.21. Company K and Company L plants pooled: rate ratio (case defn 1) by duration and cumulative diacetyl

| | Rate Ratio (relative to baseline: 0.004) | | | | | |
|---|---|---|---|---|---|---|
| **Duration** | **Cumulative Diacetyl Exposure (ppm-yrs)** | | | | | |
| **(yrs)** | **< 0.5** | **0.5 < 2.0** | **2.0 < 3.0** | **3.0 < 5.0** | **≥ 5.0** | **All** |
| < 0.5 | 7.53 | 9.11 | 9.23 | 7.59 | — | 7.71 |
| 0.5 <1.0 | 6.59 | 6.04 | 8.13 | 8.73 | — | 6.73 |
| 1.0 < 2.0 | 5.66 | 5.12 | 4.84 | 7.43 | 13.12 | 5.74 |
| 2.0 < 4.0 | 4.03 | 3.00 | 3.28 | 4.94 | 16.10 | 4.34 |
| ≥ 4.0 | 1.27 | 0.62 | 1.42 | 1.18 | 6.44 | 1.93 |
| All | 4.96 | 2.65 | 2.80 | 3.34 | 7.57 | 3.94 |

— indicates no person-time in stratum

Table 5.22. Company G: cases (case defn 2) by duration and cumulative diacetyl

| | Observed Cases | | | | | |
|---|---|---|---|---|---|---|
| **Duration** | **Cumulative Diacetyl Exposure (ppm-yrs)** | | | | | |
| **(yrs)** | **< 0.5** | **0.5 < 2.0** | **2.0 < 3.0** | **3.0 < 5.0** | **≥ 5.0** | **All** |
| < 0.5 | 2 | 1 | 0 | 0 | 0 | 3 |
| 0.5 <1.0 | 0 | 0 | 1 | 0 | 0 | 1 |
| 1.0 < 2.0 | 1 | 0 | 0 | 1 | 1 | 3 |
| 2.0 < 4.0 | 0 | 0 | 0 | 0 | 2 | 2 |
| ≥ 4.0 | 0 | 0 | 0 | 0 | 10 | 10 |
| All | 3 | 1 | 1 | 1 | 13 | 19 |

Table 5.23. Company G: rate ratio (case defn 2) by duration and cumulative diacetyl

| | Rate Ratio (relative to baseline: 0.0046) | | | | | |
|---|---|---|---|---|---|---|
| **Duration** | **Cumulative Diacetyl Exposure (ppm-yrs)** | | | | | |
| **(yrs)** | **< 0.5** | **0.5 < 2.0** | **2.0 < 3.0** | **3.0 < 5.0** | **≥ 5.0** | **All** |
| < 0.5 | 5.39 | 6.54 | 1.91 | 1.15 | — | 5.67 |
| 0.5 <1.0 | 4.39 | 6.22 | 6.57 | 7.70 | 5.39 | 5.59 |
| 1.0 < 2.0 | 4.26 | 3.72 | 7.00 | 7.63 | 6.98 | 6.22 |
| 2.0 < 4.0 | 2.54 | 4.43 | 4.70 | 5.61 | 7.74 | 6.63 |
| ≥ 4.0 | 0.83 | 0.85 | 3.15 | 1.57 | 5.11 | 4.33 |
| All | 4.22 | 4.89 | 5.85 | 6.17 | 5.85 | 5.35 |

— indicates no person-time in stratum

Table 5.24 (page 1 of 2). Incidence rate models using linear relative rate model with term for transient high-risk group at Company G (case defn 2)

| Model No. | Parameter | Estimate | RR | LRT | *P* value |
|---|---|---|---|---|---|
| 1 | −2ln(L) = 353.53 | | | | |
| | intercept | −10.9 | Baseline rate = $6.5\times10^{-3}$ | | |
| | smoke_ever | −0.879 | 0.42 | | |
| | Ind:female | 1.097 | 2.30 | | |
| | age-40 | 0.029 | 1.03 | | |
| | $(age\text{-}40)^2$ | −0.002 | 0.998 | | |
| | packyrs | 0.273 | 1.27 | | |
| | cum(DA) | 0.156 | 1.16 | 1.74 | 0.187 |
| 2 | −2ln(L) = 350.77 | | | | |
| | Intercept | −10.5 | Baseline rate = $9.9\times10^{-3}$ | | |
| | smoke_ever | −0.714 | 0.49 | | |
| | Ind:female | 0.915 | 2.50 | | |
| | age-40 | 0.043 | 1.04 | | |
| | $(age\text{-}40)^2$ | −0.002 | 0.998 | | |
| | Packyrs | 0.155 | 1.16 | | |
| | HRX | 0.536 | 1.54 | 4.50 | 0.034 |

| Model No. | Parameter | Estimate | RR | LRT | *P* value |
|---|---|---|---|---|---|
| 3 | -2ln(L) = 347.27 | | | | |
| | intercept | −15.5 | Baseline rate = $6.8\times10^{-5}$ | | |
| | smoke_ever | −0.795 | 0.45 | | |
| | Ind:female | 1.019 | 2.77 | | |
| | age-40 | 0.037 | 1.04 | | |
| | $(age-40)^2$ | −0.002 | 0.998 | | |
| | packyrs | 21.16 | 22.2 | | |
| | cum(DA) | 16.42 | 17.4 | 3.50 | 0.061 |
| | HRX | 79.60 | 80.6 | 6.26 | 0.012 |
| 4 | −2ln(L) = 345.75 | | | | |
| | intercept | −15.48 | Baseline rate = $6.9\times10^{-5}$ | | |
| | smoke_ever | −0.683 | 0.51 | | |
| | Ind:female | 0.967 | 2.63 | | |
| | age-40 | 0.041 | 1.04 | | |
| | $(age-40)^2$ | −0.002 | 0.998 | | |
| | packyrs | 17.71 | 18.7 | | |
| | cum(DA) | 12.29 | 13.3 | 2.19 | 0.139 |
| | HRX | 69.82 | 70.8 | 7.78 | 0.0053 |

LRT: likelihood ratio test

General model:

Rate = $\{\exp(\alpha + \beta smoker + \gamma sex + \delta(age-40) + \varepsilon(age-40)^2)\}\{1 + \theta packyrs + \sigma HRX + \mu cumDA\}$

Baseline rate (cases/P-Yr): 365.25exp(intercept)

RR - @ 1 pack-yr, 1 ppm at day 1 (HRX), 1 ppm-yr (cum(DA))

Model 3: HRX = $[DA]^2\exp(-0.693dur)$ – for half-life = 1.0 yr; LRT for exposure terms = 7.97 (2 df)

Model 4: HRX = $[DA]^2\exp(-0.693dur/2)$ – for half-life = 2.0 yr; LRT for exposure terms = 9.52 (2 df)

Table 5.25. Likelihood ratio tests (LRT) and *P* values for choices of parameters in HRX variable at Company G (case definition 2)

| | | LRT for cum(DA) and HRX terms (p; 2 df) | |
|---|---|---|---|
| | | Half-life, b | |
| | | 1.0 | 2.0 |
| in (Avg Exp) [a], a | 1.0 | 5.93 (0.052) | 6.37 (0.019) |
| | 2.0 | 7.97 (0.019) | 9.52 (0.0086) |

LRT: likelihood ratio test

HRX = (Avg Exp) [a] × exp(−0.693dur/b)

Table 5.26. Relative fit of selected model specifications for incidence rate (case defn 2)

| Model No. | Rate Model | Intercept | Deviance |
|---|---|---|---|
| | **Loglinear models (multiplicative exposure terms)** | | |
| 1 | $\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2)+\theta packyrs + \mu cum(DA))$ | −10.21 | 354.89 |
| 2 | $\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2)+\theta packyrs + \mu srcum(DA))$ | −10.83 | 354.52 |
| 3 | $\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2)+\theta packyrs + \mu avg(DA))$ | −11.14 | 346.41 |
| 4 | $\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2)+\theta packyrs + \eta cum(dur) + \mu cum(DA))$ | −9.66 | 348.19 |
| 5 | $\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2)+\theta packyrs + \eta(cum(dur))^2 + \mu cum(DA))$ | −10.03 | 344.33 |
| 6 | $\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2)+\theta packyrs + \eta cum(dur) + \mu srcum(DA))$ | −10.39 | 344.87 |
| 7 | $\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2)+\theta packyrs + \eta cum(dur) + \mu avg(DA))$ | −10.64 | 344.93 |
| | **Linear relative rate models (additive exposure terms)** | | |
| 8 | $\{\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2))\}\{1+\theta packyrs + \mu cum(DA)\}$ | −10.93 | 353.53 |
| 9 | $\{\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2))\}\{1+\theta packyrs + \mu srcum(DA)\}$ | −15.36 | 352.04 |
| 10 | $\{\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2))\}\{1+\theta packyrs + \mu avg(DA)\}$ | −15.37 | 348.93 |
| 11 | $\{\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2))\}\{1+\theta packyrs + \mu cum(DA) + \sigma HRX\}$ | −15.47 | 345.75 |
| 12 | $\{\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2))\}\{1+\theta packyrs + \mu srcum(DA) + \sigma HRX\}$ | −14.46 | 346.99 |
| 13 | $\{\exp(\alpha+\beta smoker+\gamma sex+\delta(age-40)+\varepsilon(age-40)^2))\}\{1+\theta packyrs + \mu avg(DA) + \sigma HRX\}$ | −15.21 | 346.17 |

Smaller deviance ~ better fit

HRX = $[DA]^2\exp(-0.693dur/2)$ – for half-life = 2.0 yr

Table 5.27. Benchmark dose, based on exposure response with cum(DA) at Company G

| Percent of predicted $FEV_1$ ($ppFEV_1$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Excess Prevalence of Impairment (per thousand) | | | |
| [DA] (ppm) | cum. exp. (ppm-yrs) | | Model-predicted $ppFEV_1$ | | < 60% of predicted | < 5th percentile | < 60% of predicted | < 5th percentile |
| | 10 yr | 45 yr | 10 yr | 45 yr | 10 yr | | 45 yr | |
| 1.0 | 10.0 | 45.0 | 95.0 | 77.5 | 7.4 | 42.4 | 126.7 | 366.8 |
| 0.5 | 5.0 | 22.5 | 97.5 | 88.8 | 3.0 | 18.7 | 27.9 | 126.7 |
| 0.2 | 2.0 | 9.00 | 99.0 | 95.5 | 1.1 | 6.9 | 6.4 | 37.2 |
| 0.1 | 1.0 | 4.50 | 99.5 | 97.8 | 0.5 | 3.4 | 2.7 | 16.6 |
| 0.05 | 0.5 | 2.25 | 99.8 | 98.9 | 0.3 | 1.7 | 1.2 | 7.8 |
| 0.02 | 0.2 | 0.90 | 99.9 | 99.6 | 0.1 | 0.7 | 0.5 | 3.0 |
| 0.01 | 0.1 | 0.45 | 99.95 | 99.8 | 0.0 | 0.3 | 0.2 | 1.5 |
| 0.005 | 0.05 | 0.225 | 99.98 | 99.89 | 0.0 | 0.2 | 0.1 | 0.7 |
| 0.002 | 0.02 | 0.090 | 99.99 | 99.96 | 0.0 | 0.1 | 0.0 | 0.3 |
| 0.001 | 0.01 | 0.045 | 100.00 | 99.98 | 0.0 | 0.0 | 0.0 | 0.1 |
| 0.0005 | 0.005 | 0.0225 | 100.00 | 99.99 | 0.0 | 0.0 | 0.0 | 0.1 |
| 0.0002 | 0.002 | 0.0090 | 100.00 | 100.00 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0001 | 0.001 | 0.0045 | 100.00 | 100.00 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0000 | 0.000 | 0.0000 | 100.00 | 100.00 | 0.0 | 0.0 | 0.0 | 0.0 |

cum. exp.: cumulative exposure

Baseline prevalence for < 60% of predicated = 0.0053, <5th percentile = 0.0498

Table 5.28. Benchmark dose, based on exposure response with $(cum(DA))^{0.5}$ at Company G

| Percent of predicted $FEV_1$ ($ppFEV_1$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Excess Prevalence of Impairment (per thousand) | | | |
| Diacetyl (ppm) | cum. exp. (ppm-yrs) | | Model-predicted $ppFEV_1$ | | < 60% of predicted | < 5th percentile | < 60% of predicted | < 5th percentile |
| | 10-yr | 45-yr | 10-yr | 45-yr | 10-yr | | 45-yr | |
| 1.0 | 3.2 | 6.7 | 91.2 | 81.4 | 18.2 | 88.3 | 81.5 | 271.2 |
| 0.5 | 2.2 | 4.7 | 93.8 | 86.9 | 10.4 | 55.5 | 38.6 | 159.0 |
| 0.2 | 1.4 | 3.0 | 96.1 | 91.7 | 5.5 | 31.4 | 16.6 | 82.1 |
| 0.1 | 1.0 | 2.1 | 97.2 | 94.1 | 3.5 | 21.0 | 9.6 | 51.8 |
| 0.05 | 0.71 | 1.5 | 98.0 | 95.8 | 2.4 | 14.3 | 5.9 | 33.7 |
| 0.02 | 0.45 | 0.95 | 98.8 | 97.4 | 1.4 | 8.7 | 3.3 | 19.8 |
| 0.01 | 0.32 | 0.67 | 99.1 | 98.1 | 1.0 | 6.1 | 2.2 | 13.5 |
| 0.005 | 0.22 | 0.47 | 99.4 | 98.7 | 0.7 | 4.2 | 1.5 | 9.3 |
| 0.002 | 0.14 | 0.30 | 99.6 | 99.2 | 0.5 | 2.7 | 0.9 | 5.7 |
| 0.001 | 0.10 | 0.21 | 99.7 | 99.4 | 0.3 | 1.9 | 0.7 | 4.0 |
| 0.0005 | 0.071 | 0.15 | 99.8 | 99.6 | 0.2 | 1.3 | 0.5 | 2.8 |
| 0.0002 | 0.045 | 0.095 | 99.88 | 99.7 | 0.2 | 0.9 | 0.3 | 1.8 |
| 0.0001 | 0.032 | 0.067 | 99.91 | 99.8 | 0.1 | 0.6 | 0.2 | 1.3 |
| 0.0000 | 0.000 | 0.000 | 100.00 | 100.00 | 0.0 | 0.0 | 0.0 | 0.0 |

cum. exp.: cumulative exposure

Baseline prevalence for < 60% of predicated = 0.0055, <5th percentile = 0.0500

Table 5.29. Empirical benchmark dose, $FEV_1$ based on exposure response with cum(DA) at Company G

| $FEV_1$—Empirical BMD | | | | |
|---|---|---|---|---|
| Diacetyl (ppm) | cum(DA) (ppm-yrs) | | Excess prevalence < lower limit of normal per thousand | |
| | 10-yr | 45-yr | 10-yr | 45-yr |
| 1 | 10.0 | 45.0 | 68.1 | 532.5 |
| 0.5 | 5.0 | 22.5 | 28.9 | 202.9 |
| 0.2 | 2.0 | 9.0 | 10.5 | 58.7 |
| 0.1 | 1.0 | 4.5 | 5.5 | 25.7 |
| 0.05 | 0.5 | 2.3 | 2.6 | 12.3 |
| 0.02 | 0.2 | 0.90 | 1.1 | 4.8 |
| 0.01 | 0.1 | 0.45 | 0.4 | 2.5 |
| 0.005 | 0.05 | 0.23 | 0.2 | 1.3 |
| 0.002 | 0.02 | 0.090 | 0.2 | 0.4 |
| 0.001 | 0.01 | 0.045 | 0.1 | 0.2 |
| 0.0005 | 0.005 | 0.023 | 0.1 | 0.2 |
| 0.0002 | 0.002 | 0.0090 | 0.1 | 0.1 |
| 0.0001 | 0.001 | 0.0045 | 0.1 | 0.1 |

Table 5.30. Empirical benchmark dose, $FEV_1$/FVC based on cum(DA) response at Company G

| $FEV_1$/FVC—Empirical BMD | | | | |
|---|---|---|---|---|
| X | cum(DA) (ppm-yrs) | | Excess prevalence < lower limit of normal per thousand | |
| (ppm) | 10-yr | 45-yr | 10-yr | 45-yr |
| 1 | 10.0 | 45.0 | 30.4 | 220.5 |
| 0.5 | 5.0 | 22.5 | 14.1 | 82.4 |
| 0.2 | 2.0 | 9.0 | 6.4 | 27.4 |
| 0.1 | 1.0 | 4.5 | 3.4 | 12.1 |
| 0.05 | 0.5 | 2.3 | 2.4 | 6.8 |
| 0.02 | 0.2 | 0.90 | 0.9 | 3.2 |
| 0.01 | 0.1 | 0.45 | 0.4 | 2.1 |
| 0.005 | 0.05 | 0.23 | 0.4 | 1.0 |
| 0.002 | 0.02 | 0.090 | 0.2 | 0.4 |
| 0.001 | 0.01 | 0.045 | 0.1 | 0.3 |
| 0.0005 | 0.005 | 0.023 | 0.1 | 0.2 |
| 0.0002 | 0.002 | 0.0090 | 0.1 | 0.1 |
| 0.0001 | 0.001 | 0.0045 | 0.1 | 0.1 |

Table 5.31. Excess Prevalence of impairment based on exposure response estimated in pooled Company K and Company L populations

| | | | Empirical BMD – Pooled Company K, L Populations Excess Prevalence, per thousand | | | | | |
|---|---|---|---|---|---|---|---|---|
| | cum(DA) (ppm-yrs) | | $FEV_1$ < LLofN | | $FEV_1$ < 60 % | | $FEV_1/FVC$ < LLofN | |
| | 10-yr | 45-yr | 10-yr | 45-yr | 10-yr | 45-yr | 10-yr | 45-yr |
| 1 | 10. | 45. | 854.2 | 892.5 | 550.5 | 994.7 | 877.5 | 898.1 |
| 0.5 | 5.0 | 23. | 491.4 | 892.5 | 108.5 | 994.4 | 550.9 | 898.1 |
| 0.2 | 2.0 | 9.0 | 134.8 | 823.6 | 16.7 | 443.4 | 135.3 | 863.9 |
| 0.1 | 1.0 | 4.5 | 54.9 | 428.0 | 5.86 | 84.7 | 52.0 | 471.2 |
| 0.05 | 0.5 | 2.3 | 23.6 | 158.4 | 2.45 | 20.4 | 24.3 | 160.6 |
| 0.02 | 0.2 | 0.90 | 9.00 | 48.2 | 0.88 | 5.09 | 9.09 | 45.6 |
| 0.01 | 0.1 | 0.45 | 4.41 | 21.2 | 0.42 | 2.17 | 5.29 | 21.9 |
| 0.005 | 0.05 | 0.23 | 2.29 | 9.88 | 0.21 | 1.00 | 3.09 | 10.1 |
| 0.002 | 0.02 | 0.090 | 0.88 | 4.15 | 0.08 | 0.38 | 1.15 | 4.50 |
| 0.001 | 0.01 | 0.045 | 0.35 | 1.94 | 0.04 | 0.18 | 0.71 | 3.00 |
| 0.0005 | 0.005 | 0.023 | 0.18 | 1.15 | 0.02 | 0.09 | 0.44 | 1.32 |
| 0.0002 | 0.002 | 0.0090 | 0.09 | 0.18 | 0.00 | 0.03 | 0.18 | 0.35 |
| 0.0001 | 0.001 | 0.0045 | 0.09 | 0.35 | 0.00 | 0.01 | 0.18 | 0.62 |

5.32. Empirical BMD for exposure response based on average diacetyl estimated in Company G population

| Average Diacetyl (ppm) | Excess prevalence < lower limit of normal per thousand | |
|---|---|---|
| | $FEV_1$ | $FEV_1/FVC$ |
| 1 | 19.23 | 23.5 |
| 0.5 | 9.18 | 10.9 |
| 0.2 | 3.88 | 4.94 |
| 0.1 | 1.59 | 3.00 |
| 0.05 | 0.97 | 1.50 |
| 0.02 | 0.35 | 0.71 |
| 0.01 | 0.18 | 0.35 |
| 0.005 | 0.09 | 0.18 |
| 0.002 | < 0.09 | 0.18 |
| 0.001 | < 0.09 | 0.09 |
| 0.0005 | < 0.09 | < 0.09 |
| 0.0002 | < 0.09 | < 0.09 |
| 0.0001 | < 0.09 | < 0.09 |

Table 5.33. High-risk subpopulation (case defn 1) at Company G

| Duration (yrs) | Cum(DA) – ppm-yr | | Total |
|---|---|---|---|
| | < 0.5 | 0.5 < 2.0 | |
| **Observed Cases (case defn 1)** | | | |
| < 0.5 | 5 | 2 | |
| 0.5 <1.0 | 3 | 0 | |
| 1.0 < 2.0 | 2 | 0 | |
| 2.0 < 4.0 | 1 | 0 | |
| All | 11 | 2 | 13 |
| **Predicted Total Cases** | | | |
| < 0.5 | 5.43 | 1.95 | |
| 0.5 <1.0 | 1.55 | 1.66 | |
| 1.0 < 2.0 | 1.29 | 0.49 | |
| 2.0 < 4.0 | 0.66 | 0.17 | |
| All | 8.93 | 4.27 | 13.2 |
| **Predicted Excess Cases (Baseline Rate = 0.022)** | | | |
| < 0.5 | 3.47 | 1.30 | |
| 0.5 <1.0 | 0.95 | 1.07 | |
| 1.0 < 2.0 | 0.78 | 0.27 | |
| 2.0 < 4.0 | 0.33 | 0.07 | |
| All | 5.53 | 2.71 | 8.24 |

Table 5.34. Composite BMD for $FEV_1$ (vs. LLofN) based on empirical BMD with cum(DA) and high risk group (case definition 1) at Company G

| | Excess prevalence (per thousand) | | | | | | |
|---|---|---|---|---|---|---|---|
| Diacetyl | 10-yr | | | | 45-yr | | |
| ppm | e-BMD | HR group | total | total×4.5 | e-BMD | HR group | total |
| 1 | 68.1 | 33.0 | 101.1 | 455.0 | 532.5 | 148.5 | 681.0 |
| 0.5 | 28.9 | 16.5 | 45.4 | 204.3 | 202.9 | 74.3 | 277.2 |
| 0.2 | 10.5 | 6.6 | 17.1 | 77.0 | 58.7 | 29.7 | 88.4 |
| 0.1 | 5.5 | 3.3 | 8.8 | 39.6 | 25.7 | 14.9 | 40.6 |
| 0.05 | 2.6 | 1.7 | 4.3 | 19.4 | 12.3 | 7.4 | 19.7 |
| 0.02 | 1.1 | 0.7 | 1.8 | 8.1 | 4.8 | 3.0 | 7.8 |
| 0.01 | 0.4 | 0.3 | 0.7 | 3.2 | 2.5 | 1.5 | 4.0 |
| 0.005 | 0.2 | 0.2 | 0.4 | 1.8 | 1.3 | 0.7 | 2.0 |
| 0.002 | 0.2 | 0.1 | 0.3 | 1.4 | 0.4 | 0.3 | 0.7 |
| 0.001 | 0.1 | 0.0 | 0.1 | 0.5 | 0.2 | 0.1 | 0.3 |

e-BMD—benchmark doses derived from BMD procedure with empirical distribution
HR—excess cases arising from high-risk population, assuming linear average exposure dependence

Table 5.35. Life-table derived excess lifetime risk based on incidence rate model with term for transient high-risk group (case definition 2; see Table 5.24, model 4) at Company G

| | Excess Lifetime Risk (per thousand) | | | | | |
|---|---|---|---|---|---|---|
| | 10 yr | | | 45 yr | | |
| Diacetyl ppm | Model 1 | Model 2 for nonsmokers | Model 2 for smokers | Model 1 | Model 2 for nonsmokers | Model 2 for smokers |
| 1 | 99.0 | 320.9 | 69.5 | 248.8 | 659.5 | 164.6 |
| 0.5 | 51.3 | 175.7 | 36.3 | 140.7 | 424.0 | 94.2 |
| 0.2 | 21.0 | 74.3 | 14.9 | 60.8 | 199.9 | 41.0 |
| 0.1 | 10.5 | 37.9 | 7.5 | 31.2 | 105.8 | 21.1 |
| 0.05 | 5.3 | 19.1 | 3.8 | 15.8 | 54.5 | 10.7 |
| 0.02 | 2.1 | 7.7 | 1.5 | 6.4 | 22.2 | 4.3 |
| 0.01 | 1.1 | 3.9 | 0.8 | 3.2 | 11.2 | 2.2 |
| 0.005 | 0.5 | 1.9 | 0.4 | 1.6 | 5.6 | 1.1 |
| 0.002 | 0.2 | 0.8 | 0.2 | 0.6 | 2.2 | 0.4 |
| 0.001 | 0.1 | 0.4 | 0.1 | 0.3 | 1.1 | 0.2 |
| 0.0005 | 0.1 | 0.2 | 0.0 | 0.2 | 0.6 | 0.1 |
| 0.0002 | 0.0 | 0.1 | 0.0 | 0.1 | 0.2 | 0.0 |
| 0.0001 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |

Case defn: $FEV_1$ < LLof N and $FEV_1/FVC$ < LLof N
Model 1: no smoking terms in model of case incidence
Model 2: [Table 5.24, model 4) smoking terms in model of case incidence but XLTR calculated separately for nonsmokers and smokers: 1 pack/day

Table 5.36. Excess lifetime risk of mortality due to $FEV_1$ deficit arising from diacetyl exposure

| Diacetyl (ppm) | XLTR (45 yrs) |
|---|---|
| 1.0 | 221.6 |
| 0.5 | 121.1 |
| 0.2 | 51.2 |
| 0.1 | 26.1 |
| 0.05 | 13.2 |
| 0.02 | 5.30 |
| 0.01 | 2.65 |
| 0.005 | 1.33 |
| 0.002 | 0.53 |
| 0.001 | 0.27 |
| 0.0005 | 0.13 |
| 0.0002 | 0.05 |
| 0.0001 | 0.03 |

Based on multiple regression predicting fall in percent predicted $FEV_1$ with diacetyl exposure (0.5% per ppm-yr diacetyl) and on 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 $FEV_1$.

Table 5.37. Risk assessment synthesis: excess prevalence or lifetime risk (per thousand) for 45-yr exposure to diacetyl

| | Method | | | | | | |
|---|---|---|---|---|---|---|---|
| | BMD Excess Prevalence (/1000) | | | Life-table Excess Lifetime Risk (/1000) (case defn 2) | | | All-cause Mortality |
| Diacetyl ppm | $FEV_1$ (LLofN) | $FEV_1$/FVC (LLofN) | $FEV_1$-HRg (LLofN) | All | for nonsmokers | for smokers | All |
| 0.05 | 12.3 | 6.8 | 19.7 | 15.8 | 54.5 | 10.7 | 13.2 |
| 0.02 | 4.8 | 3.2 | 7.8 | 6.4 | 22.2 | 4.3 | 5.3 |
| 0.01 | 2.5 | 2.1 | 4.0 | 3.2 | 11.2 | 2.2 | 2.7 |
| 0.005 | 1.3 | **1.0** | 2.0 | 1.6 | 5.6 | **1.1** | 1.3 |
| 0.004 | **1.1** | 0.8 | 1.7 | 1.3 | 4.5 | 0.9 | **1.1** |
| 0.003 | 0.6 | 0.6 | **1.0** | **1.0** | 3.4 | 0.7 | 0.8 |
| 0.002 | 0.4 | 0.4 | 0.7 | 0.6 | 2.2 | 0.4 | 0.5 |
| 0.001 | 0.2 | 0.3 | 0.3 | 0.3 | **1.1** | 0.2 | 0.3 |

$FEV_1$-HRg - BMD composite including high-risk cases
case defn 2: $FEV_1$ < LLofN and $FEV_1$/FVC < LLofN
BMD: Based on benchmark dose procedures, the predicted number of individuals with $FEV_1$ or $FEV_1$/FVC < lower limit of normal that would be prevalent in a population of 1000 with 45 yr exposure
Excess Lifetime Risk: Based on life-table analysis, the predicted number of new cases in a population of 1000 starting with exposure at age 20 through 65
1/1000 risk exposures in **bold**.

Table 5.38. Risk assessment synthesis: 45 yr exposures to diacetyl (ppm) predicting levels of excess prevalence or lifetime risk (per thousand)

| | Method | | | | | | |
|---|---|---|---|---|---|---|---|
| | BMD: Excess Prevalence | | | Life-table: Excess Lifetime Risk | | | |
| | Impairment | | | Case onset, definition 2 | | | Mortality |
| Excess Risk | $FEV_1$ (LLofN) | $FEV_1$/FVC (LLofN) | $FEV_1$-HRg* (LLofN) | all | for non-smokers | for smokers | all |
| 1/10 | 0.30 | 0.60 | 0.20 | 0.30 | 0.10 | 0.50 | 0.40 |
| 1/100 | 0.04 | 0.08 | 0.03 | 0.03 | 0.01 | 0.05 | 0.04 |
| **1/1000** | **0.004** | **0.005** | **0.003** | **0.003** | **0.0009** | **0.005** | **0.004** |
| 1/10000 | 0.0004 | 0.0005 | 0.0003 | 0.0002 | 0.00009 | 0.0004 | 0.0004 |
| 1/100000 | 0.00004 | 0.00005 | 0.00003 | 0.00002 | 0.000009 | 0.00004 | 0.00004 |

* $FEV_1$-HRg - BMD composite including high-risk group cases

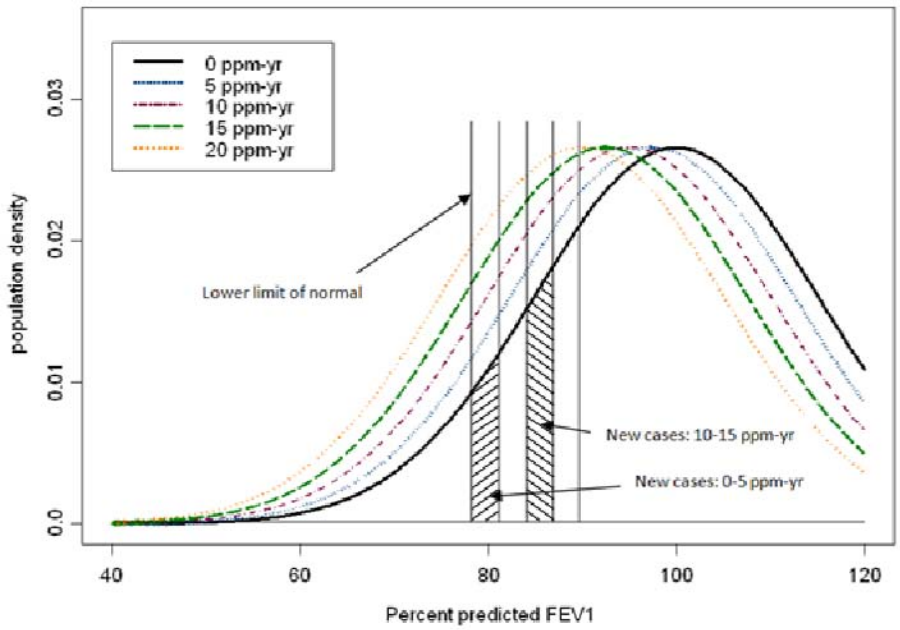
case defn 2: $FEV_1$ < LLofN and $FEV_1$/FVC < LLofN

BMD: Based on benchmark dose procedures, the exposure for 45 yr predicted to confer the specified excess prevalence of $FEV_1$ or $FEV_1$/FVC < lower limit of normal

Excess Lifetime Risk: Based on life-table analysis, the exposure at age 20 through 65 predicted to confer the specified excess life-time risk

1/1000 risk exposures in **bold**.

Figure 5.1. New cases expected from an hypothetical population with uniform susceptibility to diminishing percent predicted $FEV_1$ with increasing cumulative exposure to diacetyl



# Chapter 6: Quantitative Risk Assessment Based on Animal Data

## 6.1 Diacetyl

### *6.1.1 Introduction*

There are useful but limited animal effects data on diacetyl and 2,3-pentanedione. NIOSH has assessed these data to determine whether they support the estimate of human risk described in Chapter 5. NIOSH has evaluated the quantitative risk assessment conducted by Bruce C. Allen in the reports titled, "A Quantitative Risk Assessment for Diacetyl Based on Respiratory Tract Lesions in Mice" and "Report on Model Averaging Analysis and Results for Diacetyl Mouse Data Sets" prepared under OSHA contract number DOLQ059622303 (2009) Task Order 50. On the basis of this evaluation it was determined that the risk assessment was adequate to use as a quantitative comparison with the NIOSH epidemiology-based quantitative risk assessment. Therefore, no separate animal-based quantitative risk assessment was conducted. The full contractor reports [Allen 2009a, b] can be found in Appendix 5. A summary of the risk assessment extracted from these reports is included in this chapter. NIOSH interpretation of the findings and implications for occupational exposure recommendations for diacetyl are described below and in Chapter 7, Basis for the Standard.

Experimental animal studies designed to evaluate the effects of exposure to butter flavoring vapor or of diacetyl alone have demonstrated a relationship between exposure and respiratory effects. In rats exposed by inhalation to butter flavoring vapor for 6 hours, (diacetyl concentrations ranged from 203 to 352 ppm) rhinitis (at the lowest exposure concentration) and bronchitis (at the higher two exposure concentrations) were observed one day after exposure [Hubbs et al. 2002]. In a follow-up study rats were exposed by inhalation to diacetyl (intermittently or continuously for up to 6 hours), which resulted in various adverse respiratory effects including epithelial necrosis and inflammation in the nose, larynx, trachea, and bronchi [Hubbs et al. 2008]. The nasal region was observed to be the most sensitive.

Morgan et al. reported similar adverse respiratory effects in mice exposed by inhalation to diacetyl for up to 12 weeks [Morgan et al. 2008]. Adverse nasal and lung effects were observed with the latter found in the peribronchial, bronchial, and peribronchiolar regions. Because the Morgan et al. study had the longest exposure durations among all experimental animal studies, it was used in the dose-response analysis to derive benchmark doses (BMDs), the lower bound on the BMDs (BMDLs), and corresponding human equivalent concentrations (HECs) as discussed below.

### *6.1.2 Methods*

#### 6.1.2.1 Analytical Approach

Allen [2009] considered a variety of dosimetric adjustments to the results reported by Morgan et al. to examine the impact of various assumptions on the final risk estimates [Allen 2009a; Morgan et al. 2008]. These are briefly described below with the complete analysis found in Appendix 5. Once the dose metric values corresponding to each exposure group were derived, the suite of dichotomous dose-response models from the BMDS software version 2.1 [EPA 2011] were fitted for the selected endpoints and BMDs, and their lower bounds (BMDLs) were estimated. The BMD and BMDL estimates are presented along with the HECs (in ppm) corresponding to the estimated BMDs and BMDLs. Human dosimetric considerations analogous to those applied to the mouse were used to determine HECs.

#### 6.1.2.2 Data

The response data that were analyzed were obtained from the experimental mouse study reported by Morgan et al. [Morgan et al. 2008]. Male C57Bl/6 mice were exposed to diacetyl vapors at various concentrations and durations (both in terms of hours per day and number of weeks of exposure). Among mice exposed to 200 or 400 ppm for 5 days, the responses included death, necrotizing rhinitis, necrotizing laryngitis, and bronchitis. The responses analyzed were those most relevant to longer-term exposures, i.e., those from the subchronic portion of the study that included constant exposures of 25, 50, and 100 ppm for 6 hours/day. Responses were reported for animals treated either for 6 weeks

or for 12 weeks. The endpoints that were observed and modeled for the dose-response analysis were as follows:

- **Nasal Lesions:**
  - Inflammation (chronic, active)
  - Necrosis and ulceration of respiratory epithelium
  - Metaplasia (squamous) of respiratory epithelium
- **Lung Lesions:**
  - Peribronchial lymphocytic inflammation
  - Bronchial epithelial atrophy and denudation
  - Peribronchiolar lymphocytic inflammation

Each of those responses was observed in more than 50% (almost always 100%) of the animals in the 100 ppm exposure group at the 6-week time point. They were selected from among the endpoints reported because they were considered to be representative of the adverse responses observed and because the dose-response patterns were expected to span the range of those that would be obtained from all the endpoints. They included the apparently most-sensitive responses for both the nasal and lung regions.

The data were examined to determine if similar health endpoint-specific patterns were observed for the 6- and 12-week experiments and if the resulting BMDs could be distinguished statistically. A likelihood ratio test was performed of the null hypothesis that, conditional on exposure concentrations and a specified dose-response model, the response rates could be accounted for by the same probability of response across the two time periods. When that null hypothesis was not rejected for a given endpoint, the 6- and 12-week data were combined for the dose-response analysis.

#### 6.1.2.3 Dose-Response Modeling

Dose-response modeling is used in risk assessment to determine the relationship between the amount of exposure to the hazard and the health effect of concern. Once the

relationship is modeled, it can be used to predict risks at lower exposures (when data type permit) or as a point of departure for applying uncertainty factors to determine a "safe" level of exposure. In this case, using data from diacetyl-exposed animals, modeling the relationship between the amount of diacetyl exposure and nasal and lung lesions allows investigators to predict lung and nasal health effects at much lower levels than the experimental doses. The quantal (i.e., presence/absence) response data (Table 6.1) were modeled using the BMDS software (version 2.1) provided by the EPA [EPA 2011].

Table 6.1. Quantal data included in dose-response analysis (from Morgan et al. 2008)

| Endpoint (Site, Response) | Duration (Weeks of Exposure) | 0 ppm | 25 ppm | 50 ppm | 100 ppm |
|---|---|---|---|---|---|
| A. Nasal, Inflammation | 6 | 2/5[a] | 2/4 | 4/5 | 5/5 |
| | 12 | 1/5 | 4/5 | 5/5 | 5/5 |
| B. Nasal, Necrosis and Ulceration | 6 | 0/5 | 0/4 | 2/5 | 5/5 |
| | 12 | 0/5 | 0/5 | 1/5 | 5/5 |
| C. Nasal, Metaplasia | 6 | 0/5 | 1/4 | 3/5 | 5/5 |
| | 12 | 0/5 | 2/5 | 4/5 | 5/5 |
| D. Lung, Peribronchial Inflammation | 6 | 0/5 | 3/5 | 5/5 | 5/5 |
| | 12 | 0/5 | 2/5 | 4/5 | 5/5 |
| E. Lung, Bronchial Atrophy and Denudation | 6 | 0/5 | 0/5 | 1/5 | 5/5 |
| | 12 | 0/5 | 0/5 | 0/5 | 5/5 |
| F. Lung, Peribronchiolar Inflammation | 6 | 2/5 | 0/5 | 1/5 | 3/5 |
| | 12 | 0/5 | 0/5 | 0/5 | 3/5 |

[a]Number of animals with lesion / Number examined.

The quantal models that were fitted were as follows:

- Gamma

- Logistic
- Log-Logistic
- Log-Probit
- Multistage
- Probit
- Weibull

Equations describing these dose-response functions are shown in Appendix 5.

From among the models that had a goodness-of-fit *P* value greater than 0.1 (indicating a satisfactory fit of the model to the data), the model with the lowest Akaike Information Criterion (AIC) value was selected for BMD estimation. The AIC is a measure that can be used to compare any set of models fit to the same data set; it accounts for the fit of the models to the data and for the number of parameters used to obtain that fit; lesser values of the AIC are better for that model comparison. This approach to model selection is the default procedure recommended by EPA in its BMD guidance [EPA 2000].

In addition to the best-fitting model, the BMD and BMDL values were also estimated using a model averaging procedure described by Wheeler and Bailer [Wheeler and Bailer 2007]. This analysis was provided in a separate report titled, "Report on Model Averaging Analysis and Results for Diacetyl Mouse Data Sets" prepared by Bruce C. Allen under OSHA contract number DOLQ059622303 [Allen 2009b].

The notations BMD(10) and BMDL(10) are used to denote the calculated dose and its lower bound corresponding to an extra risk of 0.1 (1 in 10), BMD and BMDL(1) for an extra risk of 0.01 (1 in 100), and BMD(0.1) and BMDL(0.1) for an extra risk of 0.001 (1 in 1000). The BMDLs are the 95% lower bounds on the associated BMD estimates.

#### 6.1.2.4 Dose Metrics for Modeling

Several dose metrics were calculated and used for the dose-response modeling, rather than simply using the exposure levels themselves in the models. In the following

description of the dose metric derivations, it is helpful to consider that the metrics can be viewed as a function of three terms:

- exposure concentration
- reduction in concentration due to scrubbing (i.e., absorption of the chemical) in higher respiratory tract regions
- "effective dose" measure

When diacetyl is inhaled, some of it is absorbed and removed from the respiratory tract by the cells/lining of the respiratory tract higher than the site of action. The EPA [EPA 1994] has developed procedures for factoring in such scrubbing as part of its methods for deriving inhalation reference concentrations. All calculated scrubbing factors reflect the proportion of diacetyl remaining in the respiratory tract. A scrubbing factor close to 1, therefore, indicates that little diacetyl is removed; conversely, a scrubbing factor closer to zero indicates that more diacetyl has been removed from the respiratory tract. It should be noted that the scrubbing factor for mice was derived using experimental minute volumes measured in the National Institute of Environmental Health Sciences (NIEHS) study rather the EPA default value.

For nasal lesions, there is no associated scrubbing. The nasal region (also referred to as the extrathoracic, or ET, region) is the first region that is encountered by an inhaled vapor and thus, when considering lesions in the ET (nasal) region, an implicit scrubbing factor of 1 will always be applied.

The other component that should be estimated is an "effective dose" (or "target dose") once the scrubbing has occurred. As was the case with the scrubbing factors, alternative effective dose measures are considered based on the EPA default model and the Morris/Hubbs computational fluid dynamics (CFD) model approaches.
In general, the effective dose measure will be of two types. The first is based on the minute volume relative to the surface area in the affected region; this is the EPA default approach. The EPA default approach is based on a fractional penetration model and

predicts the amount of chemical (normalized to surface area) that penetrates the different regions of the respiratory tract (see Appendix I of EPA 1994). This approach is referred to as the regional penetration approach. The second approach utilizes tissue concentrations, estimated by the previously cited CFD model predictions. The CFD/PBPK model [Morris and Hubbs 2009] represents the upper respiratory tract and includes tissue compartments with terms for metabolic and systemic clearance that allow predictions of tissue concentration, which are the basis for the tissue concentration approach.

The various combinations of scrubbing terms and the effective dose measures lead to several different alternative metrics for each exposure group. Results from modeling for all the various scenarios are included in Appendix 5.

#### 6.1.2.4.1 Dose metric calculations

The methods described above include several options for scrubbing factor and effective dose estimations. The combinations that were included in the analysis are summarized in [Allen 2009a] (Appendix 5). Each combination accounts in some manner for the removal of diacetyl before the target site and an effective measure of dose at the target site. Note that for the dose metrics that use the regional penetration effective dose measure, the ppm exposure concentrations were converted to µg/ml concentrations (1 ppm = 0.00352 µg/mL on the basis of diacetyl's molecular weight of 86.09). Moreover, all the metrics were multiplied by the daily duration of exposure (360 minutes). The resulting units for such dose metrics are $\mu g/cm^2$ for those using the regional penetration effective dose measure and µg/mL-min for those using the tissue concentration metric.

#### 6.1.2.4.2 Converting benchmark doses to human equivalent concentrations

The dose-response modeling described above used the dose metrics derived as appropriate for the mice in the bioassay under consideration. Thus the BMDs obtained from that modeling are in the units of the input dose metrics and should be converted to HECs to be relevant to ascertaining risks in humans exposed to different exposure concentrations. The process by which those BMDs were converted to HECs is described

here. As in the case of the mouse-estimated dose metrics, the conversions consider both diacetyl scrubbing and the effective dose measure.

Regardless of the particular methods for HEC estimation, two assumptions were made throughout.

The region that is associated with the observed human response (obliterative bronchiolitis) is the tracheobronchial (TB) region. Effective dose estimates relevant to this region (or to bronchiolar tissue concentrations) are the focus of the HEC conversions. The relevant scrubbing factors are for the ET region (or ET and trachea in the case of bronchiolar tissue concentration measures).

No matter what region of the respiratory tract is affected in mice (nasal or TB), it is assumed that the dose-response relationship in humans is proportional regardless of the affected region in humans. This means that the relationship between any dose metric and the responses for that region is assumed to hold in humans, between the same type of metric (e.g., with effective dose proportional to the minute volume to surface area ratio and accounting for scrubbing) and the response that might occur in the human-affected region. In other words, the value of the dose metric giving risk of X in a region of the mouse respiratory tract is assumed to give the same risk to the affected human respiratory tract region, whatever that region may be; no site concordance is assumed.

In all cases, the minute volume (VE) for a human worker was assumed to be 20 Lpm (20,000 mL/min). This corresponds to a value of 9.6 $m^3$ breathed per 8-hour work shift, a value used by NIOSH and OSHA for previous risk assessments. In addition, it was assumed (as the base case) that there was 50% mouth breathing. This affects the estimated scrubbing in the ET region; it is assumed that no ET scrubbing occurs during mouth breathing, so that the scrubbing factor for the ET region is set to 1 when mouth breathing is in effect. As a test of sensitivity, rates of 0 and 100% mouth breathing have also been examined.

Surface area for the TB region in humans was assumed to be the EPA default of 3200 $cm^2$ [EPA 1994].

The scrubbing factors examined for humans are the EPA default human scrubbing factor, the CFD model-based ET scrubbing factor, and the CFD model-based ET and tracheal scrubbing factor.

#### 6.1.2.4.3 Human effective doses

Two effective dose measures were considered: TB penetration and tissue concentration. For the TB penetration approach, as in the case of the mouse dose metric calculations, the option of a regional penetration approach was based on the EPA [1994] default.

The tissue concentration effective dose corresponds to any of the mouse dose metrics where the effective dose was tissue concentration. The manuscript by Morris and Hubbs does not present CFD model-predicted bronchial tissue concentrations [Morris and Hubbs 2009]. In their absence, calculations were performed to derive such concentration estimates (see Appendix 5 for details).

It was assumed that the factor that converts airway concentration to tissue concentration is the same for bronchial mucosa tissues and for tracheal mucosa.

Thus, given the conversion factors based on CFD model predictions in the trachea (Table 6.2), a conversion factor was derived that was assumed to apply to the TB region as a whole and to bronchial tissues within that region.

Table 6.2. CFD model predicted inhalation concentrations and tissue concentrations

| Species | Inhalation Concentration (ppm)[a] | Converted Concentration (μg/ml) | Anterior Ventral Mucosal Concentration (mM)[a] | Converted Anterior Ventral Mucosal Concentration (μg/ml) | Concentration Exiting UR Tract (ppm)[a] | Converted Concentration Exiting UR Tract (μg/ml) | Anterior Tracheal Mucosal Concentration (mM)[a] | Converted Anterior Tracheal Mucosal Concentration (μg/ml) |
|---|---|---|---|---|---|---|---|---|
| Rat | 100 | 0.352 | 1.6 | 138 | 67 | 0.236 | 1.2 | 103 |
| | 200 | 0.704 | 3.2 | 275 | 136 | 0.479 | 2.5 | 215 |
| | 300 | 1.056 | 4.9 | 422 | 206 | 0.725 | 3.8 | 327 |
| Human | 100 (nose breathing) | 0.352 | 1.4 | 121 | 82 | 0.289 | 1.2 | 103 |
| | 100 (mouth breathing) | 0.352 | -- | -- | 100 | 0.352 | 1.5 | 129 |

[a]From [Morris and Hubbs 2009], Table 3. Other columns are conversions based on molecular weight of diacetyl of 86.09.

6.1.2.4.4 Duration adjustment and final human equivalent concentration conversions

An additional adjustment that completes the conversion of mouse BMDs to human exposure concentrations is a factor of 480 minutes to account for the daily duration of exposure assumed for workers. No other duration adjustments were applied (experimental exposures occurred 5 days per week, for example, so no adjustment was made for frequency of exposure during a week). Although ideally, a lifetime bioassay would be preferred for purposes of risk assessment, in this document the subchronic exposure period (up to 12 weeks) was considered adequate to characterize the extent of lesions for longer-term exposures. This conclusion was supported by the fact that the 6- and 12-week experiments had response rates that could be modeled together (i.e., the duration of the experiment could be ignored) for all the lesions analyzed; there did not appear to be a progression toward higher rates of response or more severe responses when the exposure level remained the same but the duration of exposure was increased from 6 to 12 weeks. However, because of the limited study design, increasing toxicity with increasing duration of exposure could also not be ruled out.

### *6. 1.3 Results of Modeling*

The tests for consistency of the lesions across the 6-week and 12-week experiments were uniform in indicating that the observations from the two experiments could be combined. That is, assuming a single probability of response for each exposure concentration provided nearly as good a description of the observed results as assuming two probabilities per exposure concentration, one per length of exposure. There was insufficient evidence to reject (at the 0.05 level) the hypothesis of one rate per exposure level for any endpoint. As a result, all dose-response modeling was done on the combined results of the two experiments, and the results presented here are only for such combinations. The combination of the 6- and 12-week data tends to reduce uncertainties in the estimates (BMDs) by increasing the number of animals per dose.
The most sensitive endpoints by site (nasal and lung) are nasal inflammation and peribronchial inflammation. As mentioned in the Methods section, this determination is the same no matter what dose metric is used for the modeling. This finding is not surprising given the response rates shown in Table 6.1; those inflammation endpoints had the highest incidence rates (by site) for the combined 25 ppm group. The BMDs and BMDLs estimated for the other endpoints were consistently and uniformly greater than those estimated for the nasal and peribronchial inflammation endpoints (results not shown).

The BMDs and BMDLs for the baseline assumptions and the best-fitting models are as shown in Table 6.3. The BMDs in this table are not directly comparable to the epidemiology-based BMDs because the units (regional penetration and tissue concentration) are not equivalent to the inhaled concentrations of the epidemiology study. The corresponding graphs are shown in Figures 6.1 through 6.4. The probit model provided the best fit (smallest AIC) to the nasal inflammation data using the tissue concentration metric. The multistage model was the best fitting for the other endpoint/dose metric combinations. The BMD and BMDL predictions from these models were not very different across the two inflammation endpoints for a fixed choice of effective dose measure. The BMDLs for the peribronchial inflammation endpoint were

always less than those for the nasal inflammation endpoint, with a bigger difference between the BMDLs across endpoints observed for the tissue concentration effective dose measure (slightly more than a factor of 2). This relationship holds for the other scrubbing factors (i.e., for those alternatives not used in the baseline calculations shown in Table 6.3). For the BMDs, the pattern is not so simple. The BMDs for nasal inflammation are greater than those for peribronchial inflammation, when the effective dose measure is regional penetration; the BMDs for peribronchial inflammation are greater than those for nasal inflammation when tissue concentration is the effective dose measure, and in that case the relative difference increases as the risk level decreases.

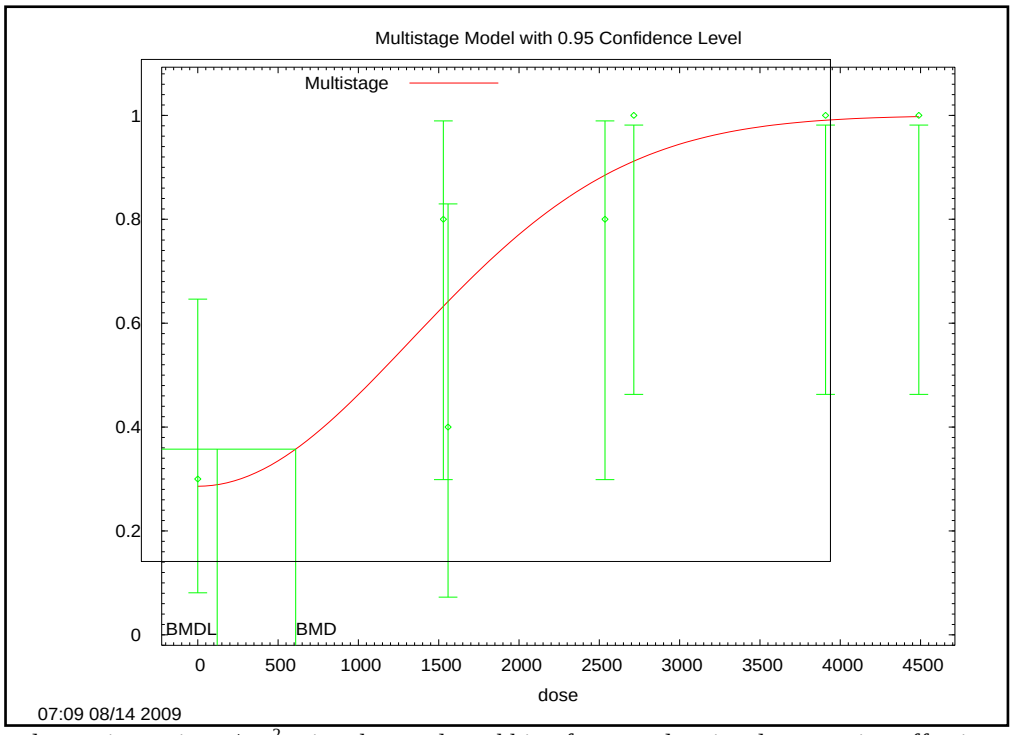
Table 6.3. BMDs and BMDLs for the sensitive endpoints; baseline dose metric calculations, best-fitting models[a]

| Endpoint | Effective Dose Measure | *P* Value[b] | Risk Level 0.1 BMD | 0.1 BMDL | 0.01 BMD | 0.01 BMDL | 0.001 BMD | 0.001 BMDL |
|---|---|---|---|---|---|---|---|---|
| **Nasal Inflammation** | **Regional Penetration ($\mu g/cm^2$)** | 0.85 | 585.9 | 100.0 | 181.0 | 9.541 | 57.10 | 0.9498 |
| | **Tissue Conc. (μg/ml-min)** | 0.84 | 2428 | 1606 | 252.3 | 162.9 | 25.34 | 16.31 |
| **Peribronchial Inflammation** | **Regional Penetration ($\mu g/cm^2$)** | 0.94 | 370.8 | 79.58 | 114.5 | 7.591 | 36.14 | 0.7557 |
| | **Tissue Conc. (μg/ml-min)** | 0.91 | 2713 | 721.9 | 396.3 | 68.86 | 42.93 | 6.855 |

[a]Best fitting model is the Probit model for nasal inflammation with tissue concentration metric; it is the Multistage model in all other cases.

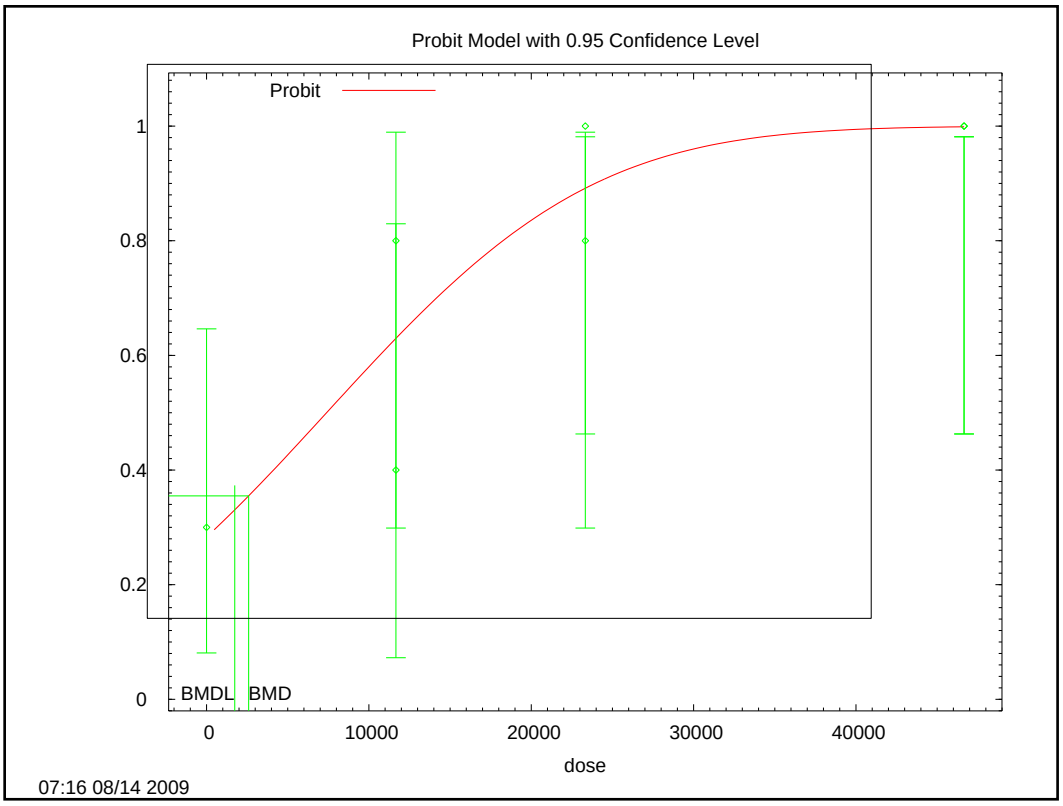
[b]*P* value is the goodness-of-fit *P* value with values closer to 1 indicating better fit (values greater than 0.1 are generally accepted as indicating satisfactory fit).

Figure 6.1. Benchmark dose modeling of the fraction of animals affected at each dose. Best fitting model for nasal inflammation; nasal penetration dose metric



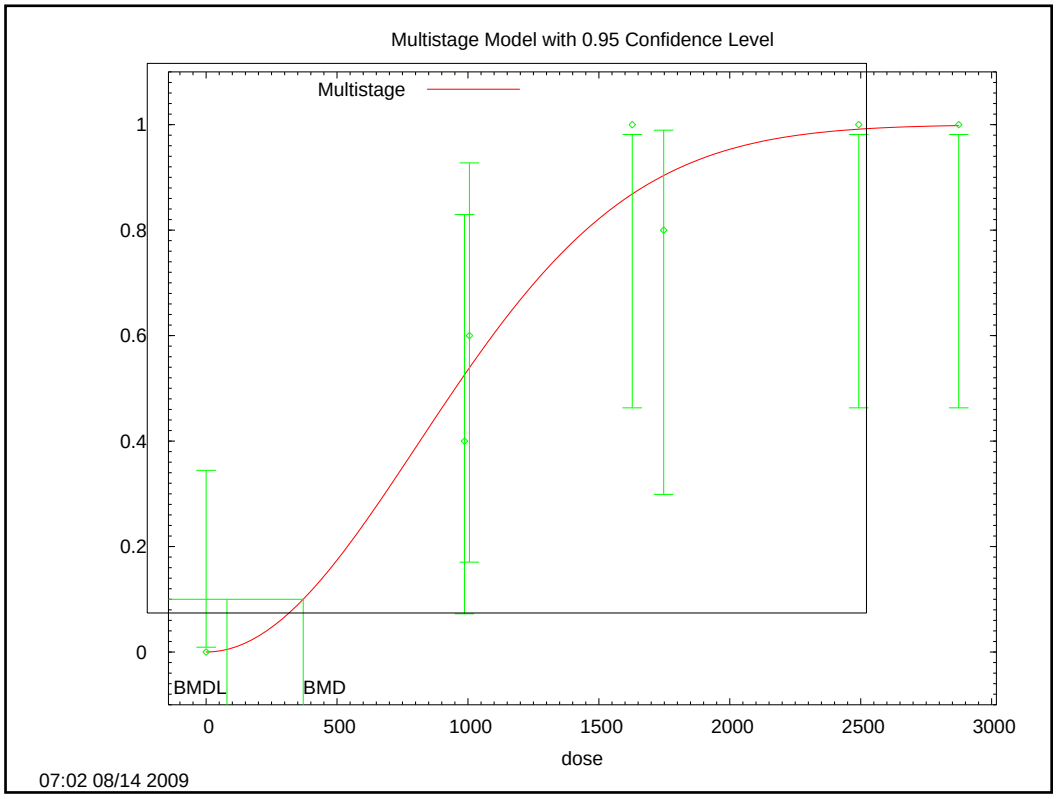

The dose units are in ug/cm$^2$ using the nasal scrubbing factor and regional penetration effective dose calculation as shown in Table 11 in Appendix 5.

Figure 6.2. Benchmark dose modeling of the fraction of animals affected per dose. Best fitting model for nasal inflammation; tissue concentration effective dose metric



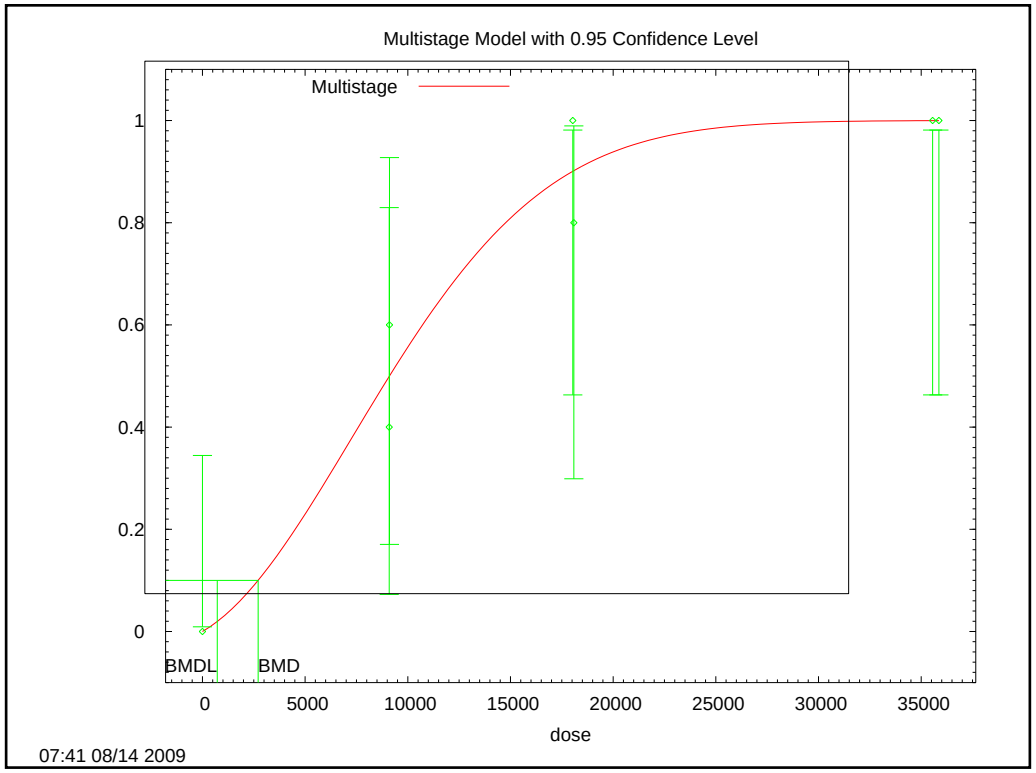

The dose units are in ug/ml-min using the nasal scrubbing factor and tissue concentration effective dose calculation 2b as shown in Table 11 of Appendix 5.

Figure 6.3. B Benchmark dose modeling of the fraction of animals affected per dose. Best fitting model for peribronchial inflammation; tracheobronchial penetration dose metric




The dose units are in ug/cm$^2$ using the dose metric calculation based on scrubbing factor 2b and TB regional penetration effective dose as shown in Table 11 of Appendix 5.

Figure 6.4. Benchmark dose modeling of the fraction of animals affected per dose. Best Fitting model for peribronchial inflammation; tissue concentration effective dose metric




The dose units are in ug/ml-min using the dose metric calculation based on scrubbing factor 3b and tissue concentration effective dose 2b as shown in Table 11 of Appendix 5.

The impact of the choice of effective dose measure can only be determined when the BMDs and BMDLs are converted to HEC ppm concentrations (Table 6.4), because the units are different for the two measures, and conversion from BMDs and BMDLs to the human equivalents is therefore dependent on the dose measure. Of note here is the fact that the HECs derived using the tissue dose metric are consistently much less than the corresponding HECs derived using the regional penetration effective dose measure. For the HECs based on BMDLs, the difference ranged from about a factor of 3 (for nasal inflammation) to a factor of about 6 (for peribronchial inflammation). The differences across dose measures were even greater for the HECs based on the BMDs, with differences up to about a 120-fold difference (e.g., for nasal inflammation and the lowest risk level of 0.001, the BMD for regional penetration of 5.940 compared to the BMD for the tissue concentration dose metric of 0.04731).

Table 6.4. HECs (ppm atmospheric concentration) corresponding to BMDs and BMDLs for the sensitive endpoints; baseline dose metric calculations and human conversion approaches, best fitting models

| Endpoint | Effective Dose Measure | Benchmark Response 0.1 BMD | 0.1 BMDL | 0.001 BMD | 0.001 BMDL |
|---|---|---|---|---|---|
| **Nasal Inflammation** | **Regional Penetration** | 60.95 | 10.41 | 5.940 | 0.09881 |
| | **Tissue Conc.** | 4.532 | 2.997 | 0.04731 | 0.03045 |
| **Peribronchial Inflammation** | **Regional Penetration** | 38.58 | 8.279 | 3.759 | 0.07861 |
| | **Tissue Conc.** | 5.064 | 1.347 | 0.08012 | 0.01280 |

The HECs correspond directly to the BMDs and BMDLs shown in Table 6.4. Values in this table are derived from those in Table 6.4 by dividing by the values in the second row of Table 12 in Appendix 5, for the regional penetration effective dose measure or by the third row of Table 12 in Appendix 5 for the tissue concentration effective dose measure.

Of course, there were variations in the BMD and BMDL estimates across all of the models fit to these data. The best fitting models were not unique in providing a satisfactory description of the observed dose-response patterns; the goodness of fit assessment for all the dichotomous models suggested that none of them would be excluded solely on the basis of fit (all the *P* values for goodness of fit were greater than 0.65, with *P* values greater than or equal to 0.1 typically considered to indicate a satisfactory fit). Thus, the model selection criterion based on AIC was what defined the best-fitting model. For each of the four endpoint/dose measure combinations, the BMDs across the dichotomous models differed by as much as a factor of nearly 150, the range

being larger for the lower risk levels (especially for 0.001 risk). The range of BMDLs was much greater; for the 0.001 risk level the range of BMDL estimates over the seven models was greater than 3700-fold for nasal inflammation and the tissue dose metric. Other variations related to the dose calculations that were discussed in the Methods section (defining how the scrubbing factor is estimated or how the tissue concentration is extrapolated) have much less impact on the HECs. For either dose metric, the HEC estimates varied from 0.91 to 1.38 times the baseline HEC values shown in Table 6.5 (about a 9% reduction to about a 38% increase) when the assumptions about the scrubbing factor were changed (as reflected in the options laid out in Table 11 of Appendix 5). For the tissue concentration dose measures, altering the conversion from airborne concentration to tissue concentration (alternatives also reflected in the last two columns of Table 11 of Appendix 5) had the effect of increasing the HECs by 7% for nasal inflammation and by 10% for peribronchial inflammation.

One of the greater impacts on the HEC calculations is the fact that the VE values used in the current analysis are much greater than one would have calculated using EPA defaults. On average, the VEs for the dose groups are slightly more than about 3.5 times greater than the default VE for mice. Changing to the lower VE would have a slight impact on scrubbing, decreasing from an average of about 0.97 to 0.92, indicating greater removal, i.e., greater scrubbing efficiency, with lower ventilation rates. But the biggest effect would be the 3.5-fold reduction in the dose measure, VE/SA. Together this would decrease the BMDs and BMDLs (and therefore the HECs) by about a factor of 4 for the regional penetration dose measure. The effect of a lower VE value on the HECs derived using the tissue concentration dose measure is unclear because the impact of inspiratory flow rate on the inhaled and tissue concentrations was not explicitly evaluated using the CFD model [Morris and Hubbs 2009]. However, the VE/SA ratio for the ET region in the CFD model simulations reported for the rat (VE/SA = 30) and human (VE/SA = 69) were in the range of those based on experimental VE values in mice (VE/SA between 31 and 49).

The effect of altering assumptions about converting effective dose measures to ppm exposure concentrations in humans (Table 12 in Appendix 5) had relatively minor effects on the HECs. Changing the assumed rate of human scrubbing in the ET region to the EPA default value (0.995) would reduce the HEC by slightly less than 9% for the regional penetration dose measure and about 12% for the tissue concentration dose measure. In fact, assuming 100% mouth breathing (for which there is no ET scrubbing) induced essentially those same reductions in the HEC estimates. Conversely, if it were assumed that workers were able to breathe through their noses 100% of the time, the HEC would only increase by 11% for the regional penetration dose measure or 7% for the tissue concentration measure.

A slightly greater effect on the HECs is attributable to the assumed rate of breathing of the workers. If the EPA default rate of 13890 mL/min were used for the HEC calculations (in place of the 20000 mL/min assumed for the current analysis), the HEC would increase by slightly more than 44% if one assumes the effective dose measure is expressed in terms of the regional penetration measure. The decreased VE value directly affects the dose measure (VE/SA(TB)) as well as slightly increasing the scrubbing efficiency, allowing greater exposure concentrations to be associated with the risk-specific doses estimated from the animal dose-response analysis. The effect would be almost negligible for the tissue concentration dose measure, because the slight decrease in diacetyl reaching the TB region associated with increased scrubbing efficiency would have only a minor effect on the airborne concentration reaching the TB region and therefore on the tissue concentrations.

### *6.1.4 Discussion*

The baseline assumptions and approaches to calculating HECs corresponding to risk-specific doses (doses corresponding to risk levels of 10%, 1%, and 0.1%) are based on the available data and predictions, for both test species and humans, of the fluid dynamics of inhaled diacetyl. Unfortunately, the test species that have relevant dose-response data

is the mouse, whereas the test species for which fluid dynamic predictions are available is the rat. The approaches that were pursued in this analysis attempt to compensate for that mismatch by adjusting defaults that would otherwise be used for mice in a way that is consistent with the adjustments suggested as being appropriate for rats and/or humans. The Methods section in Allen [2009] (Appendix 5) describes the various alternative assumptions that could be made in pursuing those adjustments; the Results section shows that the quantitative impact of those alternatives is generally not too great [Allen 2009a]. On the other hand, the biggest impact on the HEC calculations is the choice of effective dose measure, either the regional penetration predicted by the EPA default model or the tissue concentration predicted from the CFD model (Table 6.5). The former is generic and would be the same for any category 1 gas or vapor. The latter is specific to diacetyl through the parameterization of the CFD model. However, the tissue concentration measure has been extrapolated not only from CFD model predictions for rats and humans, but also from predictions restricted to the trachea (whereas tissue concentrations relevant to the bronchial subregion are desired). The lack of experimental data on causes uncertainty about diacetyl uptake in the respiratory tract and metabolism in both mice and humans, the species from which and to which the dose-response results are being extrapolated. Uncertainties also exist in relation to species differences in toxicodynamics and the related issue of exposure-response behavior at low doses (whether or not a threshold may exist for the diacetyl-induced respiratory tract effects observed in humans). Because of these uncertainties, it is not possible to definitively state that one effective dose measure is to be preferred over the other nor to determine toxicologically what dose-response relationship should be expected. Consequently, results for both effective dose measures are presented.

More general uncertainties that affect this and most other risk assessments include the limited number of observations (five per dose group), the relatively small number of doses examined, and the relatively short period of follow-up. The risk assessment described here took advantage of the observation that no statistically discernable progression was observed in response from 6 to 12 weeks of exposure in mice and that

the responses at week 18 (6 weeks after the end of exposure) were also comparable to those seen at 6 and 12 weeks [Morgan et al. 2008]. This allowed the 6- and 12-week studies to be combined to increase the power of these very small studies. However, because the concern for affected workers spans periods substantially greater than 6 or 12 weeks and the small study size limits the ability to observe a progression of effect, there is insufficient information to rule out an impact of duration of exposure as an important factor in determining the human risks of diacetyl exposure.

So, as is typical of quantitative risk assessment applications, the estimation of human risk associated with diacetyl exposures is subject to a number of uncertainties. The HECs calculated (Table 6.5) represent a reasonable basis for informing decisions related to occupational exposures to diacetyl and complement the epidemiologic investigations on the exposure-response of diacetyl in occupational settings. Of course, when adequate human data are available, they are always preferred for establishing recommended exposure limits. However, animal data serve a vital purpose in allowing risk assessors to examine the toxicity of a compound with greater consideration for mode of action. In the case of diacetyl, this animal-based risk assessment provides pathology data on lung injury in two species. If these data were the only data available to support a recommendation, the BMDL(0.1) corresponding to 1:1000 risk derived from this risk assessment are in the range of 0.01 to 0.1 ppm. Because of the limited study design, it would be a reasonable to use uncertainty factors to account at least for less-than-lifetime exposure. The resulting recommendation would be very close to what has been derived from the human data, increasing confidence in the human risk estimates.

#### 6.1.4.1 Comparison with other animal-based risk assessments

The HEC estimates derived here can be compared to the estimates that have been or might be provided by other assessments. An assessment completed by TERA [IDFA 2008] has considered the same dose-response data [Morgan et al. 2008] and estimated HECs based on BMDLs for 10% risk, the BMDL(10)s. Those HECs are compared below to the HECs derived in this analysis, also based on the one-in-ten BMDL(10)s. In

addition, estimates like a reference dose or a linearly extrapolated dose from the BMDL(10)s can be computed; such estimates are compared to the HECs derived in this analysis from the BMDL(1)s and BMDL(0.1)s.

TERA [IDFA 2008] examined the data related to the respiratory effects of diacetyl in humans and in experimental species. They concluded that the mouse data used in the analyses above [Morgan et al. 2008] were the best basis for a quantitative risk assessment. TERA excluded the nasal lesions from consideration prior to their analysis, stating that the evidence of upper respiratory symptoms in humans exposed to diacetyl was inconsistent and that those symptoms lacked reliable concentration-response information. In this assessment, it has been assumed that the dose-response relationship in a test species, rather than the lesion site, is the best criteria for choosing which endpoints to model for quantitative risk estimation. Thus, site concordance is not a requirement because the once the dose has been adequately adjusted (and ideally, once toxicodynamic considerations have been carefully considered), a valid dose-response relationship at any respiratory tract site/lesion in a test species is a reasonable basis for characterizing human risk. Additionally, exact site concordance across species would not be expected after exposure to diacetyl because of the physical interaction of the chemical with the walls of the respiratory tract. It appears that bronchii or nasal passages of a specific diameter (regardless of where they occur in the respiratory tract of a particular species) are particularly sensitive to the effects of diacetyl. For mice, this diameter passage is found in the larger bronchii and nasal passages. For rats, slightly deeper and for humans, deeper still. For further discussion of this issue, see Chapter 4.
TERA [IDFA 2008] estimated HECs using the EPA default methods [EPA 1994] modified by the same CFD model predictions [Morris and Hubbs 2009] used in the assessment presented above. However, rather than using the relationships between the default and CFD-model-predicted scrubbing factors to refine a mouse, diacetyl-specific estimate of scrubbing, they assumed that mice were exactly like the CFD-modeled rats (i.e., used the CFD model predictions for the rats as if they were equally relevant to mice). Furthermore, TERA used the air exiting the trachea to estimate upper respiratory

scrubbing. This assessment does not include tracheal scrubbing as part of a dosimetric adjustment, where the effective dose measure is a regional (tracheobronchial) penetration metric because the trachea represents the upper portion of the presumed region of action. Moreover, for the effective dose (regional penetration) measure calculated by TERA, the default mouse ventilation rates were used. As noted elsewhere, the experimentally measured ventilation rates were substantially greater than the default (by a factor of 3 to 5) and this would have a major impact on the HEC estimates (TERA's estimates would be about 3 to 5 times greater, because the major effect of changing the ventilation rate is on the effective dose measure, VE/SA, rather than the scrubbing).

TERA's analysis resulted in estimates of HECs that were 9 and 2 ppm, corresponding to the estimated BMD(10) and BMDL(10), respectively, from their dose-response analysis of the peribronchial inflammation endpoint from Morgan et al. [Morgan et al. 2008]. Those values are low compared to the HECs derived in the current analysis using the same type of regional penetration dose measure; the BMD(10) and BMDL(10) for that dose measure are 39 ppm and 8 ppm, respectively (Table 6.4). On the other hand, the current analysis obtained values of 5 ppm and 1 ppm for the BMD(10) and BMDL(10) when based on a tissue concentration dose measure (Table 6.4). The nasal inflammation endpoint yielded estimates of BMD(10) and BMDL(10) that were even slightly greater than those for the peribronchial inflammation endpoint.

The TERA assessment suggested that a composite uncertainty factor of 10 should be used to adjust those HECs downward to an occupational exposure limit (OEL). That factor of 10 was the product of a factor of 3 for interspecies differences and another factor of 3 for human variability [IDFA, 2008]. These factors of 3 are well-accepted uncertainty factors commonly used by EPA and others in risk assessment. Their recommended OEL was therefore 0.2 ppm (as an 8-hour TWA). In comparison, the HECs derived above for 1% and 0.1% extra risk were 0.8 ppm and 0.08 ppm (using the BMDL values) when the regional penetration effective dose measure was used. If extrapolation to humans were

based on the tissue concentration metric (using the BMDL values), the corresponding HECs were estimated to be 0.1 for 1% risk and 0.01 for 0.1% risk.

If a linear extrapolation from the HECs based on BMDL(10)s were considered, then the HECs for 1% and 0.1% extra risk would be 10 and 100 times less, respectively, than the HECs for 10% risk. In that case, the 1% and 0.1% HECs would be, respectively, 0.83 and 0.083 ppm (using the regional penetration dose metric) or 0.13 ppm and 0.013 ppm (using the tissue concentration metric). Those estimates are very similar to the model-based estimation of the HECs associated with those risk levels. This indicates that, at least for the best-fitting models, the bounds for BMDs are exhibiting a linear dose-response relationship. At the levels of risk under consideration (1% and 0.1%) the data in hand cannot rule out a "bounding" linear relationship for the best-fitting models, even though the best estimates (i.e., BMD(1) and BMD(0.1)) suggest that the dose-response is concave up at these risk levels.

Finally, in the context of comparing the HECs to those that might be derived in alternative risk assessments, one can consider the use of the ppm exposure concentrations rather than some calculated effective dose measure. Using the ppm exposures and adjusting only for daily duration of exposure (6 hours for the test species and the assumed 8 hours for occupationally exposed workers), the HECs for 10%, 1% and 0.1% extra risk (using the BMDL values) would be 2.6 ppm, 0.25 ppm, and 0.025 ppm, respectively. These were obtained from the best-fitting (multistage) model fit to the peribronchial inflammation endpoint. Those HECs are about a factor of two greater than the corresponding estimates obtained using the tissue concentration dose measure and about a factor of three less than estimates using the fractional penetration dose measure (Table 14 of Appendix 5).

#### 6.1.4.2 Conclusion

The mouse inhalation exposure dose-response data have provided a basis for estimating occupational diacetyl HECs corresponding to various levels of extra risk. Dosimetric

adjustments have been considered that attempt to adjust for species differences in scrubbing and uptake of diacetyl.

It was determined that the measure of effective dose had the greatest impact on the HEC estimates, with the metric based on CFD-model-predicted tissue concentrations [Morris and Hubbs 2009] yielding HECs about a factor of 6 less than the metric based on regional penetration (as expressed via EPA's default regional dose approaches [1994]). All other alternative assumptions related to dose metric calculations had much less impact (typically resulting in changes in HECs estimates on the order of 10%). Alternative (extreme) assumptions about rates of mouth breathing among workers also affected HEC estimates by only about 10%.

While there are uncertainties associated with the various assumptions used in the assessment, the variability in the estimates associated with different choices for models and modeling approaches was substantially greater than the variability associated with choice of effective dose, leading to BMDs varying over orders of magnitude. However, the best-fitting models selected for each of the modeled endpoints appear to adequately characterize the observed dose-response patterns and provide HEC estimates consistent with those of the alternatives.

For the risk levels examined (10%, 1%, 0.1%) the most sensitive endpoint was peribronchial inflammation. Nasal inflammation was also observed and was modeled as part of the quantitative assessment. The HECs for these two endpoints were fairly similar, suggesting that decisions based on HECs derived from analysis of the peribronchial inflammation endpoint are not sensitive to a single, potentially outlier, set of observations of adverse response.

The HECs corresponding to the 95% lower confidence limits on the dose associated with estimates of 1% and 0.1% extra risk are 0.8 ppm and 0.08 ppm, respectively, based on

the analysis that uses the regional penetration dose metric. If the dose metric is defined on the basis of tissue concentration, the corresponding HECs are 0.1 ppm and 0.01 ppm. The HECs described above are for comparative purposes and should be considered complementary to rather than as an equivalent to or a replacement for the epidemiology-derived REL. The animal studies strengthen our confidence in the causal association between exposure to diacetyl and respiratory tract effects because animals (unlike humans who work with flavorings) are exposed experimentally to pure diacetyl. However, predicting human health effects from the available animal studies are limited by the size of the exposure groups and the length of the studies. An REL is designed to protect workers exposed for a working lifetime, or up to 45 years of exposure. When animal studies are used to predict human risk, more confidence is provided in risk assessments using data from lifetime (2-year) bioassays, than 6- or 12-week studies. The small exposure group sizes in these studies are also cause for concern when extrapolating to humans.

If the animal data were the only data available upon which to base an REL, it would be reasonable to include an additional uncertainty factor to account for the incomplete animal database. However, even without additional uncertainty factors and without accounting for duration of exposure, the tissue concentration-based HECs are within an order of magnitude of the risk estimates derived from the epidemiologic data. NIOSH concludes that the animal-based risk assessment supports the epidemiologic assessment presented earlier both by demonstrating a causal link between diacetyl exposure and respiratory health effects and by showing a clear dose-response relationship in exposed animals as was observed in workers exposed to diacetyl in the epidemiologic assessment.

### 6.2 2,3-Pentanedione

Toxicological data for 2,3-pentanedione are limited to a single 2-week pilot study using small numbers of animals [Morgan et al. 2010]. Although these limited data may be insufficient for quantitative risk estimation and extrapolation to lifetime occupational exposure, it is possible to compare the toxicity produced by 2,3-pentanedione to that produced by diacetyl under similar conditions, and thus estimate the relative potency of 2,3-pentanedione in comparison to diacetyl. Therefore, the limited toxicological data for 2,3-pentanedione are not used directly to establish a REL for 2,3-pentanedione, but only to develop a rough estimate of the toxic potency of 2,3-pentanedione relative to that of diacetyl.

#### *6.2.1 Methods*

##### 6.2.1.1 Data Used

A summary of a pilot study of 2,3-pentanedione toxicity was reported by Morgan et al. [Morgan et al. 2010]. Individual animal data from this study were graciously provided for this analysis by Dr. Daniel Morgan, NIEHS (personal communication to Dr. Lauralynn Taylor McKernan, NIOSH, November 30, 2010). These data describe the pathological responses of male and female Wistar-Han rats and B6C3$F_1$ mice exposed to 2,3-pentanedione by inhalation for 6 hours per day, 5 days per week, for 2 weeks plus 2 days. The exposure concentrations were 0 ppm, 50 ppm, 100 ppm, and 200 ppm, with six animals per dose group; nasal, tracheal, and pulmonary endpoints were assessed.
The 2,3-pentanedione data were compared to data for diacetyl from the same laboratory, as described by Morgan et al. [Morgan et al. 2008]. These data describe the pathological responses of female C57Bl/6 mice exposed to diacetyl by inhalation for 6 hours per day, 5 days per week, for either 6 or 12 weeks. The exposure concentrations were 0 ppm, 25 ppm, 50 ppm, and 100 ppm, with five animals per dose group. Nasal, tracheal, and pulmonary endpoints similar to those examined in the 2,3-pentanedione study were assessed. In addition to the data in the Morgan et al. publication[Morgan et al. 2008] , tables of individual animal's responses were provided by Dr. Daniel Morgan, NIEHS

(personal communication to Dr. Christine Sofge, NIOSH, November 18, 2008, and November 20, 2008).

### *6.2.2 Qualitative Comparison of Pathology Induced by 2,3-Pentanedione and Diacetyl*

Morgan et al. [2008] reported extensive respiratory pathology in mice subsequent to inhalation exposures to diacetyl, including suppurative rhinitis with chronic active inflammation, foci of respiratory mucosal ulceration and/or necrosis, and squamous metaplasia of the nasal cavity, plus peribronchial lymphocytic infiltrates, atrophy, denudation, and regeneration within the large bronchi [Morgan et al. 2008]. Strikingly similar upper respiratory tract pathology was induced by inhalation of 2,3-pentanedione , as noted by Morgan et al. 2010 [Morgan et al. 2010]. Although not detailed in the Morgan et al. [2010] abstract, the individual animal pathology report provided by the authors lists mucosal inflammation; suppurative exudation; respiratory ulceration, necrosis, and squamous metaplasia of the nasal cavity; and inflammation, ulceration, necrosis, and regeneration of the bronchi in female mice exposed to 2,3-pentanedione for 2 weeks plus 2 days [Morgan et al. 2010]. Therefore, 2,3-pentanedione appears to target the same areas of the respiratory tract that are damaged by diacetyl and to cause similar pathological changes in female mice. The qualitative similarity of the target tissue sites and the pathological changes induced by 2,3-pentanedione and diacetyl suggest that a quantitative comparison of the effects is warranted.

### *6.2.3 Quantitative Comparison of Toxicity Induced by 2,3-Pentanedione and Diacetyl*

The strategy employed for quantitative comparison of 2,3-pentanedione and diacetyl toxicity was to conduct a benchmark dose analysis of pathological endpoints induced by both compounds in female mice. Because the available data for both chemicals are from pilot studies using small numbers of animals, some difficulty was encountered in identifying endpoints with partial response data (required for benchmark dose analysis) for both 2,3-pentanedione and diacetyl. One suitable nasal endpoint and one suitable

bronchial endpoint were identified, suppurative exudate of the nasal lumen and bronchial inflammation. The quantitative data for these endpoints is shown in Table 6.6. Although the original pathological scoring was categorical in nature, for modeling purposes the data were dichotomized by counting responses of any degree of severity, from mild to marked, as a "response."

A complicating factor in conducting this analysis is that the available pilot study data for 2,3-pentanedione and diacetyl followed different exposure protocols. As noted above, the 2,3-pentanedione exposures were 6 hours/day, 5 days/week, for 2 weeks and 2 days. The diacetyl exposures were 6 hours/day, 5 days/week, for either 6 or 12 weeks. Therefore, comparing 2,3-pentanedione toxicity to diacetyl toxicity entails a comparison of a 2,3-pentanedione study with 12 inhalation exposures to diacetyl studies with either 30 or 60 inhalation exposures. As noted in the toxicologically-based diacetyl risk assessment, the toxicity induced by diacetyl did not increase when the exposure duration was extended from 6 to 12 weeks. Similarly, a study in which mice were exposed to diacetyl for 1 hour/day, 5 days/week for either 2 or 4 weeks, also reported by Morgan et al. [2008], did not demonstrate increased toxicity in the 4-week exposure group, in comparison to the 2-week exposure group. These data suggest that the toxicity of inhaled diacetyl is not strongly dependent on the duration of exposure for exposures of 2–12 week duration. No data are currently available on the relationship between 2,3-pentanedione toxicity and duration of exposure, but given the chemical similarity of 2,3-pentanedione and diacetyl, and the strikingly similar pathology induced by the two chemicals, it is reasonable to assume that the toxicity of 2,3-pentanedione is not greatly dependent on duration of exposure over the 2–12 week time period, either. Therefore, the strategy adopted for this analysis is to compare the benchmark doses estimated from the 2 week plus 2 day 2,3-pentanedione study to either the 6-week or the combined 6- and 12-week diacetyl data, with no adjustment for the differing exposure durations. It is acknowledged that the lack of data on the relationship between duration of 2,3-pentanedione exposure and respiratory toxicity is an area of uncertainty, which cannot be addressed with the currently available data.

#### 6.2.3.1 Benchmark dose analysis methods

The quantal model suite of the EPA benchmark dose modeling software (BMDS), version 2.12, was used for BMD modeling. The limited amount of data available for BMD modeling imposes constraints on the models that can be employed. Specifically, as shown in Table 6.5, the 2,3-pentanedione data include only one dose group with a partial response, with the other dose groups showing either a 0% or 100% response for each of the two endpoints selected for modeling. Such data are inadequate for fitting most of the models in the BMDS quantal model suite, in that solutions are only obtainable when one of the model parameters (either the slope term or the power term) reaches an arbitrary boundary set by the programmer or implied by the model. Under these circumstances most of the models in the BMDS quantal modeling suite do not converge and thus parameter estimates for the model and BMD estimates cannot be obtained. Because BMD estimates obtained under such circumstances may be misleading, the strategy was employed of fitting only the multistage model with these data, as this model is inherently constrained in power and does not reach an arbitrary boundary in slope. Further, because the two partial response data points for 2,3-pentanedione are either at or near 50%, the BMD modeling was conducted for a response rate of 50% (BMD50) (equivalent to the median effective concentration, or EC50) rather than the conventional 10%, on the grounds that a BMD estimated from such limited data is less model dependent for points near the response rate of the observed data than at response rates requiring a greater degree of model-based extrapolation. BMD50 estimates were constructed for the 2,3-pentanedione data and compared to BMD50 estimates constructed from either the 6-week diacetyl data or the combined 6- and 12-week diacetyl data. These data sets were selected as either the diacetyl data set most comparable in duration to the 2,3-pentanedione data, in the case of the 6-week study, or the diacetyl data set offering the largest number of animals for BMD modeling, in the case of the combined 6- and 12-week data.

Table 6.5. Diacetyl and 2,3-pentanedione data used for benchmark dose modeling.

| 2,3-Pentanedione (2 week plus 2 days exposure) | | | | |
|---|---|---|---|---|
| Concentration (ppm) | 0 | 50 | 100 | 200 |
| Bronchial inflammation | 0/6[a] | 4/6 | 6/6 | 5/5 |
| Nasal suppurative exudate | 0/6 | 3/6 | 6/6 | 6/6 |
| Diacetyl (6-week exposure) | | | | |
| Concentration (ppm) | 0 | 25 | 50 | 100 |
| Bronchial inflammation | 0/5 | 3/5 | 5/5 | 5/5 |
| Nasal suppurative exudate | 0/5 | 1/4 | 4/5 | 5/5 |
| Diacetyl (6- and 12-week exposures)[b] | | | | |
| Concentration (ppm) | 0 | 25 | 50 | 100 |
| Bronchial inflammation | 0/10 | 5/10 | 9/10 | 10/10 |
| Nasal suppurative exudate | 0/10 | 1/9 | 9/10 | 10/10 |

[a]Number of animals responding/number of animals examined. Responses of any degree of severity, from mild to marked, were combined.

[b]Pooled data from 6-week and 12-week diacetyl studies.

6.2.3.2 Test for dose-response equality between 2,3-pentanedione and diacetyl

A test for equality of the dose-response relationship between 2,3-pentanedione and diacetyl was performed. In this comparison it was assumed that the control response (either nasal suppurative exudate or bronchial inflammation) was the same between the two studies; consequently, a test for equivalent dose response curves was conducted on the linear regression parameter. The test assumed that a logistic link function was adequate in describing the dose-response relationship and that only a linear term was necessary. Neither assumption was rejected by the data. A Wald test was then performed in order to assess the significance of the difference of the regression parameters obtained for 2,3-pentanedione and diacetyl.

*6.2.4 BMD50 Modeling Results.*

BMD50 estimates for 2,3-pentanedione and diacetyl were developed using benchmark dose methods, as described in Methods. The BMD50 estimates are shown below, in Table 6.6. As shown in Table 6.6, the BMD50 estimates for the two pathological endpoints are similar, for 2,3-pentanedione . The BMD50 estimates for diacetyl are lower than the BMD50 estimates for 2,3-pentanedione , and the BMD50 estimates for diacetyl did not vary greatly between the 6-week diacetyl data set and the pooled 6-week and 12-week diacetyl data sets.

Table 6.6. BMD50 estimates (ppm) for diacetyl and 2,3-pentanedione.

| | BMD50[a] | 95% LCL[b] | 95%UCL[c] |
|---|---|---|---|
| 2,3-Pentanedione (2 week plus 2 days exposure) | | | |
| Bronchial inflammation | 42.9 | 14.7 | 59.9 |
| Nasal suppurative exudate | 49.4 | 23.3 | 68.3 |
| Diacetyl (6-week exposure) | | | |

| | | | |
|---|---|---|---|
| Bronchial inflammation | 22.7 | 8.1 | 33.4 |
| Nasal suppurative exudate | 36.3 | 17.5 | 54.3 |
| Diacetyl (6- and 12-week exposures)[d] | | | |
| Bronchial inflammation | 25.0 | 13.1 | 34.8 |
| Nasal suppurative exudate | 33.3 | 25.6 | 44.0 |

[a]Estimated median effective concentration (ppm) to produce toxicity.

[b]Lower 95% confidence limit of BMD50 estimate.

[c]Upper 95% confidence limit of BMD50 estimate.

[d]Pooled data from 6-week and 12-week diacetyl studies.

#### 6.2.4.1 Comparison of 2,3-pentanedione and diacetyl toxicity

The relative toxicity of 2,3-pentanedione in comparison to diacetyl can be estimated by comparing the BMD50 estimates for the two compounds. Because the BMD50 is the estimated dose corresponding to 50% toxicity, the ratio of the toxicities at the BMD50 dose level is simply the inverse ratio of the BMD50 estimates. Therefore, for the bronchial inflammation endpoint, the toxicity of 2,3-pentanedione relative to diacetyl is estimated as 22.7/42.9, or 53%, based on the 6-week diacetyl data, and 25.0/42.9, or 58%, based on the combined 6-week and 12-week diacetyl data. Similarly, for the nasal suppurative exudation endpoint, the toxicity of 2,3-pentanedione relative to diacetyl is estimated as 36.3/49.4, or 74%, based on the 6-week diacetyl data, and 33.3/49.4, or 67%, based on the combined 6-week and 12-week diacetyl data. However, as shown in Table 6.6, the confidence limits for the BMD50 estimates for 2,3-pentanedione and diacetyl are quite broad and overlapping, so that the possibility that 2,3-pentanedione is equipotent with diacetyl cannot be ruled out.

#### 6.2.4.2 Comparison of 2,3-pentanedione and diacetyl dose-response

A test for equality of the dose-response relationship between 2,3-pentanedione and diacetyl was performed, as described in section 6.2.3.2. Comparing the 2-week + 2-day

2,3-pentanedione data to the 6-week diacetyl data, as shown in Table 6.6, the *P* value for comparison of the bronchial inflammation dose-response is 0.34. For comparison of the suppurative inflammation data the *P* value is 0.28. Therefore, the dose-response relationships for 2,3-pentanedione and diacetyl are not significantly different.

### *6.2.5 Discussion*

The current toxicological data for 2,3-pentanedione and diacetyl are extremely limited; however, some preliminary conclusions regarding the relative toxicities of the two compounds can be drawn. First, 2,3-pentanedione and diacetyl appear to target the same anatomical regions in mice exposed by inhalation, and they produce similar pathological changes. Second, the toxic potency of the two materials appears to be roughly comparable in mice exposed by inhalation. The mouse BMD50 estimates for the two endpoints with adequate data for benchmark dose analysis indicate that 2,3-pentanedione may be slightly less potent than diacetyl, though likely within a factor of two of diacetyl in potency. However, given the sparseness of the current data the BMD50 estimates for 2,3-pentanedione and diacetyl are broad and overlapping, so that the possibility that 2,3-pentanedione is equipotent with diacetyl cannot be ruled out. Given the limited data currently available for 2,3-pentanedione and diacetyl, substantial uncertainties exist in this comparison. First, the quantitative data for diacetyl are limited to one sex of one species, female C57Bl/6 mice. Data for 2,3-pentanedione were obtained in a different mouse strain, $B6C3F_1$, so that strain differences may introduce some uncertainty into the comparison – though it should be noted that the two strains are related because the C57Bl/6 strain is one of the parent strains of the $B6C3F_1$ hybrid. No chronic or subchronic toxicity data are currently available for diacetyl in other species, so that the sensitivity of the toxicity to the choice of species cannot be assessed. Furthermore, the experimental protocols for the diacetyl and 2,3-pentanedione studies differed in duration of exposure. The available data for diacetyl suggest that diacetyl toxicity is not strongly dependent on duration of exposure for subchronic exposures, but no such data are available for 2,3-pentanedione . Lack of information on the sensitivity of 2,3-

pentanedione toxicity to the duration of exposure also adds uncertainty to the comparison of diacetyl and 2,3-pentanedione toxicity. Therefore, although the BMD50 analysis detailed above suggests that 2,3-pentanedione may be slightly less toxic than diacetyl by inhalation, given the limitations of the current data equal toxic potency for the two materials cannot be ruled out.

# Chapter 7: Basis of the Recommended Standards for Diacetyl and 2,3-Pentanedione

In the Occupational Safety and Health Act of 1970 (Public Law 91–96), Congress mandated that NIOSH develop and recommend criteria for identifying and controlling workplace hazards that may result in occupational illness or injury. In fulfilling this mandate, NIOSH has reviewed the relevant human and/or animal data to assess the health effects of diacetyl and 2,3-pentanedione; assessed the risks of occupational exposure; characterized anticipated worker exposures; and developed recommended criteria for exposure limits, exposure monitoring, engineering and work practice controls, and medical monitoring. The basis for the RELs is described in this chapter.

## 7.1 Health Effect Studies of Workers Exposed to Diacetyl

As detailed in Chapter 3, medical evaluations of workers exposed to diacetyl who developed progressive shortness of breath while working at several microwave popcorn plants and flavoring plants have shown findings consistent with the severe irreversible lung disease bronchiolitis obliterans. Bronchiolitis obliterans, a potentially fatal lung disease characterized by fixed airway obstruction, has been described in workers in the microwave popcorn and flavor-manufacturing industries [CDC 2002, 2007; Kanwal et al. 2006]. Some affected workers have experienced extremely rapid declines in lung function, with severe airways obstruction occurring within several months of the start of exposure to flavoring chemicals in some cases [Akpinar-Elci et al. 2004; NIOSH 1985]. Workers as young as 22 years old have been affected. Some affected workers have been placed on lung transplant waiting lists by their physicians because of the severity of their disease [Akpinar-Elci et al. 2004]. The findings from investigations and studies conducted at multiple plants presented in Chapter 3 have revealed a link between exposure to diacetyl and risk for severe occupational lung disease. These findings meet the criteria that are often used to determine if the results of multiple studies indicate that an exposure is the likely cause of specific health effects [Gordis 1996; Hill 1965].

Investigations of severe lung disease consistent with constrictive bronchiolitis obliterans among diacetyl-exposed workers have provided substantial evidence of a causal relationship between diacetyl exposure and development of this disease.

### 7.2 Toxicological Studies of Diacetyl

In rats, acute exposures to diacetyl or diacetyl-containing butter flavoring vapors cause necrosis in the epithelial lining of nasal and pulmonary airways. Rats inhaling vapors of butter flavoring that contained diacetyl developed multifocal necrotizing bronchitis one day after a 6-hour exposure. The mainstem bronchus was the most affected intrapulmonary airway. However, nasal airways were more affected than intrapulmonary airways. Necrosuppurative rhinitis was seen in rats inhaling butter flavoring vapors at concentrations that did not cause damage in intrapulmonary airways [Hubbs et al. 2002]. As a single agent acute exposure in rats, diacetyl caused epithelial necrosis and inflammation in bronchi at concentrations of $\geq$294.6 ppm and caused epithelial necrosis and inflammation in the trachea and larynx at concentrations of $\geq$224 ppm [Hubbs et al. 2008]. In a pattern reminiscent of airway damage from diacetyl-containing butter flavoring vapors, diacetyl causes greater damage to nasal airways than to intrapulmonary airways in rats [Hubbs et al. 2008].

In mice, inhaling diacetyl at concentrations of 200 or 400 ppm for 6 hours/day for up to 5 days caused respiratory tract changes similar to those seen in rats inhaling diacetyl or diacetyl-containing butter flavoring vapors [Morgan et al. 2008]. Subchronic diacetyl inhalation caused significant histopathological changes in mice at all concentrations studied. Peribronchial lymphocytic infiltrates were seen at terminal sacrifice at 12 weeks in all subchronically exposed mice inhaling 100 ppm diacetyl and in some mice inhaling 25 or 50 ppm diacetyl. Using a CFD-PBPK model, the rodent pathologic changes, though higher in the respiratory tract, were consistent with the human bronchiolar pathology once differential nasal scrubbing, size of airway, and target organ doses were accounted for [Gloede et al. 2011; Morris and Hubbs 2009]. In rats in which nasal scrubbing was

bypassed by administering a single dose of 125 mg/kg diacetyl via intratracheal instillation, histopathological alterations characteristic of bronchiolitis obliterans ensued, including damage to airway epithelium [Palmer et al. 2011].

NIOSH concludes that the toxicological responses to diacetyl observed in animal studies support the conclusions of the epidemiologically-based risk assessment for this compound. Further, the animal-based risk assessment presented in Chapter 6 supports the epidemiologic assessment by demonstrating a causal link between diacetyl exposure and respiratory health effects and by showing a clear dose-response relationship in exposed animals as was observed in workers exposed to diacetyl in the epidemiologic assessment.

### 7.3 Quantitative Risk Assessment for Deriving the Recommended Exposure Limit

NIOSH has reviewed the literature on diacetyl toxicology and exposures in the workplace and subsequently conducted a quantitative risk assessment. Results from this comprehensive review demonstrate a causal relationship between diacetyl exposure and development of severe occupational lung disease. The quantitative risk assessment used to derive the REL was based solely on human (worker) data, but the results were informed and supported by animal risk assessments. On the basis of a quantitative risk assessment of data collected in a series of NIOSH health hazard evaluations (full description in Chapter 5), NIOSH has concluded that worker exposure to diacetyl is associated with a reduction in lung function. Specifically, a statistically significant exposure-associated reduction in the $FEV_1$/FVC ratio and percent predicted $FEV_1$ and an exposure-associated increase in the number of individuals with obstructive lung disease were observed. NIOSH quantified these exposure-response relationships and determined the exposure levels that correspond to a variety of risks (Chapter 5, Table 5.36). Risks in the range of 1:1000 corresponded to working lifetime diacetyl exposure of approximately 5 ppb. Once the risks were characterized, NIOSH examined the analytical methods (OSHA Methods 1012 and 1016) and available engineering controls and determined that they supported establishing an REL at that level.

### 7.4 Objectives

The NIOSH objective in establishing RELs for diacetyl and 2,3-pentanedione is to reduce the risk of decreased lung function and the severe irreversible lung disease constrictive bronchiolitis obliterans associated with occupational exposure to these chemicals. In addition, maintaining exposures below the RELs will help prevent other adverse health effects including but not limited to irritation of the skin, eyes, and respiratory tract in exposed workers. The recommendation to limit exposure to diacetyl and 2,3-pentanedione is based upon data from human and animal studies and the quantitative risk assessment. Additional considerations include sampling and analytical feasibility and the achievability of engineering controls.

A variety of risk estimates were evaluated and presented in Chapter 5, Table 5.36. NIOSH has historically targeted risks predicted to be in the range of approximately 1 per 1000 in establishing RELs(see Chapter 5, Table 5.36 for a table of risk estimates). In occupational exposure to diacetyl, the health effect of concern is bronchiolitis obliterans. Bronchiolitis obliterans is a debilitating, sometimes fatal, and irreversible effect. There are validated analytical methods that can be used to effectively measure worker exposures at the selected level. Additionally, information from OSHA sponsored site visits [Eastern Research Group 2009c] that the REL is achievable with engineering controls where diacetyl is used or handled.

### 7.5 Recommended Exposure Limits

#### *7.5.1 Recommended Exposure Limit for Diacetyl*

On this basis, NIOSH recommends an REL of 5 ppb for diacetyl (as a TWA for up to 8 hours/day during a 40-hour workweek). NIOSH has determined that workers exposed to diacetyl at this level for 8 hours a day, 40 hours a week for a 45-year working lifetime should have no more than a 1/1000 chance of suffering from reduced lung function associated with diacetyl exposure.

To ensure that worker exposures are routinely below the REL for diacetyl, NIOSH also recommends using an action level (AL) of 2.6 ppb with the exposure monitoring program to ensure that all control efforts (engineering controls, medical surveillance, and work practices) are in place and working properly. When exposures exceed the AL, employers should take immediate action (determine the source of exposure, identify methods for controlling exposure) to ensure that exposures are maintained below the REL. NIOSH has concluded that the use of an AL in conjunction with periodic monitoring of worker exposures (described in Chapter 10) is helpful to protect workers.

NIOSH is also recommending a short-term exposure limit (STEL) for diacetyl of 25 ppb for a 15-minute time period. The establishment of a STEL is based on the concern that peak exposures may have greater toxicity than the same total dose spread out over a longer period of time. Some limited evidence of this type of dose-rate effect is available in animal studies [Hubbs et al. 2008]. On the basis of general industrial hygiene principles, the STEL, which is five times the REL, would serve to reduce peak exposures and tend to reduce overall worker exposures to diacetyl. The selection of a STEL that is five times the REL is based upon past precautionary practice. In the absence of a STEL in workplaces complying with the NIOSH REL for diacetyl of 5 ppb TWA, workers could theoretically be exposed to 2400 ppb diacetyl for 1 minute or 480 ppb for 5 minutes in an 8-hour day with no additional exposure the remaining part of their 8-hour shift. The STEL for diacetyl of 25 ppb would limit those exposures to a possible peak of 375 ppb for 1 minute and 75 ppb for 5 minutes.

### *7.5.2 Recommended Exposure Limit for 2,3-Pentanedione*

2,3-Pentanedione is an alpha-diketone that has received attention as a substitute for diacetyl. 2,3-Pentanedione is structurally very similar to diacetyl because it is a 5-carbon alpha-diketone, and diacetyl is a 4-carbon alpha-diketone. Published reports on the toxicity of 2,3-pentanedione suggest that in rats 2,3-pentanedione causes airway epithelial damage similar to that produced by diacetyl [Hubbs et al. 2010; Morgan et al. 2010].

Individual animal data from this study were provided by Dr. Daniel Morgan, NIEHS (personal communication to Dr. Lauralynn Taylor McKernan, NIOSH, November 30, 2010). These data describe the pathological responses of male and female Wistar-Han rats and B6C3F$_1$ mice exposed to 2,3-pentanedione by inhalation for 6 hours per day, 5 days per week, for 2 weeks plus 2 days. The exposure concentrations were 0 ppm, 50 ppm, 100 ppm, and 200 ppm, with six animals per dose group; nasal, tracheal, and pulmonary endpoints were assessed.

Although the current toxicological data for 2,3-pentanedione are limited, some preliminary conclusions regarding the relative toxicities between diacetyl and 2,3-pentanedione can be drawn. First, 2,3-pentanedione and diacetyl appear to target the same anatomical regions in mice exposed by inhalation, and they produce very similar pathological changes. Second, the toxic potency of the two materials appears to be roughly comparable in mice exposed by inhalation (see Chapter 6.2 for a full discussion). Given the structural similarity between diacetyl and 2,3-pentanedione and the evidence published to date, NIOSH would prefer to recommend an identical REL for diacetyl and 2,3-pentanedione. However, OSHA Method 1016, the validated analytical method available for 2,3-pentanedione, can only reliably quantify 2,3-pentanedione at a concentration 9.3 ppb and above. Therefore the NIOSH REL for 2,3-pentanedione, while informed by the toxicological potential, is based upon the capabilities of the analytical method and is established at 9.3 ppb. This REL for 2,3-pentanedione will result in a residual risk of lung disease simialar to diacetyl, but may be higher. It does not imply that 2,3-pentanedione is safer than diacetyl. Because the REL is established at the reliable quantitation level, no AL is established for 2,3-pentanedione.

Because of their structural similarity, concerns for short term exposures to 2,3-pentanedione also apply. Accordingly, a STEL for 2,3-pentanedione is established at 31 ppb ( i.e., the lowest concentrations the method can sample accurately during a 15-minute time period). The NIOSH REL for 2,3-pentanedione of 9.3 ppb and STEL of 31 ppb would limit exposures to a possible peak of 465 ppb for 1 minute and 93 ppb for 5

minutes. Because of the concern for potential dose-rate effects, NIOSH recommends STELs for diacetyl and 2,3-pentanedione to reduce peak exposures to workers.

Maintaining diacetyl and 2,3-pentanedione concentrations at or below the RELs and STELs requires the implementation of a comprehensive safety and health program that includes routine medical surveillance, exposure monitoring, engineering controls, and worker training in good work practices. Specific recommendations for these components can be found in Chapters 2, 8, 9, and 10 of this document.

### 7.6 Rationale for the Recommended Exposure Limit

The recommendation to limit occupational exposures to diacetyl to an 8-hour TWA of 5 ppb is based on data from human quantitative risk assessment with additional rationale provided by animal toxicological studies. From the human studies, 5 ppb represents a reasonable summary of estimates from several concordant approaches to risk assessment. Although smoking affects the excess life-time risk estimates, a full treatment for the purpose of developing separate REL recommendations on smoking status would require including interactions between smoking and diacetyl exposure histories for which we believe there us insufficient statistical power to implement. NIOSH also recommends an AL of 2.6 ppb to help protect workers from exposure to diacetyl above the 5 ppb REL and a STEL of 25 ppb to limit peak exposures and protect against dose-rate effects. Engineering controls and work practices are available to control diacetyl exposures below the REL (and the AL) in workplaces. OSHA Method 1012 is a validated analytical method that can be used to effectively measure worker exposures to diacetyl. Establishing the recommended exposure limits for diacetyl is consistent with the mission of NIOSH mandated in the Occupational Safety and Health Act of 1970.

### 7.7 Controlling Diacetyl and 2,3-Pentanedione Exposures in the Workplace

In general, many industries have implemented engineering controls to reduce exposure and risk of disease among their workers. Many of the processes used where diacetyl and

2,3-pentanedione are manufactured, handled, or used are similar to other industries and may allow for common approaches to reducing employee exposure. These processes include blending, mixing, and handling of flavoring ingredients in liquid and powder form. In addition to the NIOSH studies presented in this document, Eastern Research Group, Inc. (ERG) conducted an evaluation comparing airborne diacetyl levels before and after the installation of engineering controls in a buttered popcorn production facility. Their measurements indicated reductions in personal breathing zone measurements on a TWA and a short-term basis from 83.8% to 99.4%. TWA measurements were reduced to below the LOD (generally about 3 ppb) [Eastern Research Group 2009c].

The design concepts required for working with hazardous materials include specification of general ventilation, local exhaust ventilation (LEV), maintenance, cleaning and disposal, personal protective equipment, exposure monitoring, and medical surveillance [Naumann et al. 1996]. Bag emptying, bag filling, charging tanks, benchtop weighing and handling, and drum filling and emptying are a few of the production processes of concern. Other more specialized processes (for example, candy panning, a process in which candy pieces in a rotating drum are sprayed with chocolate or other flavoring ingredients) may also result in worker exposure. Special attention should be given to manual handling of flavoring chemicals, particularly in heated processes, and when spraying flavoring ingredients. Research into various food industries has led to the development of engineering controls that may help reduce worker exposure to diacetyl, 2,3-pentanedione, and other chemicals. Chapter 8 describes engineering controls for the industries where diacetyl is handled or used within products.

NIOSH acknowledges that the frequent use of personal protective equipment (PPE), including respirators, may be required for some workers who handle diacetyl, 2,3-pentanedione, diacetyl-containing flavorings or flavored products. The frequent use of PPE may be required during job tasks for which (1) routinely high airborne concentrations of diacetyl or 2,3-pentanedione (e.g., pouring, mixing, packaging) exist, (2) the airborne concentration of diacetyl or 2,3-pentanedione is unknown or

unpredictable, and (3) job tasks are associated with highly variable airborne concentrations because of environmental conditions or the manner in which the job task is performed. In all work environments where diacetyl, 2,3-pentanedione, diacetyl-containing flavorings or flavored products are found, control of exposure through engineering controls should be the highest priority.

### 7.8 Hazards Associated with Diacetyl Substitutes

Much has been made of the possible removal/substitution of diacetyl and 2,3-pentanedione from the flavor manufacturing, flavoring industries, or food production industries. A health benefit from substitution can only be realized if the substitute is safer than diacetyl or 2,3-pentanedione. However, the current knowledge on toxicity of available substitutes is limited; few if any have OELs, and therefore exposure to substitutes should be controlled.

There is reason to think that, like diacetyl, other alpha-dicarbonyl compounds would have a tendency to cause protein cross-links [Miller and Gerrard 2005]. The reactivity of the alpha-dicarbonyl compounds is enhanced by electron-attracting groups and decreased by electron donors [Roberts et al. 1999]. Alpha-dicarbonyl compounds can inactivate proteins, principally through reactions with the amino acid, arginine [Epperly and Dekker 1989; Saraiva et al. 2006]. The related alpha-dicarbonyl flavoring, 2,3-pentanedione, has been reported to be even more reactive with arginine groups than with diacetyl [Epperly and Dekker 1989].

While the focus of this document is on diacetyl and 2,3-pentanedione, NIOSH has concern about other flavoring substitutes with structural similarities to diacetyl or moieties that are biologically active and capable of producing similar toxic effects as diacetyl. Therefore, NIOSH recommends that such exposures also be considered and controlled as low as reasonably achievable.

The guidance recommendations presented in Chapter 8 regarding control of exposures are applicable not only to diacetyl and 2,3-pentanedione, but also to their substitutes and other flavorings and flavoring chemicals used in this industry. The control of exposures is discussed in detail in Chapter 8, but a couple of LEV systems described have been shown to be particularly effective in controlling diacetyl and would be expected to work well for other compounds as well. Ventilated back-draft workstations used for small batch mixing have been evaluated in two field studies conducted in flavoring production plants. The field studies showed reductions in exposure of 90%–97% when performing mixing tasks using these stations [NIOSH 2008a]. Also, the use of controls to reduce worker exposure during pouring and mixing of ingredients in a commercial mixer has been evaluated in a flavoring production plant [NIOSH 2008d]. The use of LEV at the mixing tank helps to maintain the vessel at a negative pressure and contain evaporative emissions. NIOSH evaluated the impact of a ventilated tank lid on the exposure of a worker during the mixing of a food flavoring [NIOSH 2008d]. The use of the ventilated tank lid resulted in a reduction of approximately 76% exposure compared to the same operation without the ventilated tank lid. Most of the exposure during the evaluated mixing process was attributed to tasks performed outside of the hood. Ventilated tank lids have also been recommended by the British Health and Safety Executive to contain vapors during the mixing of liquids with other liquids or solids [Health and Safety Executive 2003d].

### 7.9 Summary

The following points summarize the relevant information used as the basis for the NIOSH assessment for occupational exposure to diacetyl and 2,3-pentanedione:

- Airborne exposures to diacetyl have been characterized as potentially hazardous on the basis of a review of the available literature regarding both human exposure and animal studies.
- Human health and animal data indicate a causal relationship between diacetyl exposure and development of bronchiolitis obliterans. Studies show a progressive

shortness of breath for workers at several microwave popcorn plants and flavoring plants as well as workers who have experienced rapid declines in lung function.

- Toxicology data from rats show that acute exposures to diacetyl or diacetyl-containing butter flavoring vapors cause necrosis in the epithelium lining the nasal and pulmonary airways and multifocal necrotizing bronchitis. Inhalation studies on mice produced similar results.
- Published reports on the toxicity of 2,3-pentanedione suggest that in rats 2,3-pentanedione causes airway epithelial damage similar to that produced by diacetyl [Hubbs et al. 2010; Morgan et al. 2010].
- Risk assessment analysis using data from both animal and inhalation human studies indicates that a diacetyl REL of 5 ppb as a TWA for up to 8 hours/day during a 40-hour workweek would be appropriate. Further, NIOSH recommends a STEL of 25 ppb to limit peak exposures and protect against dose-rate effects. An AL of 2.6 ppb is recommended to ensure that worker exposures are routinely below the REL for diacetyl and to ensure that all control efforts (engineering controls, medical surveillance, and work practices) are in place and working properly.
- Given preliminary evidence and the structural similarity of 2,3-pentanedione to diacetyl, NIOSH recommends a 2,3-pentanedione REL of 9.3 ppb as a TWA for up to 8 hours/day during a 40-hour workweek. The REL for 2,3-pentanedione is based upon the reliable quantitation limit for the analytical method and should not be misconstrued to infer that 2,3-pentanedione is of lower toxicity than diacetyl. Further, NIOSH recommends a STEL of 31ppb to limit peak exposures on the same basis of analytic method limitation.
- Data gathered on diacetyl exposure demonstrated that engineering controls and work practices currently available can control diacetyl exposures below the REL. A validated analytical method can be used to effectively measure worker exposures at these levels.

# Chapter 8: Hazard Prevention and Control of Exposures to Diacetyl and 2,3-Pentanedione

## 8.1 Introduction

Worker exposure to air contaminants can best be reduced by a combination of efforts to minimize air contaminant generation through good work practices and control of the emissions at their source through process changes or engineering controls. Traditionally, a hierarchy of controls has been used as a means of determining how to implement feasible and effective controls. One representation of this hierarchy can be summarized as follows:

- Elimination/Substitution
- Engineering controls
- Work practice controls
- Personal protective equipment (PPE)

The idea behind this hierarchy is that the control methods at the top of the list are potentially more effective, protective, and economical (in the long run) than those at the bottom. Following the hierarchy normally leads to the implementation of inherently safer systems, ones where the risk of illness or injury has been substantially reduced.

The first item in the hierarchy is elimination/substitution. The intention of eliminating a flavoring or chemical in the workplace is to remove the exposure by removing the source. Similarly, the goal of substitution is to substitute a flavoring or chemical with another of lower toxicity. The removal of diacetyl and 2,3-pentanedione from the flavor manufacturing or flavoring industries would be practical only with the substitution of an alternative butter flavor chemical, which is currently being done in some situations. However, the current knowledge on toxicity of available substitutes is limited, and exposure to substitutes also needs to be controlled. Therefore, elimination and substitution do not provide a feasible control and are not discussed in detail. The

recommendations that follow are applicable not only to diacetyl and 2,3-pentanedione, but also to other flavorings and flavoring chemicals used in this industry.

Engineering controls, as discussed below, are mechanical techniques for removing contaminants from the workplace. For instance, local exhaust ventilation can be used to capture and remove emissions from a hazardous or nuisance source. A major advantage of this type of system is that, when properly designed, it requires little user effort or training.

Work practices are procedures followed by employers and workers to control hazards in the workplace. The use of good work practices, incorporated into the facility's standard operating procedures, can help reduce exposures to diacetyl, 2,3-pentanedione, and other flavoring chemicals while at the same time maximizing efficiency and product quality. Work practices include housekeeping and cleaning, storage and use procedures, work clothes, labels and postings, hazard training, and procedures for use of engineering controls.

The use of respirators (a form of personal protective equipment) is discussed because this control, while not favored, is in common use in some facilities. As the discussion demonstrates, considerable effort is required in the proper selection and use of respiratory protection in the workplace. Finally, the protection of skin, eyes and face is discussed.

### 8.2 Engineering Controls

Currently, there is no model or standard guidance for engineering controls for flavoring and food production processes. If it is not possible to eliminate toxic compounds from the workplace or replace them with less toxic substances, then the use of engineering controls and work practices to minimize exposures is the next level of controls for the necessary reduction of exposure. The use of respirators and other PPE, as mentioned above, is a less

desirable, less effective, and more expensive technique to reduce exposures that is normally considered as the last line of defense to reduce exposures. PPE effectiveness has the greatest reliance on individual worker compliance of use and work practices of all the controls to be an effective control. Another drawback is that PPE provides worker protection to only one individual at a time, whereas all the other controls can be effective for exposure reduction for an area or group of workers. Given that the toxicity potential of many substitutes is unknown, implementing the most effective and reliable feasible controls to minimize the exposure is of paramount importance.

### *8.2.1 General Considerations*

A properly designed supply air ventilation system can provide plant ventilation, building pressurization, and exhaust air replacement. When local exhaust ventilation (LEV) is installed in production areas, it is important to consider the need for replacement air. In general, it is necessary to balance the amount of exhausted air with a nearly equal amount of supply air. Without replacement air, uncontrolled drafts will exist at doors, windows, and other openings; doors become difficult to open because of the high pressure difference, and exhaust fan performance may degrade. Good supply air design consists of ducted supply with air discharge registers about 10 feet above floor level [ACGIH 2010].

It is important to confirm that the LEV system is operating as designed by periodically measuring exhaust airflows. A standard measurement, hood static pressure, provides important information on the hood performance, because any change in airflow results in a change in hood static pressure. For hoods designed to prevent exposures to hazardous airborne contaminants, the American Conference of Governmental Industrial Hygienists (ACGIH®) Operation and Maintenance Manual recommends the installation of a fixed hood static pressure gauge [ACGIH 2010].

In addition to routine monitoring of the hood static pressure, additional system checks should be completed periodically to ensure adequate system performance, including smoke tube testing, hood slot/face velocity measurements, and duct velocity measurements using an anemometer. These system evaluation tasks should become part of a routine preventative maintenance schedule to check system performance. It is important to note that the collection and release of air contaminants may be regulated; companies should contact agencies responsible for local air pollution control to ensure compliance with emissions requirements when implementing new or revised engineering controls.

To minimize exposure and reduce the risk of flavoring-related lung disease, a few standard precautions should be followed in areas where flavoring-related exposures may occur:

- Isolate rooms where flavorings or flavoring chemicals are handled from the rest of the plant with walls, doors, or other barriers.

- Maintain flavoring mixing rooms and other areas where flavorings are handled under negative air pressure relative to the rest of the plant.

- Install hood static pressure gauges (manometers) near hoods to provide a way to verify proper hood performance.

- Consider installing a control "on/off" light to indicate the status of the exhaust fan.

- When possible, place hoods away from doors, windows, air supply registers, and aisles to reduce the impact of cross drafts.

- Provide supply air to production rooms to replace most of the exhausted air.

- Direct exhaust air discharge stacks away from air intakes, doors, and windows.

### *8.2.2 Primary Production Processes and Controls*

The food and flavoring production industries have several primary processes that may result in increased potential for worker exposure to diacetyl, 2,3-pentanedione, and other flavoring chemicals. These may be grouped, from an exposure standpoint, into a few general categories including production operations, packaging operations, cleaning, and maintenance operations [Eastern Research Group 2008e]. Workers in each of these job categories may potentially be exposed to flavoring chemicals, including diacetyl and 2,3-pentanedione. Table 8.1 displays a list of job categories and work activities associated with these manufacturing processes. For each activity, the section of this document that discusses relevant exposure control and the figure(s) at the end of this chapter that shows relevant LEV systems are indicated. Other job categories may potentially be exposed to flavoring chemicals. These include supervisory personnel, laboratory and quality controls personnel, and cleaning and maintenance personnel. When these personnel are in production areas, they should comply with recommended control procedures and wear appropriate the PPE posted for that specific area.

Table 8.1. Controls for job categories and major activities in the food and flavor production industries

| **Job Category** | **Major Activities** | **See Section** | **See Figure** |
|---|---|---|---|
| Production Operator | Bag emptying | 8.2.2.2 | 8.4 |
| | Charging tanks | 8.2.2.4 | 8.7, 8.8, 8.9, 8.10 |
| | Benchtop weighing and handling | 8.2.2.1 | 8.1, 8.2, 8.3 |
| | Manually handling flavorings or flavoring chemicals | - - - - | 8.2, 8.7, 8.8, 8.9 |
| Packaging Personnel | Bag filling | 8.2.2.3 | 8.5, 8.6 |
| | Drum filling and emptying | 8.2.2.5 | 8.11, 8.12 |

Many different industries have implemented engineering controls to reduce exposure and risk of disease among their workers. Many of the processes used in the flavoring and food manufacturing industries are similar to those of other industries and may allow for common approaches to reducing employee exposure. These processes include blending, mixing, and handling of flavoring ingredients in liquid and powder form. The design concepts required for working with hazardous materials include specification of general ventilation, LEV, maintenance, cleaning and disposal, PPE, exposure monitoring, and medical surveillance [Naumann et al. 1996]. Bag emptying, bag filling, charging tanks, benchtop weighing and handling, and drum filling and emptying are a few of the production processes of concern. Other more specialized processes (for example, candy panning, a process in which candy pieces in a rotating drum are sprayed with chocolate or other flavoring ingredients) may also result in worker exposure. Special attention should be given to manual handling of flavoring chemicals, particularly in heated processes, and when spraying flavoring ingredients.

Research into various food industries has led to the development of potential engineering controls to help reduce worker exposure to diacetyl, 2,3-pentanedione and other chemicals. The following sections describe the primary production processes used in the food and flavoring industries and discuss engineering controls that can be used to minimize worker exposure to diacetyl, 2,3-pentanedione and other potential airborne hazards.

#### 8.2.2.1 Benchtop Weighing and Handling

Small-scale weighing and handling of ingredients are common tasks used in flavoring production, bakeries, dairy production, and snack food manufacturing. The tasks of weighing out both dry and wet food ingredients can lead to worker exposure primarily through the scooping, pouring, and dumping of these materials. Studies in bakeries have shown that the workers exposed to dusts (commonly from flour) are those who perform mixing and weighing tasks [Elms et al. 2003]. In addition, a recent survey at a commercial bakery showed that mixer operators were exposed to diacetyl when they

measured and added an artificial butter flavor to a dough mixer [Eastern Research Group 2008b, d]. Because weighing and pouring are often performed on a benchtop workstation, the addition of slotted backdraft ventilation for both the bench and the weighing area is recommended. This approach can also be applied to larger-scale operations.

The application of engineering controls to reduce worker exposure to chemicals during mixing and weighing has been evaluated in flavoring production. In flavoring production facilities, compounders measure and pour flavoring ingredients on a bench and then transfer these mixtures to open tanks for liquid flavoring production or to blenders used for powdered flavoring production. The use of ventilated backdraft workstations, adapted from welding bench designs available in the ACGIH® Industrial Ventilation Design Manual (Figure 8.1) [ACGIH 2010] has been evaluated by NIOSH in two field studies conducted in flavoring production plants.

Ventilated back-draft workstations used for small batch mixing have been evaluated in two field studies conducted in flavoring production plants (Figure 8.2). These stations were designed to maintain an air velocity of 100–150 feet per minute (fpm) at the face of the enclosure. The field studies showed reductions in exposure of 90%–97% when performing mixing tasks using these stations [NIOSH 2008a]. The key design parameters are to enclose as much of the activity as possible and to use properly sized exhaust slots to maintain a uniform air velocity across the face of the station.

Other groups have also produced designs that may be amenable to the control of exposure during benchtop mixing and weighing activities. The British Health and Safety Executive (HSE) has developed a series of control approaches based on common processes in a variety of industries. One approach is similar to the one evaluated by NIOSH in flavoring facilities and recommends a control velocity of 100–200 fpm (0.5–1 meter per second [m/s]) at the face of the workstation when working with flour improvers (Figure 8.3) [Health and Safety Executive 2003g].

The selection of proper control velocity should be made on the basis of the material being used (powder versus liquid), plant conditions (background drafts), and momentum of contaminant source (pouring versus spraying or vigorous mixing). The use of baffles on the side and top of these workstations to better enclose the process provides improved control and minimizes the deleterious effects of cross drafts on contaminant control. Plastic curtains can provide reasonable enclosure while allowing improved access to the bench area. The proper positioning of these workstations away from doors, windows, air supply registers, and aisle ways will also help to reduce the impact of cross drafts.

#### 8.2.2.2 Bag Dumping/Emptying

Manual handling of solid powders is a process used in many industries, including food and flavoring production. The opening and dumping of bags of powdered ingredients is commonly performed by workers in the production of flavorings, dairy products, snack foods, and in bakeries. Typically, a worker cuts open bags of material (e.g., 50-lb bags) and dumps the ingredients into a hopper, and then stacks or disposes of the empty bags. In powdered flavoring production, these hoppers are commonly outfitted onto blenders used to load the base starch ingredient for dry flavor blends. In snack food production, they may be used to load spices and flavors for application to the product via open drum coaters just before packaging. These open-ended devices typically are used to coat larger, more irregularly shaped materials such as cereal flakes or expanded snacks. Coatings may be applied as a slurry or as a dry mix following spray application of oil or lecithin. The drums rotate as the flavoring is being applied to allow for even coverage of the snacks. This process can cause worker exposure to the powdered flavoring; a case of BOOP was reported in a spice process technician whose primary responsibility was to manually dump spices from bags into a slurry for application to potato chips [Alleman 2002].

Technology used to control dusts during bag dumping has been in place for many years. The standard control—a ventilated bag dump station—consists of a hopper outfitted with an exhaust ventilation system to pull dusts away from workers as they open and dump bags of powdery materials. The designs for these devices are examples available from

several sources of industrial ventilation guidance. The British HSE has developed a Control Approach for a ventilated station for emptying bags of solid materials. The control includes the specification of a face velocity of 200 fpm (1.0 m/s) and includes a waste bag collection chute (Figure 8.4) [Health and Safety Executive 2003e].

Research into the effectiveness of these types of devices has shown that they can effectively reduce worker exposure to dust and vapors. A review of commercially available units showed that their use controlled dust levels to between 1–2 mg/m$^3$ [Heitbrink and McKinnery 1986]. However, dust contamination on the surface of the bag and handling/disposal of bags caused increased worker exposure. An integral pass through to a bag disposal chute/compactor will help reduce dust exposure resulting from bag handling. Further studies in mineral processing plants showed that the use of an overhead air supply also significantly decreased worker exposure [Cecala et al. 1988].

The ACGIH Ventilation Manual also has two designs that are applicable to the control of powder materials during bag dumping. Design plate VS-15-20, Toxic Material Bag Opening, is similar in design to the HSE station described above but recommends a slightly higher control velocity of 250 fpm at the face of the station opening. In addition, Design plate VS-50-10, Bin and Hopper Ventilation, requires a hood face velocity of 150 fpm. In general, higher velocities may be needed to adequately capture dusts in a plant environment. Air velocities around 200 fpm into the hood should provide reasonable contaminant removal for these operations [ACGIH 2010].

#### 8.2.2.3 Bag Filling

The process by which bags are filled with products is typically done by flavor manufacturers and other producers of powder materials. Powder flavorings are typically mixed with industrial blenders or produced by a spray drying process. For the blending process, a powdered starch or other carbohydrate is combined with a liquid or paste flavoring agent. When the blending is completed, the powder product may be discharged into a bulk tote or packaged into smaller containers. In the spray-drying process, a mixture of liquid and powder ingredients (slurry) is sprayed within a large sealed tank.

Heat within the tank dries the slurry droplets, leaving a powder as the finished product. This powder is then collected and packaged in product containers.

Studies conducted at flavoring production facilities have shown that intermittent peak exposures to dust and flavoring volatile ingredients occur when powder products are being packaged following blending or spray drying [NIOSH 2007a, 2008a, b, 2009d]. The use of a ventilated collar-type hood around the discharge point can help minimize worker exposure to dust and vapors. The British HSE has developed a Control Approach for an exhaust hood for the filling of bags with solid materials. The control includes the specification of a ventilated enclosure around the powder discharge outlet and has applicability to the filling of smaller product bags as well as intermediate bulk containers (Figure 8.5) [Health and Safety Executive 2003c, f]. This design guidance recommends an inward air velocity of 200 fpm (1.0 m/s) into the enclosure. The ACGIH Industrial Ventilation Manual, Design plate VS-15-02, Bag Filling, is similar in design to the HSE exhaust hood but specifies an overall hood flow rate of 400–500 cubic feet per minute (cfm) for nontoxic dust or 1000–1500 cfm for toxic dust with a maximum inward air velocity of 500 fpm.

In addition to ventilation solutions, other dust control approaches have been used in a variety of industries and should be applicable for food and flavoring production. For example, an inflatable seal can be used to create a dust tight seal on the discharge outlet of an industrial blender (Figure 8.6). The outlet spout can be fitted with an inflatable seal that prevents dust from escaping during the bag filling process. The seal inflates during the product transfer from the blender to the packaging bag (providing the seal) and deflates once the transfer is completed to allow removal of the packaging bag. These systems are available on many commercially available bulk bag filling systems [Hirst et al. 2002].

Another system that can be used is the continuous liner system. Polypropylene liners are often used when products are discharged from the industrial blenders into the final

product container. In this operation, a sleeve of polypropylene liners is stowed around the circumference of the discharge outlet. The first liner, the bottom having been sealed, is pulled down into the overpack (usually a 5-gallon bucket or a cardboard box). Product is discharged into the liner through a butterfly valve on the blender outlet. Once full, the top of the first liner sleeve is closed using tape or a fastener, or it is heat sealed and cut. The product is sealed within the poly-lined container, and a new sealed poly liner is pulled down to start discharge into the next container. This continuous process seals off the primary leak paths for dust during unloading of an industrial blender or other equipment.

These systems are commonly used in the pharmaceutical industry and may provide cost-effective alternatives to traditional local exhaust ventilation control systems for food and flavoring production.

#### 8.2.2.4 Charging/filling tanks and mixers

The addition of solid and liquid ingredients into tanks and other mixing vessels can cause exposure to dusts and vapors due to the displacement of air in the vessel. Medical and environmental surveys conducted in the microwave popcorn manufacturing industry have showed that workers who mixed butter flavorings into soybean oil had the highest exposures to diacetyl and the highest risk of developing severe irreversible lung disease. These workers measured out artificial butter flavoring in open containers and poured the flavoring into heated mixing tanks filled with oil. Real-time monitoring of a mixer at one plant measured a diacetyl peak of more than 80 ppm over several minutes as he poured flavorings into the mixing tank. NIOSH investigations at a plant where many exposed workers developed severe lung disease also showed that the implementation of LEV for heated tanks of oil and flavorings and general dilution ventilation for production areas reduced diacetyl concentrations by three orders of magnitude [NIOSH 2006]. Exposures to diacetyl were also recorded at a plant that produced flavorings and other products in workers who added flavors to mixing and spray dryer feed tanks while the tanks were being filled. One worker who was adding diacetyl-containing starter distillate and starch to a spray dryer slurry feed tank was exposed to elevated levels of volatile organic

compounds including diacetyl for a sustained period of time [NIOSH 2009d]. In addition, elevated concentrations of volatile contaminants were measured as a worker poured diacetyl-containing starter distillate from a collection vessel into a bulk container.

The use of controls to reduce worker exposure during pouring and mixing of ingredients in a commercial mixer has been evaluated in a flavoring production plant [NIOSH 2008d]. The use of LEV at the mixing tank helps to maintain the vessel at a negative pressure and contain evaporative emissions. NIOSH evaluated the impact of a ventilated tank lid on the exposure of a worker during the mixing of a food flavoring (Figure 8.7) [NIOSH 2008d]. The use of the ventilated tank lid resulted in a reduction of approximately 76% compared to the same operation without the ventilated tank lid. Most of the exposure during the evaluated mixing process was attributed to tasks performed outside of the hood. Ventilated tank lids have also been recommended by the British HSE to contain vapors during the mixing of liquids with other liquids or solids (Figure 8.8) [Health and Safety Executive 2003d].

Another approach evaluated by NIOSH at a flavoring manufacturing facility was the use of a ventilated mixing booth. This booth allows a large portable mixing tank to be rolled inside so that chemical vapors emitted during pouring and mixing of flavoring ingredients in the tank are captured and exhausted outdoors (Figure 8.9). However, the booth provides some flexibility and can also be used for other production tasks such as large pouring and product packaging activities. The use of slots across the booth plenum helps evenly distribute the flow across the height and width of the booth. A field study showed hood capture efficiencies of greater than 95% based on tracer gas tests [Dunn et al. 2008]. An important design consideration is to make the booth deep enough to fully contain the process.

Other approaches to controlling exposure during filling of mixing vessels and tanks include the use of a simple exhaust hood near the opening of fixed tanks. This approach is highlighted in the HSE Control Approach 210, titled "Charging Reactors and Mixers

from a Sack or Keg" (Figure 8.10). This design calls for the use of a local exhaust hood near the tank opening with an inward velocity of at least 200 fpm. Another design provided by the HSE and ACGIH for mixers and tanks includes the use of rim exhausts placed around the edge of the mixer/tank. These designs take the shape of an annular slotted hood, which pulls air away from workers as they add ingredients or operate the mixer (Figure 8.11) [ACGIH 2010; Health and Safety Executive 2003e]. An annular exhaust provides a semicircular ventilation ring around the edge of the tank to capture contaminants as they evaporate or are displaced during pouring/mixing. Typical rim exhausts, however, are limited in the area where they can provide adequate capture velocity and should not be used to capture contaminants beyond approximately 24 inches from the hood face [Goodfellow and Tähti 2001].

#### 8.2.2.5 Drum filling and emptying

In some cases, manually operated and powered pumps have been used to transfer liquids from barrels to mixing and feed tanks. Although the use of these devices can reduce exposure by reducing the amount of open handling, care should be taken when filling and emptying drums of flavoring ingredients. The use of ventilation at the barrel opening has been recommended for capture of vapors during transfer of chemicals. The HSE has developed two engineering control approaches for drum filling and emptying (Figure 8.12) [Health and Safety Executive 2003a, b]. For drum filling, the guidance recommends the use of an annular exhaust hood around the interface between the drum and feed pipe (at the bung hole). The recommended airflow is a minimum of 100 fpm across the drum cap/bung hole. For flammable liquids, suitable fans and equipment as well as appropriate grounding schemes should be used to prevent the buildup and discharge of static electricity. The ACGIH Ventilation Manual also has developed a design plate with several different implementation options based on the process [ACGIH 2010]. In all cases, when transferring flammable liquids, grounding and bonding requirements should be met to prevent sparks and explosions [NFPA 2007].

### 8.3 Work Practice Controls

Work practices, sometimes called administrative controls, are procedures followed by employers and workers to control hazards in the workplace. The use of good work practices, incorporated into the facility's standard operating procedures, can help reduce exposures to diacetyl, 2,3-pentanedione, and other flavoring chemicals while at the same time maximizing efficiency and product quality. Work practices include housekeeping and cleaning, storage and use procedures, work clothes, labels and postings, hazard training, and procedures for use of engineering controls, many of which are discussed here.

The emission of the volatile components in each flavoring mixture can be minimized by preventing spillage. To the extent possible, containers used to mix and store flavoring chemicals should be covered when not in use. This practice will minimize the evaporation of chemicals into the workplace air. Manual handling of chemicals also provides a potentially significant source of worker exposures and emissions. Use of closed transfer processes, where feasible, significantly reduces exposure. Also, slow careful pouring/handling of chemicals can reduce splashing, spillage, and exposure during this activity [Boylstein et al. 2006]. Reduction in spills and elimination of leakage from vessels aid in reducing the overall emission of chemicals into the workplace and lower worker exposure.

#### *8.3.1 Good Housekeeping Practices*

An organized, clean workplace enables faster and easier production, improves quality assurance, and reduces the potential for slips, trips, and falls. It is important to maintain good general housekeeping practices so that leaks, spills, and other process integrity problems are readily detected and corrected. Proper practices regarding spills include:

- Allowing only individuals wearing appropriate protective clothing and equipment who are properly trained, equipped, and authorized for response to

enter the affected area until the cleanup has been completed and the area properly ventilated

- Using HEPA-filtered vacuums, wet sweeping, or a properly enclosed wet vacuum system for cleaning up dust that contains diacetyl or 2,3-pentanedione
- Cleaning work areas regularly with HEPA-filtered vacuums or with wet sweeping methods to minimize the accumulation of dust
- Cleaning up spills promptly
- Limiting accumulations of liquid or solid materials on work surfaces, including floors, to reduce contamination of products and the work environment

### *8.3.2 Closed Transfers, Containers, and Processes*

Because of the volatile nature of diacetyl, 2,3-pentanedione and other flavoring chemicals, proper handling to limit the duration of exposure to vapors is essential. The use of closed vessels and closed transfer procedures is one technique to promote proper handling. To limit exposure time:

- Whenever possible, avoid open pouring, measuring, and transfer of diacetyl, 2,3-pentanedione, and other flavoring chemicals on the Flavor and Extract Manufacturers Association priority list [FEMA 2004].
- Add diacetyl, 2,3-pentanedione and other priority chemicals into tanks last, when possible, to minimize the time during which volatilization can occur.
- Keep tanks and containers of flavoring chemicals/ingredients sealed at all times.
- Maintain and use volatile flavoring chemicals at the lowest possible temperature within the manufacturers' recommended temperature range for each chemical to minimize volatility.

Some manufacturing processes may be enclosed to keep airborne diacetyl, 2,3-pentanedione and other priority flavoring chemicals contained and separated from workers by:

- Isolating mixing and other high-exposure processes from the rest of the workplace.
- Maintaining the isolated work areas under negative air pressure.
- Ensuring that workers take special precautions and if necessary use appropriate PPE on entry into production work areas where diacetyl, 2,3-pentanedione, and other flavoring chemicals are handled.

When production processes that utilize flavorings or flavoring chemicals are not enclosed or contained, workers performing other work tasks in the vicinity should be informed and required to use appropriate PPE to prevent incidental exposures.

### *8.3.3 Hygiene Procedures*

Good personal hygiene is important to limit not only inhalation exposures to diacetyl, 2,3-pentanedione, and other flavoring chemicals, but also exposure from ingestion and dermal absorption. Important hygiene considerations include:

- Employers should not allow workers to smoke, eat, or drink in work areas where diacetyl, 2,3-pentanedione, and other flavoring chemicals are used.
- Employers should provide appropriate personal protective clothing such as gloves, long-sleeved shirts, and aprons to all workers who may have hazardous dermal exposures during normal work activities.
- Workers should wash their hands and exposed skin before eating, drinking, or smoking.
- For some processes, employers may need to provide workers with showers and require them to shower before leaving work.

### *8.3.4 Reduced Process Temperatures for Priority Flavoring Chemicals*

To minimize volatilization, the temperature of diacetyl, 2,3-pentanedione, and other flavoring chemicals in heated tanks should be maintained as low as production processes will allow, even when using closed systems. Employers should make sure that:

- All temperature-related equipment such as thermometers and automatic shut-off mechanisms are regularly checked to ensure that they are in good working order.
- Tank thermometers and thermostats are calibrated at least monthly or as recommended by the manufacturer.
- Workers take periodic manual temperature readings with a stem thermometer inserted just below the surface of the heated agents or with an infrared thermometer.

### *8.3.5 Cleaning Practices for Equipment and Tools*

Where possible, cold water should be used to clean out tanks and blenders to reduce the volatilization of chemicals into plant air. Workers involved in cleaning or working nearby should use appropriate PPE including respiratory protection, eye, and skin protection.

### *8.3.6 Limit Access to Priority Flavoring Chemicals*

Employers should structure work tasks to minimize the amount of time workers spend near priority chemicals and production processes that involve these chemicals. Employers should limit access to areas where diacetyl, 2,3-pentanedione, or other priority flavoring chemicals are used to only those workers who are essential to the process or operation. These areas should be clearly marked with signage.

### *8.3.7 Hazard Training and Communication*

All containers of food flavorings fall under the labeling requirements of the OSHA Hazard Communication Standard (HCS) unless they are covered under the Federal Food, Drug and Cosmetic Act or the Virus-Serum-Toxin Act of 1912 [29 CFR 1910.1200 (b)(5)]. Updated MSDSs and product labels should be consulted for necessary information for employee training on safety and health hazards, personal protective equipment and emergency procedures. MSDSs for the same substance from different suppliers can vary in the information provided. The employer is required to maintain in the workplace copies of the required MSDSs for each hazardous chemical, and should ensure that they are readily accessible during each work shift to employees when they are in their work area(s). Employers are required to provide employees with training on the methods used to detect the presence or release of a hazardous chemical; the physical and health hazards of the chemical; and control measures including work practices, emergency procedures, and personal protective equipment [29 CFR 1910.1200 (h)(3)].

Just as employees need to be trained in the proper performance of various production tasks in the workplace, they should also be trained to recognize potential hazards and respond appropriately. Employers should establish a safety and health training program for all workers who manufacture, use, or handle diacetyl or 2,3-pentanedione or perform other activities that bring them into contact with diacetyl, 2,3-pentanedione, or other priority flavoring chemicals. Employers should ensure that workers are trained on the operation and use of ventilation systems and other engineering controls for reducing diacetyl, 2,3-pentanedione, and other flavoring chemical exposures.

As part of the training, employers should:

- Inform all potentially exposed workers (including temporary and contract workers) about diacetyl or 2,3-pentanedione associated health risks such as skin and eye irritation and respiratory disease.

- Teach workers to report to management any eye or skin problems that may be associated with exposure to flavoring chemicals, and any persistent or worsening respiratory symptoms such as cough, shortness of breath, or wheezing.
- Train workers to detect hazardous situations.
- Inform workers about practices or operations that may generate high airborne diacetyl or 2,3-pentanedione concentrations (e.g., mixing).
- Establish procedures for reporting hazards and giving feedback about actions taken to correct them.
- Train workers in the proper use and maintenance of implemented engineering controls to protect them from hazardous exposures.
- Train workers in the proper use and maintenance of PPE.
- Inform workers about other priority flavoring chemicals that may pose occupational exposure hazards.

To communicate hazard information effectively to workers, employers should:

- Post appropriate labeling on all flavoring product containers.
- Post warning labels and signs describing the health risks associated with flavoring chemical exposures at entrances to work areas and inside work areas where diacetyl, 2,3-pentanedione, or other flavoring chemicals are used.
- Post warning labels and signs describing any needs for PPE in the work area.
- If respiratory protection is required, post the statement: "Respiratory Protection Required in this Area."
- Print all labels and warning signs in both English and the predominant language of workers who do not read English.
- Verbally inform workers about the hazards and instructions printed on the labels and signs if they are unable to read them.

- Follow the recommendations of the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) for diacetyl, 2,3-pentanedione, and other flavoring chemicals. The OSHA website has additional information on GHS at [http://www.osha.gov/dsg/hazcom/ghs.html].

### 8.4 Respiratory Protection

Respirators should not be used as the primary means of controlling worker exposures to inhalation hazards for routine operations. Whenever possible, techniques discussed above are preferred. Respirators may be needed and can be used during the implementation of engineering controls and work practices, during some short-duration maintenance procedures, and during emergencies. Respirators should be used for exposure situations when engineering controls cannot control exposures below concentrations that are still associated with risk.

Employers need to monitor work processes to accurately determine exposure levels of airborne chemicals. Respiratory protection should be provided when that assessment indicates exposures may exceed the NIOSH REL of 5 ppb TWA or 25 ppb STEL for diacetyl; when exposures may exceed the NIOSH REL of 9.3 ppb TWA or 31 ppb STEL for 2,3-pentanedione; when occupational exposure limits of other chemicals may be exceeded; or when exposures of concern to diacetyl substitutes without OELs occur. When respiratory protection is used, employers need to establish a written respiratory protection program that meets the requirements of the OSHA respiratory protection standard 29 CFR 1910.134. The program should be administered by a suitably trained program administrator and updated to reflect changes in workplace conditions that affect respirator use [29 CFR 1910.134].

A respiratory protection program should include the following elements:

- Procedures for selecting respirators for use in the workplace
- Medical evaluations of employees required to use respirators

- Fit testing procedures for tight-fitting respirators
- Procedures for proper use of respirators in routine and reasonably foreseeable emergency situations
- Procedures and schedules for cleaning, disinfecting, storing, inspecting, repairing, discarding, and otherwise maintaining respirators
- Procedures to ensure adequate air quality, quantity, and flow of breathing air for atmosphere-supplying respirators
- Training for employees in the respiratory hazards to which they are potentially exposed during routine and emergency situations
- Training for employees in the proper use of respirators, including putting on and removing them, any limitations on their use, and their maintenance
- Procedures for regularly evaluating the effectiveness of the program

If an air-purifying respirator with cartridge/canister for the protection against gases and vapors does not have an end-of-service-life indicator, then the employer should implement a cartridge/canister change schedule based on objective information that will ensure the cartridges/canisters are changed before the end of their service life. A cartridge's useful service life is how long it provides adequate protection from the harmful chemicals in the air which are identified in the respirator approval. A change schedule to establish the time period for replacing respirator cartridges and canisters is the part of the written respirator program that says how often cartridges should be replaced. Data and information relied upon to establish the schedule should be included in the respirator program. The use of warning properties cannot be used as the sole basis for determining change schedules. However, respirator users should be trained to understand that they should leave the area if abnormal odor or irritation is experienced. The respirator should be checked to see if the odor or irritation is evidence that respirator cartridges need to be replaced or the respirator facepiece needs adjustment for better face seal fit.

The following table indicates which types of respirators are recommended for use against diacetyl and 2,3-pentanedione and the maximum use concentrations for diacetyl and 2,3-pentanedione, calculated using the OSHA-assigned protection factors for each type of respirator listed [29 CFR 1910.134 (d)(3)(i)(A)]. For escape, use a gas mask with a full facepiece and OV-P100 canisters or self-contained breathing apparatus.

Table 8.2. OSHA assigned protection factors and maximum use concentrations of respirators for diacetyl and 2,3-pentanedione

| Type of Respirator | OSHA Assigned Protection Factor | Maximum Use Concentration for Diacetyl* | Maximum Use Concentration for 2,3-Pentanedione* |
|---|---|---|---|
| Full facepiece air purifying, w/OV-P100 cartridge(s) or canister(s) | 50 | 0.25 ppm (250 ppb) | 0.46 ppm (460 ppb) |
| PAPR, full facepiece w/OV-HE cartridge(s) or canister(s) | 1000 | 5 ppm (5000 ppb) | 9.3 ppm (9300 ppb) |
| PAPR, hood or helmet w/OV-HE cartridge(s) or canister(s) | 25/1000† | 0.12 / 5 ppm (120 / 5000 ppb) | 0.23 / 9.3 ppm (230 / 9300 ppb) |
| PAPR, loose fitting facepiece w/OV-HE cartridge(s) or canister(s) | 25 | 0.12 ppm (120 ppb) | 0.23 ppm (230 ppb) |
| SAR, positive pressure mode, full facepiece | 1000 | 5 ppm (5000 ppb) | 9.3 ppm (9300 ppb) |
| SAR, hood or helmet | 25/1000† | 0.12 / 5 ppm (120 / 5000 ppb) | 0.23 / 9.3 ppm (230 / 9300 ppb) |
| SAR, loose fitting facepiece | 25 | 0.12 ppm (120 ppb) | 0.23 ppm (230 ppb) |

PAPR = Powered air-purifying respirator
SAR = Supplied air respirator
OV-HE = Organic vapor-high efficiency particulate
*Maximum use concentrations will be lower than shown when those concentrations are equal to or exceed immediately dangerous to life and health levels.
†The employer should have evidence provided by the respirator manufacturer that testing of these respirators demonstrates performance at a level of protection of 1,000 or greater to receive an APF of 1,000. Absent such evidence, these respirators receive an APF of 25.

All respirators selected for use should be approved by NIOSH under the provisions of 42 CFR Part 84, as required by OSHA regulations. The current listing of NIOSH certified respirators can be found in the *NIOSH Certified Equipment List*, which is available on the NIOSH website [NIOSH 2010a].

Selection of a specific respirator within a given class of recommended respirators depends on the particular situation; this choice should be made only by qualified personnel. There is no formal certification requirement for a respiratory protection program manager. Worker activity and worker location in a hazardous environment need to be considered in respirator selection, as well as the time period of use, and the type of respirator application, such as for routine, nonroutine, emergency or rescue use.

Additional information on the selection and use of respirators can be found in the *NIOSH Respirator Selection Logic* [NIOSH 2004c].

### 8.5 Dermal, Eye, and Face Protection

Diacetyl can cause skin and eye irritation. Chemical resistant gloves or sleeves or other appropriate protection for exposed skin should be used when handling liquid, paste, or powdered flavoring ingredients containing diacetyl that could cause dermal injury [29 CFR 1910.138]. Tight-fitting chemical goggles, used in conjunction with a face shield or other appropriate eye and face protection should also be used.

Eye and face protection should be provided when there is a hazard from flying particles, molten metal, liquid chemicals, acids or caustic liquids, chemical gases or vapors, or potentially injurious light radiation. OSHA regulations at 29 CFR 1910.133 contain the specific requirements. Protective eye and face devices purchased after July 5, 1994, should comply with ANSI Z87.1-1989, "American National Standard Practice for Occupational and Educational Eye and Face Protection," which is incorporated by

reference in the OSHA regulations [29 CFR 1910.133]. The ANSI standard was revised in 2003 [ANSI 2003]. The current edition also includes respirators that cover the eyes and face as approvable under the standard.

Goggles for chemical splash should be used for eye protection for workers with potential exposures to diacetyl, 2,3-pentanedione, or food flavorings containing these compounds who are not also required to wear a respirator with a full facepiece, hood, or helmet. Face shields can also be used in conjunction with goggles to shield the wearer's face, or portions thereof, in addition to the eyes for protection from liquid splash. Face shields should be worn only in conjunction with spectacles and goggles, as required by ANSI Z87.1-2003. A face shield with a polyethylene terephthalate visor should provide good chemical resistance against diacetyl, 2,3-pentanedione, or food flavorings containing these compounds.

Gloves and protective clothing such as aprons made from butyl rubber, Teflon™, or Tychem™ are effective in reducing skin contact with ketones to prevent skin irritation [OSHA 2007]. Diacetyl and 2,3-pentanedione are diketones and certain food flavorings containing either may contain other ketones or diketones. Glove suppliers should be contacted to ensure that appropriate glove materials are selected for the specific chemicals involved [OSHA 2002].

An analysis should be performed on each operation involving diacetyl, 2,3-pentanedione, or other food flavoring compounds to assess the potential exposures and to establish specific guidance about when to use skin, eye, and face protection.

1 Figure 8.1. Welding ventilation bench hood.*

2

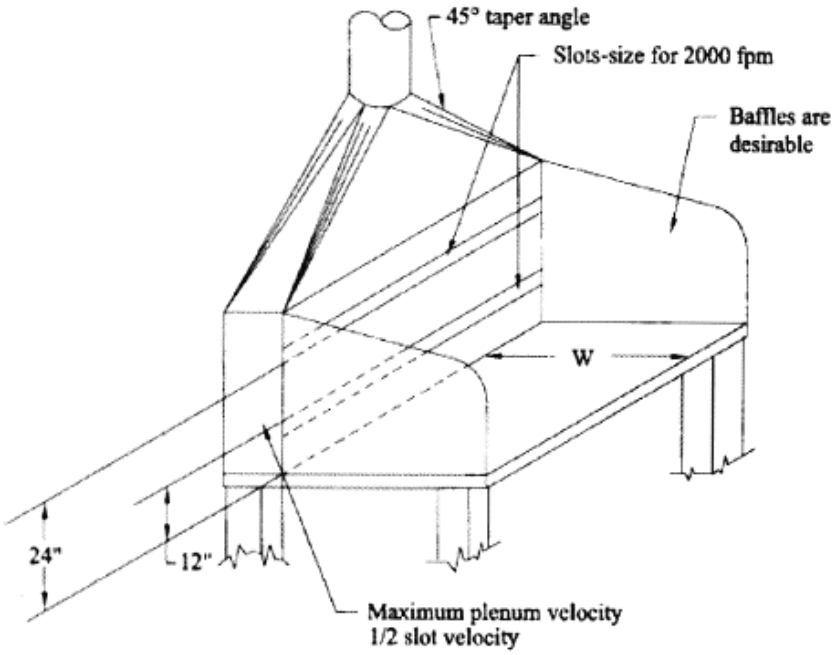



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Figure 8.2. Ventilated small batch mixing workstation

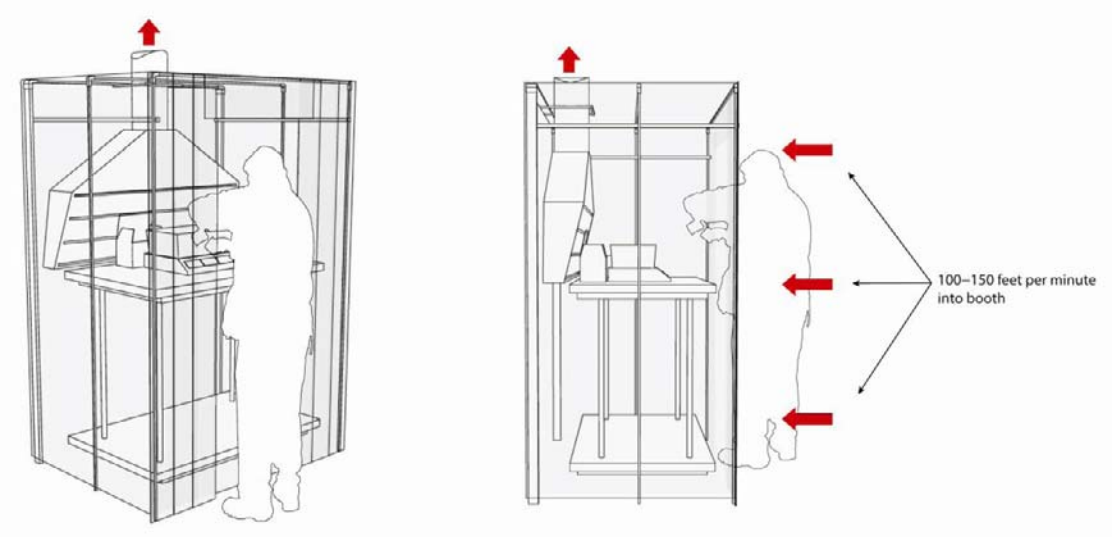

Figure 8.3. Benchtop ventilation for weighing/handling powders 0.5 to 1 mps = 100 to 200 fpm.

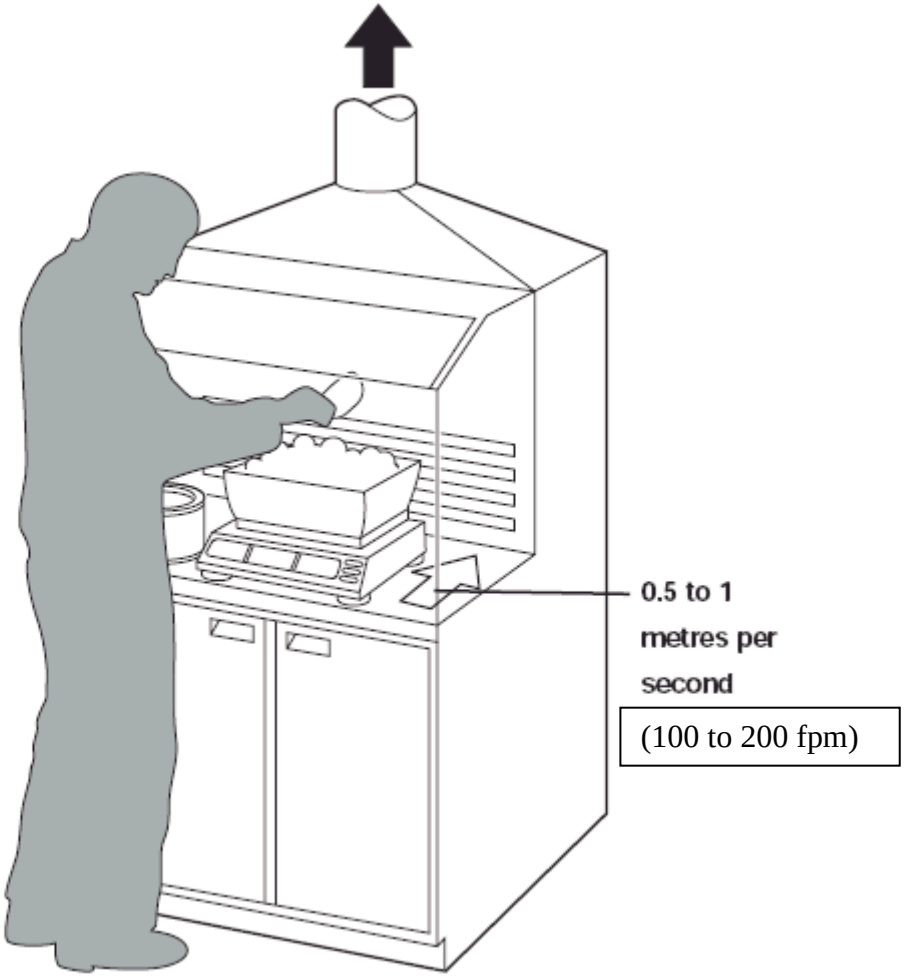

Figure 8.4. Ventilated bag dumping/emptying station*1.0 mps = 200 fpm.

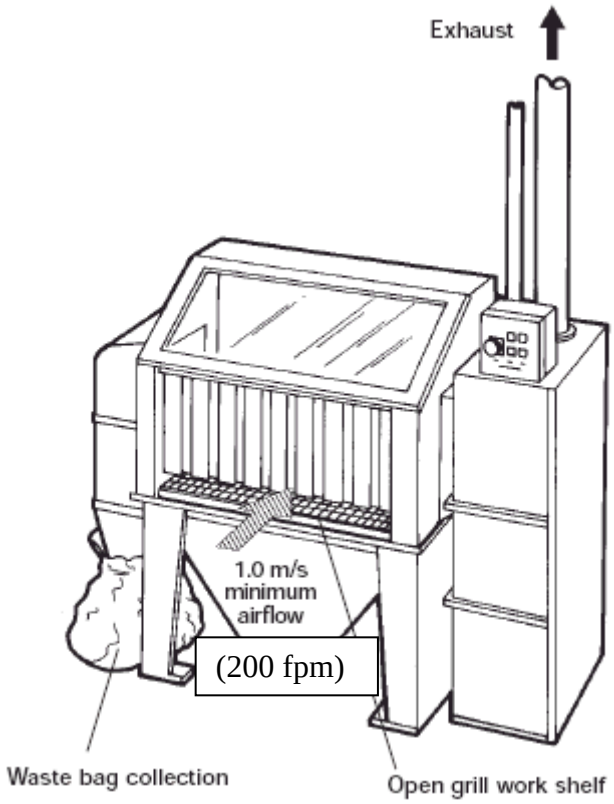


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Figure 8.5. Ventilation for bag filling*

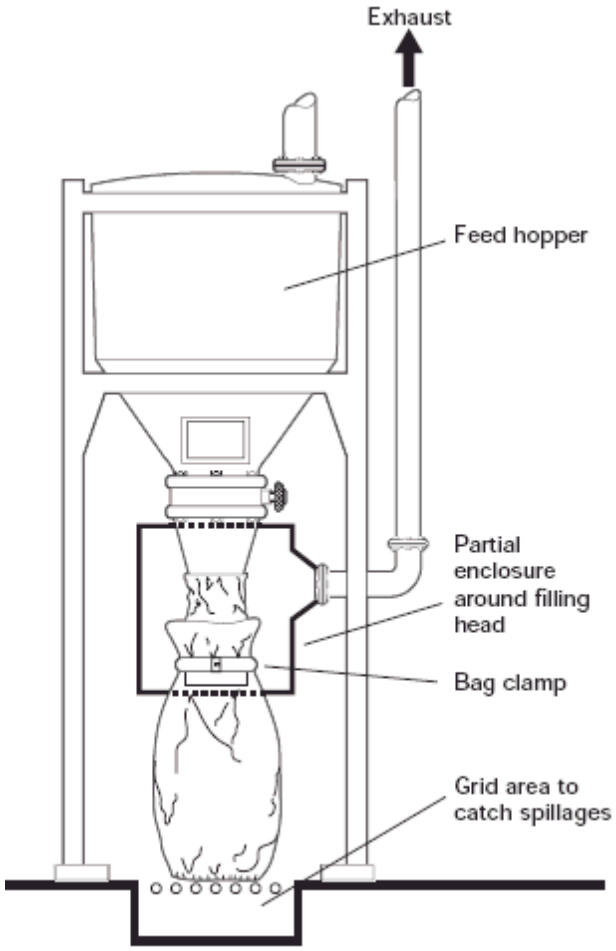


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Figure 8.6. Dust control during bag filling operation

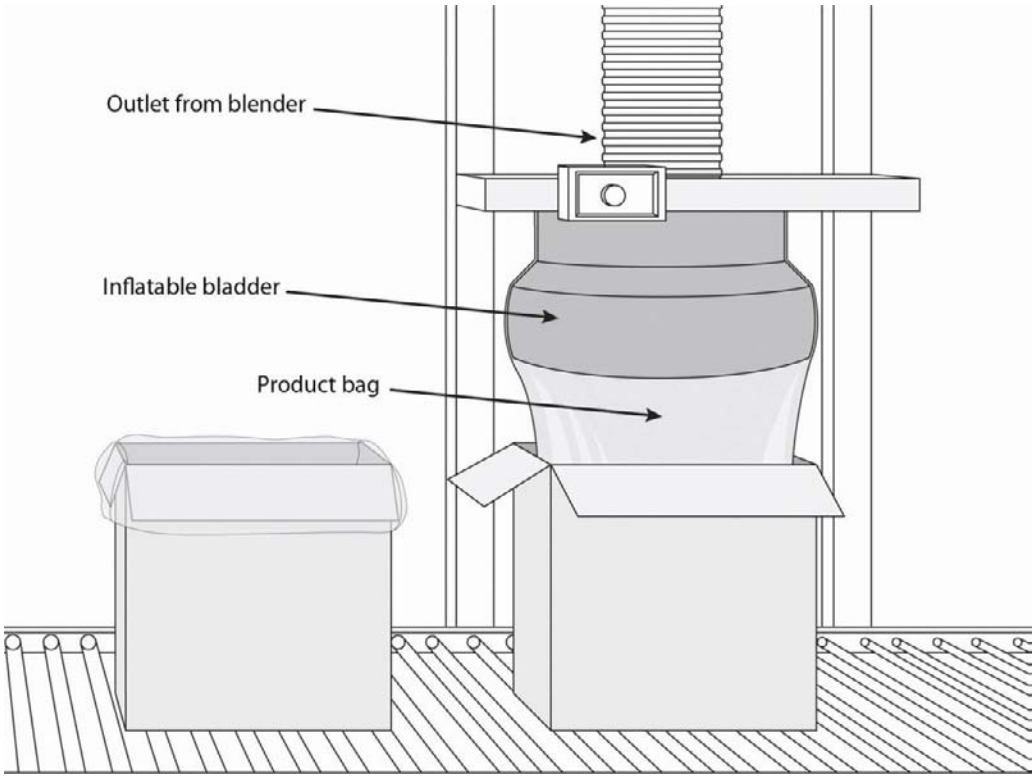

Figure 8.7. Mixing vessel with a ventilated hinged tank lid [*]

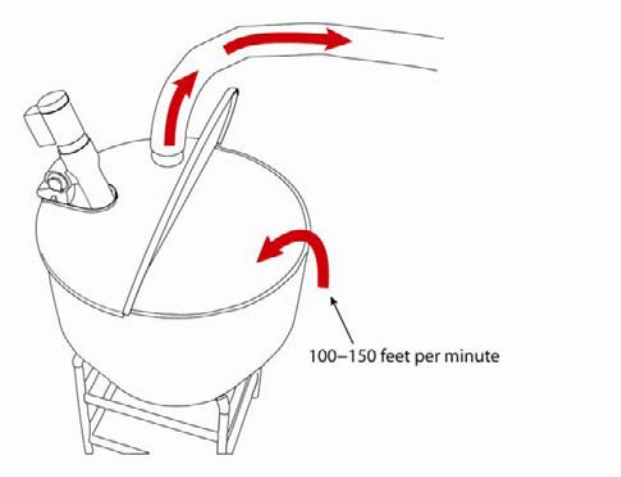


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Figure 8.8. Ventilated booth for large batch mixing

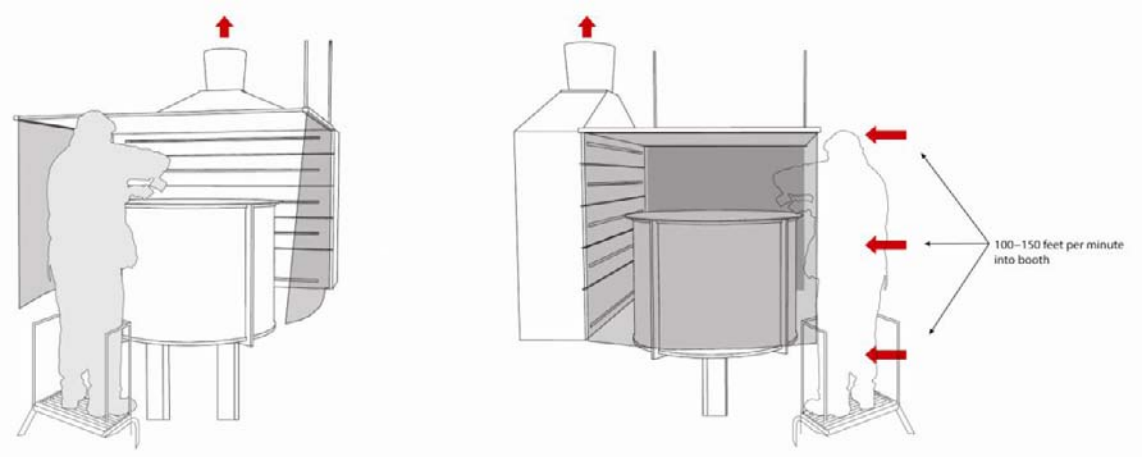

Figure 8.9. Charging reactors and mixers from a sack or keg.[*]
1 m/s = 200 fpm.

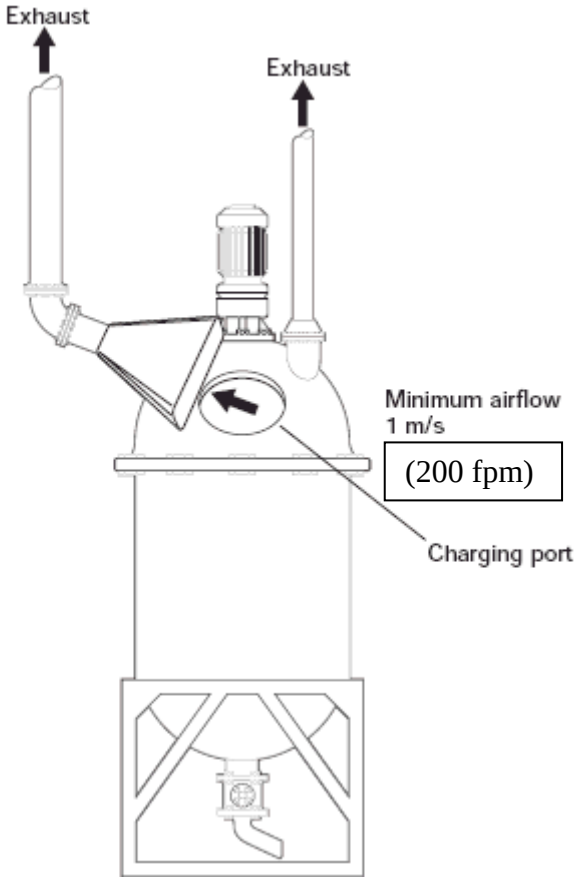


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Figure 8.10. Annular exhaust for capturing dusts/vapors from mixers (HSE 215)

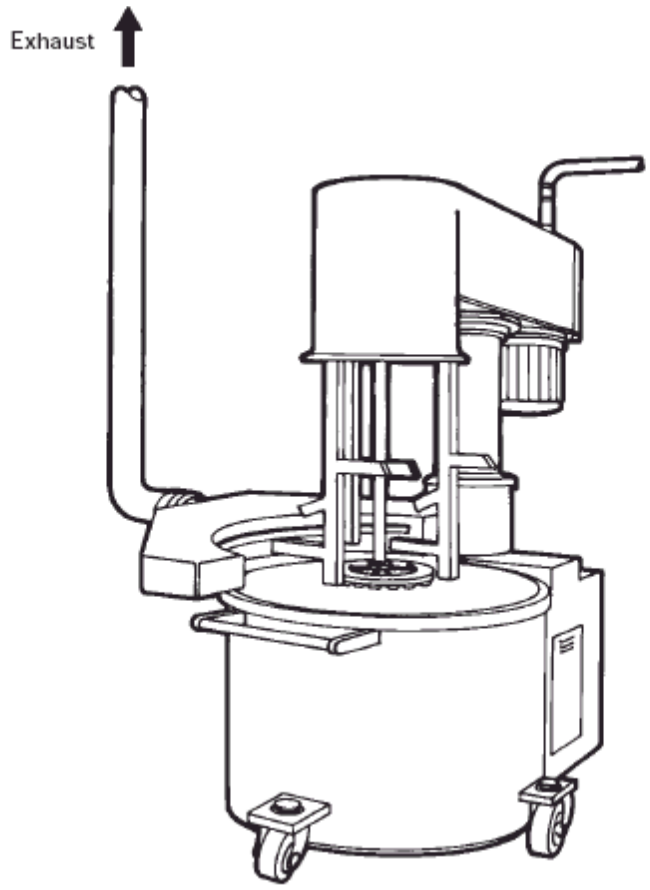

Figure 8.11. Annular exhaust for capturing vapors during drum filling*

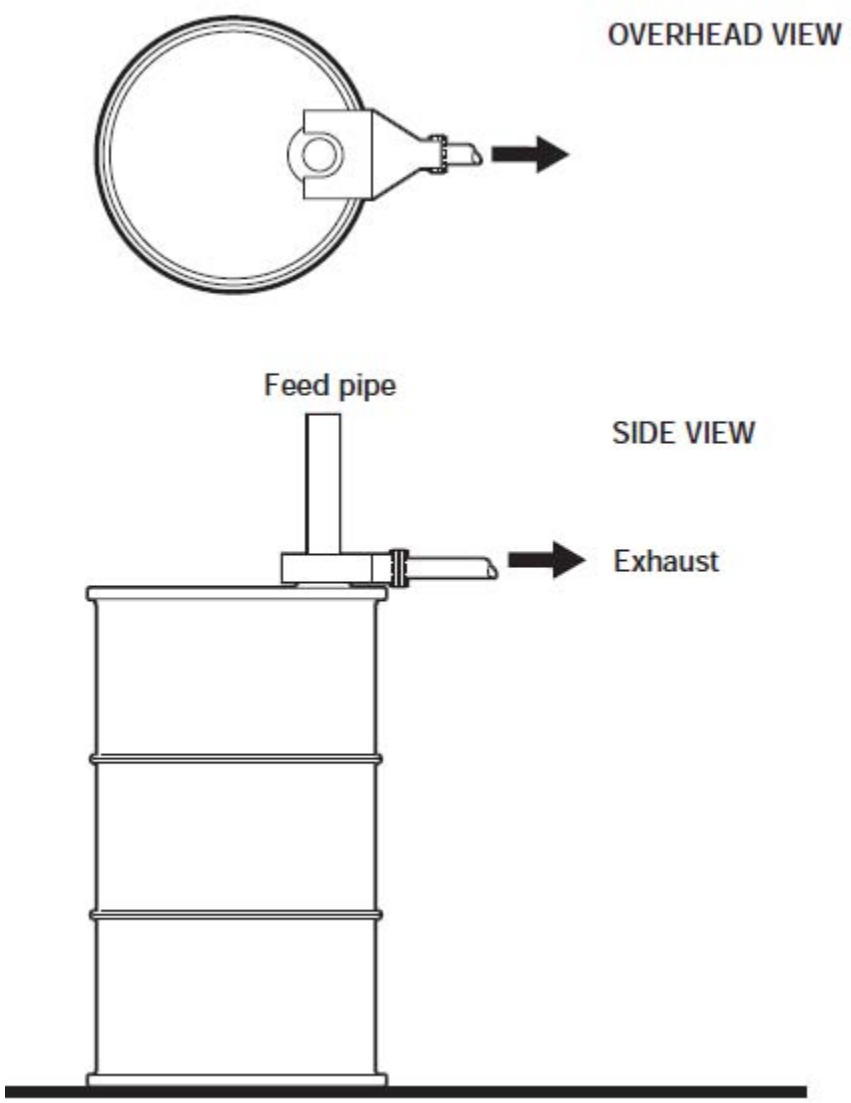


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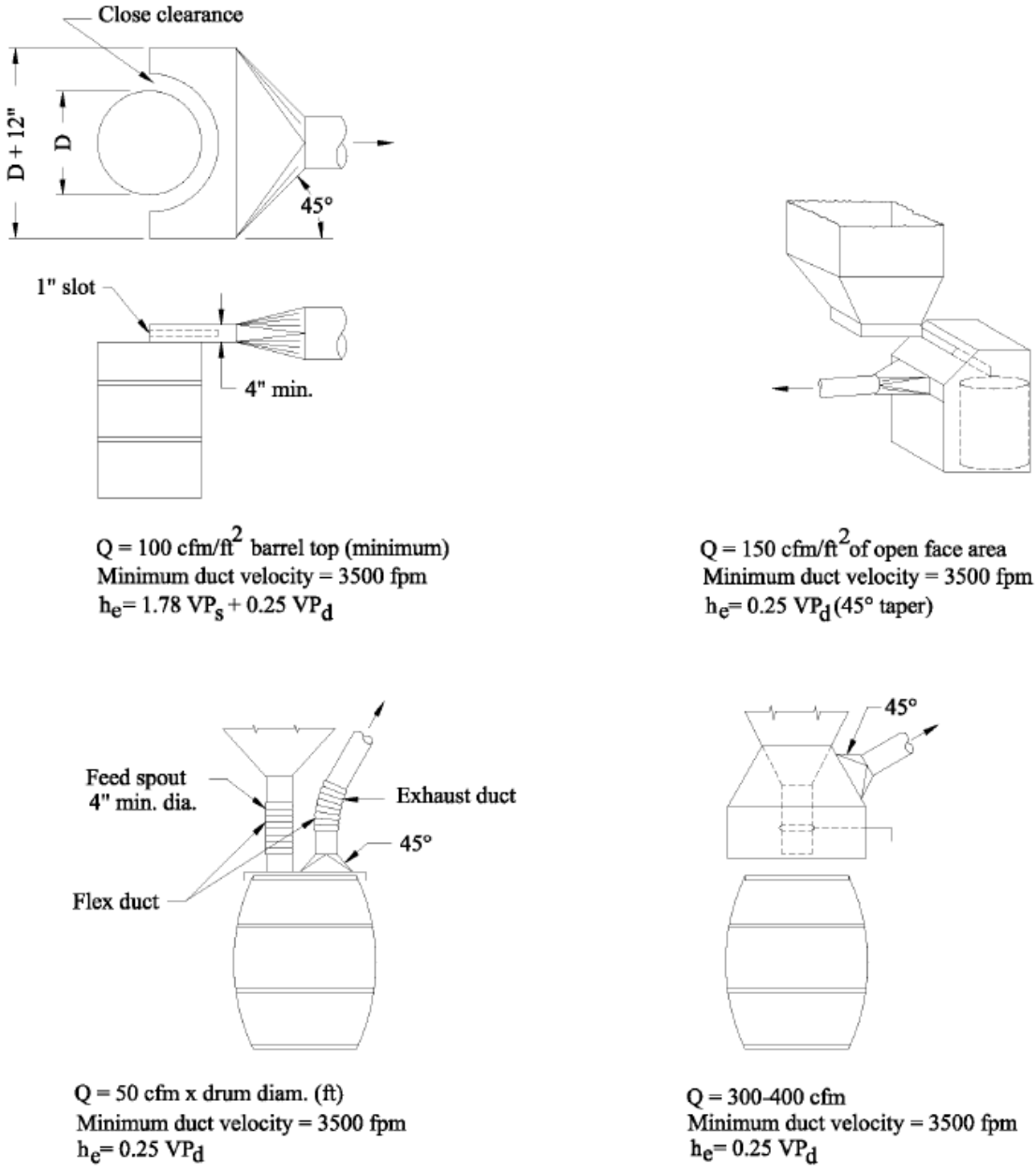
Figure 8.12. Ventilation design options for capturing vapors during drum filling*




Note 1: Air displaced by material feed rate may require higher exhaust flow rates.

Note 2: Excessive air flow can cause loss of product.

Note 3: When transferring flammable or combustible liquids, bonding and grounding requirements of NFPA Code 77 should be followed.

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# Chapter 9: Medical Monitoring and Surveillance of Exposed Workers

Despite attempts to control exposure to diacetyl, 2,3-pentanedione and similar flavoring chemicals, some workers may develop health effects as a result of insufficient control, susceptibility, or unrecognized hazardous exposures. Medical monitoring provides a safety net for flavoring-exposed workers by incorporating screening and surveillance goals. The screening goal is to identify early disease in workers so that steps can be taken to prevent disease progression. This approach constitutes secondary prevention in that it attempts to ameliorate (or at least halt progression of) health effects that have already occurred. Evidence of early disease through medical screening serves as a sentinel event or warning that other workers might be at risk for the same outcome. This warning should stimulate efforts to evaluate the workplace to identify possible risk factors for exposures that can be controlled. Such an approach addresses the goal of primary prevention, preventing disease from developing in the first place. For medical monitoring to serve surveillance purposes, screening should include both medical tests and collecting questionnaire information or linking administrative data on all of the employees screened. Epidemiologic analysis of medical results and questionnaire/administrative data on possible risk factors can result in understanding what actions need to be prioritized to decrease the risk of all workers and can document the effectiveness of interventions over time in preventing flavoring-related health effects. The systematic analysis of aggregated results over time constitutes medical (epidemiologic) surveillance of trends in symptoms or functional changes which can be assessed in relationship to jobs, tasks, and exposures [Silverstein 1990]. Both primary and secondary prevention are important for workers exposed to diacetyl and similar flavor ingredients, as these workers are at risk of rapidly developing severe irreversible lung disease.

The rapid onset and progression of diacetyl-related lung disease requires that more frequent medical monitoring evaluations be done than with slowly progressive occupational lung diseases such as silicosis and coal worker's pneumoconiosis. The most important component of an effective medical monitoring program for workers exposed to

diacetyl and similar flavor ingredients is the careful comparison of spirometry test results over time to identify rapid declines in lung function [California Department of Public Health 2007b]. Spirometry tests should be high quality to allow valid interpretation of lung function changes over time. This chapter provides information on how to conduct effective medical monitoring of these workers. The chapter also provides examples that illustrate how medical surveillance can identify workplace risk factors.

### 9.1 Medical Monitoring Program Director

The Medical Monitoring Program Director should be a licensed physician with training and experience in identifying and preventing occupational lung disease or other qualified practitioner (as determined by appropriate state laws and regulations. This individual (hereafter referred to as "the medical monitoring director") should adhere to American Thoracic Society (ATS) guidelines for conducting high quality spirometry tests [Miller et al. 2005]. The employer should consider a board-certified occupational medicine physician or a board-certified pulmonary medicine physician for this position and ensure that the physician is familiar with the natural history of flavoring-related lung disease and knowledgeable about spirometry accuracy and test validity. (The employer should provide the medical monitoring director with a copy of the NIOSH Alert, *Preventing Lung Disease in Workers Who Use or Make Flavorings* [NIOSH 2003c] and a copy of this criteria document.) The medical monitoring director should review and interpret questionnaire and spirometry results, including assessing spirometry quality. During a worker's scheduled visit for a medical monitoring program evaluation, the medical monitoring director should inquire about the worker's knowledge of the potential risk from exposure to diacetyl or similar flavor ingredients and of how to minimize the risk. The medical monitoring director should educate workers as needed [California Department of Public Health 2007b], and encourage workers to report any new persistent respiratory symptoms to their supervisor or the monitoring physician. At the end of each evaluation visit, the medical monitoring director should provide the worker with a written report describing the following items:

- The results of any medical tests performed on the worker
- The medical monitoring director's opinion regarding any abnormalities detected during the evaluation and recommendations for further evaluation and treatment
- Recommendations (if necessary) for reducing the worker's exposure to diacetyl and similar flavor chemicals

The medical monitoring director should inform the employer in writing of any recommendations for limiting the worker's exposures and that the worker was informed of any abnormalities detected during the evaluation and given recommendations for further evaluation and treatment. Any aspect of the worker's medical history that has no bearing on whether the worker should continue to work in areas where diacetyl and similar flavor chemicals are handled should not be revealed to the employer.

### 9.2 Workers to Include in the Medical Monitoring Program

All workers (permanent, temporary, and contract workers) who regularly work in or enter areas where diacetyl and similar flavor ingredients (or products that contain these ingredients) may benefit by being included in the medical monitoring program. In addition to production workers, this includes workers who are periodically exposed such as supervisors, warehouse workers, laboratory workers, quality assurance/control workers, shipping and receiving workers, maintenance workers, and janitorial workers. Office workers who frequently enter areas where these substances are used should also be included in the program. Workers with past experience in such jobs or performing such duties should be included in the monitoring program for one year and longer if abnormalities are present [California Department of Public Health 2007b].

To achieve the intent of primary and secondary prevention, employers should have an interest in attaining a high rate of worker participation in regular medical monitoring. Participation should be encouraged.

### 9.3 Components of Medical Monitoring

The medical monitoring evaluation should include a questionnaire to obtain health and exposure information and spirometry to assess lung function. Newly-hired workers and current workers should have a baseline evaluation before they are allowed to work in or enter areas where diacetyl and similar flavor ingredients (or products that contain these ingredients) are used or produced. The questionnaire data from all workers in a medical monitoring program should be entered into a database (along with spirometry results) for use in epidemiologic analyses for medical surveillance. These analyses may reveal associations between health outcomes and exposure variables (e.g., work tasks and practices) that can be addressed to decrease lung disease risk (see Section 9.8).

#### *9.3.1 Questionnaire*

The purpose of the questionnaire is to obtain standardized information on demographics, work history, exposures, personal risk factors (such as smoking) and health history. The medical monitoring director can use information from the questionnaire when assessing the worker at each evaluation.

Work history questions should allow workers to correctly indicate the specific job titles they have held at their current employer. For each job title, the questionnaire should collect information on specific work tasks and practices that may affect the worker's exposure to diacetyl and similar flavor ingredients. For example, for a worker whose job requires direct handling of diacetyl-containing flavorings, specific questions might address how often a particular task is performed, the amounts of flavorings used, whether open or closed containers of flavorings are used, and whether respiratory protection is used (including the type of respirator used and when it is worn). To help the medical monitoring director develop appropriate questions on jobs and exposures, the employer should provide the medical monitoring director with the specific job titles of potentially exposed workers, a description of the work tasks for each job that may be associated with

potential for exposure to diacetyl and similar flavor ingredients, and the types of personal protective equipment (e.g., respirators) and other measures that workers have available to them to minimize exposures in each job. A visit to the plant by the medical monitoring director to view the production process may provide additional useful information for questionnaire development.

The questionnaire should contain questions on the presence or absence of respiratory symptoms such as shortness of breath on exertion, cough, and wheezing; respiratory illnesses such as asthma, emphysema, chronic bronchitis, and COPD; and the dates of diagnosis. Additional questions might inquire about work-related nasal, ocular, and dermal symptoms. The American Thoracic Society Respiratory Symptom Questionnaire [Ferris 1978b] or the NHANES III questionnaire [CDC 1994] can provide standardized questions. Examples of questions NIOSH has used in HHE medical surveys of flavoring-exposed workers can be found in NIOSH HHE reports at [http://www.cdc.gov/niosh/hhe/].

While respiratory symptom information is important in the assessment of workers exposed to diacetyl and similar flavor ingredients (or products that contain these ingredients), the medical monitoring director should not conclude that a worker's exposures are below harmful levels solely by the absence of respiratory symptoms. Workers may not experience respiratory symptoms early in the course of rapid lung function decline. NIOSH medical surveys of flavoring-exposed workers have identified airways obstruction [Kreiss et al. 2002] and excessive declines in lung function [NIOSH 2008b] in workers who did not report respiratory symptoms.

The medical monitoring director should counsel workers identified as having pre-existing lung disease on their initial medical monitoring evaluation regarding the potential risks of working in areas where they may be exposed to diacetyl and other flavor ingredients. The medical monitoring director should also explain that it may be hard to determine the role of work exposures or pre-existing lung disease with regard to any future abnormal lung

function declines. Such workers should also be referred to their personal physician for additional evaluation and recommendations regarding potential exposure to these substances.

### *9.3.2 Spirometry*

Every worker in the medical monitoring program should have a spirometry test at each evaluation irrespective of respiratory symptom status. Evaluation of lung function over time is the most important component of medical monitoring for identifying possible work-related lung disease in workers exposed to diacetyl and similar flavor ingredients (see Section 9.5). High quality spirometry tests are necessary to allow the medical monitoring director to correctly interpret the results and make appropriate recommendations to the worker and the employer. Accuracy of spirometry measurements depend on four key elements: (1) a trained technician who can obtain valid test results, (2) a reliable and accurate spirometer, (3) an approved testing protocol, and (4) a spirometry quality assurance program directed by a laboratory supervisor or the medical monitoring director.

#### 9.3.2.1 Persons administering the spirometry examination

Each person administering spirometry examinations shall successfully complete a NIOSH-Approved Spirometry Training Course (information at [http://www.cdc.gov/niosh/topics/spirometry/training.html] and maintain valid certificates. The medical monitoring director may also benefit from this training. The American Thoracic Society [Miller et al. 2005] and The American College of Occupational and Environmental Medicine (ACOEM) [ACOEM 2009] endorse NIOSH-Approved Spirometry Training and also recommend refresher training for spirometry technicians. Both the ATS/ERS and ACOEM recommend ongoing review of spirometry tests for quality after training to identify and correct any aspects of the technician's performance that have resulted in poor quality tests. Certification of acceptable spirometry test administration is a supplementary means of addressing quality concerns

(National Board for Respiratory Care; American Association for Respiratory Care [AARC 2011]).

#### 9.3.2.2 Spirometer specifications

Spirometry testing equipment shall meet the American Thoracic Society/European Respiratory Society (ATS/ERS) guidance for Standardization of Spirometry (or most recent equivalent) [Miller et al. 2005] specifications for spirometer accuracy and precision, and real-time display size and content. The electronic output format shall meet the most current ATS/ERS specifications for spirometers. Written verification from a third party testing laboratory (not the manufacturer or distributor) that the model of spirometer being used has successfully passed its validation checks as required by the most current ATS protocol should be requested from the spirometer manufacturer.

#### 9.3.2.3 Spirometry testing protocol and reporting information

Administration of spirometry examinations should follow the American Thoracic Society/European Respiratory Society (ATS/ERS) guidance for Standardization of Spirometry (or most recent equivalent) [Miller et al. 2005]. These guidelines outline the criteria to follow to ensure overall test results are valid (Table 9.1). The technician should be able to view real-time testing displays as specified in the most recent ATS/ERS spirometry standardization. On-site back-up of the results shall include spirometry test reports and retention of all spirometry examination results in printed or electronic format. Spirometry examination reports for the employee's health record shall contain, at a minimum, the employee's age, height, gender, race and weight, numerical values and volume-time and flow-volume spirograms for at least the 3 best valid expiratory maneuvers, normal reference value set used, employee position during testing (standing or sitting), dates of test and last calibration check, ambient temperature and barometric pressure (volume spirometers), and the technician's unique identification number or initials. The name, postal mailing and contact e-mail addresses, and telephone and fax numbers of the facility completing the spirometry examination results and forms shall also be recorded.

Table 9.1. Spirometry guidelines for testing procedures and interpretation.

| | |
|---|---|
| Testing Procedures | 1. Spirometer calibration checks should be performed using a currently calibrated (per manufacturer recommendations) 3-liter syringe on each day of testing.[a] A copy of the spirometer calibration report should be maintained in either electronic or hard copy form.<br>2. Spirometry should be performed in the same documented position (either sitting or standing) during the baseline and all subsequent tests.<br>3. A minimum of three forced exhalation maneuvers producing "acceptable curves" on the spirometry report should be characterized by the following:<br>• Lack of hesitation (back-extrapolation volume should be less than 5% of FVC or 150 ml, whichever is larger)<br>• No cough in the first second<br>• No evidence of airflow cessation, variable effort, leak, obstructed mouthpiece, or extra breath(s)<br>• Acceptable end-of-test criteria ($\leq$ 25 ml increase in volume for 1 second or a maneuver longer than 15 seconds)<br>4. Less than 150 ml difference between the two highest FVC measurements and the two highest $FEV_1$ measurements is the goal. |
| Spirometry Predicted Values | If spirometry software allows a choice of predicted values, NHANES III or the most recent equivalent should be used[b] as they are based on a large sample of the US population. Since predicted values are not available from NHANES III for Asian people born in the United States, these predicted values may be estimated by multiplying the NHANES III Caucasian predicted values for $FEV_1$ and FVC by 0.94[c] or by 0.88.[d] Asian-specific equations or corrections will be available from NHANES Plus data. If spirometry software does not include lower limits of normal values, the spirometry reference value calculator at [http://www.cdc.gov/niosh/topics/spirometry/RefCalculator.html] can be used to calculate lower limits of normal for NHANES III reference values. All predicted values and lower limits of normal used should be from the same reference paper. |

[a] [Miller et al. 2005]

[b] [Hankinson et al. 1999]

[c] [Pellegrino et al. 2005]

[d] [Hankinson et al. 2010]

### 9.3.2.4 Spirometry quality assurance

A comprehensive spirometry quality assurance program is necessary to minimize the rate of invalid test results. This program should include all of the following components: instrumentation calibration checks, automated maneuver and test session quality checks, and ongoing monitoring of test quality. Testing personnel shall be fully familiar with and adhere to the most current ATS/ERS guidelines for instrument calibration check procedures. Calibration check procedures shall include daily (day of testing) leak and volume accuracy checks and according to the frequency established by the most current ATS/ERS spirometry standardization statement. Instrument calibration check records shall be maintained by the provider for as long as the related employees' medical reports are maintained. Spirometer software shall automatically perform quality assurance checks on expiratory maneuvers during each spirometry testing session. Messages shall alert the technician to maneuver acceptability errors and test session non-repeatability. Each spirometry test session shall have the goal of obtaining 3 acceptable with 2 repeatable forced expiratory maneuvers, as defined by the most current ATS/ERS spirometry standardization statement. Since all spirometry software packages are not able to identify all the possible technical errors encountered during testing, NIOSH developed a poster that provides guidance to identify and correct common testing errors and improve spirometry test quality [NIOSH 2011a]. This document has been translated into several languages for non-English speaking technicians and can be accessed at [http://www.cdc.gov/niosh/docs/2011-135/]. Providers shall utilize physicians or other qualified health care professionals with expertise in evaluation and interpretation of spirometry to conduct ongoing monitoring of test quality. Determination of quality requires review of the flow-volume and volume-time curves for each acceptable maneuver and comparison of the two highest $FEV_1$ and FVC measurements [Townsend 2011]. If suboptimal quality (< 80% of tests do not meet the most current ATS/ERS recommendations for test session acceptability and repeatability) is identified, the reviewing physician or other appropriate health care professional shall provide feedback to the appropriate technician(s) along with suggestions for improvement. Some studies have found evidence that providing regular feedback to technicians improves test quality and decreases variability. In two studies where extensive feedback was provided to

technicians on the quality of their tests, the investigators found lower measures of variability for their test measurements than in other studies where extensive feedback to technicians was not provided [Enright et al. 1991; Malmstrom et al. 2002]. In these studies, the technicians received immediate feedback from the spirometry device on the acceptability of a forced exhalation maneuver and on the overall quality of the test. The investigators also provided ongoing review of the quality of their tests and gave feedback to the technicians; additional technician training was provided as needed. Test quality in these studies was graded using an A, B, C, D, F scale. In a study of a workplace spirometry testing program, use of a new spirometer that provided technicians with feedback during the test led to increases in the mean $FEV_1$ and mean FVC of the study group, compared to use of an older spirometer without feedback capability [Banks et al. 1996].

With poor quality tests, some workers' results may be considered abnormal which are truly normal, and employers may incur costs for lost work time in follow-up testing and clinical evaluation. In addition, employees may suffer needless worry, risks of unnecessary medical tests, and may be subject to workplace discrimination or even job loss. An example of an incorrect interpretation due to a poor quality test is the finding of a restrictive abnormality because the test subject did not exhale long enough during the maneuver; this results in a falsely low FVC. High quality spirometry tests are also necessary for comparison of spirometry results over time, an important consideration for flavoring-exposed workers. Low quality spirometry has greater variability in test results; over time, decreased precision may cause the medical monitoring director to incorrectly identify whether a worker has had an excessive decline in lung function from one test to the next.

In reviewing the quality of spirometry tests performed for employers by private healthcare providers, NIOSH has identified instances where the quality of most tests performed by a private health provider was poor and thus not useful for assessing lung function changes over time [Kanwal et al. 2011; Kreiss et al. 2011; NIOSH 2004b, 2006].

High quality spirometry minimizes the variability in the results caused by technical aspects (i.e., how the test was conducted) so that changes in spirometry measurements over time reflect true changes in lung function more accurately. In California public health surveillance, only one of 13 commercial providers of surveillance spirometry for flavoring workers who reported results to the California Department of Public Health met a minimum quality criterion of 80% of test sessions with $FEV_1$ of good quality [Kreiss et al. 2011]. Employers of flavoring-exposed workers can motivate provision of good quality spirometry by use of independent audits of spirometry quality and withholding contractual payment for spirometry that does not meet prespecified quality requirements.

### 9.4 Frequency of Medical Monitoring Evaluations

Workers in the medical monitoring program should be evaluated with a questionnaire and spirometry every 6 months. If a worker exposed to diacetyl or similar flavor ingredients is identified as likely having lung disease due to this exposure, then all workers who perform similar job tasks or have a similar or greater potential for exposure should be evaluated every 3 months. When work-related risks of excessive $FEV_1$ decline are apparent from epidemiologic surveillance, more frequent 3-monthly monitoring is appropriate for workers with excessive decline and their similarly exposed co-workers. Although interpretation of excessive decline is challenging for short intervals between testing because of measurement error, the increased numbers of tests may facilitate improvement of spirometry quality and monitoring physician confidence in trends that may be occurring. The 3-month schedule should be maintained until factors that may have led to excessive exposure have been corrected and 12 months have passed during which no additional workers with likely flavoring-related lung disease are identified. Workers should be instructed to report to their occupational health service or supervisor any new persistent or worsening shortness of breath, cough, wheezing, or other respiratory symptoms lasting more than 6 weeks. Such workers should be immediately evaluated by the medical monitoring director. All workers who have been in the

monitoring program should have a final evaluation at the end of employment [California Department of Public Health 2007b].

### 9.5 Early Identification of Affected Workers

Early recognition of workers with lung disease due to exposure to diacetyl or similar flavor ingredients is essential to prevent rapid progression to severe irreversible disease. Identifying affected workers will also stimulate prevention efforts so that risk to coworkers is minimized. The most effective means for identifying affected workers early is careful evaluation of results of serial spirometry tests of workers in the medical monitoring program. Symptom reports alone are not a reliable indicator of early disease, as many workers with early disease will be asymptomatic.

At each evaluation of a worker in the medical monitoring program, the medical monitoring director should compare the results of the current spirometry test to the baseline (pre-exposure) test, or to the test with the highest values if post-hire spirometry values were higher than at baseline. The most important finding that may indicate development of lung disease from exposure to diacetyl or similar flavor ingredients is an abnormal decline in the $FEV_1$. A worker's test may reveal an abnormal decline in $FEV_1$ compared to baseline even though overall test values may still be above the LLN calculated from the reference population. While such test results might not meet the criteria for an abnormality such as airways obstruction, an abnormal decline in $FEV_1$ may indicate early disease in this case and should be further evaluated. Additionally, any new abnormality on spirometry compared to baseline should prompt further evaluation. Flavoring-exposed workers with obstructive abnormalities ($FEV_1$/FVC ratio and $FEV_1$ less than the LLN) need additional medical tests to assess whether they have bronchiolitis obliterans. Workers with restrictive abnormalities (FVC less than LLN and normal $FEV_1$/FVC ratio) also need additional medical tests to differentiate between lung causes and other causes of spirometric restriction.

The criteria for an abnormal rapid decline in the $FEV_1$ depend on the quality of the spirometry tests performed as part of the medical monitoring program and the time period of follow up. Both ATS and ACOEM have stated that a decline in $FEV_1$ over one year should exceed 15% before being considered clinically meaningful [ACOEM 2009; Pellegrino et al. 2005]. By this criterion, someone with a baseline $FEV_1$ of 4 liters would have to experience a decline of at least 600 mL for the results to be considered abnormal. Recent studies indicate that when ATS criteria for spirometry quality are followed and high standards of quality are achieved, a threshold less than 15% can indicate an abnormally rapid decline in $FEV_1$ in a year. In a study that used data from a spirometry surveillance program for coal miners, Wang et al. [Wang and Petsonk 2004] found that the $5^{th}$ percentile for $FEV_1$ declines over 6 months in all workers studied, in stable workers (those workers whose $FEV_1$ slope over 5 years was less than 90 mL/year), and in healthy workers (those workers without symptoms or methacholine responsiveness over 5 years) were 320 ml (7.8%), 300 ml (7.1%), and 280 ml (6.5%), respectively. The quality of spirometry data in this study reflected a within-person variation of 3% which is rarely achievable. In another study that used data (with a within-person variation of 4%) from a spirometry surveillance program for thousands of workers at a large chemical company, Wang et al. [Wang et al. 2006] found that the $5^{th}$ percentile values for $FEV_1$ decline for testing at one-year intervals were 380 mL (10.4%) in men and 280 mL (10.6%) in women. These studies suggest that in a medical monitoring program that follows ATS criteria and achieves high quality spirometry, an $FEV_1$ decline of 10% or higher in one year or less can be considered abnormal and be followed by further medical evaluation of the worker. ACOEM now accepts this 10% criterion after allowing for expected average annual loss due to aging when the relationship between longitudinal results and endpoint disease is clear, as in flavoring-exposed workers [Townsend 2011]. Lower quality spirometry programs have the disadvantage of only being able to detect larger declines in $FEV_1$ as abnormal.

NIOSH has developed a computer program (Spirometry Longitudinal Data Analysis [SPIROLA] Software) that measures the precision of a spirometry provider's serial

spirometry data (an indication of spirometry quality across the provider's program) and provides a limit of longitudinal decline (LLD) for each individual tested, adjusted for the quality of the provider's spirometry program [NIOSH 2010b]. The LLD allows the spirometry provider to determine if an individual's serial spirometry results suggest a rapid decline in lung function and allows higher quality programs to identify smaller changes in lung function as abnormal. Details on this free software are available at [http://www.cdc.gov/niosh/topics/spirometry/spirola-software.html]. The advantage of using relative lower limits of longitudinal decline and fifth percentile approaches over the 15% criterion in microwave popcorn workers has been demonstrated [Chaisson et al. 2010].

Companies may change medical providers of medical monitoring services. Subsequent providers typically do not have previous spirometry records for employees with which to assess whether abnormal declines have occurred in comparison to baseline measurements at the beginning of employment. Contracts to medical providers of monitoring services should anticipate the need for transfer of employees' personal medical information to a subsequent provider so that early detection of abnormal declines is possible. This may require obtaining consent from employees as testing services are provided.

### 9.6 Medical Evaluation of Workers with Abnormalities on Medical Monitoring Spirometry

The first step in evaluating a worker whose medical monitoring spirometry test shows either a rapid decline in $FEV_1$ (even if overall results are still above the LLN) or a new abnormality compared to baseline is to repeat the test within one month to confirm the change. If the repeat spirometry test confirms a rapid decline in $FEV_1$ or other abnormality, the worker should be referred for more extensive pulmonary function tests (described below). The medical monitoring director can request these and other necessary tests or refer the worker to a pulmonary medicine physician. The employer should inform the medical monitoring director of its agreement with this approach for medical evaluation of suspect cases, and enable the physician to request additional tests or refer

the worker to a pulmonary medicine physician at no cost to the worker. If the worker is referred to another physician, the medical monitoring director should ensure that the other physician is aware of the nature of flavoring-related lung disease (e.g., provide the physician with a copy of the NIOSH Alert, *Preventing Lung Disease in Workers Who Use or Make Flavorings* [NIOSH 2003c] and this Criteria Document).

The referred worker should receive complete pulmonary function tests (PFTs) which include spirometry with an assessment of bronchodilator response, diffusing capacity for carbon monoxide ($DL_{CO}$), and static lung volumes. Most workers who have developed lung disease while being exposed to diacetyl and similar flavoring chemicals have not had a response to bronchodilator (i.e., they had fixed airways obstruction). $DL_{CO}$ in affected workers has usually been normal, although some individuals with advanced disease have had a low $DL_{CO}$. Lung volume measurements have shown a normal or elevated total lung capacity (TLC) and an increased residual volume, consistent with air trapping [Akpinar-Elci et al. 2004]. Individuals with moderate to severe airways obstruction may have a mixed obstructive/restrictive (reduced $FEV_1$, $FEV_1$/FVC ratio and FVC) pattern of spirometry because air trapping decreases the FVC. The actual underlying physiology can be clarified by determining lung volumes.

Workers found to have fixed airways obstruction or other abnormalities on complete PFTs should have additional evaluation with a high resolution computerized tomography (HRCT) scan of the chest with inspiratory and expiratory views. Heterogeneous air trapping during expiration has been the most common finding in flavoring-exposed workers with fixed airways obstruction. Other common findings include cylindric bronchiectasis, bronchial wall thickening, and a mosaic pattern of attenuation. Patchy ground glass opacities have been observed less commonly. These findings may not be present in flavoring-exposed workers who have early or mild obstructive disease [Kim et al. 2010]. HRCTs have not been systematically performed in flavoring-exposed workers with restrictive pulmonary function abnormalities or with excessive $FEV_1$ declines within

the normal range of $FEV_1$. Specialist consideration of the diagnostic or baseline utility of this test is suggested.

Final determination of the likely nature of lung disease in a worker exposed to diacetyl or similar flavor ingredients should take into account the changes identified in medical monitoring spirometry tests, the results of complete PFTs and of HRCT scans of the chest, the course of the worker's illness over time, and medical, work, and personal risk factor history. A worker exposed to diacetyl or similar flavor ingredients who has normal pre-exposure spirometry and subsequently develops fixed airways obstruction, has evidence of air trapping on complete PFTs or on HRCT scan, and does not improve after exposure cessation likely has clinical bronchiolitis obliterans due to this exposure. (As explained in Chapter 3, the term *clinical bronchiolitis obliterans* has been used for patients who have medical test findings consistent with the disease *constrictive bronchiolitis obliterans* but do not have confirmatory biopsy results.) Even without other findings of clinical bronchiolitis obliterans, post-hire fixed airways obstruction (or a rapid decline in $FEV_1$) in an exposed worker that does not resolve after exposure cessation is likely work related unless medical evaluation reveals an alternative cause. In exposed workers who smoke, fixed airways obstruction should not be attributed to smoking if there is no evidence of emphysema on medical tests (e.g., a low $DL_{CO}$). Clinically significant emphysema occurs in a subset of smokers after many years of smoking; it is uncommon in smokers less than 50 years old [Wise 2008]. In middle-aged and older smoking workers, work history, clinical course and medical tests are important in attempting to differentiate between smoking-related chronic obstructive pulmonary

disease (COPD) and flavoring-related obstruction. In older persons, smoking is the key factor in development of COPD. Smoking explains about 80% of COPD in the United States, with about 15% attributable to work exposures. Smoking diacetyl-exposed workers appear to have lower excess risk of obstruction than never-smoking flavoring-exposed workers (Kreiss et al 2002).

While some physicians might desire biopsy confirmation, the patchy nature of constrictive bronchiolitis obliterans and lack of familiarity of some pathologists with the techniques necessary to identify bronchiolar lesions may prevent identification of the disease on biopsy. In an exposed worker with evidence of clinical bronchiolitis obliterans on PFTs or HRCT scans and no other identifiable cause for the disease, lack of biopsy confirmation would not rule out that the worker had lung disease due to exposure to diacetyl or similar flavor ingredients. The non-invasive clinical findings alone are sufficient to conclude that an exposed worker likely has clinical bronchiolitis obliterans and should no longer be exposed to diacetyl and similar flavor chemicals. The physician evaluating such a worker should consider the limitations of lung biopsy before recommending that the worker undergo an invasive procedure such as an open lung or thoracoscopic lung biopsy (transbronchial biopsies are not useful for evaluating clinical bronchiolitis obliterans). Physicians caring for lung transplant patients use a similar approach. Constrictive bronchiolitis obliterans commonly occurs after patients receive a lung transplant. Because this disease is difficult to identify on biopsy, the International Society for Heart and Lung Transplantation developed a clinical description for the disease termed *bronchiolitis obliterans syndrome*. The syndrome refers to graft deterioration secondary to persistent airflow obstruction as defined by pulmonary function changes with or without biopsy confirmation [Estenne et al. 2002].

Another possible outcome of occupational exposure to diacetyl and similar flavor ingredients is work-related asthma (new onset asthma or exacerbation of pre-existing asthma). A worker with no past asthma history who experiences post-hire recurrent respiratory symptoms and has airways obstruction responsive to bronchodilator on PFTs (reversible airways obstruction) may have new onset asthma due to diacetyl or similar flavor chemicals. If a worker with asthma symptoms does not have changes over time on medical monitoring spirometry, a methacholine challenge test may be necessary to determine if the worker has airways hyperresponsiveness as occurs in asthma. Worsening symptoms in a worker with stable pre-existing asthma may be due to exposure to diacetyl, similar flavor chemicals, or other agents in the workplace. An important consideration for diacetyl-exposed workers with worsening pre-existing asthma or new onset reversible airways obstruction is that this may actually reflect early disease that may ultimately progress to clinical bronchiolitis obliterans. A worker at a California flavoring plant who had stable pre-existing asthma (no symptoms at time of hire) developed progressive shortness of breath and was found to have severe fixed airways obstruction on PFTs; a lung biopsy showed evidence of bronchiolitis obliterans [NIOSH 2007a]. Workers with worsening pre-existing asthma or new onset reversible airways obstruction should be evaluated with an HRCT scan of the chest to determine if findings consistent with clinical bronchiolitis obliterans are present. However, since HRCT abnormalities are insensitive in detecting early or mild disease, such asthmatic workers require careful and frequent follow up.

Although some medical surveys of flavoring-exposed workers have revealed an increased prevalence of an isolated restrictive pattern on spirometry (i.e., without concurrent airways obstruction), static lung volume measurements of TLC have not been available in these studies to confirm restrictive lung disease. Workers who develop restrictive abnormalities or who have excessive parallel $FEV_1$ and FVC declines should have assessment of lung volumes, diffusing capacity, and x-rays to differentiate between restrictive lung disease and other causes of restrictive spirometric patterns. Further

evaluation of restrictive lung disease is necessary for a diagnosis and may require bronchoalveolar lavage and biopsy.

### 9.7 Response to Identification of Work-related Lung Disease

Workers with abnormalities identified on medical monitoring spirometry should be removed from exposure pending further medical evaluation. Workers who receive a formal diagnosis of flavoring-related lung disease or who have findings on medical evaluation that indicate likely clinical bronchiolitis obliterans or other lung disease due to exposure to diacetyl or similar flavor ingredients should not be allowed any further exposure to flavor chemicals and other substances in the workplace that may cause their lung disease to worsen. Use of respiratory protection is not equivalent to removal from further exposures as workers may still be exposed because of incomplete compliance, selection of an inappropriate respirator, or respirator malfunction [California Department of Public Health 2007b]. Affected workers who require transfer to another work area that has minimal or nonexistent exposures should retain seniority, wages, and benefits.

Employers of a worker with confirmed or likely flavorings-related lung disease should arrange for an industrial hygiene evaluation of the plant areas where the worker had been assigned. The evaluation may identify aspects of the production process or work practices where control strategies can be implemented to minimize exposures. This may prevent additional workers from developing work-related lung disease. Medical monitoring evaluations of workers in these areas should increase in frequency from every 6 months to every 3 months, with a return to 6-month intervals after factors that may have led to excessive exposure have been corrected and 12 months have passed during which no additional workers with likely flavoring-related lung disease are identified (see section 9.4).

The employer should record all lung disease cases and other worker injuries related to exposure to diacetyl or similar flavoring chemicals in the OSHA Log of Work-Related Injuries and Illnesses (OSHA Form 300).

### 9.8 Medical Surveillance Analyses

A workplace assessment conducted after identification of a sentinel case of work-related lung disease may reveal sources of uncontrolled exposures from particular aspects of production processes and work practices that can be improved to prevent other workers from becoming affected. However, this approach may not identify all such risk factors for hazardous exposure in a given workplace. Additional risk factors may be identified through medical surveillance, which is the use of epidemiologic techniques for analyses of aggregated data obtained from medical monitoring evaluations of all workers in a medical monitoring program. Such analyses show trends and distributions of health outcomes by exposure variables such as work area, job category, and work task. In some instances, the results of such analyses may provide early evidence of risk factors that can be addressed before workers develop significant lung disease. Because production processes and work practices in manufacturing plants that use diacetyl or similar flavor ingredients (or products that contain these ingredients) vary from plant to plant, medical surveillance may also allow identification of risk factors unique to a particular plant. For these reasons, medical surveillance should be a component of medical monitoring programs for workers exposed to diacetyl or similar flavor ingredients. If the medical monitoring director is not able to conduct such analyses, the employer or medical monitoring director should arrange for consultants with expertise in epidemiology to undertake this task. Two examples below show how medical surveillance can help to identify lung disease risk factors in the workplace.

Example 1. At the plant where microwave popcorn workers were first identified as being at risk for severe fixed airways obstruction consistent with clinical bronchiolitis obliterans from exposure to butter flavoring vapors (index plant), four known affected

former workers had worked in the mixing room as mixers of oil and butter flavorings, and four other affected former workers had worked on the packaging lines near the mixing room. A medical survey of current workers showed that the prevalence of airways obstruction on NIOSH spirometry tests was 3.3 times higher than expected in comparison to U.S. population data, a finding that was consistent with the known disease in former workers. The environmental assessment showed that air concentrations of the butter flavoring chemical diacetyl were highest in the mixing room. The next highest exposures were in the packaging line area because of contamination from the mixing room, which was not isolated from the rest of the plant. Diacetyl air concentrations in other parts of the plant were lower. Analyses of the medical and environmental data showed a dose-response relationship between abnormal spirometry and quartiles of estimated cumulative exposure to diacetyl [Kanwal et al. 2011; Kreiss et al. 2002; NIOSH 2006].

Additional analyses of the medical survey data revealed an unexpected finding: Among current workers, the highest prevalence of airways obstruction was found in quality control (QC) laboratory workers, five of six (83%) of whom had airways obstruction [Kreiss et al. 2002]. These workers popped approximately 100 bags of microwave popcorn in microwave ovens per 8-hour shift. The mean time-weighted average diacetyl air concentration in the QC laboratory was 0.8 parts per million parts air (ppm) compared to approximately 57.2 ppm in the mixing room and 2.8 ppm for machine operators in the packaging line area. QC laboratory workers may be at risk for lung disease because they experience intermittent peak exposures to vapors of diacetyl from microwave popcorn bags during and after popping in microwave ovens; mixers experience similar intermittent peaks when they add butter flavorings to tanks of heated oil [NIOSH 2003c]. Another possible explanation is that the much higher temperatures that occur in microwave popping (compared with the temperatures in heated tanks of oil and butter flavorings) increase the volatilization of other chemicals. QC laboratory workers' exposures may be substantially different from those of other production workers; diacetyl air concentrations alone may not be a satisfactory predictor of risk for these workers. Because of this evidence of risk to QC laboratory workers, NIOSH recommended

implementing exposure controls in the QC laboratory in addition to the mixing room and packaging line area [Kanwal et al. 2011; NIOSH 2006].

In evaluations at five other microwave popcorn plants, NIOSH found evidence of affected mixers in four plants and evidence of affected packaging line workers in one plant [Kanwal et al. 2006]. No other plant had an elevated prevalence of airways obstruction in QC workers. Fewer bags of microwave popcorn were popped per worker per day in those plants, and the mean time-weighted average diacetyl air concentrations in the QC laboratories were lower than at the index plant.

Example 2. At a microwave popcorn plant where a young mixing room worker developed moderately severe fixed airways obstruction and other findings consistent with clinical bronchiolitis obliterans, management had put a mandatory respirator use policy for mixing room workers in place soon after the company first started production. (In addition to using respirators, the company had also ventilated and isolated the mixing room from the rest of the plant and had local exhaust ventilation for tanks of heated oil and butter flavorings. Butter flavorings were handled in open containers as they were at other microwave popcorn plants.) The respirators used were full facepiece respirators with organic vapor cartridges and particulate filters. Included in the questionnaire that NIOSH administered to current workers during a medical survey at the plant were questions about respirator use for the following work tasks: (1) weighing or handling open containers of flavorings, (2) pouring flavorings into tanks in the mixing room, (3) pouring other ingredients into tanks in the mixing room, (4) checking the levels in the tanks, and (5) other duties in the mixing room. Thirteen current workers reported ever having worked as a mixer (six had abnormal lung function on NIOSH spirometry tests). The reported percentages of time these workers used respirators during these activities ranged from 0% to 100%. The median reported percentage was 20% for all activities, except for three where the median was 50% [NIOSH 2003c]. These results showed that workers were not fully compliant with management's respirator use policy; management was able to address this problem through worker education and enforcement of the

policy. Had the company become aware of this problem earlier by regularly collecting and evaluating information on respirator use during medical monitoring evaluations, it could have increased compliance with respirator use and thus minimized some workers' exposures to butter flavoring chemicals. (Before 2001 when NIOSH informed microwave popcorn companies of the risk of severe lung disease to workers exposed to butter flavorings, the company had been unaware of the respiratory toxicity potential of diacetyl; the company had implemented a mandatory respirator use policy for mixing room workers many years earlier to prevent severe eye irritation that workers had experienced when handling certain flavorings.)

# Chapter 10: Exposure Monitoring in Occupational Safety and Health Programs

Employers should develop and implement comprehensive occupational safety and health programs to prevent occupational injuries, illnesses, and deaths. To be successful, safety and health programs should be developed and implemented as part of an employer's management system, with strong management commitment, worker involvement, and occupational safety and health expertise. A safety and health program designed to protect workers from the adverse effects of exposure to diacetyl, 2,3-pentanedione, and other flavoring chemicals should include mechanisms to identify all risk factors for exposure to flavoring substances. Just as medical monitoring is part of an overall occupational safety and health programs, so is exposure monitoring. Exposure monitoring should be conducted whenever there is workplace exposure diacetyl or 2,3-pentanedione and should (1) determine worker exposure to diacetyl, 2,3-pentanedione, and other flavoring chemicals used in the workplace, (2) evaluate the effectiveness of work practices and engineering controls, and (3) facilitate selection of appropriate personal protective equipment, if appropriate.

## 10.1 Exposure Monitoring Program Goals

A workplace exposure monitoring program should have clear, stated goals [Mulhausen and Damiano 1998]. In addition to routine monitoring of airborne contaminant concentrations, the monitoring strategy should assess the effectiveness of engineering controls, work practices, PPE, training, and other factors in controlling exposures to flavoring chemicals. The monitoring program should also identify areas or tasks that are associated with higher exposures to flavoring compounds where additional control efforts and/or sampling are needed. The program should also determine how changes in production (processes, chemicals and other substances used, and products) affect worker exposures.

## 10.2 Exposure Monitoring Program Elements

Proper measurement of contaminants in the environment involves a variety of program elements. The sampling and analytical methods referred to in this chapter include an outline of tested and validated procedures that produce statistically reliable data when used in the manner prescribed. Several of the more significant elements of a monitoring program are described below [Gross and Pechter 2002; Milz et al. 2003; Soule 2000].

Where possible, a written sampling strategy or protocol should be developed prior to sampling; this protocol should guide all aspects of the sampling process. The protocol should contain a description of (1) the objectives of sampling, (2) what to sample, (3) whom and where to sample, (4) how to sample, (5) when to sample, (6) how long to sample, (7) how many samples to collect, and (8) how to handle, store and ship samples [Gross and Pechter 2002; Milz et al. 2003; Soule 2000]. A walk-through survey or preliminary worksite visit is often useful in developing the sampling strategy [Jennison et al. 1996] and knowledge of the data-keeping system to be used to store and retrieve subsequent information can also have an effect.

### *10.2.1 Objectives of Sampling*

Sampling as part of an exposure monitoring program for diacetyl, 2,3-pentanedione, and other flavoring substances has several objectives. Often, this sampling is part of comprehensive assessment to identify and quantify exposure hazards throughout a designated plant or work area to protect workers' health. The frequency of monitoring will depend on the purpose and rationale of the sampling campaign. Specific sampling objectives can include:

1. Characterizing (qualitatively or quantitatively) the flavoring chemicals present in workplace air or in bulk materials
2. Ensuring compliance with existing OELs
3. Assessing the effectiveness of engineering controls, work practices, PPE, training, or other methods used for exposure control

4. Identifying areas, tasks, or jobs with higher exposures that require additional exposure control
5. Evaluating exposures related to production process changes and from changes in products made or materials used
6. Evaluating specific high risk job categories to ensure that exposures do not exceed exposure standards or guidelines
7. Measuring exposures of workers who report symptoms or illnesses

Sampling can also be used to access any fugitive emissions from plant processes into the surrounding community.

Exposure monitoring should be conducted by qualified industrial hygiene personnel. The sampling strategy should provide an opportunity to determine each worker's exposure, either by direct measure or through reasonable estimates based on the sampling of similar work tasks or jobs. Sampling strategies that group workers according to exposure zones, uniform job titles, or functional job categories have been used in some industries to reduce the number of required samples while increasing the confidence that all workers at similar risk will be identified [Mulhausen and Damiano 1998]. Area sampling may also be useful in exposure monitoring for determining sources of airborne contaminants and assessing the effectiveness of engineering controls.

When sampling to determine whether worker exposures are below an OEL, a focused sampling strategy that targets workers perceived to have the highest exposure concentrations may be more useful than random sampling. A focused strategy is most efficient for identifying exposures above the REL if maximum-risk workers and time periods are accurately identified. Focused sampling may help identify short-duration tasks involving high airborne concentrations that could result in elevated exposures over a full work shift and also tasks that result in exposures over the STEL.

### *10.2.2 What to Sample (Specific Agents and Physical States)*

Because flavorings can consist of many chemicals in addition to diacetyl and 2,3-pentanedione, deciding what to sample often requires preliminary knowledge of the specific flavoring chemicals being produced or used, or that are present in flavorings or other food ingredients used in the workplace, and the known exposure hazards posed by each. Information on possible food and flavoring chemicals present in workplace air can be obtained from reviews of product ingredient lists, flavor or food recipes, MSDSs, and other information provided by the employer or flavor manufacturer [Gross and Pechter 2002]. In the flavor manufacturing industry, the recipe for each flavoring indicates the chemicals, solvents, and other ingredients used in the formulation. In the food manufacturing industry, this information may be available directly from the company or from MSDSs for all flavorings and other ingredients used, although usually flavoring MSDSs do not list all potentially hazardous chemicals that may be present. Additional information may be needed from the flavoring manufacturers. Often, qualitative characterization may be useful prior to quantitative measurement to better guide the selection of substances to measure in the workplace. A review of any past exposure assessment reports from the target workplace, or similar workplaces, may also be helpful in selecting which agents to sample. In either case, a list of substances to which workers will potentially be exposed should be developed to help determine which of those compounds are the most critical to sample [Mulhausen and Damiano 1998]. In instances where a company has stopped using diacetyl and 2,3-pentanedione, in a flavor or food product, this list should include the butter flavor substances substituted for diacetyl or 2,3-pentanedione. Determining which chemicals to measure and sample should be based upon the chemical, physical, and toxicological properties as well as the chemical quantities in use. Diacetyl, 2,3-pentanedione, and other flavoring chemicals can be present in air as solids, liquids, gases/vapors, or a combination of these. The physical state of the flavoring chemical in air influences decisions about sampling [NIOSH 1977].

### *10.2.3 Whom and Where to Sample*

Selecting whom or where to sample depends in part on the sampling objectives as previously described. When sampling to determine whether worker exposures are below existing OELs, a focused sampling strategy that targets workers perceived to have the highest exposures may be more efficient than other strategies if maximum-risk workers and time periods can be accurately identified. Focused sampling may also help identify short-duration tasks involving high flavoring chemical concentrations that could result in peak exposures or contribute to elevated exposures over a full work shift. The sampling protocol should include sampling during the production of foods or flavorings with higher diacetyl, 2,3-pentanedione, or other food flavoring content. Sampling considerations include (1) distance from a diacetyl, 2,3-pentanedione, or flavoring chemical exposure source, (2) worker mobility, (3) air movement patterns, (4) specific tasks or work patterns, (5) individual work habits, and (6) exposure controls, [NIOSH 1977]. When a sampling strategy is selected that groups workers according to similar exposure potential, uniform job titles, or functional job categories, the industrial hygienist should select at random a predetermined number of workers from each group for personal air sampling to represent the exposures of those groups [Mulhausen and Damiano 1998; NIOSH 1977]. Area sampling may also be useful for determining sources of airborne contaminants and identifying the worst-case chemical concentrations in various locations or processes. When sampling for other purposes, logic should dictate the selection of which workers or work locations are selected.

### *10.2.4 How to Sample*

A variety of methods are available to sample for diacetyl, 2,3-pentanedione, or other food and flavoring substances. These include (1) gas and vapor air methods, (2) methods to sample particulates in air, (3) direct reading and real-time methods for gases/vapors and for particulates, (4) evacuated container sampling methods, (5) particle size distribution methods, (6) bulk air methods, and (7) bulk material methods. Selecting appropriate sampling and analytical methods and using professionally accepted techniques maximize

the validity of measurements of flavoring compounds in the work environment. While the state of the art in measuring diacetyl and 2,3-pentanedione is currently in flux, the methods with the most veracity at the time of publication of this document are OSHA Methods 1012 and 1013 for diacetyl and OSHA Method 1016 for 2,3-pentanedione.

Some sampling and analytical methods for diacetyl, 2,3-pentanedione, and other flavoring compounds are published by NIOSH and OSHA [http://www.cdc.gov/niosh/nmam/] and [http://www.osha.gov/dts/sltc/methods/index.html], are described in detail in Chapter 2 of this document, and are presented in Appendices 1 and 2. These methods include recommendations on sampling media, flow rate, duration, storage, shipment, sampling and analytical equipment, and procedures. A typical protocol for measuring diacetyl and 2,3-pentanedione is presented in Appendix 6.

To minimize the likelihood of inaccurate results, sampling equipment should be maintained in reliable working order through proper care and maintenance. All equipment should be regularly inspected and cleaned; periodically it may be necessary to have equipment repaired. Sampling pumps should be calibrated before and after each use. Because differences in pressure drop across the sampler affect flow rate, each sampling pump should be precalibrated and postcalibrated with the specific type of sampling media used for sampling. Busy industrial hygiene departments or consultant groups may wish to use additional staff (such as technicians) to help with these tasks and to ensure that sampling equipment is properly maintained. Such staff can also be responsible for stocking or procuring any necessary field sampling media or other necessary items and can help to prevent equipment loss by recording the names of the equipment users and the location where the equipment is to be used.

Careful record keeping in the field is also important. A detailed description of the work tasks conducted and the processes and materials involved is essential. Pertinent information such as sampling location, job category or task, air temperature, relative

humidity, possible interfering compounds in air, should be documented. To avoid confusion in the laboratory, samples should be carefully labeled and accompanied by accurate paperwork. The exact sampling duration should be known to accurately calculate the sampled volume. Determining the sampling duration from the recorded start and stop times assumes that the pump functions properly over the entire sampling period. Occasional spot checks to verify proper sampler operation should be made throughout the sampling period.

Personnel performing field sampling should not overlook quality assurance procedures. The field sampling parameters, such as calibration checks and accurate timing, often affect precision and accuracy of the final result more than the measurement's parameters. Field personnel should devote time to learning the sampling and analytical methods and sampling equipment operation procedures prior to arriving at the sampling site. These methods usually specify the proper sampling media to be used, the correct flow rate and sample volume, as well as special precautions of sample handling, shipping, and possible interferences.

Because many modern analytical techniques are extremely sensitive, contaminating field samples should be carefully avoided. Samples should not be stored or shipped with bulk materials that might spill or otherwise contaminate the field samples. The glassware or other containers used in sampling and shipping should be cleaned as recommended in the analytical method. For many sampling methods, the analytical laboratory requires submission of a specific number of blank samples with each set of samples to be analyzed; this number of samples is specific to the method. Blanks are used to mitigate the potential for unrecognized contamination due to media or sample handling [NIOSH 1994]. The two types of sample blanks are field blanks and media blanks. Field blanks are unopened new samplers or media taken to the sampling site and handled in every way like the actual samples, except that no air is drawn through them. Media blanks are simply unopened new samplers or media that are submitted to the laboratory with the samples (these blanks are not usually taken to the field). Additional blind field blanks,

labeled as field samples, should be sent along with the field samples as a further check on the analysis. Another occasionally used quality control practice is to include spiked samples—samples with known amounts of flavoring substance added—along with the other field samples sent to the laboratory for analysis. These spiked samples are often prepared by a separate laboratory and then included with the other field samples sent to the analytical laboratory. They are labeled as field samples so that the analytical laboratory is blinded to their identity as spiked samples.

The variety of types of direct-reading methods available for monitoring specific gases and vapors, as well as general contaminant concentration, is large and expanding. Detector tubes (short-term and long-term), also referred to as colorimetric indicator tubes, are widely used sampling devices for obtaining immediate, quantitative measures of gas or vapor concentrations in air. Also, aerosol monitors, integrating passive monitors for certain gases, and portable instrumentation for gas chromatography or infrared spectroscopy, are becoming more commonly used for measuring exposures to flavoring chemicals [ACGIH 2001; Soule 2000]. Many direct-reading instruments now used for personal or area measurements have evolved from laboratory or process control instruments. These types of monitoring techniques have significant advantages, although to date none of these methods has been validated for monitoring diacetyl, 2,3-pentanedione, or other flavoring compounds in the work environment.

### *10.2.5 When to Sample*

Because of the considerable variation in exposure during the production of food or flavoring products, individuals conducting air sampling should coordinate with plant management to ensure that sampling is conducted when food or flavoring products of particular interest are being manufactured. Sampling several products or production runs may be necessary to better characterize exposures. Additionally, some products may be produced infrequently, and production schedules may change rapidly, so the timing of sampling can be challenging. Exposure monitoring should be conducted whenever

changes in production processes, controls, work practices, or other conditions indicate a potential change in exposure conditions.

In order to determine compliance with STEL criteria, sampling should be done during tasks which are considered likely to produce the highest short term exposures. A series of sequential or overlapping samples can be taken for 15 minute intervals to determine the maximum exposures.

### *10.2.6 How Long to Sample*

In general, TWA exposures should be determined by collecting samples over a full work shift, normally 8 hours, for comparison with OELs and other toxicological data. Information on allowable sampling duration is given in validated sampling and analytical methods; depending on the method, in some instances it is necessary to collect multiple shorter-term samples to obtain an integrated 8-hour sample. Work shifts that exceed 8 hours require extended sampling duration.

When the potential for exposure to diacetyl, 2,3-pentanedione, or flavoring chemicals is sporadic throughout a work shift, short-term or task-based sampling may be needed to replace or supplement full-shift sampling. Short-term sampling periods are typically 5 to 120 minutes. Data from these short-term measurements can provide valuable perspective on task-based exposures and on the effectiveness of various control techniques. They can also be used to evaluate exposures relative to a short-term exposure limit, [Milz et al. 2003] such as the STEL values recommended for diacetyl and 2,3-pentanedione.

### *10.2.7 How Many Samples to Collect*

The numbers of samples to collect is important in that it relates to the confidence that can be placed in the exposure estimate. However, no predetermined number of samples should be taken to adequately determine a worker's flavoring chemical exposure, OEL

compliance, an average task-associated analyte concentration, or chemical point source; often occupational health decisions are made with limited numbers of samples. The number of samples needed for an accurate and reliable exposure assessment depends on the purpose of the sampling, the number of processes, work tasks or jobs to be evaluated, the variability inherent in the measured contaminant concentrations, sampling and analytical variability, and other factors. In most instances, time and budget constraints are major factors determining sample size. Statistical methods are available for calculating the minimum sample size needed to characterize a maximum risk employee exposure subgroup or to achieve a set degree of statistical confidence in the representativeness of an exposure measurement [NIOSH 1977, 1994; Snedecor and Cochran 1967; Soule 2000]. Recently, exposure control banding and Bayesian decision analysis have been used to help support exposure assessment decisions with more limited sample numbers [Hewett et al. 2006].

### *10.2.8 Sample Handling, Storage, and Shipment*

Following sampling, appropriate sample handling, storage, and shipping methods should be used. Some flavoring chemical analytes such as diacetyl are light sensitive and should be protected from light not only during sample collection but they should also be stored in the dark prior to analysis. Many volatile flavoring substance analytes should be stored and shipped refrigerated to ensure sample stability; this necessitates access to field refrigeration dedicated to sample storage. Some flavoring substance analytes/methods may have requirements for timely analysis or desorption to ensure analyte stability. Working closely with the analytical laboratory before sampling to determine the handling, storage, and shipping methods required for each analyte is advised. An American Industrial Hygiene Association or other accredited analytical laboratory should analyze collected samples. Consulting with the analytical laboratory before sampling to ensure that the measurement methods available can meet the defined sampling needs is essential.

## 10.3 Outcomes of Exposure Monitoring

### *10.3.1 Interpretation*

As stated above, a monitoring strategy should assess the effectiveness of various methods used to control airborne flavoring substance concentrations and to identify areas or tasks that are associated with higher exposures to flavoring substance. A common technique for evaluating the effectiveness of controls is to compare the outcome of environmental measurements made prior to the installation of those controls with measurements made following that installation. A control technique can be judged, for example, to be 50% efficient if the post-installation contaminant concentration is half of the pre-installation concentration.

The TWA and STEL measurements of exposure to flavoring substances, made with the collection of personal breathing zone air samples, can be used to assess workers' exposures relative to an OEL. As discussed in the section of this document describing the development of the RELs, a TWA measurement in excess of 5 ppb diacetyl or 9.3 ppb 2,3-pentanedione indicates that the worker in question was at a greater risk of developing occupationally induced illness. A short-term exposure in excess of 25 ppb diacetyl or 31 ppb 2,3-pentanedione during task based personal sampling would be interpreted similarly.

If monitoring indicates that exposures have increased over past measurements, or exposures exceed the selected OELs, a thorough investigation of controls to identify problems and guide remedial actions is needed. Regular routine monitoring (e.g., yearly) will help ensure the continued effectiveness of controls.

### *10.3.2 Notification of Workers*

Employers should establish procedures for the timely notification of workers of their environmental monitoring results, any identified exposure hazards, and any subsequent

actions taken based on this monitoring to reduce their exposures. Workers should be informed about any products or processes that may generate high concentrations of diacetyl, 2,3-pentanedione, or other flavoring chemicals and any PPE and changes in work practices needed in response. Employers should ensure that workers understand this information and their role in helping to maintain a healthful workplace. Information should be conveyed in English and other languages as needed to ensure that all workers receive and comprehend this information.

# Chapter 11: Research Needs

In this chapter, information gaps pertaining to diacetyl, 2,3-pentanedione and the area of flavoring-induced lung disease are identified. General areas of need include environmental analysis to better measure exposures; field studies to develop information on prevention, epidemiology and control of flavoring substances; research related to personal protective equipment; and toxicological studies concerning the etiology of related diseases.

Research in this area should address such questions as:

- Do larger powder particles tend to retain diacetyl more readily than smaller powder particles?
- Can a more sensitive analytic method be developed for 2,3-pentanedione that is comparable to the sensitivity and lower limit of quantification for diacetyl? Can more sensitive analytical methods be developed for other flavoring chemicals?
- How does one effectively measure exposure to airborne particulate for diacetyl and other flavoring chemicals?
- Can a real-time, portable sampling device be developed which will allow for both full-shift and peak exposure measurements for diacetyl and other flavoring agents?
- Is canister sampling with GC-MS analysis comparable to thermal desorption GC-MS for flavoring volatile organic compounds?
- Is the asthma excess in flavoring workers a misdiagnosis of fixed obstruction or part of the range of flavoring-related diseases or their natural history?
- What flavoring chemicals are responsible for the excess in restrictive spirometry seen in flavoring manufacturing worker populations? Does the spectrum of diacetyl-related lung disease include restrictive lung disease?
- Do biomarkers of flavoring exposure or lung injury exist that could be used in worker screening or diagnosis?
- Are there genetic markers for susceptibility for diacetyl-related respiratory effects?

- Can longitudinal examination of spirometry in flavoring-exposed workers for excessive declines be effective in primary and secondary prevention of lung impairment in flavoring workers? What are minimum quality requirements in spirometry equipment, technician performance, interpretation, and physician follow-up are necessary for flavoring-exposed worker medical surveillance?
- What proportions of excess obstructive lung disease in food production workers and in cooks are attributable to flavorings exposures?
- Can the effectiveness of a proposed standard (given the limitations of risk assessment) be substantiated by worker medical surveillance?
- Can flavoring-exposed workers undertake their personal medical surveillance with peak flow meters or portable spirometers?
- Could cohort mortality studies or a population-based registry of flavoring workers elucidate other flavoring-related risks, e.g., kidney toxicity, burden of respiratory mortality?
- What is the prevalence of fixed obstructive lung disease in workers making scented candles, hard candies, snack foods, dairy products, baked goods, fragrances, etc.?
- What non-flavoring volatile chemicals have similar inhalation toxicity for workers in industries already shown to have excess obstructive lung disease in population-based studies such as NHANES?

There is a need for field studies to assess exposures associated with various job tasks in the food production industries and to develop and validate control measures to reduce exposures to potentially harmful substances. Research in this area should address such questions as:

- What are appropriate variability estimates for occupational exposures in food production facilities?

- What jobs have peak exposures that may be pertinent to health risks of flavoring exposures?
- What are the major food production processes involving flavorings that require engineering controls?
- What flavoring chemicals should be of most concern when developing engineering controls?
- Is there a concern for low-level mixed exposures to flavorings?
- What are the exposures for the downstream workers in food production processes or workplaces?
- What work practice interventions most effectively reduce worker exposure?

Also needed is further research for developing guidance for respirators and personal protective equipment that will continue to have an important role in worker protection. Other research needs have been identified in the area of respirators and other personal protective equipment.

- What methodology should be used for respirator selection?
- What gloves should be used in the workplace and how frequently should they be changed?
- What guidance can be provided regarding change-out schedules for organic vapor cartridges used in flavoring production?
- What are the end-of-service indicators for respirators used in mixed environments?

Regarding the health effects of diacetyl and 2,3-pentanedione, unanswered questions include the following:

- What is the chronic respiratory toxicological pathology and pathophysiology of diacetyl inhalation?
- Can a CFD-PBPK model of diacetyl or 2,3-pentanedione absorption during chronic exposure be developed?

- What is the role of metabolism and adhesion molecules in diacetyl toxicity?
- What is the role of immunotoxicity in diacetyl toxicity?
- Do inhalation-related and lung transplant-associated bronchiolitis obliterans share common mechanisms of injury?
- What role do other components of butter flavoring play on diacetyl-induced respiratory tract injury?
- What is the respiratory toxicity of substitutes for diacetyl and 2,3-pentanedione?
- What are the pathophysiological mechanisms of acute and chronic diacetyl toxicity?
- Which butter flavoring powders cause the least and the most airway injury?
- Can in vitro models of acute and chronic exposures provide information useful to risk assessment?

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# APPENDIX 1

# APPENDIX 1
# SAMPLING AND ANALYTICAL METHODS FOR DIACETYL AND 2,3-PENTANEDIONE

Currently validated sampling and analytical methods to determine airborne concentrations of diacetyl include OSHA Method PV2118, OSHA Method 1012, and 1013 OSHA Method. OSHA 1016 is validated for 2,3-pentanedione. These methods are presented in this appendix.

NIOSH Method 2549 for the qualitative determination of volatile organic compounds has also been used extensively in diacetyl research discussed in this document and is therefore also presented here.

**APPENDIX 1**

**TABLE OF CONTENTS**

# Diacetyl

*Related Information: Chemical Sampling - Diacetyl*

| | |
|---|---|
| Method no.: | PV2118 |
| Control no.: | T-PV2118-01-0301-CH |
| Target concentration: | 25 ppm ( 88 mg/m$^3$) |
| Procedure: | Samples are collected by drawing a known volume of air through two silica gel sampling tubes connected in series. Samples are extracted with ethyl alcohol: water (95:5) and analyzed by GC using a flame ionization detector (FID). |
| Recommended sampling time and sampling rate: | 60 min at 0.05 L/min (3 L) |
| Reliable quantitation limit: | 0.28 ppm (1.00 mg/m$^3$) |
| Special requirements: | Samples are collected on two silica gel tubes in series. The second tube is used as a backup for the first tube. Samples should be protected from the light after sampling. |
| Status of method: | Partially evaluated method. This method has been subjected to established evaluation procedures of the Method Development Team and is presented for information and trial use. |
| Date: | January 2003 (revised September 2006) |
| Chemist: | Yogi C. Shah |

Chromatography Team
Industrial Hygiene Chemistry Division
OSHA Salt Lake Technical Center
Salt Lake City UT 84115-1802

1. General Discussion
   1.1 Background
      1.1.1 History

The purpose of this evaluation was to develop a sampling procedure for diacetyl that gave a better storage stability than did the NIOSH Method 2557, which used SKC Anasorb CMS as the sampling media[1].The NIOSH method requires that the samples be refrigerated immediately after sampling, and the analysis be performed within 7 days. A more stable sampling media was desired for OSHA samples. The following media were tested at SLTC but all gave poor storage stability: coconut shell charcoal Lot 2000, 4-*tert*-butylcatechol coated charcoal, XAD-7, and OVS-7. Silica gel tubes (150mg/75 mg) were tried next and had an average storage recovery of 94.9% for samples stored at room temperature for 14 days. A sampling train of two silica gel tubes in series was necessary because a significant amount of the diacetyl was found on the smaller, backup section of the first tube in the retention study. A second tube in series insures that all of the sample will be collected on the sampling train. The desorbing solvent of 95:5 ethyl alcohol:water with 0.25 µL/mL *p*-cymene internal standard gave an average recovery of 99.1% over the concentration range of 26.5 to 529 µg of diacetyl.

1.1.2 Toxic Effects[2] (This section is for information only and should not be taken as the basis of OSHA policy.)

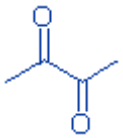
In 2002, the CDC published a report in the Morbidity and Mortality Weekly Report (MMWR) on employee exposures at a microwave popcorn factory in Missouri. A group of former employees had developed fixed airways obstructive lung disease. All eight had a respiratory illness that resembled a rare lung disease called bronchiolitis obliterans. Some of the cases had such

severe illness they were candidates for lung transplants. The main volatile organic chemical (VOC) found in the workplace atmospheres was diacetyl, which was used in a mixture of heated soybean oil, salt and flavorings to impart a butter flavoring to the popcorn. During NIOSH's investigation of the facility, diacetyl was chosen as a marker compound for VOC exposure. The MMWR publication reported that the geometric mean air concentration of diacetyl was 18 ppm in the room where the mixing tank was located, 1.3 ppm in the packaging area, and 0.02 in other areas of the plant. Of the eight former employees with severe respiratory illness, four were mixers and four worked in packaging. The report concluded that "workers exposed to flavorings at microwave popcorn factories are at risk for developing fixed obstructive lung disease."

1.1.3 Workplace exposure[3,4]

Diacetyl is a naturally occurring chemical in bay and other oils, beer, butter, coffee, vinegar, and other food products. It is an artificial flavoring which adds the flavor of butter, cream or creaminess, and butterscotch.

1.1.4 Physical properties and other descriptive information[3,4]

| | | | |
|---|---|---|---|
| CAS number: | 431-03-8 | IMIS: | D740[5] |
| RTECS number: | EK2625000 | molecular weight | 86.09 |
| melting point | -3ºC | boiling point: | 88ºC |
| appearance: | green-yellow liquid | molecular formula: | $C_4H_6O_2$ |
| odor: | characteristic buttery | flashpoint: | 6ºC |
| autoignition temperature: | 365ºC | density (g/mL): | 0.99 |
| solubility: | ether; alcohol; acetone. DMSO | | |
| synonyms: | 2,3 -butanedione; 2,3 -diketobutane; dimethyl diketone; dimethylglyoxal | | |
| structure: |  | | |

This method was evaluated according to the OSHA SLTC "Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis"[6]. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations and acceptance criteria. The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters. Air concentrations listed in ppm are referenced to 25°C and 101.3 kPa (760 mmHg).

1.2 Detection Limit of the Overall procedure (DLOP) and Reliable Quantitation Limit (RQL)

The DLOP is measured as mass per sample and expressed as equivalent air concentrations, based on the recommended sampling parameters. Ten samplers were spiked with equal descending increments of analyte, such that the highest sampler loading was 3.7 µg diacetyl. This is the amount spiked on a sampler that would produce a peak approximately 3 times the response for a sample blank. These spiked samplers were analyzed with the recommended analytical parameters, and the data obtained used to calculate the required parameters (standard error of estimate and slope) for the calculation of the DLOP. The slope was 13.89 and the SEE was 41.82. The RQL is considered the lower limit for precise quantitative measurements.

Table 1.2
Detection Limit of the Overall Procedure for Diacetyl

| Mass per sample (µg) | area counts (µV-s) |
|---|---|
| 0.00 | 293 |
| 1.3 | 504 |
| 1.58 | 631 |
| 1.85 | 658 |
| 2.11 | 676 |
| 2.38 | 700 |
| 2.64 | 734 |
| 2.90 | 759 |
| 3.17 | 788 |
| 3.43 | 816 |
| 3.70 | 838 |

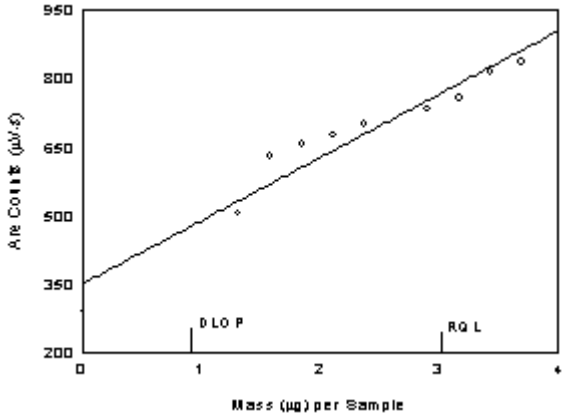



**Figure 1.2.1 Plot of data to determine the DLOP/RQL for diacetyl. (Y=139X+349)**

RQL is determined from the regression line parameters obtained for the calculation of the DLOP, providing 75% to 125% of the analyte is recovered. The DLOP and RQL were 0.902 µg and 3.01 µg respectively

Below is chromatogram of the RQL level.

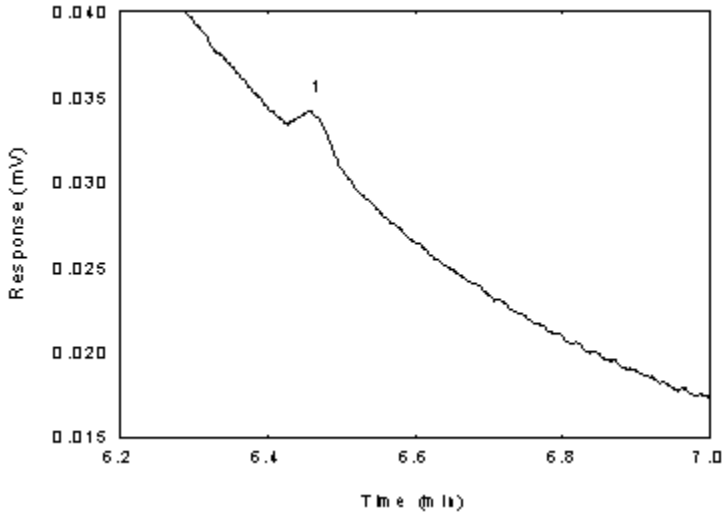



**Figure 1.2.2 Chromatogram of the diacetyl standard near RQL (key: (1) diacetyl).**

2. Sampling Procedure

All safety practices that apply to the work area being sampled should be followed. The sampling equipment should be attached to the worker in such a manner that it will not interfere with work performance or safety.

2.1 Apparatus

2.1.1 Samples are collected using a personal sampling pump calibrated, with the sampling device attached, to within ±5% of the recommended flow rate.

2.1.2 Silica gel tubes: glass tube with both ends flame sealed, 70 mm × 6-mm i.d. containing 2 sections of 20/40 mesh silica gel

separated by a 2-mm portion of urethane foam. The adsorbing section contains 150 mg of silica gel, the backup section 75 mg. A 3-mm portion of urethane foam is placed between the outlet end of the tube and the backup section. A plug of silane-treated glass wool is placed in front of the front section( SKC No. 226-10) tubes or equivalent was used in this evaluation.

2.2 Reagents

None required.

2.3 Technique

2.3.1 Immediately before sampling, break off the ends of the flame-sealed tube to provide an opening approximately half the internal diameter of the tube. Wear eye protection when breaking ends. Use tube holders to minimize the hazard of broken glass. All tubes should be from the same lot.

2.3.2 Connect two tubes in series to the sampling pump with flexible tubing. The smaller sections of the silica gel tubes should be positioned nearer the sampling pump. The tube closer to the pump is used as a backup. A minimum amount of tubing is used to connect the two sampling tubes together. Position the sampling pump, tube holder and tubing so they do not impede work performance or safety.

2.3.3 Draw the air to be sampled directly into the inlet of the tube holder. The air being sampled is not to be passed through any hose or tubing before entering the sampling tube

2.3.4 After sampling for the appropriate time, remove the adsorbent tube and seal it with plastic end caps. Seal each sample end-to-end with an OSHA-21 form as soon as possible.

2.3.5 Submit at least one blank sample with each set of samples. Handle the blank sample in the same manner as the other samples except draw no air through it.

2.3.6 Record sample air volumes (liters), sampling time (minutes) and sampling rate (mL/min) for each sample, along with any potential interferences on the OSHA-91A form.

2.3.7 Submit the samples to the laboratory for analysis as soon as possible after sampling. If delay is unavoidable, store the samples at refrigerator temperature. Ship any bulk samples separate from the air samples.

2.4 Extraction efficiency

The extraction efficiency was determined by liquid-spiking silica gel tubes with diacetyl at 0.1 to 2 times the target concentration. These samples were stored overnight at ambient temperature and then extracted for 30 minutes with occasional shaking and analyzed. The mean extraction efficiency over the studied range was 99.1%. The wet extraction efficiency was determined at the target concentration by liquid spiking the analyte on the front, larger, section of the first silica gel tube of the sampling train of two silica gel tubes in series, and drawing 3 L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2ºC) through them. The mean recovery for the wet samples was 100.2 %

Table 2.4
Extraction Efficiency (%) of Diacetyl

| *level* | | *sample number* | | | | | |
|---|---|---|---|---|---|---|---|
| ×target concn | µg per sample | 1 | 2 | 3 | 4 | 5 | mean |
| 0.1 | 26.5 | 105.0 | 105.0 | 105.8 | 100.3 | 100.8 | 103.4 |
| 0.25 | 66.5 | 110.4 | 98.6 | 100.5 | 97.7 | 100 | 101.4 |
| 0.5 | 133 | 91.0 | 90.8 | 90.8 | 90.6 | 95.1 | 91.7 |
| 1.0 | 265 | 98.8 | 100.3 | 99.1 | 98.9 | 99.6 | 99.3 |
| 2.0 | 529 | 100.8 | 101.3 | 99.2 | 98.7 | 99.6 | 99.9 |
| 1.0 (wet) | 265 | 104.2 | 101.6 | 99.6 | 93.3 | 102.2 | 100.2 |

2.5 Retention efficiency

Six silica gel tubes were spiked with 0.265 mg (25.ppm) of diacetyl and allowed to equilibrate for 6 h at room temperature in a drawer.. The spiked tubes were placed in series with a second unspiked silica gel tube and had 3 L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2ºC) pulled through them at 0.05 L/min. The samples were extracted and analyzed. The mean retention recovery was 94.3%. There was no analyte found on the backup section of any of the tubes.

Table 2.5
Retention Efficiency (%) of Diacetyl

| | *sample number* | | | | | | |
|---|---|---|---|---|---|---|---|
| section | 1 | 2 | 3 | 4 | 5 | 6 | mean |
| front (a+b) | 94.3 | 94.1 | 96.8 | 93.7 | 93.8 | 93.1 | 94.3 |
| rear (a) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| rear (b) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total | 94.3 | 94.1 | 96.8 | 93.7 | 93.8 | 93.1 | 94.3 |

2.6 Sample storage

Nine silica gel tubes were spiked with 0.265 mg (25.ppm) of diacetyl and allowed to equilibrate for 6 h at room temperature in a drawer. The tubes were placed in series with a second unspiked silica gel tube and had 3 L humid air (absolute humidity of 15.9 mg/L of water, about 80% relative humidity at 22.2ºC) pulled through them at 0.05 L/min. Three samples were analyzed immediately, and the rest were sealed and stored at room temperature in a drawer. Three more were analyzed after 7 days of storage and the remaining three after 14 days of storage. The amounts recovered indicate good storage stability for the time period studied.

Table 2.6
Storage Test for Diacetyl (% Recovery)

| | sample number | | | |
|---|---|---|---|---|
| time (days) | 1 | 2 | 3 | mean |
| 0 | 99.4 | 96.9 | 96.2 | 97.5 |
| 7 | 94.5 | 97.7 | 95.0 | 95.7 |
| 14 | 97.2 | 92.8 | 94.8 | 94.9 |

2.7 Recommended air volume and sampling rate.

Based on the data collected in this evaluation, 3-L air samples should be collected at a sampling rate of 0.05 L/min for 60 minutes.

2.8 Interferences (sampling)

2.8.1 There are no known compounds that will severely interfere with the collection of diacetyl.

2.8.2 Suspected interferences should be reported to the laboratory with submitted samples.

3. Analytical Procedure

Adhere to the rules set down in your Chemical Hygiene Plan. Avoid skin contact and inhalation of all chemicals and review all appropriate MSDSs.

3.1 Apparatus

3.1.1 A gas chromatograph equipped with an FID. For this evaluation, an Agilent 6890 Plus gas Chromatograph equipped with a 7683 Automatic Sampler was used.

3.1.2 A GC column capable of separating diacetyl from the desorption solvent, internal standard and any potential interferences. A 60-m × 0.32-mm i.d. capillary DBWAX with a 0.5-µm df (J&W Scientific) was used in the evaluation.

3.1.3 An electronic integrator or some other suitable means of measuring peak areas. A Waters Millennium$^{32}$ Data System was used in this evaluation.

3.1.4 Amber glass vials with poly(tetrafluoroethylene)-lined caps. For this evaluation 2-mL vials were used.

3.1.5 A dispenser capable of delivering 1.0 mL of desorbing solvent to prepare standards and samples. If a dispenser is not available, a 1.0-mL volumetric pipet may be used.

3.1.7 Volumetric flasks - 10-mL and other convenient sizes for preparing standards.

3.1.8 Calibrated 10-µL syringe for preparing standards.

3.2 Reagents

3.2.1 Diacetyl, Reagent grade. Aldrich 99% (lot 09122TS BO) was used in this evaluation.

3.2.2 Ethyl alcohol, USP grade 190 proof. Aaper (lot 98G23BB ) was used for this evaluation.

3.2.3 *p*-Cymene, Reagent grade. Aldrich 99% (lot 306PZ) was used in this evaluation.

3.2.4 The extraction solvent was 0.25 µL/mL *p*-cymene in ethyl alcohol:water (95:5).

3.2.5 GC grade nitrogen, air, and hydrogen.

3.3 Standard preparation

3.3.1 Prepare working analytical standards by injecting micro liter amounts of diacetyl into volumetric flasks containing the extraction solvent. An analytical standard at a concentration of 0.530 mg/mL (5.3 µL/10 mL) is equivalent to 50 ppm based on a 3-L air volume. Stock standards were stored in amber vials at refrigerated temperature for stability.

3.3.2 Bracket sample concentrations with working standard concentrations. If sample concentrations are higher than the concentration range of prepared standards, prepare and analyze additional standards, at least as high a concentration as the highest sample, to ascertain the linearity of response, or dilute the sample with extracting solvent to obtain a concentration within the existing standard range. The range of standards used in this study was from 0.00132 to 0.60 mg/mL analyze additional standards, at least as high a concentration as the highest sample, to ascertain the linearity of response, or dilute the sample with extracting solvent to obtain a concentration within the existing standard range. The range of standards used in this study was from 0.00132 to 0.60 mg/mL.

3.4 Sample preparation

3.4.1 Remove the plastic end caps from the sample tubes and carefully transfer both adsorbent sections from front tube and each section of backup tube to separate labeled 2-mL amber glass vials. Discard the glass tube and glass wool plug.

3.4.2 Add 1.0 mL of extraction solvent to each vial using the same dispenser as used for preparation of standards.

3.4.3 Immediately seal the vials with poly(tetrafluoroethylene)-lined caps.

3.4.4 Place vials on shaker and agitate for 60 minutes.

3.5 Analysis

3.5.1 Analytical conditions.

| GC conditions | |
|---|---|
| injector: | 200°C |
| detector: | 250°C |
| run time: | 16 min |
| column gas flow: | 2.5 mL/min (hydrogen) |
| septum purge: | 1.9 mL/min (hydrogen) |
| injection size: | 1.0 µL (10:1 split) |
| column: | 60-m x 0.32-mm i.d. capillary DBWAX (0.5-um df) |
| column temperatures: | 50°C for 6 min, 15°C/min to 150°C final time 3 min |
| retention times: | 5.51 min ethyl alcohol, 6.48 min diacetyl, 12.46 min p-cymene |

| FID conditions | |
|---|---|
| hydrogen flow | 30mL/min |
| air flow: | 400 mL/min |
| makeup flow: | 25 mL/min (nitrogen |

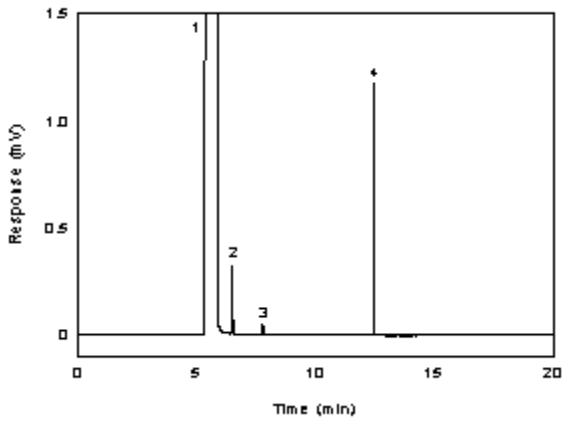



**Figure 3.5.1 A chromatogram of 268 µg/ml diacetyl in 95:5 ethyl alcohol:water with 0.25 µl of p-cymene as internal standard. (Key: (1) ethyl alcohol, (2) diacetyl, (3) impurity, and (4) p-cymene).**

3.5.2 Peak areas are measured by an integrator or other suitable means.

3.5.3 An internal standard (ISTD) calibration method is used. A calibration curve can be constructed by plotting ISTD-corrected response of standard injections versus micrograms of analyte per sample. Bracket the samples with freshly prepared analytical standards over a range of concentrations

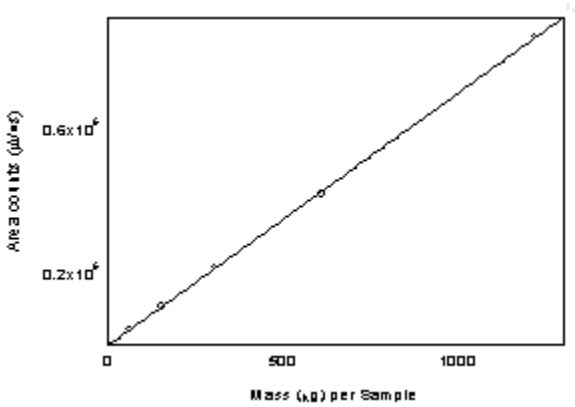



**Figure 3.5.3 Calibration curve of diacetyl.**
**(Y = 696 x - 336)**

3.6 Interferences (analytical)

3.6.1 Any compound that produces a GC response and has a similar retention time as the analyte is a potential interference. If any potential interferences were reported, they should be considered before samples are extracted. Generally, chromatographic conditions can be altered to separate an interference from the analyte.

3.6.2 When necessary, the identity or purity of an analyte peak may be confirmed by mass spectrometry or by another analytical procedure. The mass spectrum in Figure 3.6.2 was from the NIST spectral library.

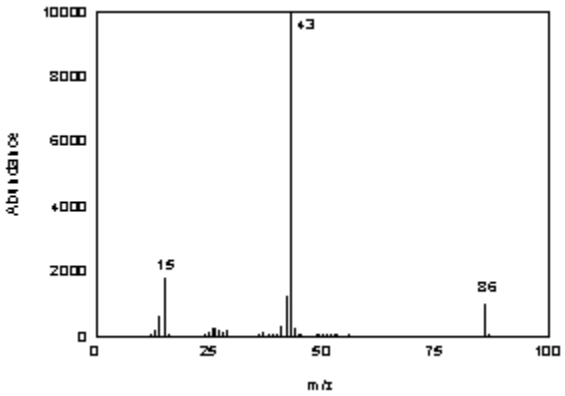



**Figure 3.6.2 Mass spectrum of diacetyl.**

3.6.3 Calculations

The amount of analyte per sampler is obtained from the appropriate calibration curve in terms of micrograms per sample, uncorrected for extraction efficiency. This total amount is then corrected by subtracting the total amount (if any) found on the blank. The air concentration is calculated using the following formulas.

$$C_M = \frac{M}{VE_E}$$

where $C_M$ is concentration by weight (mg/m$^3$)
M is micro grams per sample
V is liters of air sampled
$E_E$ is extraction efficiency, in decimal form

$$C_V = \frac{V_M C_M}{M_r}$$

where $C_V$ is concentration by volume (ppm)
$V_M$ is molar volume ate 25°C=24.46
$C_M$ is concentration by weight
$M_r$ is molecular weight = 86.09

4. Recommendations for Further Study

Several other tests need to be performed to make this a validated method.

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1. NIOSH Method 2557.

2. Simoes E., et al. (2002) "Fixed Obstructive Lung Disease in Workers at a Microwave Popcorn Factory – Missouri" 2000-2002. MMWR 51(16):345-347.

3. O'Neil, M., *The Merck Index,* 13th ed., Merck & Co. Inc.: Whitehouse Station, NJ, 2001, p 522.

4. Lewis, R., *Sax's Dangerous Properties of Industrial Materials*, 10th ed., Vol. 2, John Wiley & Sons, New York, 2000, p 595.

5. OSHA Chemical Sampling Guide.

6. Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. C. *Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis* ; OSHA Salt Lake Technical Center, U.S. Department of Labor: Salt Lake City, UT, 1999.

Acetoin
Diacetyl

| | |
|---|---|
| Method no.: | 1012 |
| Control no.: | T-1012-FV-01-0811-M |
| Target concentration: | 0.05 ppm (TWA) (0.18 mg/m$^3$) acetoin<br>0.05 ppm (TWA) (0.18 mg/m$^3$) diacetyl |
| OSHA PEL: | none acetoin<br>none diacetyl |
| ACGIH TLV: | none acetoin<br>none diacetyl |
| Procedure: | Samples are collected by drawing workplace air through two tubes containing specially cleaned and dried silica gel connected in series. Samples are extracted and derivatized with a solution of 95:5 ethyl alcohol:water containing 2 mg/mL of *O*-(2, 3, 4, 5, 6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) and analyzed by gas chromatography using an electron capture detector (GC-ECD). |
| Recommended sampling time and sampling rate: | 180 min at 0.05 L/min (9.0 L) (TWA)<br>15 min at 0.2 L/min (3.0 L) (short term) |
| Reliable quantitation limit: | 1.49 ppb (5.37 µg/m$^3$) acetoin<br>1.30 ppb (4.57 µg/m$^3$) diacetyl |
| Standard error of estimate at the target concentration: | 5.06% acetoin<br>5.11% diacetyl |
| Special requirements: | Protect samplers from the light during and after sampling with aluminum foil or opaque tape. |
| Status of method: | Evaluated method. This method has been subjected to the established evaluation procedures of the OSHA Salt Lake Technical Center Methods Development Team. |

November 2008

Mary E. Eide

Methods Development Team
Industrial Hygiene Chemistry Division
OSHA Salt Lake Technical Center
Sandy UT 84070-6406

1. General Discussion

For assistance with accessibility problems in using figures and illustrations presented in this method, please contact Salt Lake Technical Center (SLTC) at (801) 233-4900. This procedure was designed and tested for internal use by OSHA personnel. Mention of any company name or commercial product does not constitute endorsement by OSHA.

1.1 Background

1.1.1 History

On September 24, 2007 OSHA issued a Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl[1] in which diacetyl was identified as an indicator compound for hazardous exposures found at plants packaging microwave popcorn. This was based on Health Hazard Evaluations performed by NIOSH which found the occurrence of severe lung disease in some employees at microwave popcorn packaging plants and flavorings manufacturing facilities. In three NIOSH Health Hazard Evaluation reports, acetoin and diacetyl are listed as major constituents of butter flavoring and they were used as indicators of exposure to butter flavoring vapors.[2,3,4]

OSHA has a partially validated method for diacetyl, PV2118, which recommends the use of two standard sized silica gel tubes in series to collect diacetyl at 0.05 L/min for 1 hour.[5] There were three reasons a new method was needed: 1) the reliable quantitation limit of PV2118 is 0.28 ppm which is higher than the target concentration of 0.05 ppm for this method; 2) a new medium was needed to enable the industrial hygienist to sample for a longer sampling time and take fewer samples; and 3) to allow acetoin and diacetyl to be sampled and analyzed together. The new medium used in this method is a tube packed with specially cleaned and dried silica gel (600 mg) with a glass wool plug and a glass fiber filter in front of the dried silica gel bed (this medium is referred to as dried silica gel in this method). It was necessary to specially dry the silica gel to obtain a higher capacity because of the amount of water already present on the silica gel in the currently commercially available tubes. The dried silica gel tube can be used to sample diacetyl for up to 1.5 times longer than the currently available silica gel tube. There was not a capacity problem with acetoin. The powder and liquid formulated forms of acetoin and diacetyl may contain oily compounds and other base materials such as maltodrextin. These materials could affect the extraction of acetoin and diacetyl from the silica gel. The glass fiber filter in the tube serves only to trap these materials before they enter the silica gel bed. Retention studies using a powder containing acetoin and diacetyl showed that the acetoin and diacetyl can be stripped off the powder and collected on the silica gel, especially when sampling high humidity air. (Section 4.9)

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1 Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dsg/guidance/diacetyl-guidance.html (accessed 3/17/2008).

2 HETA 2001-0474-2943 American Pop Corn Company, 2004. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2001-0474-2943.pdf (accessed 3/15/2008).

3 HETA 2002-0408-2915 Agrilink Foods Popcorn Plant, 2003. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2002-0408-2915.pdf (accessed 3/15/2008).

4 HETA 2003-0112-2949 ConAgra Snack Foods, 2004. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2003-0112-2949.pdf (accessed 3/15/2008).

5 Shah, Y. C. OSHA Diacetyl (OSHA Method PV2118), 2003. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/partial/t-pv2118/t-pv2118.html (accessed 3/17/2008).

To obtain adequate sensitivity for this method, it was necessary to derivatize the acetoin and diacetyl. 2,4-Dinitrophenyl hydrazine (DNPH) was the first derivatizing agent tried, but DNPH can react with both ketone and α-hydroxy ketones[6], and while it initially formed unique derivatives of acetoin and diacetyl by reacting with the first ketone group, it eventually reacted also with the alcohol group on acetoin and the second ketone group on diacetyl, forming the same derivative. In EPA Method 556.1 *O*-pentafluorobenzyl hydroxylamine hydrochloride (PFBHA) was used to derivatize ketone and aldehyde groups.[7] Unique derivatives of acetoin and diacetyl are formed by reacting them with PFBHA. The first ketone group on diacetyl reacts within four hours with PFBHA, but the second ketone group takes 36 hours to reach completion. Acetoin reacts within 3 hours. In this method, samples are extracted and derivatized in an extraction solution containing PFBHA. This is accomplished by first rotating the samples for 60 min and then allowing the samples to stand at room temperature for an additional 36 hours for the derivatization reaction to reach completion.

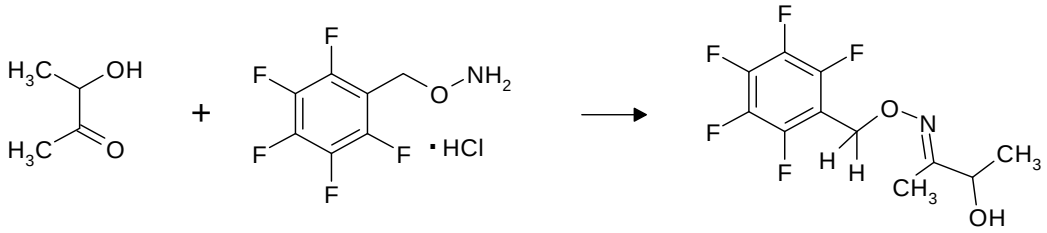

Figure 1.1.1.1. The reaction of acetoin with PFBHA to form the acetoin-PFBHA derivative.

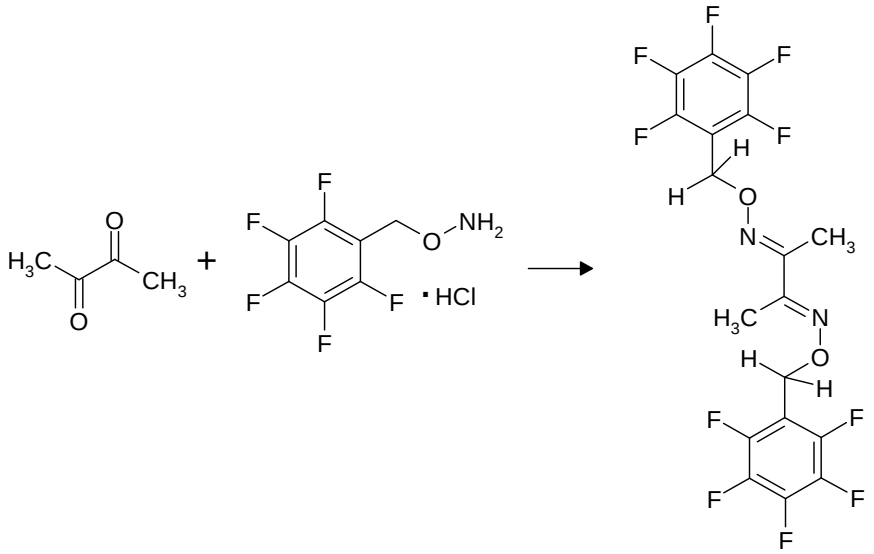

Figure 1.1.1.2. The reaction of diacetyl with PFBHA to form the diacetyl-PFBHA derivative.

This method is designed for low air concentrations of acetoin, diacetyl, and potential interferences. If high exposures are anticipated, use OSHA Method 1013[8] or increase

6 Smith, M., March, J.; *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure,* 5th ed.; John Wiley & Sons Inc.: New York, 2001, p 1193.

7 EPA Method 556.1 Determination of Carbonyl Compounds in Drinking Water by Fast Gas Chromatography, 1999. U.S. Environmental Protection Agency Web site. http://www.epa.gov/safewater/methods/pdfs/methods/met556_1.pdf (accessed 3/17/2008).

8 Simmons, M., Hendricks, W. Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).

the amount of PFBHA in the extraction solution to ensure complete derivatization. Samples extracted by OSHA Method 1013 can be derivatized and analyzed by this method to detect lower concentrations.

1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.)

NIOSH Health Hazard Evaluations (HHE) of microwave popcorn manufacturing plants found fixed airway obstruction, in some cases, consistent with bronchiolitis obliterans in some employees. [9] Acetoin, diacetyl, acetic acid, acetaldehyde, and 2-nonanone were amongst the chemicals found by NIOSH in several popcorn manufacturing plants.[10] Diacetyl was found to be present in all workplaces where the bronchiolitis obliterans was observed, and acetoin was found in some of the workplaces. Animal toxicology studies were performed by NIOSH with diacetyl, or butter flavorings containing diacetyl. Respiratory tract damage, including necrosis of the nasal and tracheal epithelium, and death were reported in rodents exposed to diacetyl, and butter flavorings containing diacetyl, at an air concentration of approximately 200 ppm of diacetyl for 6 hours. Mice exposed to 200 and 400 ppm diacetyl via inhalation for 6 hours per day over 5 days had the following health effects: death, acute necrotizing rhinitis, and erosive or necrotizing laryngitis. Mice exposed to 200 and 400 milligrams per kilogram (mg/kg) diacetyl via oropharyngeal aspiration for 6 hours per day over 5 days had bronchiolar fibrosis and death. Rats exposed to butter flavoring vapors containing 300 ppm diacetyl for 6 hours had epithelial injury in the nasal passages and pulmonary airways.[11]

1.1.3 Workplace exposure

Workers are exposed to acetoin and diacetyl in various manufacturing processes. Acetoin and diacetyl are natural flavorings that are also synthesized for use in odor and flavor manufacturing.[12,13] Acetoin and diacetyl are found in tobacco smoke, vapors from garbage, vapors from liquid and solid animal wastes, exhaust emissions from petroleum based fuels, vapors from moldy buildings, charcoal production, vapors from latex-polyurethane backed carpet, and as chemical reagents and in chemical reactions.[14] Diacetyl is also used as an anti-microbial preservative, modifier of radiation responses for chemical and biological systems, and as a photoinitializer in polymerization of plastics.

Occupational exposure to acetoin and diacetyl in microwave popcorn manufacturing has been studied since the first reported case of severe obstructive lung disease in 2000.[15] NIOSH identified acetoin and diacetyl as useful indicator compounds that can

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[9] Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dsg/guidance/diacetyl-guidance.html (accessed 3/17/2008).

[10] Flavorings-Related Lung Disease: Health Hazard Evaluations. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www.cdc.gov/niosh/topics/flavorings/hhe-eval.html (accessed 3/17/2008).

[11] Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dsg/guidance/diacetyl-guidance.html (accessed 3/17/2008).

[12] *Fenarolli's Handbook of Flavor Ingredients,* 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 11.

[13] *Fenarolli's Handbook of Flavor Ingredients,* 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 411.

[14] Chemical Information Review Document for Artificial Butter Flavoring and Constituents Diacetyl (CAS No. 431-03-8) and Acetoin (CAS No. 513-86-0), 2007. Department of Health and Human Services, National Toxicology Program Web site. http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumpdf/ Artificial_butter_flavoring.pdf (accessed 3/17/2008).

[15] HETA 2000-0401-2991 Gilster-Mary Lee Corporation, 2000. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www2a.cdc.gov/hhe/select.asp?PjtName=40422&bFlag=1&ID=1 (accessed 3/17/2008).

be used to represent exposure to butter flavorings. Areas of concern were the flavor production rooms, mixing/blending rooms, packaging/production rooms, rooms where the mixing tanks were located, maintenance and cleaning operations, and quality control labs.[16]

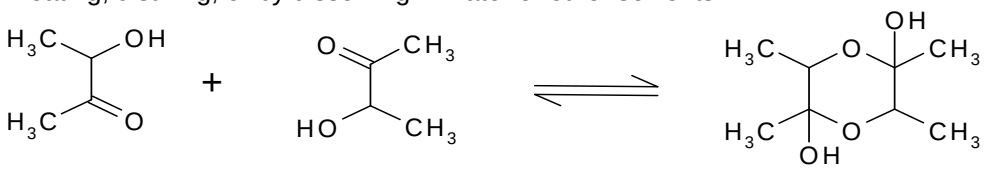
Acetoin is used as an aroma carrier and as a flavor ingredient to impart a creamy taste in fragrances and flavorings.[17] Acetoin annual use in food and flavors manufacturing in 2004 was 34,000 pounds. Acetoin is used as a flavor ingredient for butter, milk, yogurt, and strawberry flavors. The FDA maximum allowable concentration for acetoin in beverages is 5 ppm, and in food is 50 ppm. Acetoin is naturally found in fresh apple, cooked apple, leek, cooked leek, corn, honey, cocoa, butter, roasted coffee, cheeses, yogurt, milk, wines, beer, fermented tea, scallops, crowberry, quince, and other sources. Acetoin is used in manufacturing alcoholic beverages, baked goods, breakfast cereals, cheese, chewing gum, condiments and relishes, confections and frostings, fats and oils, frozen dairy products, fruit juices, gelatins and puddings, gravies and mixes, hard candy, imitation dairy products, meat products, milk products, nonalcoholic beverages, grains, reconstituted vegetables, seasonings and flavorings, snack foods, soft candy, soups, and sweet sauce.

Diacetyl is used as a fragrance and flavor ingredient to give products a buttery or creamy odor and flavor.[18] Diacetyl annual use in food and flavor manufacturing in 2004 was 153,500 pounds. The FDA maximum allowable concentration for diacetyl in beverages is 5 ppm, and in food is 50 ppm. Diacetyl naturally occurs in butter, milk products, yogurt, grains, meat, wines, beer, oils of pine, oil of angelica, oils of lavender and other flowers, many flowers, raspberries, strawberries, citrus, ligonberry, guava, cabbage, peas, tomato, vinegar, cheeses, chicken, beef, mutton, pork, cognac, whiskies, tea, and coffee. Diacetyl is used in manufacturing as a flavoring in alcoholic beverages, baked goods, cheese, chewing gum, fats and oils, frozen dairy products, gelatins and puddings, gravies, hard candy, soft candy, imitation dairy, meat products, milk products, nonalcoholic beverages, and snack foods.

### 1.1.4 Physical properties and other descriptive information

acetoin[19,20,21]

Acetoin is found as the liquid monomer and the solid dimer. The pure monomer forms the dimer at room temperature. The monomer can be formed from the dimer by heating, distilling, or by dissolving in water or other solvents.



[16] HETA 2001-0474-2943 American Pop Corn Company, 2001. Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health Web site. http://www2a.cdc.gov/hhe/select.asp?PjtName=36271&bFlag=0&ID=2 (accesed 3/17/2008).

[17] *Fenarolli's Handbook of Flavor Ingredients,* 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 11.

[18] *Fenarolli's Handbook of Flavor Ingredients,* 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 411.

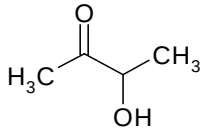
[19] Budavari, S., Ed; *The Merck Index*, 13th ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 2001; p 68.

[20] Material Safety Data Sheet: Acetoin, Chemwatch, Victoria, Australia (accesed 3/17/08).

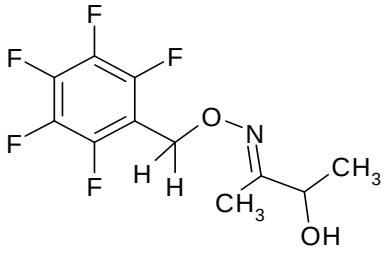
[21] Acetoin MSDS. SigmaAldrich Web site. http://www.sigmaaldrich.com/catalog/search/ProductDetail/ALDRICH/A17951 (accessed 3/17/2008).

| | |
|---|---|
| synonyms: | acetyl methyl carbinol; 2,3-butanolone; 2-butanone, 3-hydoxy-; 2-butanol-3-one; dimethylketol; $\gamma$-hydroxy-$\beta$-oxobutane; 3-hydroxybutan-2-one; 3-hydroxy-2-butanone; 1-hydroxyethyl methyl ketone; methyl acetyl carbinol |
| IMIS[22]: | A624 |
| CAS number: | 513-86-0 (monomer); 23147-57-1 (dimer)[23] |
| boiling point: | 148 ºC (298 ºF) (monomer) |
| melting point: | 15 ºC (59 ºF) (monomer); 90 ºC (194 ºF) (dimer) |
| density: | 1.005 g/mL @ 20/20 (monomer) |
| molecular weight: | 88.11 (monomer) |
| flash point: | 50.6 ºC (123 ºF) (closed cup) (monomer) |
| autoignition temperature: | 370 ºC (773.8 ºF) |
| appearance: | clear to light yellow liquid (monomer); light cream to light yellow crystals (dimer) |
| vapor density: | >1 (air = 1) |
| molecular formula: | $C_4H_8O_2$ (monomer); $C_8H_{16}O_4$ (dimer) |
| odor: | pleasant buttery odor |
| solubility: | soluble in water; miscible with alcohol; sparingly soluble in ether and petroleum ether |
| reactive hazards: | acetoin is light sensitive [24] (Section 4.9) |

structural formula:
(monomer)




structural formula:
(acetoin-PFBHA derivative)




[22] Acetoin (OSHA Chemical Sampling Information), 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_217010.html (accessed 3/17/2008).

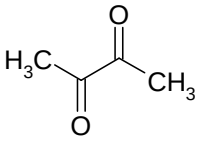
[23] CID: 90884 Acetyl Methyl Carbinol Dimer, 2008. Department of Health and Human Services, National Institutes of Health, National Center for Biotechnology Information. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=90884&loc=ec_rcs (accessed 3/17/2008).

[24] Material Safety Data Sheet: Acetoin, 2008. The Good Scents Company Web site. http://www.thegoodscentscompany.com/msds/md102388.html (accessed 3/17/2008).

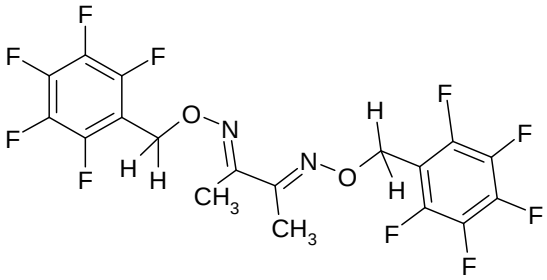
diacetyl[25,26,27,28]

| | |
|---|---|
| synonyms: | biacetyl; 2,3-butanedione; 2,3-butadione; 2,3-diketobutane; dimethyldiketone; dimethylglyoxal; glyoxal, dimethyl-; |
| IMIS[29]: | D740 |
| CAS number: | 431-03-8 |
| boiling point: | 88 ºC (190 ºF) |
| melting point: | 3-4 ºC (37.4-39.2 ºF) |
| density: | 0.99 g/mL @ 15/15 |
| molecular weight: | 86.09 |
| vapor pressure: | 7 kPa @ 20 ºC |
| flash point: | 26.7 ºC (80 ºF) (closed cup) |
| appearance: | yellow to yellow-green liquid |
| vapor density: | 3 (air = 1) |
| molecular formula: | $C_4H_6O_2$ |
| odor: | butter in lower concentrations, quinone odor or chlorine-like odor in higher concentrations |
| solubility: | 4 parts water; miscible with alcohol, ether |
| reactive hazards: | diacetyl is light sensitive (Section 4.9); vapors may ignite when pouring or pumping due to static electricity |
| autoignition temperature: | 285 ºC (545 ºF) |

structural formula:




structural formula:
(diacetyl-PFBHA derivative)




[25] *The Merck Index*, 13th ed.; Budavari, S., Ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 2001; p 522.

[26] Material Safety Data Sheet: Diacetyl, Chemwatch, Victoria, Australia (accessed 3/17/2008).

[27] Material Safety Data Sheet: 2,3-Butanedione, 2007. Fisher Scientific Web site. https://fscimage.fishersci.com/msds/03275.htm (accessed 3/17/2008).

[28] Material Safety Data Sheet: 2,3-Butanedione, 2007. Chem Service Inc Web site. http://www.chemservice.com/msds/msds_detail.asp?catnum=O-816 (accessed 3/17/2008).

[29] Diacetyl (OSHA Chemical Sampling Information), 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_231710.html (accessed 3/17/2008).

This method was evaluated according to the OSHA SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[30]. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations, and acceptance criteria. The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters. Air concentrations in ppm are referenced to 25 °C and 101.3 kPa (760 mmHg).

1.2 Limit defining parameters

1.2.1 Detection limit of the analytical procedure

The detection limit of the analytical procedure is 0.17 pg for acetoin and 0.11 pg for diacetyl. These are the amounts of analyte that will give a detector response that is significantly different from the response of a reagent blank. (Section 4.1)

1.2.2 Detection limit of the overall procedure

The detection limit of the overall procedure is 14.5 ng (0.447 ppb or 1.61 μg/$m^3$) for acetoin and 12.3 ng (0.389 ppb or 1.37 μg/$m^3$) for diacetyl. These are the amounts of analyte spiked on the sampler that will give detector responses that are significantly different from the responses of the respective sampler blanks. (Section 4.2)

1.2.3 Reliable quantitation limit

The reliable quantitation limit is 48.4 ng (1.49 ppb or 5.37 μg/$m^3$) for acetoin and 41.1 ng (1.30 ppb or 4.57 μg/$m^3$) for diacetyl. These are the amounts of analyte spiked on the samplers that will give detector responses that are considered the lower limits for precise quantitative measurements. (Section 4.2)

1.2.4 Instrument calibration

The standard error of estimate is 0.019 μg/sample for acetoin over the range of 0.41 to 3.28 μg/sample. The standard error of estimate is 0.052 μg/sample for diacetyl over the range of 0.40 to 3.16 μg/sample. This range corresponds to 0.25 to 2 times the TWA target concentration. (Section 4.3)

1.2.5 Precision

The precision of the overall procedure at the 95% confidence level for the ambient temperature 18-day storage test at the target concentration from dried silica gel tubes was ±9.9% for acetoin and ±10.0% for diacetyl. These each include an additional 5% for sampling pump variability. (Section 4.4)

1.2.6 Recovery

The recoveries of acetoin and diacetyl from samples used in the 18-day storage test remained above 98.4% for acetoin and 98.0% for diacetyl when the samples were stored at 23 °C. (Section 4.5)

[30] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M.; Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/index.html (accessed 3/15/2008).

1.2.7 Reproducibility

Six samples were collected from a controlled test atmosphere and submitted for analysis by the OSHA Salt Lake Technical Center. The samples were analyzed according to a draft copy of this procedure after being stored at 4 ºC for 20 days and at -12 °C for an additional 19 days. No individual sample result deviated from its theoretical value by more than the precision reported in Section 1.2.5. (Section 4.6)

2. Sampling Procedure

All safety practices that apply to the work area being sampled should be followed. The sampling equipment should be attached to the worker in such a manner that it will not interfere with work performance or safety.

2.1 Apparatus

Samples are collected with two tubes in series. The tubes consist of 110-cm × 7-mm o.d. glass sampling tubes packed with one section (600 mg) of specially cleaned and dried silica gel. From the front to back, the sampler consists of a silane-treated glass wool plug, glass fiber filter, 600 mg specially cleaned silica gel, and a second silane-treated glass wool plug. The silica gel should be cleaned and dried as described in Appendix A of OSHA Method 1013.[31] The tubes used in this evaluation were labeled front and back tube. The front tube is connected to the back tube with a piece of tubing to form the sampling train. For this evaluation commercially prepared sampling tubes containing the speacially dried silica gel were purchased from SKC, Inc. (Catalog no. 226-183, lot no. CPM112907-001).

Samples are collected using a personal sampling pump calibrated, with the sampling device attached, to within ±5% of the recommended flow rate.

Use aluminum foil, opaque tape, or a tube holder, such as SKC, Inc. Cover D (catalog no. 244-29D), to protect samples from light.

2.2 Reagents

None required

2.3 Technique

Immediately before sampling, break off both ends of the flame-sealed tube to provide an opening approximately half the internal diameter of the tube. Wear eye protection when breaking the tube. Use tube holders to minimize the hazard of broken glass and to protect tubes from light exposure during sampling. All tubes should be from the same lot.

A sampling train is created by attaching two tubes in series with a small section of tubing so that the front opening of the back tube is close to the back opening of the front tube. The front of each tube contains glass wool followed by a glass fiber filter, and the back of the tube contains only the glass wool.

The back tube is used as a back-up and is positioned nearest the sampling pump. Attach the tube holder to the sampling pump so that the adsorbent tube is in an approximately vertical position with the inlet in the breathing zone. Position the sampling pump, tube holder, and tubing so they do not impede work performance or safety. Use a tube holder or wrap the tubes

[31] Simmons, M., Hendricks, W. Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).

in aluminum foil to insure that both sampling tubes are protected from light exposure. Light will decompose the acetoin and diacetyl.

Draw the air to be sampled directly into the inlet of the tube holder. The air being sampled is not to pass through any hose or tubing before entering the sampling tube.

After sampling for the appropriate time, remove the sampling train, separate the tubes, and seal each tube with plastic end caps. Wrap each tube in aluminum foil or opaque tape, and then seal each sample end-to-end with a Form OSHA-21 seal as soon as possible.

Submit at least one blank sample with each set of samples. Handle the blank sample in the same manner as the other samples except draw no air through it.

Record sample air volumes (liters), sampling time (minutes), and sampling rate (L/min) for each sample, along with any potential interferences on the Form OSHA-91A.

Submit the samples to the laboratory for analysis as soon as possible after sampling. As a precaution, store the samples at refrigerator temperature if a delay in shipment is unavoidable. Ship any bulk samples separate from the air samples.

2.4 Sampler capacity (Section 4.7)

The sampling capacity was determined using test atmospheres containing the analytes. The concentrations of the test atmospheres were: 0.101 ppm (0.365 mg/$m^3$) acetoin, and 0.101 ppm (0.355 mg/$m^3$) diacetyl with an average relative humidity (RH) of 80% at 23 ºC. The samples were collected at 0.05 L/min. The 5% breakthrough air volumes were determined to be 12.1 L for diacetyl and greater than 24 L for acetoin.

There was no acetoin or diacetyl on the back-up tube when a 15 min sample was taken at 0.2 L/min. The 5% breakthrough air volumes for a flow rate of 0.2 L/min were determined to be 11.98 L for diacetyl and greater than 13 L for acetoin.

2.5 Extraction efficiency (Section 4.8)

It is the responsibility of each analytical laboratory to determine the extraction efficiency of the analyte from the media because the adsorbent material, internal standard, reagents and laboratory techniques may be different than those listed in this evaluation and influence the results.

The mean extraction efficiencies from dry silica gel over the range of RQL to 2 times the target concentration were: 102.0% (0.022 to 3.28 µg/sample) for acetoin and 97.6% (0.01 to 3.16 µg/sample) for diacetyl. The extraction efficiency was not affected by the presence of water.

Extracted samples remain stable for at least 24 h.

2.6 Recommended sampling time and sampling rate

Sample with dried silica gel tubes for up to 180 min at 0.05 L/min (9 L) to collect TWA (long-term) samples, and for 15 min at 0.2 L/min (3 L) to collect short-term samples.

When short-term samples are collected, the air concentration equivalent to the reliable quantitation limit becomes larger. For example, the reliable quantitation limits for dried silica gel tubes for a 15 min sample taken at 0.2 L/min are 0.0044 ppm (0.016 mg/$m^3$) for acetoin and 0.0042 ppm (0.015 mg/$m^3$) for diacetyl.

2.7 Interferences, sampling (Section 4.9)

Retention efficiency

The mean retention efficiency was 96.7% for acetoin and 96.9% for diacetyl when dried silica gel tubes containing 0.819 µg of acetoin and 0.808 µg of diacetyl were allowed to sample 6.75 L of contaminant-free air having an average relative humidity of 80% at 23 ºC. (Section 4.9)

Low humidity

The ability of dried silica gel tubes to collect the analytes from a relatively dry atmosphere was determined by sampling an atmosphere containing two times the target concentration and at an average relative humidity of 20% RH at 23 ºC. The mean recoveries (% of theoretical) were 98.7% for acetoin and 98.5% for diacetyl. (Section 4.9)

Low concentration

The ability of dried silica gel tubes to collect the analytes at low concentrations was tested by sampling an atmosphere at 0.1 times the target concentration with at an average relative humidity of 80% RH at 23 ºC. The mean recoveries (% of theoretical) were 99.0% for acetoin and 98.4% for diacetyl. (Section 4.9)

Sampling interference

The ability of dried silica gel tubes to collect the analyte when other potential interferences are present was tested under two separate series of tests. The first test was an atmosphere similar to ones found at some popcorn manufacturing plants consisting of acetoin and diacetyl at the target concentration with an interference mixture of acetaldehyde, acetic acid, and methyl ethyl ketone at an average humidity of 80% at 23 ºC. All three of these interferences can react with PFBHA. The concentrations of the analytes in this test atmosphere were: 0.051 ppm (0.184 mg/m$^3$) acetoin and 0.051 ppm (0.180 mg/m$^3$) diacetyl, 1.01 ppm (1.82 mg/m$^3$) acetaldehyde, 1.05 ppm (2.58 mg/m$^3$) acetic acid, and 1.02 ppm (3.01 mg/m$^3$) methyl ethyl ketone. Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The mean recoveries (% of theoretical) were: acetoin 97.9% and diacetyl 98.2%.

The second series of tests was with acetoin and diacetyl at the target concentration and each of the interferences listed above individually at their PEL concentration following the guidelines in SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[32]. The concentrations of these interferences are much higher than would normally be expected in a food or flavoring manufacturing workplace. The PFBHA extraction solution needed to be modified to 18 mg/mL PFBHA (72.1 µmoles/mL) to insure that there was enough PFBHA to derivatize all the analytes. These interferences and acetoin react fully within 4 hours of extraction, but the diacetyl requires 36 hours to fully react. These three test atmospheres each contained the one of the following concentrations of interference: 190 ppm (350 mg/m$^3$) acetaldehyde, 9.49 ppm (23.3 mg/m$^3$) acetic acid, or 190 ppm (560 mg/m$^3$) methyl ethyl ketone. These three compounds were chosen because they can collect onto the dried silica gel tubes and can react with the PFBHA. For each test, three sampling trains had contaminated air (air containing the analytes and an interference) drawn through them at 0.05 L/min for 180 min for each test. All of the samples were immediately analyzed. The average recoveries (% of theoretical) with 190 ppm acetaldehyde were 97.8% for acetoin and 95.5% for diacetyl. The average recoveries (% of theoretical) with 9.49 ppm acetic acid were 97.3% for acetoin and

[32] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/index.html (accessed 3/15/2008).

98.2% for diacetyl. The average recoveries (% of theoretical) with 200 ppm methyl ethyl ketone were 98.4% for acetoin and 97.6% for diacetyl. These interferences were not a sampling interference, but under normal sample analysis, these levels of interferences would be analytical interferences. (Section 4.9)

Light

Acetoin and diacetyl are light-sensitive. The interference of light during sampling was tested using three foil-wrapped sampling trains and three uncovered sampling trains. An atmosphere containing twice the target concentration at an average relative humidity of 78% at 23ºC was sampled for 180 min at 0.05 L/min, and the samples were extracted that day. The average recovery for acetoin of the foil-wrapped samplers was 98.5% and the uncovered samplers had an average recovery of 93.9%. The average recovery for diacetyl of the foil-wrapped samplers was 98.9% and the uncovered samplers had an average recovery of 94.3%. An additional three sampling trains were collected at the same time, and were protected from the light by aluminum foil. After collection, these samplers had the foil removed and were placed on the counter at ambient temperature under room light. These samples were analyzed 24 h after sampling during which they were exposed to the room light for 14 of the 24 h. The average recoveries were 81.3% for acetoin and 80.0% for diacetyl. Light is a significant interference; therefore, both tubes in the sampling train need to be covered by aluminum foil or opaque tape during and after sampling. (Section 4.9)

Powder form

The powder form of acetoin and diacetyl tested consisted of starch coated with acetoin and diacetyl. Three tests were performed on this powder. The first consisted of a sampling train of a pre-weighed PVC filter in a conical cassette in series with two dried silica gel tubes. The two dried silica gel tubes were used to collect any vapors of acetoin and diacetyl which would strip off from the powder. Known amounts of the powder were placed onto the PVC filter, and 9 L of air at an average relative humidity of 78% at 22 °C were pulled through the sampling trains at 0.05 L/min. The recovery of acetoin and diacetyl on the pre-weighed PVC filters was 0% to 1.9% for acetoin and 0% to 2.3% for diacetyl. The recovery on the dried silica gel tubes was 96.6% for acetoin and 97.8% for diacetyl. The acetoin and diacetyl recoveries were calculated from the percentages obtained from analysis of the powder and the amounts of powder weighed out. The second and third tests consisted of a sampling train of two dried silica gel tubes in series, with the powder spiked on the front glass wool of the front tube. The two tests had 9 L of air drawn through the sampling trains at 0.05 L/min, the first test used air at an average relative humidity of 20% at 22 °C, and the other test used air at an average relative humidity of 78% at 22 °C. At 20% RH most of the acetoin and diacetyl were found on the front glass wool and glass fiber filter, but at 78% RH most of the acetoin and diacetyl were found on the dried silica gel beds. These tubes can collect particulates, but cannot be used as a particulate sampler at 0.05 L/min. (Section 4.9)

3. Analytical Procedure

Adhere to the rules set down in your Chemical Hygiene Plan[33]. Avoid skin contact and inhalation of all chemicals and review all MSDSs before beginning this analytical procedure.

3.1 Apparatus

Gas chromatograph equipped with an electron capture detector. An Agilent Model 6890 GC equipped with a Chemstation, an automatic sample injector, and a μ-electron capture detector (μECD) was used in this evaluation.

[33] Occupational Exposure to Hazardous Chemicals in Laboratories. *Code of Federal Regulations*, Part 1910.1450, Title 29, 2003.

A GC column capable of separating the PFBHA derivatives of acetoin and diacetyl from the PFBHA extraction solution, potential interferences, and internal standard. A 30-m × 0.32-mm i.d. fused silica capillary column (DB-5 0.25-µm df) (Agilent Technologies, Santa Clara CA) was used in this evaluation.

An electronic integrator or other suitable means of measuring GC detector response. A Waters Empower 2 Data System was used in this evaluation.

Amber glass vials with PTFE-lined caps. Amber 2 and 4-mL vials were used in this evaluation.

A dispenser capable of delivering 2.0 mL of PFBHA extraction solution to prepare standards and samples. If a dispenser is not available, 2.0-mL volumetric pipettes can be used.

Class A volumetric flasks of appropriate sizes such as 10-mL and other convenient sizes for preparing standards.

Calibrated 10-µL syringe for preparing standards.

.

Micro-analytical balance capable of weighing at least 0.001 mg. An Ohaus Galaxy 160D was used in this evaluation.

Rotator. A Fisher Roto Rack was used to extract the samples.

3.2 Reagents

Acetoin, [CAS no. 513-86-0], reagent grade or better. Acetoin used in this evaluation was 99+% (lot no. 05025DH) purchased from Sigma-Aldrich (Milwaukee, WI).

Diacetyl, [CAS no. 431-03-8], reagent grade or better. Diacetyl used in this evaluation was 97% (lot no. 10815TD) purchased from Sigma-Aldrich (Milwaukee, WI).

Ethyl alcohol, [CAS no. 64-17-5], 95% v/v (190 proof) A.C.S. Spectrophotometric grade. Ethyl alcohol used in this evaluation was 95% (lot no. B0513970) purchased from Acros (Morris Plains, NJ).

*O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride, [CAS no. 57981-02-9] (PFBHA), reagent grade or better. PFBHA used in this evaluation was 99+% (lot no. 1242759 54706063) purchased from Fluka, a subsidiary of Sigma-Aldrich (Milwaukee, WI).

4-Bromobenzylbromide, [CAS no. 589-15-1], reagent grade or better. 4-Bromobenzylbromide used in this evaluation was 98% (lot no. A0251708) purchased from Acros (Morris Plains, NJ).

DI water, 18 MΩ-cm. A Barnstead NanoPure Diamond system was used to purify the water for this evaluation.

The PFBHA extraction solution used for this evaluation consisted of 20 µg/mL 4-bromobenzylbromide in the 95:5 ethyl alcohol:water with 2 mg/mL PFBHA. The 4-bromobenzylbromide was added to 95:5 ethyl alcohol:water as an internal standard. Other internal standards can be used provided they are fully tested. Store this solution in a tightly sealed container in a refrigerator that does not contain solutions of aldehydes, acids, or ketones. This solution can absorb formaldehyde, other aldehydes, ketones, and acids out of the air. These compounds will react with the PFBHA, decreasing the amount available to react with acetoin or diacetyl. This solution can be stored in the refrigerator for 1 week.

3.3 Standard preparation

Prepare stock solution of acetoin and diacetyl in water. Acetoin is usually sold as the dimer, which will disassociate in water to the monomer as the solid dimer dissolves. This stock solution will remain stable for four weeks if stored in an amber bottle in the refrigerator.[34]

Freshly prepare analytical standards from the stock solutions for each analysis. These analytical standards are prepared for each of the analytes by injection of microliter amounts of a stock solution into 2-mL volumetric flasks and diluting with the PFBHA extraction solution over a concentration range of 0.02 to 6 µg/sample. For example: a target concentration standard of 1.60 µg/sample acetoin and 1.56 µg/sample diacetyl was prepared by injecting 16 µL of a stock solution containing 0.10 µg/mL acetoin and 0.10 µL/mL (0.0975 µg/mL) diacetyl in water into a 2-mL volumetric flask containing about 1.75 mL of PFBHA extraction solution and then diluting to the mark with PFBHA extraction solution (this is equivalent to 0.80 µg/mL acetoin or 0.049 ppm based on a 2-mL extraction and 9 L air volume, and 0.78 µg/mL diacetyl or 0.049 ppm based on a 2-mL extraction and 9 L air volume). Standards must be allowed to react with the PFBHA at room temperature for 36 hours.

Bracket sample concentrations with standard concentrations. If upon analysis, sample concentrations fall outside the range of prepared standards, prepare and analyze additional standards to confirm instrument response, or dilute high samples with PFBHA extraction solution and reanalyze the diluted samples.

3.4 Sample preparation

Remove the plastic end caps from the sample tube and carefully transfer the section of the adsorbent from each tube into separate 4-mL amber vials. Normally the front glass wool plug and glass fiber filter are discarded. If the industrial hygienist requests the analysis, the front glass wool plug and the glass fiber filter should be placed into a separate 4-mL amber vial. Discard the glass tubes and back glass wool plugs.

Add 2.0 mL of PFBHA extraction solution to each vial and immediately seal the vials with PTFE-lined caps.

Place the samples on a mechanical rotator and rotate at approximately 40 rpm for 60 min. Do not use a shaker to extract samples, as the recoveries will be lower.

Allow the samples to stand at room temperature for an additional 36 hours for the derivatization reaction to reach completion.

Transfer each solution from the 4-mL vial to a labeled amber 2-mL glass autosampler vial and seal with a PTFE-lined cap.

If more sensitivity is desired for samples prepared by OSHA Method 1013[35], they can be derivatized by the PFBHA solution and analyzed by GC-ECD. The samples in OSHA 1013 are extracted with 2 mL 95:5 ethyl alcohol:water. The samples can be derivatized by the following procedure: add 0.5-mL of sample and 0.5-mL of PFBHA extraction solution into a labeled 2-mL vial, and react for 36 hours, and then analyze by GC-ECD following the analytical conditions in this method. Standards prepared by OSHA Method 1013 are derivatized following the same procedure. The RQL will be a factor of 2 higher due to this dilution of the samples.

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[34] Simmons, M., Hendricks, W., Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).

[35] Simmons, M., Hendricks, W., Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).

3.5 Analysis

3.5.1 Analytical conditions:

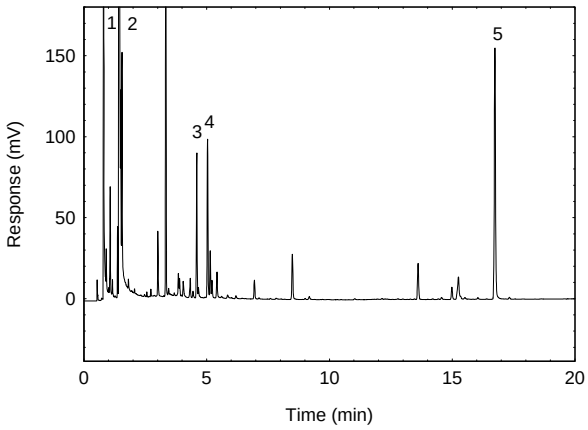


Figure 3.5.1. A chromatogram of the PFBHA derivatives of 1.60 µg/sample acetoin and 1.56 µg/sample diacetyl in the extraction solution. (Key: (1) ethyl alcohol; (2) PFBHA; (3) 4-bromobenzylbromide (ISTD); (4) acetoin-PFBHA; and (5) diacetyl-PFBHA; all other peaks are from PFBHA and its breakdown products)

GC conditions:

| | |
|---|---|
| column: | initial 100 ºC, hold 1 min, program at 5 ºC/min to 200 ºC, hold 0 min |
| injector: | 250 ºC |
| detector: | 250 ºC |
| run time: | 20 min |
| column gas flow: | 3.0 mL/min (hydrogen) |
| column mode: | constant pressure |
| column pressure: | 6.8 psi |
| injection size: | 1.0 µL (40:1 split) |
| column: | 30-m × 0.32-mm i.d. capillary column (DB-5 df = 0.25 µm) |
| retention times: | 0.85 min ethyl alcohol<br>1.44 min PFBHA<br>4.60 min 4-bromobenzylbromide<br>5.04 min acetoin-PFBHA<br>16.75 min diacetyl-PFBHA |

ECD conditions:

| | |
|---|---|
| makeup flow: | 40 mL/min (nitrogen) |

Peak areas are measured with an integrator or other suitable means.

3.5.2 An internal standard (ISTD) calibration method is used. A calibration curve can be constructed by plotting response of standard injections versus micrograms of analyte per sample. Bracket the samples with freshly prepared analytical standards over the range of concentrations.

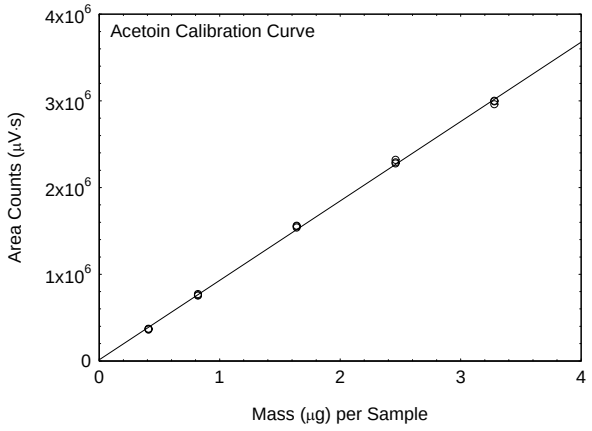


Figure 3.5.2.1. Calibration curve for acetoin. (y = 9.16E5x + 1.44E4)

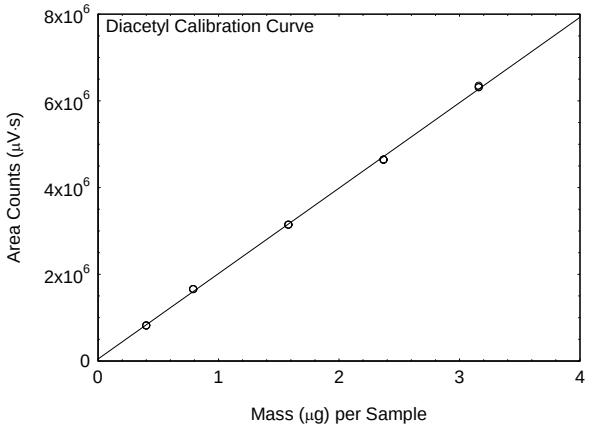


Figure 3.5.2.2. Calibration curve for diacetyl. (y = 1.97E6x + 4.59E4)

3.6 Interferences (analytical)

Any compound that produces a GC-ECD response and has a similar retention time as the analyte is a potential interference. If any potential interferences were reported, they should be considered before samples are extracted. Generally, chromatographic conditions can be altered to separate an interference from the analyte.

3.7 Calculations

The amount of analyte per sampler is obtained from the appropriate calibration curve in terms of micrograms of analyte per sample, uncorrected for extraction efficiency. The front amount found is then corrected by subtracting the total amount (if any) found on the front blank. The back amount found is then corrected by subtracting the total amount (if any) found on the back blank. The amount found on the back dried silica gel tube is added to the front tube for the total loading on each sample. The back-up tube is analyzed separately to determine the extent of analyte saturation to determine if breakthrough occurred. Even though the analytes are analyzed as the PFBHA derivatives and the calibration and results are as the amount of analyte. The air concentration is calculated using the following formulas.

$$M = [M_{front} - M_{front\ blank}] + [M_{back} - M_{back\ blank}]$$

where
- $M$ is total micrograms per sample
- $M_{front}$ is micrograms found on front tube
- $M_{back}$ is micrograms found on back tube
- $M_{\text{front blank}}$ is micrograms found on front blank tube
- $M_{\text{blank}}$ is micrograms found on back blank tube

$$C_M = \frac{M}{VE_E}$$

where
- $C_M$ is concentration by weight ($mg/m^3$)
- $M$ is micrograms per sample
- $V$ is liters of air sampled
- $E_E$ is extraction efficiency, in decimal form

$$C_V = \frac{V_M C_M}{M_r}$$

where
- $C_V$ is concentration by volume (ppm)
- $V_M$ is 24.46 (molar volume at NTP)
- $C_M$ is concentration by weight ($mg/m^3$)
- $M_r$ is molecular weight of analyte (acetoin = 88.11 and diacetyl = 86.09

4. Backup data

General background information about the determination of detection limits and precision of the overall procedure is found in the "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatography Analysis".[36] The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations, and acceptance criteria.

4.1 Detection limit of the analytical procedure (DLAP)

The DLAP is measured as the mass of analyte introduced onto the chromatographic column. Ten analytical standards were prepared with equally descending increments with the highest standard containing 97.9 ng/mL acetoin, and for diacetyl the highest standard was 95.5 ng/mL.

[36] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/index.html (accessed 3/15/2008).

These are the concentrations that would produce peaks at least 10 times the response of a reagent blank near the elution time of the analyte. These standards, and the reagent blank were analyzed with the recommended analytical parameters (1-µL injection with a 40:1 split), and the data obtained were used to determine the required parameters (slope and standard error of estimate) for the calculation of the DLAP. For acetoin, the slope and standard error of estimate, respectively, were 3818 and 219. For diacetyl, the slope and standard error of estimate, respectively, were 9595 and 366.

Table 4.1.1
Detection Limit of the Analytical Procedure for Acetoin

| concentration (ng/mL) | mass on column (pg) | area counts (µV•s) |
|---|---|---|
| 0 | 0 | 0 |
| 9.79 | 0.245 | 863 |
| 19.6 | 0.490 | 1679 |
| 29.4 | 0.735 | 2588 |
| 39.2 | 0.980 | 3443 |
| 49.0 | 1.23 | 4167 |
| 58.7 | 1.47 | 5301 |
| 68.5 | 1.71 | 6084 |
| 78.3 | 1.96 | 7465 |
| 88.1 | 2.20 | 8098 |
| 97.9 | 2.45 | 9529 |

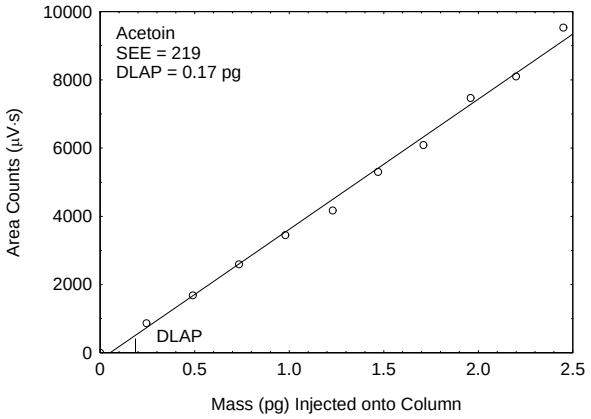


Figure 4.1.1. Plot of data to determine the DLAP for acetoin. (y = 3818x - 202)

Table 4.1.2
Detection Limit of the Analytical Procedure for Diacetyl

| concentration (ng/mL) | mass on column (pg) | area counts (µV•s) |
|---|---|---|
| 0 | 0 | 0 |
| 9.55 | 0.238 | 2824 |
| 19.1 | 0.478 | 5099 |
| 28.7 | 0.718 | 7020 |
| 38.2 | 0.955 | 9587 |
| 47.8 | 1.20 | 11701 |
| 57.3 | 1.43 | 13790 |
| 66.9 | 1.67 | 15745 |
| 76.4 | 1.91 | 18523 |
| 86.0 | 2.15 | 20511 |
| 95.5 | 2.39 | 23882 |

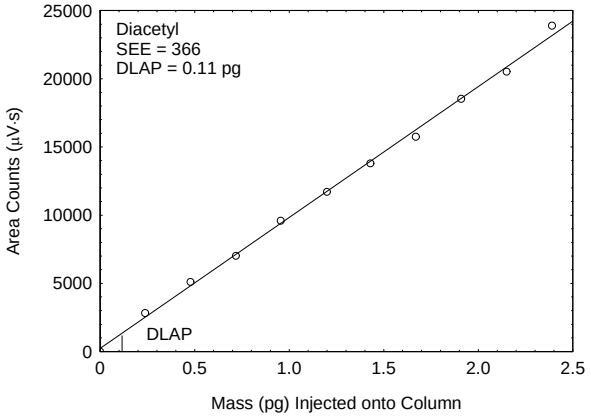


Figure 4.1.2. Plot of data to determine the DLAP for diacetyl. (y = 9595x + 238)

4.2 Detection limit of the overall procedure (DLOP) and reliable quantitation limit (RQL)

DLOP is measured as mass per sample and expressed as equivalent air concentrations, based on the recommended sampling parameters. Ten samplers were spiked with equally descending increments of analyte. The highest amount is the amount spiked on the sampler that would produce a peak approximately 10 times the response of a sample blank. These spiked samplers and the sample blank were analyzed with the recommended analytical parameters, and the data obtained used to calculate the required parameters (slope and standard error of estimate) for the calculation of the DLOP. For acetoin, the slope and standard error of estimate, respectively, were 46.9 and 227. For diacetyl, the slope and

standard error of estimate, respectively, were 121 and 497. For acetoin, the DLOP was 14.5 ng and the RQL was 48.4 ng. For diacetyl, the DLOP was 12.3 ng and the RQL was 41.1 ng.

Table 4.2.1
Detection Limit of the Overall Procedure for Acetoin

| mass per sample (ng) | area counts (μV•s) |
|---|---|
| 0 | 0 |
| 19.6 | 866 |
| 39.2 | 1901 |
| 58.7 | 2927 |
| 78.3 | 3421 |
| 97.9 | 4158 |
| 117 | 5543 |
| 137 | 6002 |
| 157 | 7399 |
| 176 | 8221 |
| 196 | 9373 |

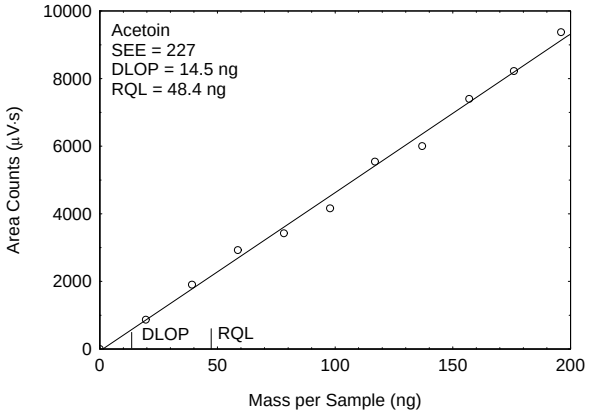


Figure 4.2.1. Plot of data to determine the DLOP/RQL for acetoin. (y = 46.9x - 63.1)

Table 4.2.2
Detection Limit of the Overall Procedure for Diacetyl

| mass per sample (ng) | area counts (μV•s) |
|---|---|
| 0.0 | 0 |
| 19.1 | 2758 |
| 38.2 | 5554 |
| 57.4 | 7690 |
| 76.4 | 10101 |
| 95.5 | 11743 |
| 115 | 13988 |
| 134 | 15701 |
| 153 | 18651 |
| 172 | 21621 |
| 191 | 23995 |

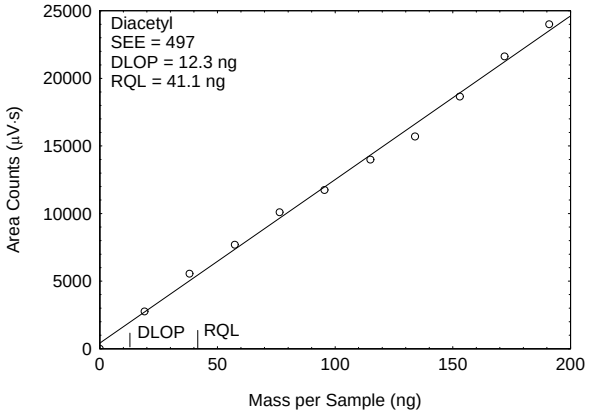


Figure 4.2.2. Plot of data to determine the DLOP/RQL for diacetyl. (y = 121x + 407)

The RQL is considered the lower limit for precise quantitative measurements. It is determined from the regression line parameters obtained for the calculation of the DLOP, providing 75% to 125% of the analyte is recovered. The RQLs are listed in Table 4.2.3.

Table 4.2.3
Reliable Quantitation Limits

| analyte | ng | ppb | μg/m$^3$ | $E_E$ |
|---|---|---|---|---|
| acetoin | 48.4 | 1.49 | 5.37 | 102.3 |
| diacetyl | 41.1 | 1.30 | 4.57 | 97.3 |

$E_E$ = extraction efficiency

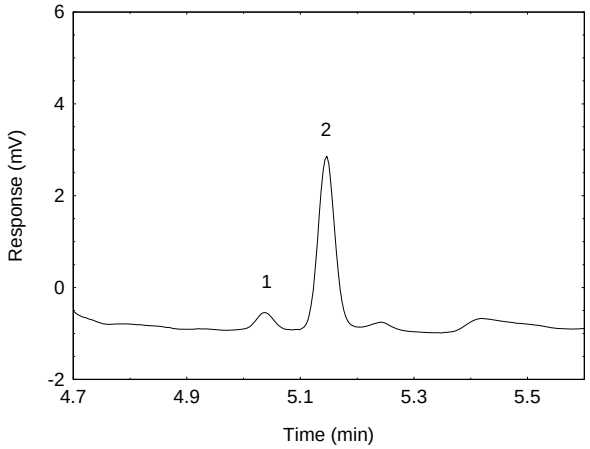


Figure 4.2.3. A chromatogram of the RQL of acetoin. (Key: (1) acetoin-PFBHA, (2) interference)

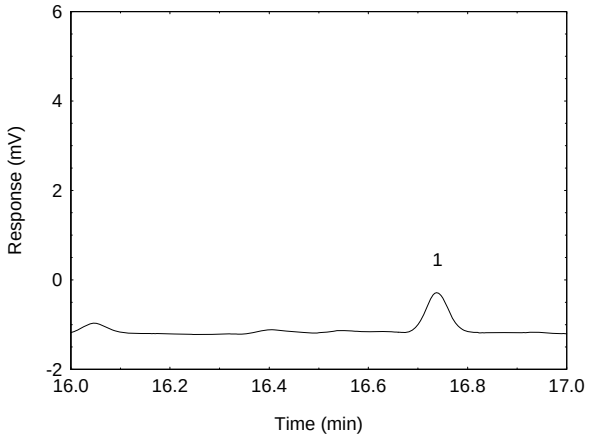


Figure 4.2.4. A chromatogram of the RQL of diacetyl. (Key: (1) diacetyl-PFHBA)

## 4.3 Instrument calibration

The standard error of estimate was determined from the linear regression of data points from standards over a range that covers 0.25 to 2 times the TWA target concentration. Calibration curves were constructed and shown in Section 3.5.2 from the three injections each of five standards. The standard errors of estimates were 0.019 µg for acetoin and 0.052 µg for diacetyl.

Table 4.3.1
Instrument Calibration for Acetoin

| standard concn (µg/sample) | area counts (µV·s) | | |
|---|---|---|---|
| 0.41 | 367186 | 360667 | 370276 |
| 0.82 | 759141 | 752935 | 771533 |
| 1.64 | 1550965 | 1559979 | 1538639 |
| 2.46 | 2318162 | 2277568 | 2290341 |
| 3.28 | 2993893 | 2999180 | 2959244 |

Table 4.3.2
Instrument Calibration for Diacetyl

| standard concn (µg/sample) | area counts (µV·s) | | |
|---|---|---|---|
| 0.40 | 818644 | 817236 | 817895 |
| 0.79 | 1658619 | 1654024 | 1658622 |
| 1.58 | 3140780 | 3142807 | 3140857 |
| 2.37 | 4604360 | 4645231 | 4644018 |
| 3.16 | 6349382 | 6315236 | 6309791 |

## 4.4 Precision (overall procedure)

The precision at the 95% confidence level is obtained by multiplying the standard error of estimate by 1.96 (the z-statistic from the standard normal distribution at the 95% confidence level). In Section 4.5, 95% confidence intervals are drawn about their respective regression lines in the storage graph figures. The precisions of the overall procedure were obtained from the ambient temperature 18 day storage tests were ±9.9% for acetoin and ±10.0% for diacetyl.

## 4.5 Storage test

Storage samples for acetoin and diacetyl were prepared using dried silica gel tubes from controlled test atmospheres using the recommended sampling conditions. The concentrations were 0.051 ppm (0.184 mg/m$^3$) acetoin and 0.050 ppm (0.180 mg/m$^3$) diacetyl at an average relative humidity of 80% at 23 ºC. Thirty-three storage samples were prepared. Three samples were analyzed on the day of generation. Fifteen of the tubes were stored at reduced temperature (4 ºC) and the other fifteen were stored in a closed drawer at ambient temperature (about 23 ºC). At 3 to 4-day intervals, three samples were selected from each of the two storage sets and analyzed. Recoveries are not corrected for extraction efficiency.

Table 4.5.1
Storage Test for Acetoin at 80% RH

| time (days) | ambient storage recovery (%) | | | refrigerated storage recovery (%) | | |
|---|---|---|---|---|---|---|
| 0 | 100.4 | 98.5 | 101.1 | | | |
| 4 | 99.1 | 100.3 | 98.9 | 100.1 | 100.4 | 98.6 |
| 7 | 99.5 | 99.1 | 98.6 | 98.9 | 99.7 | 100.8 |
| 10 | 100.5 | 98.8 | 99.4 | 98.5 | 100.1 | 99.9 |
| 14 | 97.9 | 99.3 | 98.3 | 99.9 | 99.3 | 98.6 |
| 18 | 98.5 | 99.3 | 97.6 | 99.8 | 98.3 | 99.1 |

Table 4.5.2
Storage Test for Diacetyl at 80% RH

| time (days) | ambient storage recovery (%) | | | refrigerated storage recovery (%) | | |
|---|---|---|---|---|---|---|
| 0 | 100.2 | 100.4 | 98.2 | | | |
| 4 | 99.3 | 100.1 | 98.1 | 99.4 | 100.1 | 97.3 |
| 7 | 99.8 | 98.7 | 97.2 | 100.3 | 99.3 | 97.1 |
| 10 | 97.3 | 99.8 | 98.9 | 97.5 | 100.0 | 99.8 |
| 14 | 99.7 | 99.1 | 97.6 | 99.7 | 98.9 | 96.6 |
| 18 | 98.7 | 97.7 | 96.8 | 98.6 | 97.7 | 96.5 |

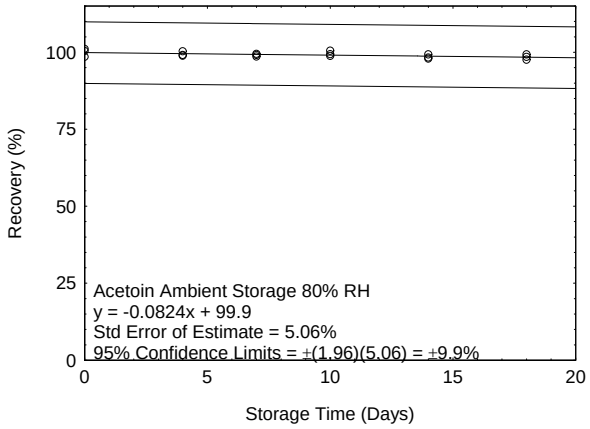


Figure 4.5.1. Ambient storage test for acetoin at 80% RH.

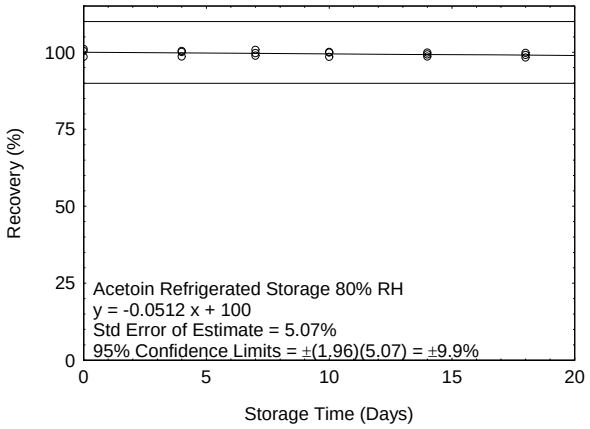


Figure 4.5.2. Refrigerated storage test for acetoin at 80% RH.

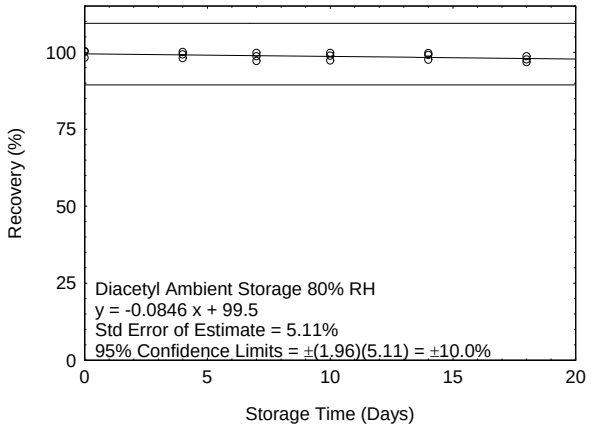


Figure 4.5.3. Ambient storage test for diacetyl at 80% RH.

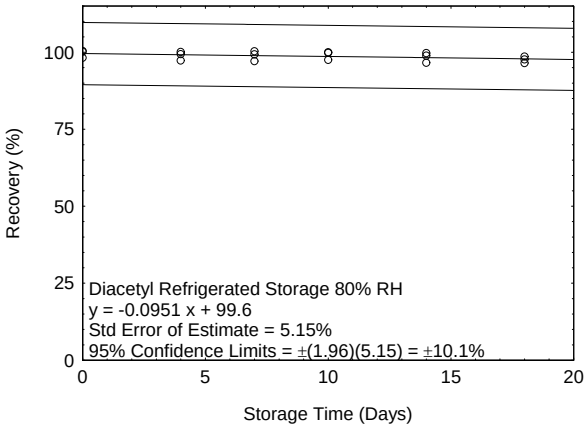


Figure 4.5.4. Refrigerated storage test for diacetyl at 80% RH.

Storage studies were also performed using tubes packed with 400/200 mg sections of dried silica gel, at an average relative humidity of 22% RH at 23 °C to determine the effects of low humidity on storage and on migration. The concentrations were 0.051 ppm (0.184 mg/m$^3$) acetoin and 0.050 ppm (0.180 mg/m$^3$) diacetyl. Thirty-three storage samples were prepared. Three samples were analyzed on the day of generation. At 3 to 4-day intervals, three samples were selected from each of the two storage sets and analyzed. Fifteen of the tubes were stored at reduced temperature (4 °C) and the other fifteen were stored in a closed drawer at ambient temperature (about 23 °C). At 22% RH ambient and refrigerated storage samples showed no migration for acetoin or diacetyl. Recoveries are not corrected for extraction efficiency.

Table 4.5.3
Storage Test for Acetoin at 22% RH

| time (days) | ambient storage recovery (%) | | | refrigerated storage recovery (%) | | |
|---|---|---|---|---|---|---|
| 0 | 100.2 | 99.8 | 97.9 | | | |
| 4 | 99.9 | 97.4 | 98.4 | 100.1 | 97.4 | 99.6 |
| 7 | 98.2 | 100.5 | 96.9 | 99.7 | 98.8 | 97.5 |
| 10 | 99.9 | 97.7 | 97.1 | 99.4 | 97.7 | 100.3 |
| 14 | 98.9 | 99.4 | 96.8 | 98.2 | 99.9 | 96.9 |
| 17 | 99.2 | 97.3 | 95.7 | 96.2 | 98.7 | 99.3 |

Table 4.5.4
Storage Test for Diacetyl at 22% RH

| time (days) | ambient storage recovery (%) | | | refrigerated storage recovery (%) | | |
|---|---|---|---|---|---|---|
| 0 | 100.4 | 97.1 | 98.5 | | | |
| 4 | 99.9 | 98.2 | 97.0 | 99.5 | 100.1 | 97.3 |
| 7 | 99.6 | 98.8 | 97.1 | 99.9 | 98.7 | 97.4 |
| 10 | 99.9 | 98.1 | 96.9 | 99.8 | 98.9 | 97.0 |
| 14 | 99.7 | 96.5 | 98.4 | 99.5 | 98.0 | 96.8 |
| 17 | 99.0 | 98.0 | 95.7 | 98.1 | 99.3 | 96.3 |

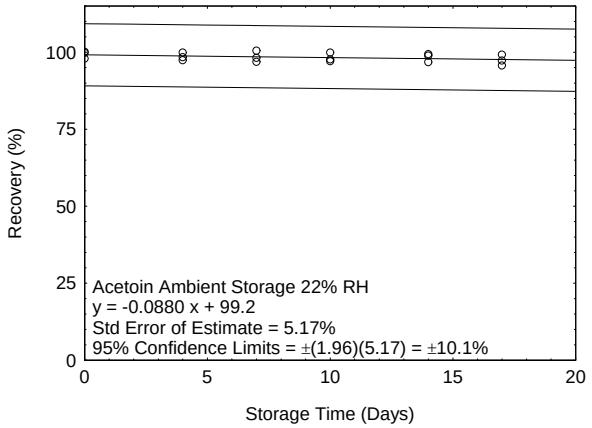


Figure 4.5.5. Ambient storage test for acetoin at 22% RH.

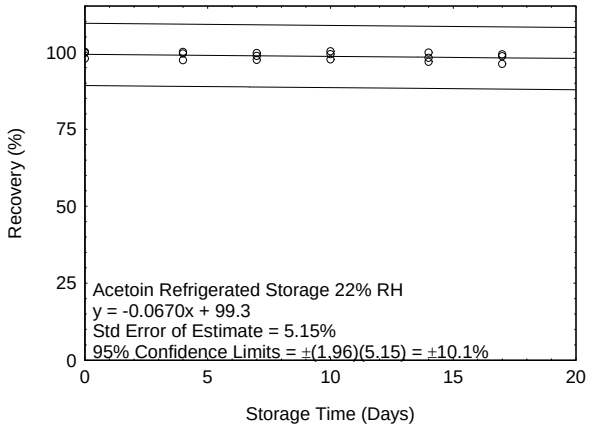


Figure 4.5.6. Refrigerated storage test for acetoin at 22% RH.

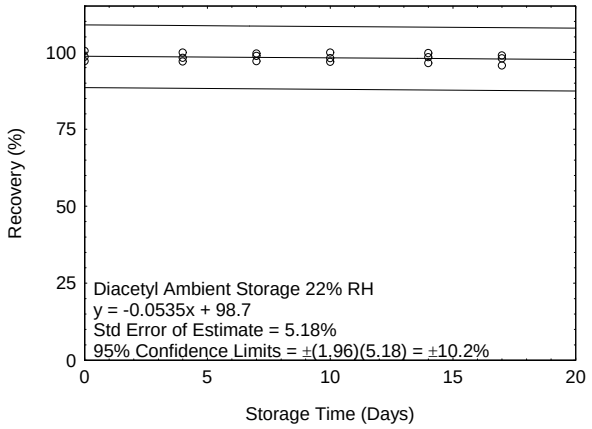


Figure 4.5.7. Ambient storage test for diacetyl at 22% RH.

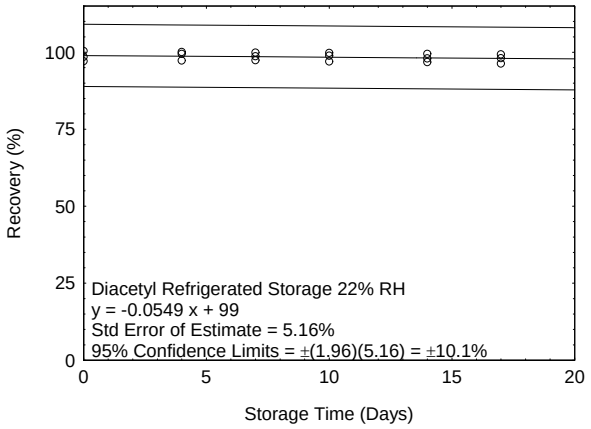


Figure 4.5.8. Refrigerated storage test for diacetyl at 22% RH.

At the beginning of this method, the SKC 226-183 tubes were available as a 400/200 mg tube. Migration studies showed that it would be necessary to use two tubes in series, so subsequent tubes were packed as a single 600 mg tube. A 600 mg section makes it easier for the analyst to prepare the samples for extraction. Migration occurs when the analyte equilibrates between the two sections of the tube after collection. There is more migration with higher humidities, due to the higher amounts of water collected. Using 400/200 mg dried silica gel tubes, at 80% RH acetoin showed no migration but the diacetyl refrigerated samples at day 18 showed a 4.5% migration and ambient showed 15.2% migration. Based on these results, a single 400/200 mg dried silica gel tube should not be used for sampling.

Table 4.5.5
Migration of Diacetyl on 400/200 mg Dried Silica Gel Tube
Sampled at 0.05 L/min for 180 min from 0.05 ppm Atmosphere

| day | ambient | | refrigerated | |
|---|---|---|---|---|
| | 400 mg % of total found | 200 mg % of total found | 400 mg % of total found | 200 mg % of total found |
| 4 | 96.1 | 3.2 | 99.4 | 0.0 |
| | 96.0 | 4.1 | 100.1 | 0.0 |
| | 94.4 | 3.7 | 97.3 | 0.0 |
| 7 | 93.4 | 5.4 | 100.3 | 0.0 |
| | 92.9 | 5.8 | 99.3 | 0.0 |
| | 91.5 | 5.7 | 97.1 | 0.0 |
| 10 | 89.1 | 8.2 | 97.5 | 0.0 |
| | 91.3 | 8.5 | 100.0 | 0.0 |
| | 90.9 | 8.0 | 99.8 | 0.0 |
| 14 | 88.0 | 11.7 | 97.9 | 1.8 |
| | 87.8 | 11.3 | 97.3 | 1.6 |
| | 86.0 | 11.6 | 95.7 | 0.9 |
| 18 | 81.2 | 17.5 | 86.9 | 4.2 |
| | 82.7 | 15.0 | 85.5 | 4.5 |
| | 83.7 | 13.1 | 83.2 | 4.8 |

## 4.6 Reproducibility

Six samples were prepared from a controlled test atmosphere at the target concentration at an average relative humidity of 78% at 23 °C. The samples were submitted to the OSHA Salt Lake Technical Center for analysis, along with a draft copy of this method. The samples were analyzed after being stored at 4 °C for 20 days and at -12 °C for an additional 19 days. Sample results were corrected for extraction efficiency. No sample result for acetoin or diacetyl had a deviation greater than the precision of the overall procedure determined in Section 4.4.

Table 4.6.1
Reproducibility Data for Acetoin

| theoretical (µg/sample) | recovered (µg/sample) | recovery (%) | deviation (%) |
|---|---|---|---|
| 1.62 | 1.59 | 98.1 | -1.9 |
| 1.65 | 1.53 | 92.7 | -7.3 |
| 1.67 | 1.54 | 92.2 | -7.8 |
| 1.66 | 1.56 | 94.0 | -6.0 |
| 1.69 | 1.64 | 97.0 | -3.0 |
| 1.64 | 1.51 | 92.1 | -7.9 |

Table 4.6.2
Reproducibility Data for Diacetyl

| theoretical (µg/sample) | recovered (µg/sample) | recovery (%) | deviation (%) |
|---|---|---|---|
| 1.62 | 1.53 | 94.4 | -5.6 |
| 1.64 | 1.48 | 90.2 | -9.8 |
| 1.60 | 1.49 | 92.5 | -7.5 |
| 1.61 | 1.50 | 93.2 | -6.8 |
| 1.66 | 1.53 | 92.2 | -7.8 |
| 1.62 | 1.50 | 92.6 | -7.4 |

Samples that are prepared and analyzed by OSHA Method 1013[37] can be derivatized and re-analyzed by this method to detect lower levels. The following samples were prepared from a controlled test atmosphere at 0.51 ppm (0.184 mg/m$^3$) acetoin and 0.50 ppm (0.180 mg/m$^3$) diacetyl at 74% RH and 24 °C. They were submitted for analysis by OSHA Method 1013 and then reanalysis by OSHA Method 1012. The average acetoin recovery of samples analyzed by OSHA Method 1013 was 99.3% and by OSHA Method 1012 was 97.1%. The average diacetyl recovery of samples analyzed by OSHA Method 1013 was 98.9% and by OSHA Method 1012 was 96.6%.

[37] Simmons, M., Hendricks, W., Acetoin Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed 11/1/2008).

Table 4.6.3
Samples for Acetoin Analyzed by OSHA Method 1013 and Then by OSHA Method 1012

| | OSHA Method 1013 GC-FID | | | OSHA Method 1012 GC-ECD | | |
|---|---|---|---|---|---|---|
| theoretical (µg/sample) | recovered (µg/sample) | recovery (%) | deviation (%) | recovered (µg/sample) | recovery (%) | deviation (%) |
| 16.5 | 16.4 | 99.4 | -0.6 | 16.2 | 98.2 | -1.8 |
| 16.4 | 16.2 | 98.8 | -1.2 | 16.0 | 97.6 | -2.4 |
| 16.6 | 16.3 | 98.2 | -1.8 | 16.1 | 97.0 | -3.0 |
| 15.9 | 16.1 | 101.3 | +1.3 | 15.6 | 98.1 | -1.9 |
| 16.5 | 16.1 | 97.6 | -2.4 | 15.8 | 95.8 | -4.2 |
| 16.3 | 16.4 | 100.6 | +0.6 | 15.6 | 95.7 | -4.3 |

Table 4.6.4
Samples for Diacetyl Analyzed by OSHA Method 1013 and Then by OSHA Method 1012

| | OSHA Method 1013 GC-FID | | | OSHA Method 1012 GC-ECD | | |
|---|---|---|---|---|---|---|
| theoretical (µg/sample) | recovered (µg/sample) | recovery (%) | deviation (%) | recovered (µg/sample) | recovery (%) | deviation (%) |
| 16.0 | 15.9 | 99.4 | -0.6 | 15.6 | 97.5 | -1.8 |
| 15.7 | 15.4 | 98.1 | -1.9 | 15.1 | 96.2 | -2.4 |
| 15.8 | 15.5 | 98.1 | -1.9 | 15.1 | 95.6 | -3.0 |
| 15.6 | 15.8 | 101.3 | +1.3 | 15.2 | 97.4 | -1.9 |
| 15.7 | 15.2 | 96.8 | -3.2 | 15.0 | 95.5 | -4.2 |
| 15.9 | 15.8 | 99.4 | -0.6 | 15.5 | 97.5 | -4.3 |

## 4.7 Sampler capacity

The sampling capacity of the front tube of two dried silica gel tubes in series was tested by sampling from a dynamically generated test atmosphere with an average relative humidity of 81% at 23ºC at concentrations of 0.101 ppm (0.365 mg/m$^3$) acetoin, and 0.101 ppm (0.355 mg/m$^3$) diacetyl. The second tube in the sampling train was changed at 1 h intervals for the first 3 hours then at 0.5 hour intervals for the rest of the sampling. The dried silica gel tube sampling trains were used to sample at approximately 0.05 L/min (each air volume listed below uses that specific tube's flow rate). The presence of analyte on the second tube was defined as breakthrough. The percentage of the amount found on the second tube of the total concentration is the % breakthrough. The % breakthrough was plotted versus the air volume sampled to determine the 5% breakthrough air volumes. The 5% breakthrough air volume for diacetyl was 12.1 L. The recommended air volume is 80% of the breakthrough air volume which is 9.68 L. Acetoin had no breakthrough after samples were collected for up to 8 hours.

Table 4.7.1
Capacity Test for Diacetyl on Dried Silica Gel Tubes at 0.101 ppm

| sampling train 1 | | sampling train 2 | | sampling train 3 | |
|---|---|---|---|---|---|
| air volume | % BT | air volume | % BT | air volume | % BT |
| 2.71 | 0.0 | 2.80 | 0.0 | 2.78 | 0.0 |
| 5.51 | 0.0 | 5.69 | 0.0 | 5.67 | 0.0 |
| 8.36 | 0.0 | 8.64 | 0.0 | 8.60 | 0.0 |
| 9.69 | 0.0 | 10.0 | 0.0 | 9.97 | 0.0 |
| 12.0 | 5.2 | 12.6 | 27.8 | 12.5 | 20.3 |

%BT = % breakthrough

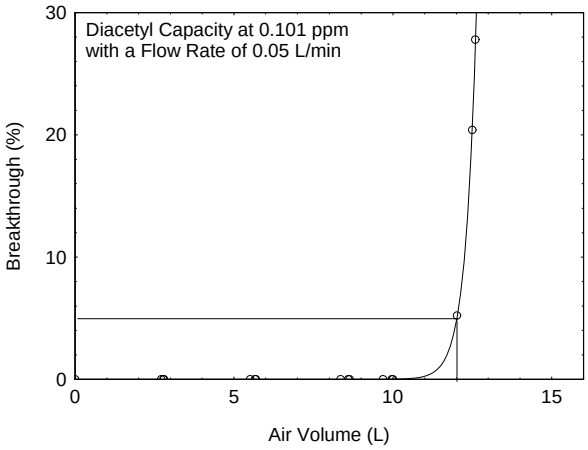


Figure 4.7.1. Five percent breakthrough test for diacetyl from a 0.101 ppm atmosphere, with a flow rate of 0.05 L/min.

A capability of collection at higher flow rates with a 15 minute short term sample was tested for breakthrough. A test atmosphere was dynamically generated with an average relative humidity of 79% at 23 ºC at concentrations of 0.101 ppm (0.365 mg/m$^3$) acetoin and 0.101 ppm (0.355 mg/m$^3$) diacetyl. A sampling train consisting of two dried silica gel tubes (400/200 mg) in series was used to test the capacity. Three sampling trains at each flow rate of 0.1 L/min or 0.2 L/min were tested. There was no acetoin or diacetyl on the second tube of any of the sampling trains. Since the short term sampling may be a time of higher exposure, two higher concentrations were also tested. The first was 0.541 ppm (1.95 mg/m$^3$) acetoin and 0.506 ppm (1.78 mg/m$^3$) diacetyl and a relative humidity of 79% at 23 ºC. The second was 23.2 ppm (83.5 mg/m$^3$) acetoin and 22.4 ppm (78.8 mg/m$^3$) diacetyl at an average relative humidity of 79% at 23 ºC. In all of these tests there was no acetoin or diacetyl on the back-up tube of the sampling train.

Table 4.7.2
15 min Capability to Sample at 0.2 L/min from an Atmosphere of 0.101 ppm Acetoin and 0.101 ppm Diacetyl

| | acetoin | | diacetyl | |
|---|---|---|---|---|
| flow rate (L/min) | front tube (%) | back tube (%) | front tube (%) | back tube (%) |
| 0.1 | 98.6 | 0.0 | 99.4 | 0.0 |
| 0.1 | 99.4 | 0.0 | 98.7 | 0.0 |
| 0.1 | 99.9 | 0.0 | 99.1 | 0.0 |
| 0.2 | 99.2 | 0.0 | 99.5 | 0.0 |
| 0.2 | 98.5 | 0.0 | 98.4 | 0.0 |
| 0.2 | 97.7 | 0.0 | 99.8 | 0.0 |

Table 4.7.3
15 min Capability to Sample at 0.2 L/min from an Atmosphere of 0.541 ppm Acetoin and 0.506 ppm Diacetyl

| | acetoin | | diacetyl | |
|---|---|---|---|---|
| flow rate (L/min) | front tube (%) | back tube (%) | front tube (%) | back tube (%) |
| 0.1 | 99.7 | 0.0 | 99.9 | 0.0 |
| 0.1 | 99.0 | 0.0 | 98.4 | 0.0 |
| 0.1 | 98.8 | 0.0 | 97.9 | 0.0 |
| 0.2 | 99.3 | 0.0 | 99.4 | 0.0 |
| 0.2 | 97.9 | 0.0 | 98.9 | 0.0 |
| 0.2 | 99.5 | 0.0 | 99.0 | 0.0 |

Table 4.7.4
15 min Capability to Sample at 0.2 L/min from an Atmosphere of 23.2 ppm Acetoin and 22.4 ppm Diacetyl

| | acetoin | | diacetyl | |
|---|---|---|---|---|
| flow rate (L/min) | front tube (%) | back tube (%) | front tube (%) | back tube (%) |
| 0.1 | 98.6 | 0.0 | 99.6 | 0.0 |
| 0.1 | 99.4 | 0.0 | 98.7 | 0.0 |
| 0.1 | 99.0 | 0.0 | 97.3 | 0.0 |
| 0.2 | 99.9 | 0.0 | 99.6 | 0.0 |
| 0.2 | 97.5 | 0.0 | 99.0 | 0.0 |
| 0.2 | 98.1 | 0.0 | 97.8 | 0.0 |

A capacity test at 0.2 L/min was performed at two test air concentrations, 0.101 ppm (0.365 $mg/m^3$) acetoin and 0.101 ppm (0.355 $mg/m^3$) diacetyl at an average relative humidity of 78% air at 22 °C; and 23.2 ppm (83.5 $mg/m^3$) acetoin and 22.4 ppm (78.8 $mg/m^3$) diacetyl at relative humidity of 77% at 22 ºC. There was no acetoin on the back-up tube after 13.9 L was sampled. The 5% breakthrough air volume for diacetyl with 0.101 ppm atmosphere was 11.98 L, and with a 22.4 ppm atmosphere was 11.64 L.

Table 4.7.5
Capacity Test for Diacetyl on Dried Silica Gel Tubes at a Flow Rate of 0.2 L/min and 0.101 ppm

| sampling train 1 | | sampling train 2 | | sampling train 3 | |
|---|---|---|---|---|---|
| air volume | % BT | air volume | % BT | air volume | % BT |
| 5.98 | 0.0 | 5.95 | 0.0 | 6.03 | 0.0 |
| 7.97 | 0.0 | 7.94 | 0.0 | 8.04 | 0.0 |
| 9.97 | 0.0 | 9.92 | 0.0 | 10.05 | 0.0 |
| 10.96 | 0.7 | 10.91 | 0.0 | 11.06 | 1.4 |
| 11.96 | 5.4 | 11.90 | 3.4 | 12.06 | 8.8 |
| 12.95 | 26.4 | 12.90 | 22.7 | 13.07 | 35.1 |

%BT = % breakthrough

Table 4.7.6
Capacity Test for Diacetyl on Dried Silica Gel Tubes
at a Flow Rate of 0.2 L/min and 22.4 ppm

| sampling train 1 | | sampling train 2 | | sampling train 3 | |
|---|---|---|---|---|---|
| air volume | % BT | air volume | % BT | air volume | % BT |
| 6.15 | 0.0 | 5.94 | 0.0 | 6.06 | 0.0 |
| 8.20 | 0.0 | 7.92 | 0.0 | 8.08 | 0.0 |
| 10.25 | 0.0 | 9.90 | 0.0 | 10.10 | 0.0 |
| 11.28 | 2.1 | 10.89 | 0.6 | 11.11 | 1.2 |
| 12.30 | 17.2 | 11.88 | 5.1 | 12.12 | 10.5 |
| 13.33 | 48.5 | 12.87 | 24.1 | 13.13 | 40.5 |

%BT = % breakthrough

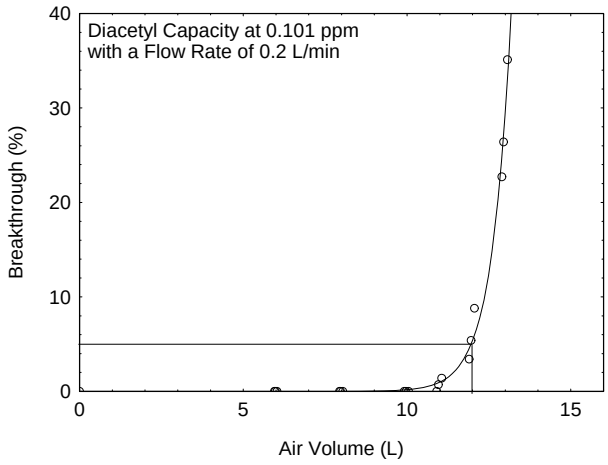


Figure 4.7.2. Five percent breakthrough test for diacetyl from a 0.101 ppm atmosphere, with a flow rate of 0.2 L/min.

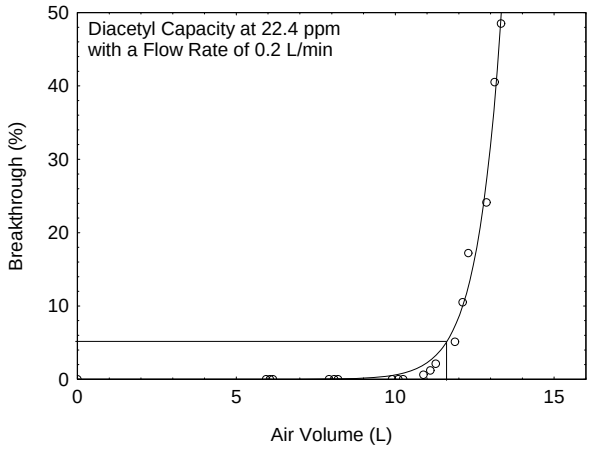


Figure 4.7.3. Five percent breakthrough test for diacetyl from a 22.4 ppm atmosphere, with a flow rate of 0.2 L/min.

## 4.8 Extraction efficiency and stability of extracted samples

The extraction efficiency is dependent on the extraction solvent as well as the internal standard. The extraction solvent used for this evaluation consisted of 95:5 ethyl alcohol:water with 2 mg/mL PFBHA and 20 µg/mL 4-bromobenzyl bromide. Other extraction solvents or internal standards may be used provided that the new extraction solution or internal standard is tested. The new extraction solvent or internal standard should be tested as described below.

### Extraction efficiency

The extraction efficiencies of acetoin and diacetyl were determined by liquid-spiking four dried silica gel tubes, at each concentration level, with the analyte from the RQL to 2 times the target concentration. These samples were stored overnight at ambient temperature and then analyzed. The samples need to be extracted on a rotator for 1 hour, and then allowed to set at room temperature for 36 hours. Do not use a shaker as recoveries will be much lower (Table 4.8.3). The mean extraction efficiency over the working range from the RQL to 2 times the target concentration is 102.0% for acetoin and 97.6% for diacetyl. The extraction efficiency for the wet samplers and samplers extracted on the shaker were not included in the overall mean because it would bias the results. The test of wet samplers was performed to determine if the amount of water that would collect under high humidity conditions at the recommended air volume would affect the extraction efficiency. Wet samplers were prepared by sampling humid

air having an average relative humidity of about 80% at 23 ºC for 180 minutes at 0.05 L/min and then liquid-spiking the sampler with the analyte. The dried silica gel tube (600 mg) collects 140 mg water at 78% RH and 23 °C when sampled for 9 L.

Table 4.8.1
Extraction Efficiency (%) of Acetoin

| level | | sample number | | | | mean |
|---|---|---|---|---|---|---|
| × target concn | µg per sample | 1 | 2 | 3 | 4 | |
| RQL | 0.022 | 104.2 | 102.1 | 101.2 | 101.6 | 102.3 |
| 0.25 | 0.41 | 103.7 | 102.3 | 102.1 | 100.8 | 102.2 |
| 0.5 | 0.82 | 100.7 | 102.4 | 101.1 | 100.9 | 101.3 |
| 1.0 | 1.64 | 102.3 | 100.5 | 103.3 | 103.5 | 102.4 |
| 1.5 | 2.46 | 102.6 | 103.1 | 100.6 | 100.8 | 101.8 |
| 2.0 | 3.28 | 103.0 | 103.3 | 101.6 | 100.4 | 102.1 |
| 1.0 (wet) | 1.64 | 101.1 | 102.9 | 103.1 | 102.2 | 102.3 |

Table 4.8.2
Extraction Efficiency (%) of Diacetyl

| level | | sample number | | | | mean |
|---|---|---|---|---|---|---|
| × target concn | µg per sample | 1 | 2 | 3 | 4 | |
| RQL | 0.02 | 96.7 | 95.7 | 97.8 | 98.9 | 97.3 |
| 0.25 | 0.40 | 97.5 | 98.0 | 99.1 | 98.5 | 98.3 |
| 0.5 | 0.79 | 98.5 | 96.8 | 99.4 | 98.0 | 98.2 |
| 1.0 | 1.58 | 96.9 | 95.3 | 96.4 | 95.4 | 96.0 |
| 1.5 | 2.37 | 99.9 | 95.9 | 96.5 | 97.8 | 97.5 |
| 2.0 | 3.16 | 97.1 | 99.6 | 99.9 | 97.5 | 98.5 |
| 1.0 (wet) | 1.58 | 98.1 | 96.6 | 95.8 | 97.1 | 96.9 |

Table 4.8.3
Extraction Efficiency (%) of Acetoin and Diacetyl at 1.0 x Target Concentration Using a Shaker

| | | sample number | | | | |
|---|---|---|---|---|---|---|
| analyte | µg per sample | 1 | 2 | 3 | 4 | mean |
| acetoin | 1.64 | 87.5 | 88.8 | 90.1 | 87.7 | 88.5 |
| diacetyl | 1.58 | 82.6 | 81.9 | 85.5 | 84.3 | 83.6 |

Stability of extracted samples

The stability of extracted samples was investigated by reanalyzing the target concentration samples 24 h after initial analysis. After the original analysis was performed, two autosampler vials were recapped with new septa while the remaining two retained their punctured septa. The samples were reanalyzed with fresh standards. The average percent change was +0.7% for acetoin and +1.6% for diacetyl when samples were resealed with new septa and -1.1% for acetoin and +0.3% for diacetyl when samples retained their punctured septa. Each septum was punctured 5 times for each analysis. The test was performed at room temperature.

Table 4.8.3
Stability of Extracted Samples for Acetoin

| punctured septa replaced | | | punctured septa retained | | |
|---|---|---|---|---|---|
| initial (%) | after one day (%) | difference (%) | initial (%) | after one day (%) | difference (%) |
| 102.3 | 101.5 | -0.8 | 103.3 | 101.9 | -1.4 |
| 100.5 | 102.7 | +2.2 | 103.5 | 102.7 | -0.8 |
| | (mean) | | | (mean) | |
| 101.4 | 102.1 | +0.7 | 103.4 | 102.3 | -1.1 |

Table 4.8.4
Stability of Extracted Samples for Diacetyl

| punctured septa replaced | | | punctured septa retained | | |
|---|---|---|---|---|---|
| initial (%) | after one day (%) | difference (%) | initial (%) | after one day (%) | difference (%) |
| 96.9 | 98.3 | +1.4 | 96.4 | 95.1 | -1.3 |
| 95.3 | 97.1 | +1.8 | 95.4 | 97.3 | +1.9 |
| | (mean) | | | (mean) | |
| 96.1 | 97.7 | +1.6 | 95.9 | 96.2 | +0.3 |

4.9 Interferences (sampling)

Retention

The ability of a dried silica gel tube to retain the analytes after they have been collected was tested by using a test atmosphere having an average relative humidity of 80% at 23 °C. The test atmosphere was dynamically generated at 0.101 ppm (0.364 mg/m$^3$) acetoin, and 0.102 ppm (0.359 mg/m$^3$) diacetyl. Six samplers had contaminated air drawn through them at 0.05 L/min for 45 min. Sampling was discontinued and three samples set aside. The generation system was flushed with contaminant-free air. Sampling resumed with the other three samples having contaminant-free air drawn through them at 0.05 L/min for 135 min and then all six samplers were analyzed. The mean recoveries for the samples in the second set divided by the first set were: 96.7% for acetoin, and 96.9% for diacetyl.

Table 4.9.1
Retention of Acetoin

| | percent recovery | | | |
|---|---|---|---|---|
| set | 1 | 2 | 3 | mean |
| first | 99.5 | 100.4 | 98.9 | 99.6 |
| second | 95.0 | 96.8 | 97.0 | 96.3 |
| second/first | | | | 96.7 |

Table 4.9.2
Retention of Diacetyl

| | percent recovery | | | |
|---|---|---|---|---|
| set | 1 | 2 | 3 | mean |
| first | 100.2 | 99.9 | 98.1 | 99.4 |
| second | 96.3 | 97.4 | 95.3 | 96.3 |
| second/first | | | | 96.9 |

Low humidity

The ability of dried silica gel tubes to collect the analytes from a relatively dry atmosphere was tested by using a test atmosphere having an average relative humidity of 20% at 23 °C. The test atmosphere was dynamically generated at 0.101 ppm (0.364 mg/m$^3$) acetoin and 0.102 ppm (0.359 mg/m$^3$) diacetyl. Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The recoveries (% of theoretical) for acetoin were: 97.0%, 101.4%, and 97.8%; and for diacetyl were: 98.3%, 96.8%, and 100.3%.

Low concentration

The ability of dried silica gel tubes to collect the analytes from a low concentration atmosphere was tested by using a test atmosphere at 0.1 times the target concentration having an average relative humidity of 80% at 23 °C. The test atmosphere was dynamically generated at 0.0051 ppm (0.0184 mg/m$^3$) acetoin and 0.0051 ppm (0.0180 mg/m$^3$) diacetyl. Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The recoveries (% of theoretical) for acetoin were: 99.8%, 99.9%, and 97.2%, and for diacetyl were: 97.3%, 98.1%, and 99.8%.

Sampling interference

The ability of dried silica gel tubes to collect the analytes from an atmosphere containing interferences was tested under two different sets of conditions. The first set of conditions was a test atmosphere of 0.051 ppm (0.0184 mg/m$^3$) acetoin and 0.051 ppm (0.0180 mg/m$^3$) diacetyl and an interference mixture of 1.01 ppm (1.82 mg/m$^3$) acetaldehyde, 1.05 ppm (2.58 mg/m$^3$) acetic acid, and 1.02 ppm (3.01 mg/m$^3$) methyl ethyl ketone at an average humidity of 80% at 23 °C. These lower concentrations were chosen for two reasons: they are similar to some of the concentrations found in plants manufacturing microwave popcorn, and all of these compounds will be derivatized by the PFBHA; therefore, there would be enough PFBHA in solution to derivatize all of the analytes that were collected (8.01 µmole/mL PFBHA). The recoveries (% of theoretical) of acetoin and diacetyl were: 95.4%, 98.5%, and 99.7% for acetoin and 95.8%, 98.9%, and 99.8% for diacetyl. There was no analyte on the backup tube of the two dried silica gel tubes in series for any of the tests.

The second series of tests was with acetoin and diacetyl at the target concentration and each of the interferences listed above individually at their PEL concentration following the guidelines in SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[38]. The concentrations of these interferences are much higher than would normally be expected in a food or flavoring manufacturing workplace. These three compounds were chosen as interferences because they collect on the dried silica gel tubes and react with the PFBHA. The extraction solution needed to be modified to 18 mg/mL PFBHA (72.1 µmoles/mL) to insure that there was enough PFBHA in solution to derivatize all the analytes. These three atmospheres each contained acetoin and diacetyl with one of the following concentrations of the interference mixture in it: 194 ppm (350 mg/m$^3$) acetaldehyde, 9.49 ppm (23.3 mg/m$^3$) acetic acid, or 190 ppm (560 mg/m$^3$) methyl ethyl ketone. Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min for each test. All of the samples were immediately analyzed. The recoveries (% of theoretical) of acetoin and diacetyl with 190 ppm acetaldehyde were: 99.8%, 95.9%, and 97.7% for acetoin and 97.2%, 93.5%, and 95.7% for diacetyl. The recoveries (% of theoretical) of acetoin and diacetyl with 9.49 ppm acetic acid were: 95.3%, 97.7%, and 98.9% for acetoin and 95.5%, 99.3%, and 99.8% for diacetyl. The recoveries (% of theoretical) of acetoin and diacetyl with 190 ppm methyl ethyl ketone were: 96.7%, 98.7%, and 99.9% for acetoin and 95.8%, 97.8%, and 99.3% for diacetyl. There was no analyte found on the backup tube of the two dried silica gel tubes in series for any of the tests. These interferences were not a sampling interference, but under normal sample analysis, these levels of interferences would be an analytical interference.

Light

Diacetyl and acetoin are light-sensitive.[39,40,41,42] The interference of light during sampling was tested using three foil-wrapped sampling trains and three uncovered sampling trains. An

[38] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/index.html (accessed 3/15/2008).

[39] Material Safety Data Sheet: Acetoin, http://www.thegoodscentscompany.com/msds/md102388.html (accessed 3/17/2008).

atmosphere containing twice the target concentration at an average humidity of 78% at 23 °C was sampled for 180 min at 0.05 L/min, and the samples were extracted that day.

Table 4.9.3
Light Interference During Sampling

| | acetoin | | diacetyl | |
|---|---|---|---|---|
| tube # | foil wrapped recovery (%) | uncovered recovery (%) | foil wrapped recovery (%) | uncovered recovery (%) |
| 1 | 98.9 | 93.7 | 97.8 | 93.3 |
| 2 | 97.0 | 92.6 | 98.9 | 94.6 |
| 3 | 99.5 | 95.4 | 99.9 | 95.0 |
| mean | 98.5 | 93.9 | 98.9 | 94.3 |

An additional three sampling trains were collected at the same time, and were protected from the light by aluminum foil. After collection, these samplers had the foil removed and were placed on the counter at ambient temperature under room light. These samples were analyzed 24 h after sampling during which they were exposed to the room light for 14 of the 24 h, and the recoveries were 80.7%, 84.7%, and 78.5% for acetoin and 79.3%, 82.4%, and 78.4% for diacetyl.

Powder form

The powder form of acetoin and diacetyl tested consisted of starch coated with acetoin and diacetyl. Three tests were performed on this powder. The first consisted of a sampling train of a pre-weighed (tared) PVC filter in a conical cassette in series with two dried silica gel tubes. Two dried silica gel tubes were used to collect any vapors of acetoin and diacetyl which would be stripped off of the powder. Known amounts of the powder were placed onto the PVC filter, and 9 L of air at an average relative humidity of 78% RH and 22 °C were pulled through the sampling trains at 0.05 L/min. The recovery of acetoin and diacetyl on the pre-weighed PVC filters was 0% to 1.9% for acetoin and 0% to 2.3% for diacetyl, with larger amounts found on the PVC filters that were spiked with larger amounts of powder. Most of the acetoin and diacetyl was stripped from the starch and collected on the dried silica gel tubes. The average recovery found on the dried silica gel tubes was 96.6% for acetoin and 97.8% for diacetyl (Table 4.9.4). The acetoin and diacetyl theoretical weights were calculated from the percentages obtained from analysis of the powder and the amounts of the powder weighed out.

The second and third tests consisted of a sampling train of two dried silica gel tubes in series, with the powder spiked on the front glass wool of the front tube. The two tests had 9 L air drawn through the sampling trains at 0.05 L/min, the first test used air at an average relative humidity of 20% at 22 °C, and the other test used air at an average relative humidity of 78% at 22 °C. At 20% RH most of the acetoin and diacetyl were found on the front glass wool and glass fiber filter, but at 78% RH most of the acetoin and diacetyl were found on the dried silica gel beds. The sampling trains with 78% RH air drawn through them had the highest amounts of acetoin and diacetyl on the glass wool and filter on the tube spiked with the highest amount of powder, which may be due to the size of the clump of powder weighed out (Table 4.9.5 and 4.9.6).

[40] Material Safety Data Sheet: Diacetyl, Chemwatch, Victoria, Australia (accesed 3/17/2008).

[41] Material Safety Data Sheet: 2,3-Butanedione, https://fscimage.fishersci.com/msds/03275.htm (accessed 3/17/2008).

[42] Material Safety Data Sheet: 2,3-Butanedione, http://www.chemservice.com/msds/msds_detail.asp?catnum=O-816 (accessed 3/17/2008).

Table 4.9.4
% Recovery of Acetoin and Diacetyl from Powder on Tared PVC Filters in a Conical Cassette in Series with Dried Silica Gel Tubes with 78% RH Air Sampled

| | | | acetoin | | | | diacetyl | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| amount of powder (µg) | powder weight found (µg) | theoretical weight (µg) | PVC filter (µg) | front tube (µg) | back tube (µg) | silica gel recovery (%) | theoretical weight (µg) | PVC filter (µg) | front tube (µg) | back tube (µg) | silica gel recovery (%) |
| 1130 | 1082 | 18.1 | 0.0 | 18.0 | 0.0 | 99.4 | 29.4 | 0.0 | 28.0 | 0.0 | 95.2 |
| 2110 | 2021 | 33.8 | 0.6 | 32.1 | 0.0 | 95.0 | 54.9 | 1.0 | 53.1 | 0.0 | 96.7 |
| 2960 | 2856 | 47.4 | 0.9 | 46.3 | 0.0 | 97.7 | 77.0 | 1.8 | 75.9 | 0.0 | 98.6 |
| 2940 | 2809 | 47.0 | 0.3 | 45.0 | 0.0 | 95.7 | 76.4 | 0.8 | 75.7 | 0.0 | 99.1 |
| 1310 | 1265 | 21.0 | 0.2 | 20.5 | 0.0 | 97.6 | 34.1 | 0.6 | 34.0 | 0.0 | 99.7 |
| 1010 | 964 | 16.2 | 0.0 | 15.3 | 0.0 | 94.4 | 26.3 | 0.0 | 25.6 | 0.0 | 97.3 |

Table 4.9.5
% Recovery of Acetoin and Diacetyl from Powder Spiked on Dried Silica Gel Tubes with 20% RH Air Sampled

| | | | acetoin | | | | diacetyl | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| amount of powder (µg) | theoretical weight (µg) | front glass wool and filter (µg) | front glass wool and filter recovery (%) | front tube (µg) | back tube (µg) | silica gel recovery (%) | theoretical weight (µg) | front glass wool and filter (µg) | front glass wool and filter recovery (%) | front tube (µg) | back tube (µg) | silica gel recovery (%) |
| 1080 | 17.3 | 16.7 | 96.5 | 0.0 | 0.0 | 0.0 | 28.1 | 26.3 | 93.6 | 1.1 | 0.0 | 3.9 |
| 1240 | 19.8 | 19.5 | 98.5 | 0.0 | 0.0 | 0.0 | 32.2 | 30.1 | 93.5 | 1.5 | 0.0 | 4.7 |
| 1750 | 28.0 | 27.4 | 97.9 | 0.0 | 0.0 | 0.0 | 45.5 | 42.8 | 94.1 | 1.8 | 0.0 | 4.0 |
| 2080 | 33.3 | 32.1 | 96.4 | 0.0 | 0.0 | 0.0 | 54.1 | 50.2 | 92.8 | 2.3 | 0.0 | 4.3 |
| 2240 | 35.8 | 34.5 | 96.4 | 0.5 | 0.0 | 1.4 | 58.2 | 53.4 | 91.8 | 2.8 | 0.0 | 4.8 |
| 2380 | 38.1 | 36.7 | 96.3 | 0.7 | 0.0 | 1.8 | 61.9 | 55.8 | 90.1 | 3.6 | 0.0 | 5.8 |

Table 4.9.6
% Recovery of Acetoin and Diacetyl from Powder Spiked on Dried Silica Gel Tubes with 78% RH Air Sampled

| | | | acetoin | | | | diacetyl | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| amount of powder (µg) | theoretical weight (µg) | front glass wool and filter (µg) | front glass wool and filter recovery (%) | front tube (µg) | back tube (µg) | silica gel recovery (%) | theoretical weight (µg) | front glass wool and filter (µg) | front glass wool and filter recovery (%) | front tube (µg) | back tube (µg) | silica gel recovery (%) |
| 1220 | 19.5 | 0.0 | 0.0 | 19.1 | 0.0 | 97.9 | 31.7 | 0.0 | 0.0 | 30.9 | 0.0 | 97.5 |
| 1760 | 28.2 | 0.0 | 0.0 | 26.9 | 0.0 | 95.4 | 45.8 | 0.0 | 0.0 | 44.2 | 0.0 | 96.5 |
| 1070 | 17.1 | 0.0 | 0.0 | 16.9 | 0.0 | 98.8 | 27.8 | 0.0 | 0.0 | 27.5 | 0.0 | 98.9 |
| 1590 | 25.4 | 0.0 | 0.0 | 24.9 | 0.0 | 98.0 | 41.3 | 0.0 | 0.0 | 40.9 | 0.0 | 99.0 |
| 2030 | 32.5 | 0.0 | 0.0 | 32.4 | 0.0 | 99.7 | 52.8 | 0.0 | 0.0 | 52.5 | 0.0 | 99.4 |
| 5020 | 80.3 | 0.7 | 0.9 | 79.4 | 0.0 | 98.9 | 130.5 | 2.2 | 1.7 | 129.9 | 0.0 | 99.5 |

## 4.10 Qualitative analysis

When necessary, the identity or purity of an analyte peak can be confirmed by GC-mass spectrometry or by another analytical procedure. The mass spectra of the acetoin-PFBHA and diacetyl-PFBHA derivative were determined by analyzing an analytical standard on an Agilent 6890 with a 5973 mass selective detector using a 30-m × 0.25-mm i.d. fused silica capillary column (DB-1-MS 0.25-µm df) capillary column at a temperature program of 50 °C, hold 2 min, program at 10 °C/min up to 180 °C hold 10 min, with injection port at 240 °C and mass spectrometer at 250 °C.

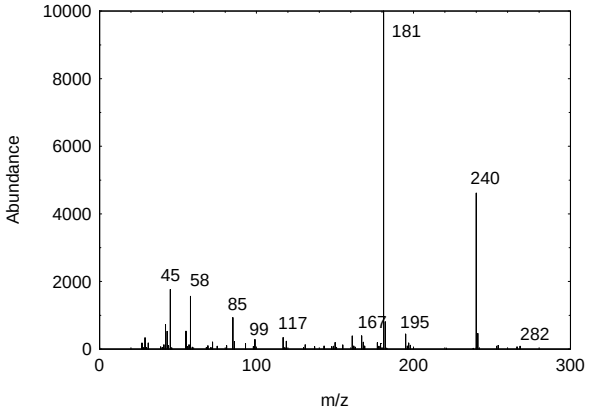


Figure 4.10.1. Mass spectrum of acetoin-PFBHA derivative.

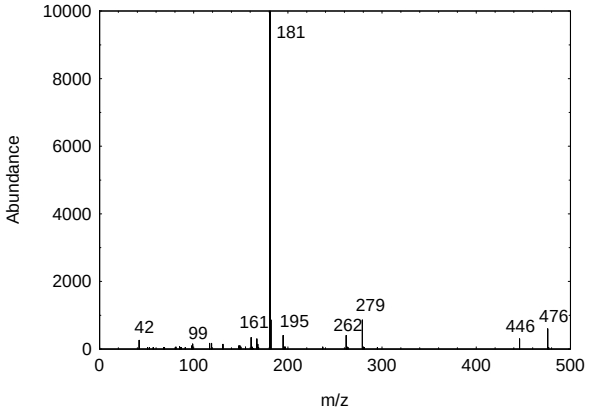


Figure 4.10.2. Mass spectrum of diacetyl-PFBHA derivative.

## 4.11 Generation of test atmospheres

The test atmosphere of acetoin and diacetyl was generated from a water solution.

The following apparatus was placed in a walk-in hood. The acetoin and diacetyl vapors were generated by pumping the solution, using the Isco pump, through a short length of 0.53-mm uncoated fused silica capillary tubing into a vapor generator where it was heated and evaporated into the dilution air stream (Figure 4.11). The vapor generator consisted of a 15-cm length of 5-cm diameter glass tubing with a side port for introduction of the capillary tubing. The glass tube of the vapor generator was wrapped with heating tape to evaporate the chemicals. The humidity, temperature, and volume of the dilution stream of air were regulated by use of a Miller Nelson Flow-Temperature-Humidity controller. The test atmosphere passed into a glass mixing chamber (76-cm $\times$ 30-cm) from the vapor generator, and then into a glass exposure chamber (76-cm $\times$ 20-cm). Active samplers were attached to glass tubes extending from the exposure chamber. The humidity and temperature were measured at the exit of the exposure chamber with an Omega Digital Thermo-hygrometer.

Generation of test atmospheres required extra heating of the air stream to vaporize the acetoin. The temperature and humidity were measured after the air had exited the sampling chamber. The air stream cooled as it passed from the mixing chamber to the sampling chamber and then out the exit. While the air coming out of the exit was 23 °C and 80% RH, the temperature measured in the front of the sampling chamber was 30 °C and 54% RH, giving similar absolute humidities of 16.4 mg/L $H_2O$.

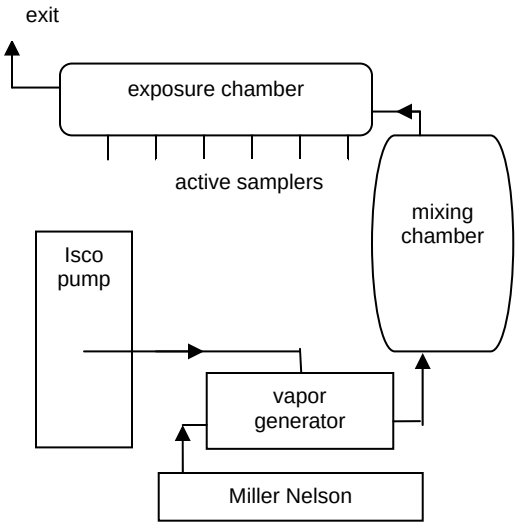


Figure 4.11. The test atmosphere generation and sampling apparatus.

| | |
|---|---|
| Method no.: | 1013 |
| Control no.: | T-1013-FV-01-0809-M |
| Target concentration: | 0.5 ppm (1.80 mg/m$^3$) acetoin<br>0.5 ppm (1.76 mg/m$^3$) diacetyl |
| OSHA PEL: | none for acetoin<br>none for diacetyl |
| ACGIH TLV: | none for acetoin<br>none for diacetyl |
| Procedure: | Samples are collected by drawing workplace air through two sampling tubes, containing specially dried and cleaned silica gel, connected in series. Samples are extracted with ethyl alcohol:water (95:5) and analyzed by gas chromatography (GC) using a flame ionization detector (FID). |
| Recommended sampling time and sampling rate: | 180 min at 0.05 L/min (9 L) (TWA)<br>15 min at 0.2 L/min (3 L) (short term) |
| Reliable quantitation limit: | 0.011 ppm (0.039 mg/m$^3$) acetoin<br>0.012 ppm (0.041 mg/m$^3$) diacetyl |
| Standard error of estimate at the target concentration: | 5.7% acetoin<br>5.2% diacetyl |
| Special requirement: | Protect samples from light exposure during sampling, shipping and analysis. |
| Status of method: | Evaluated method. This method has been subjected to the established evaluation procedures of the Methods Development Team. |

September 2008

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Methods Development Team
Industrial Hygiene Chemistry Division
OSHA Salt Lake Technical Center
Sandy UT 84070-6406

1. General Discussion

For assistance with accessibility problems in using figures and illustrations presented in this method, please contact the Salt Lake Technical Center (SLTC) at (801) 233-4900. This procedure was designed and tested for internal use by OSHA personnel. Mention of any company name or commercial product does not constitute endorsement by OSHA.

## 1.1 Background

### 1.1.1 History

In 2003 OSHA issued Method PV2118[1] for sampling and analysis of diacetyl using two silica gel sorbent tubes (150/75 mg) in series. PV2118 has a recommended sampling volume of 3 L and a reliable quantitation limit of 3 µg (0.28 ppm). In 2003 NIOSH issued Method 2557[2] for diacetyl and Method 2558[3] for acetoin. Both methods use Anasorb CMS sorbent (150/75 mg) tubes, can sample up to 10 L of air and have a limit of detection for acetoin of 1 µg and 0.6 µg for diacetyl. These two methods use slightly different acetone/methanol extraction solvents and were not optimized for simultaneous analysis of both analytes. In 2008 a note was placed on NIOSH Method 2557 indicating that high humidity is a sampling interference that results in underestimation of the true concentration.

In September of 2007, OSHA published a Hazard Communication Guidance Document[4] and a Safety and Health Information Bulletin on Respiratory Disease among Employees in Microwave Popcorn Processing Plants[5] for diacetyl. Due to the increasing concern of workplace exposure to diacetyl, two new sampling and analytical methods were validated that permitted longer sampling times and had lower quantitation limits than PV2118. The new methods were also validated for acetoin because it has been found in facilities in which diacetyl was in use.

This procedure, Method 1013, was streamlined for monitoring low ppm levels, and Method 1012[6] was optimized for ppb levels. Both methods use two 600 mg silica gel sorbent tubes in series. Both methods have a recommended sampling time of 3 hours (9 L) and both use the same solvent for sample extraction. However, in Method 1012, acetoin and diacetyl are derivatized using *O*-pentafluorobenzyl hydroxylamine hydrochloride. This derivatization results in a reliable quantitation limit approximately 10 times less than Method 1013. The disadvantage of derivatizing acetoin and diacetyl is that the derivatization step requires 36 hours; whereas, with this method sample preparation can be performed in 1 hour. Also, samples extracted and analyzed according to this procedure can then be derivatized and analyzed using Method 1012, if needed.

The silica gel used in the sampler for this method, and for Method 1012, has been specially cleaned and dried as described in Appendix A. It was found that sampler

[1] Shah, Y. C. Diacetyl (OSHA Method PV2118), 2003. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/partial/t-pv2118/t-pv2118.html (accessed July 2008).
[2] Pendergrass, S. M. Diacetyl (NIOSH Method 2557), 2003. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web site. http://www.cdc.gov/niosh/nmam/pdfs/2557.pdf (accessed July 2008).
[3] Pendergrass, S. M. Acetoin (NIOSH Method 2558), 2003. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web Site. http://www.cdc.gov/niosh/nmam/pdfs/2558.pdf (accessed July 2008).
[4] Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dsg/guidance/diacetyl-guidance.html (accessed July 2008).
[5] Respiratory Disease Among Employees in Microwave Popcorn Processing Plants, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/shib/shib092107.html (accessed July 2008).
[6] Eide, M. Acetoin and Diacetyl (OSHA Method 1012), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html (accessed September 2008).

capacity for diacetyl was not based on analyte concentration but limited by the amount of water remaining on the silica gel after cleanup and on the amount of water collected during sampling. In other words, the silica gel tube acts as a chromatography column and water elutes the collected diacetyl. By removing as much water as possible from the silica gel prior to sampling, the sampling volume for diacetyl can be increased because the time required to saturate the silica gel during sampling increases. Diacetyl was also found to gradually migrate within the sampling tube during storage resulting in the need to use a second tube in series during sampling in order to detect breakthrough. Acetoin has no capacity or migration issues on silica gel at the recommended sampling volume.

The powder and liquid formulated forms of acetoin and diacetyl may contain oily compounds and other base materials such as maltodrextin. These materials could affect the extraction of acetoin and diacetyl from the silica gel. The sampler contains a front glass wool plug followed by a glass fiber filter that serves only to trap any of these materials before they enter the silica gel bed. Retention studies using a powder containing acetoin and diacetyl showed the acetoin and diacetyl can be stripped off the powder and collected on the silica gel. These studies demonstrate that the glass fiber filter is not an efficient collector for diacetyl and acetoin, and will not normally be analyzed (see OSHA Method 1012[7], Section 4.9).

1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.)

Exposure to acetoin may result in skin, eyes, nose and throat irritation.[8]

Exposure to diacetyl "liquid or vapors can cause irritation to the skin, eyes, nose, and throat". "Animals exposed to diacetyl experienced damage to the nose and upper airways, including severe damage to cells lining the respiratory tract" and "NIOSH has reported that employees exposed to butter flavorings containing diacetyl are at risk of developing occupational lung diseases".[9]

Diacetyl, and to some extent acetoin, may be responsible for the occurrence of a rare and potentially fatal lung disease, bronchiolitis obliterans, among workers in microwave popcorn manufacturing plants and flavor manufacturing plants.[10] Symptoms of bronchiolitis obliterans include cough, shortness of breath with exertion, and spirometry test results showing fixed airways obstruction.[11]

Acetoin and diacetyl are used in the production of powdered flavorings.[12] These powdered flavorings may provide a means to deliver the substances deep into the lungs of exposed workers, however, the significance of this form of exposure is presently unknown.[13]

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[7] Eide, M. Acetoin and Diacetyl (OSHA Method 1012), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html (accessed September 2008).

[8] Acetyl Methyl Carbinol (Chemical Sampling Information), 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_217010.html (accessed July 2008).

[9] Hazard Communication Guidance for Diacetyl and Food Flavorings Containing Diacetyl, 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dsg/guidance/diacetyl-guidance.html (accessed July 2008).

[10] van Rooy, F.; et al. Bronchiolitis Obliterans Syndrome in Chemical Workers Producing Diacetyl for Food Flavoring. *Am. J. Crit. Care Med*. **2007**, 176 (5), 498-504.

[11] Kanwal, R. Bronchiolitis obliterans in workers exposed to flavoring chemicals. *Curr Opin Pulm Med*. **2008**, 14 (2), 141-6.

[12] Kanwal, R.; Kullman, G. Report on Severe Fixed Obstructive Lung Disease in Workers at a Flavoring Manufacturing Plant Health Hazard Evaluation Report #2006-0303-3043, 2007. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2006-0303-3043.pdf (accessed July 2008) pp 11-13.

[13] Boylstein, R. J.; et al. Diacetyl Emissions and Airborne Dust from Butter Flavorings Used in Microwave Popcorn Production. *J. Occup. Environ. Hyg*. **2006**, 3 (10), 530-535.

### 1.1.3 Workplace exposure

Acetoin has a somewhat creamy taste and a woody yogurt odor. It is used as an ingredient in yogurt, butter, milk and strawberry flavors. It occurs naturally in foods such as wines, chesses, fruits and vegetables.[14] Occupational exposures can occur by inhalation or skin contact in locations where it is produced, used as a food additive, or used to produce flavorings or aromas.

Diacetyl has a strong butter odor in dilute form and a chlorine-quinone odor when concentrated. It is used as an ingredient to produce a butter flavor in many foods and beverages. It occurs naturally in alcoholic and nonalcoholic beverages, dairy products, fruits, plants, vegetables, meats, and natural aromas.[15] Like acetoin, occupational exposures to diacetyl can occur by inhalation or skin contact in locations where it is produced, used as a food additive, or used to produce flavorings or aromas.

Recently, occupational exposure to butter flavorings in the production of microwave popcorn and in other industries has received much publicity. NIOSH has identified acetoin and diacetyl as useful indicator compounds that can be used to represent exposure to butter flavorings.[16] Areas of special concern include flavor production rooms, areas where mixing/blending operations occur, packing/packaging operations, areas where flavors are handled openly, rooms where mixing tanks are located, quality control laboratories, and maintenance and cleaning operations.[17, 18]

### 1.1.4 Physical properties and other descriptive information

Acetoin[19, 20]

Acetoin occurs as the liquid monomer and the solid dimer. The monomer can be formed from the dimer by dissolving in water or other solvents.

| | |
|---|---|
| synonyms: | acetyl methyl carbinol; 2,3-butanolone; dimethylketol; *γ*-hydroxy-*β*-oxobutane; 1-hydroxyethyl methyl ketone |
| IMIS[21]: | A624 |
| CAS number: | 513-86-0 (monomer) |
| boiling point: | 148 ºC (298 ºF) @ 760 mmHg (monomer) |
| melting point: | 15 ºC (59 ºF) (monomer); 91 ºC (196 ºF) (dimer) |
| density: | 1.005 (g/mL@ 25 ºC ) (monomer) |
| molecular weight: | 88.11 (monomer) |
| flash point: | 46.7 ºC (116 ºF) (closed cup) (monomer) |
| appearance: | Pale yellow to colorless as liquid or solid |
| molecular formula: | $C_4H_8O_2$ (monomer); $C_8H_{16}O_4$ (dimer) |

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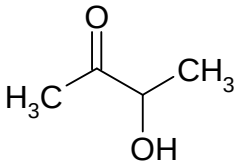
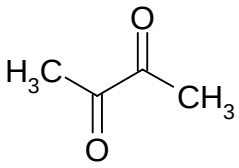
[14] Burdock, G. A. *Fenaroli's Handbook of Flavor Ingredients*, 5th ed.; CRC Press: Boca Raton, FL, 2005; pp 11-12.
[15] Burdock, G. A. *Fenaroli's Handbook of Flavor Ingredients*, 5th ed.; CRC Press: Boca Raton, FL, 2005; pp 411-412.
[16] Kanwal, R.; Boylstein, R. J.; Piacitelli, C. NIOSH Health Hazard Evaluation Report #2001-0474-2943, 2004. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2001-0474-2943.pdf (accessed July 2008) pp 8-9.
[17] Kanwal, R. Bronchiolitis obliterans in workers exposed to flavoring chemicals. *Curr Opin Pulm Med*. **2008**, 14 (2), 141-6.
[18] Kreiss, K. Flavoring-related bronchiolitis obliterans. *Curr Opin Allergy Clin Immunol* **2007**, 7 (2), 162-167.
[19] *The Merck Index*; 12th ed.; Budavari, S., Ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 1996; p 12.
[20] Material Safety Data Sheet: Acetoin, 2008. The Good Scents Company Web site. http://www.thegoodscentscompany.com/msds/md102388.html (accessed July 2008).
[21] Acetyl Methyl Carbinol (Chemical Sampling Information), 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_217010.html (accessed June 2008).

| | |
|---|---|
| solubility: | miscible with water and alcohol; sparingly soluble in ether and petroleum ether |
| structural formula: | |




Diacetyl[22,23]

| | |
|---|---|
| synonyms: | biacetyl; 2,3-butanedione; 2,3-butadione; 2,3-diketobutane; dimethyl diketone; dimethylglyoxal; glyoxal, dimethyl-; 2,3-diketobutane |
| IMIS[24]: | D740 |
| CAS number: | 431-03-8 |
| boiling point: | 88 ºC (190 ºF) |
| melting point: | 3-4 ºC (37.4-39.2 ºF) |
| density: | 0.99 (g/mL@ 15/15) |
| molecular weight: | 86.09 |
| vapor pressure: | 7 kPa @ 20 ºC |
| flash point: | 26.7 ºC (80 ºF) (closed cup) |
| appearance: | yellow to yellow-green liquid |
| vapor density: | 3 (air = 1) |
| molecular formula: | $C_4H_6O_2$ |
| odor: | quinone odor in higher concentrations, butter in lower concentrations |
| solubility: | 4 parts water; miscible with alcohol, ether |
| autoignition temperature: | 285 ºC (545 ºF) |
| structural formula: | |




[22] *The Merck Index*; 12th ed.; Budavari, S., Ed.; Merck & Co. Inc.: Whitehouse Station, NJ, 1996; p 503.
[23] Material Safety Data Sheet: Diacetyl, 2007. Chemwatch; Victoria, Australia (accessed March 2008).
[24] Diacetyl (Chemical Sampling Information), 2007. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_231710.html (accessed 2008).

This method was evaluated according to the OSHA SLTC "Evaluation Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[25]. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations and acceptance criteria. The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters. Air concentrations in ppm are referenced to 25 °C and 101.3 kPa (760 mmHg).

1.2 Limit defining parameters

1.2.1 Detection limit of the analytical procedure

The detection limit of the analytical procedure is 0.017 ng for acetoin and 0.033 ng for diacetyl. These are the amount of analytes that will give a detector response that is significantly different from the response of a calibration blank. (Section 4.1)

1.2.2 Detection limit of the overall procedure

The detection limit of the overall procedure for acetoin is 0.10 µg per sample (0.0031 ppm or 0.011 mg/m$^3$) and 0.11 µg per sample for diacetyl (0.0034 ppm or 0.012 mg/m$^3$). These are the amounts spiked onto the sampler that will give a detector response that is significantly different from the response of a sampler blank. (Section 4.2)

1.2.3 Reliable quantitation limit

The reliable quantitation limit for acetoin is 0.35 µg per sample (0.011 ppm or 0.039 mg/m$^3$ for a TWA sample) and 0.37 µg per sample for diacetyl (0.012 ppm or 0.041 mg/m$^3$ for a TWA sample). These are the amounts spiked onto the sampler that will give a detector response that is considered the lower limit for precise quantitative measurements. (Section 4.2)

1.2.4 Instrument calibration

The standard error of estimate is 0.42 µg for acetoin over the range of 3.73 µg to 31.0 µg. The standard error of estimate is 0.82 µg for diacetyl over the range of 3.58 µg to 29.9 µg. These ranges correspond to approximately 0.25 to 2 times the target concentration. (Section 4.3)

1.2.5 Precision

The precision of the overall procedure at the 95% confidence level for the ambient temperature 18-day storage test (at the target concentration) is ±11.2% for acetoin and ±10.1% for diacetyl. These include an additional 5% for sampling pump variability. (Section 4.4)

1.2.6 Recovery

The recovery from samples used in a 18-day storage test remained above 88.5% for acetoin and 102.7% for diacetyl when the samples were stored at ambient temperature. (Section 4.5)

[25] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. C. Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/chromguide.pdf (accessed November 2007).

1.2.7 Reproducibility

Six samples collected from a controlled test atmosphere were submitted for analysis by the OSHA Salt Lake Technical Center. The samples were analyzed according to a draft copy of this procedure after 20 days of storage at refrigerated temperature. No individual sample result deviated from its theoretical value by more than the precision reported in Section 1.2.5. (Section 4.6)

2. Sampling Procedure

All safety practices that apply to the work area being sampled should be followed. The sampling equipment should be attached to the worker in such a manner that it will not interfere with work performance or safety.

2.1 Apparatus

Sampler: glass tube with both ends flame sealed, 110-mm × 7-mm i.d., containing a glass fiber filter and 1 section of 20/40 mesh silica gel. From front to back, the sampling tube consists of a silane-treated glass wool plug, a glass fiber filter to collect particulate, 600 mg of silica gel and a second plug of silane-treated glass wool. The silica gel should be cleaned and dried as described in Appendix A. Sampling tubes are available for purchase through SKC, Inc. (cat. no. 226-183).

Samples are collected using a personal sampling pump calibrated, with the sampling device attached, to within ±5% of the recommended flow rate.

Use aluminum foil or a tube cover, such as SKC, Inc Tube Cover D (cat. no. 224-29D), to protect samples from light.

2.2 Reagents

None required

2.3 Technique

Immediately before sampling, break the ends off of two flame-sealed glass tubes to provide an opening approximately half the internal diameter of the tube. Wear eye protection when breaking ends. Use a tube holder to minimize the hazard of broken glass and to protect samplers from light exposure during sampling. All tubes should be from the same lot.

Connect the two silica gel sampling tubes in series, using the least amount of flexible tubing as possible between the sampling tubes, and then connect to a sampling pump with flexible tubing. The filter in the silica gel tubes should be positioned away from the sampling pump. The tube closer to the pump is used as a backup. Use a tube cover or wrap sampling tubes in aluminum foil to insure that both sampling tubes are protected from light exposure. Place the sampling tubes in a vertical position with the inlet in the breathing zone and position the sampling pump and tubing so they do not impede work performance or safety.

Draw air directly into the inlet of the sampler. The air being sampled should not pass through any hose or tubing before entering the sampler.

After sampling for the appropriate time, disconnect the tubes from the pump tubing and seal each tube with plastic end caps. Separately wrap each tube in aluminum foil and seal end-to end with a Form OSHA-21.

Submit at least one blank sample with each set of samples. Handle the blank sample in the same manner as the other samples except draw no air through it.

Record sample air volume (L), sampling time (min) and sampling rate (L/min) for each sample, along with any potential interferences on the Form OSHA-91A.

Submit the samples to the laboratory for analysis as soon as possible after sampling. If a delay is unavoidable, store the samples in a refrigerator. Ship any bulk samples separate from the air samples.

2.4 Sampler capacity (Section 4.7)

The sampling capacity of the front tube was tested by sampling a dynamically generated test atmosphere of acetoin (3.58 mg/$m^3$ or 0.99 ppm) and diacetyl (3.55 mg/$m^3$ or 1.01 ppm) with an average relative humidity of 40% at 34 °C (absolute humidity of 14.8 mg/L $H_2O$). The samples were collected at a sampling rate of approximately 0.05 L/min for 270 min. The 5% breakthrough sampling time was determined to be 248 min for diacetyl. No breakthrough was observed for acetoin. (Note: In order to volatilize acetoin the test atmosphere generation conditions were modified slightly for this method evaluation as described in the second paragraph of Section 4.11.)

2.5 Extraction efficiency (Section 4.8)

It is the responsibility of each analytical laboratory to determine the extraction efficiency because the adsorbent material, reagents and laboratory techniques may be different than those listed in this evaluation and influence the results.

The mean extraction efficiency for acetoin from dry silica gel over the range of RQL to 2 times the target concentration (0.33 to 31.0 µg per sample) was 92.9%. The extraction efficiency was not affected by the presence of water.

The mean extraction efficiency for diacetyl from dry silica gel over the range of RQL to 2 times the target concentration (0.38 to 29.9 µg per sample) was 99.6%. The extraction efficiency was not affected by the presence of water.

Extracted samples remain stable for at least 72 hr.

2.6 Recommended sampling time and sampling rate

Sample for up to 180 min at 0.05 L/min (9 L) to collect TWA (long-term) samples.

Sample for up to 15 min at 0.2 L/min (3 L) to collect short-term samples.

When short-term samples are collected, the air concentration equivalent to the reliable quantitation limit becomes larger. For example, the reliable quantitation limit is 0.032 ppm (0.12 mg/$m^3$) for acetoin and 0.035 ppm (0.12 mg/$m^3$) for diacetyl when 3 L are collected.

2.7 Interferences, sampling (Section 4.9)

Retention efficiency

The retention efficiency for all samples was 100.6% of theoretical for acetoin and 96.6% for diacetyl, when samplers containing approximately 8.3 µg of acetoin and 8.1 µg of diacetyl were allowed to sample 6.75 L of contaminant-free air having an average relative humidity of 40% at 35 °C (absolute humidity of 15.6 mg/L $H_2O$). Samples were collected at a sampling rate of 0.05 L/min.

Low humidity

The collection efficiency for all samples was 100.7% of theoretical for acetoin and 101.5% for diacetyl, when the samplers were used to sample a test atmosphere containing two times the target concentration having an average relative humidity of 8% at 33 °C (absolute humidity of 2.82 mg/L $H_2O$). Samples were collected at a sampling rate of 0.05 L/min for 180 min.

Low concentration

The collection efficiency for all samples was 91.8% of theoretical for acetoin and 95.6% for diacetyl, when the samplers were used to sample a test atmosphere containing approximately 0.1 times the target concentration having an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L $H_2O$). Samples were collected at a sampling rate of 0.05 L/min for 180 min.

The collection efficiency for all samples when taking short term samples was 106% of theoretical for acetoin and 90.6% for diacetyl, when the samplers were used to sample a test atmosphere containing approximately 0.1 times the target concentration having an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L $H_2O$). Samples were collected at a sampling rate of 0.2 L/min for 15 min.

Sampling interference

The collection efficiency for all samples was 95.5% of theoretical for acetoin and 101.8% for diacetyl, when the sampler was used to sample a test atmosphere containing approximately one times the target concentration of acetoin and diacetyl and 2.59 mg/m$^3$ of 2-nonanone and 1.88 mg/m$^3$ of 2,3-pentanedione. The test atmosphere had an average relative humidity of 38% at 34 °C (absolute humidity of 14.1 mg/L $H_2O$). Samples were collected at a sampling rate of 0.05 L/min for 181 min.

Sampler exposure to light, particularly sunlight, during sampling will result in degradation of both acetoin and diacetyl. The recovery for all samples was 67.0% of theoretical for acetoin and 6.43% for diacetyl, when the sampler was used to sample a test atmosphere containing approximately one times the target concentration of acetoin and diacetyl and then exposed to 3 h of direct sunlight (samples were covered during sampling). The test atmosphere had an average relative humidity of 40% at 35 °C (absolute humidity of 15.6 mg/L $H_2O$). Samples were collected at a sampling rate of 0.05 L/min for 180 min. See Section 4.9 for data on other light tests performed.

3. Analytical Procedure

Adhere to the rules set down in your Chemical Hygiene Plan[26]. Avoid skin contact and inhalation of all chemicals and review all appropriate MSDSs.

3.1 Apparatus

A gas chromatograph equipped with an FID. For this evaluation an Agilent Technologies 6890 Plus Gas Chromatograph equipped with a 7683 Automatic Sampler and an Agilent tapered, deactivated, split, low pressure drop liner with glass wool (catalog no. 5183-4647).

A GC column capable of separating acetoin and diacetyl from the desorption solvent, internal standard and any potential interferences. A Restek 60-m × 0.32-mm i.d. $Rt_x$-Volatiles (1.5-µm df) capillary column was used in this evaluation.

[26] Occupational Exposure to Hazardous Chemicals in Laboratories. *Code of Federal Regulations*, Part 1910.1450, Title 29, 2003.

An electronic integrator or other suitable means of measuring GC detector response. Waters Empower 2 Data System was used in this evaluation.

A dispenser capable of delivering 2.0 mL of desorbing solvent to prepare standards and samples. If a dispenser is not available, a 2.0-mL volumetric pipet can be used.

Amber glass vials with PTFE-lined caps. For this evaluation 2 and 4-mL vials were used.

Calibrated 10-µL and 25-µL syringes for preparing standards.

Water purifier. A Barnstead NANOpure Diamond system was used to produce 18.0 MΩ-cm DI water in this evaluation.

Water bath. A Precision Scientific (5 – 100 °C range) water bath was used in this evaluation.

A mechanical rotator. A Fisher Roto-Rack was used in this evaluation.

Class A 1-L volumetric flasks.

Class A 1-mL and 5-mL volumetric pipets.

3.2 Reagents and Standards

Acetoin ($C_4H_8O_2$), [CAS no. 513-86-0]. The acetoin (lot no. 05025DH) used in this evaluation was purchased from Sigma Aldrich (Milwaukee, WI).

Diacetyl ($C_4H_6O_2$), [CAS no. 431-03-8]. The diacetyl used in this evaluation was 97+% (lot no. 17823LD) purchased from Sigma Aldrich (Milwaukee, WI).

DI water, 18.0 MΩ-cm.

Ethyl Alcohol [CAS no. 64-17-5]. The ethyl alcohol used in this evaluation was 95% v/v (190 proof) A.C.S. spectrophotometric grade (lot no. B0513920) purchased from Acros Organics (Morris Plains, NJ).

3-Pentanone [Cas no. 96-22-0]. The 3-pentanone used in this evaluation was 99+% (lot no. HR 00231KF) purchased from Aldrich (Milwaukee, WI).

The extraction solvent used for this evaluation consisted of 0.007 µL/mL 3-pentanone in 95% v/v ethyl alcohol. The 3-pentanone was added to the ethyl alcohol as an internal standard (ISTD).

3.3 Standard preparation

Prepare a concentrated stock standard of acetoin and diacetyl in 18.0 MΩ-cm DI water and store in an amber vial or bottle. (**Note**: Acetoin is usually obtained as the solid dimmer and will convert back to the monomer when dissolved in water.) Acetoin will slowly dissolve in water, however, this process can be accelerated by placing the solution in a 60 ºC water bath for 10 min. Refrigerate the stock standard when not in use and remake once a month.

Prepare working analytical standards by injecting microliter amounts of the concentrated stock standard into amber 4-mL vials containing 2 mL of the extraction solvent delivered by the same dispenser used to extract samples. For example, to prepare a target level standard (16.25 µg/sample acetoin and 15.86 µg/sample diacetyl) , inject 13 µL of a stock standard containing 1.25 µg/µL acetoin and 1.22 µg/µL diacetyl into 2-mL of extraction solvent. Transfer working standards to 2-mL amber glass autosampler vials.

Bracket sample concentrations with standard concentrations. If upon analysis, sample concentrations fall outside the range of prepared standards, prepare and analyze additional standards to confirm instrument response, or dilute high samples with extraction solvent and reanalyze the diluted samples.

3.4 Sample preparation

Remove the plastic end caps from the front sample tube and carefully transfer the silica gel to a 4-mL amber glass vial. The sampling tube and the back of the glass fiber filter should be carefully inspected to insure that all the silica gel is transferred into the 4-mL vial. Remove the plastic end caps from the backup tube and carefully transfer the silica gel to a second 4-mL amber glass vial. If the industrial hygienist requests analysis of the front glass fiber filter, which is not normally analyzed, place the front glass wool plug and filter from the front tube into a third 4-mL vial. If analysis of filter is not requested then discard the front glass wool plug and filter. Discard the glass tubes and back glass wool plugs and back glass fiber filter.

Add 2.0 mL of extraction solution to each vial and immediately seal with PTFE-lined caps.

**Note**: The use of an extraction solution or internal standard other than that specified in Section 3.2 should not be used unless a full extraction efficiency study is performed using both dry and wet media as described in Section 4.8.

Place the 4-mL vials on a mechanical rotator and rotate at approximately 40 rpm for 60 min.

Transfer the extraction solution in each 4-mL vial to a 2-mL amber glass autosampler vial and seal with a PTFE-lined cap.

Analyze samples for acetoin and diacetyl as described in Section 3.5.

**Note**: If after analysis lower detection limits are needed samples can be derivatized and analyzed according to Section 3.4 of OSHA Method 1012[27].

3.5 Analysis

3.5.1 Analytical conditions

GC conditions

| | |
|---|---|
| column temperature: | Initial 60 °C, hold 4 min; ramp at 15 °C/min to 135 °C, hold 0 min; ramp at 60 °C/min to 250 °C, hold 4 min |
| zone temperatures: | 240 °C (injector); 250 °C (detector) |
| run time: | 14.75 min |
| column mode: | constant pressure |
| column pressure: | 14 psi |
| initial column gas flow: | 3.3 mL/min (hydrogen) |
| injection size: | 1.0 µL (2:1 split) |

[27] Eide, M. Acetoin and Diacetyl (OSHA Method 1012), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html (accessed September 2008).

| | |
|---|---|
| column: | Restek 60-m × 0.32-mm i.d. capillary $Rt_x$-Volatiles (df = 1.5-µm) or equivalent |
| inlet liner: | Agilent 5183-4647 or equivalent |
| retention times: | 5.2 min (diacetyl)<br>8.1 min (acetoin)<br>7.5 min (ISTD) |

FID conditions

| | |
|---|---|
| hydrogen flow: | 40 mL/min |
| air flow: | 450 mL/min |
| nitrogen makeup flow: | 45 mL/min |

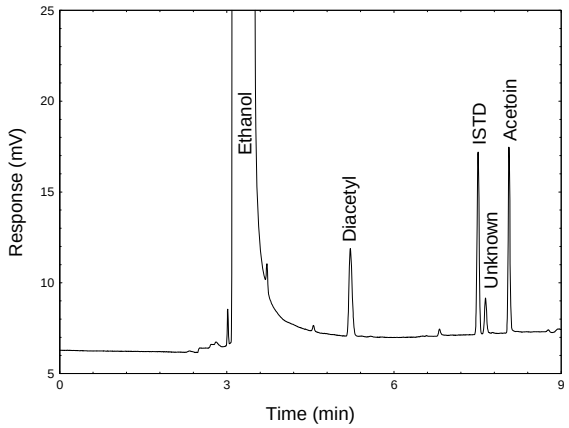



Figure 3.5.1. Chromatogram obtained at target concentrations with recommended conditions.

### 3.5.2 Calibration

An internal standard calibration method is used. A calibration curve can be constructed by plotting ISTD-corrected response of standard injections versus micrograms of analyte per sample. Bracket the samples with freshly prepared analytical standards over the range of concentrations.

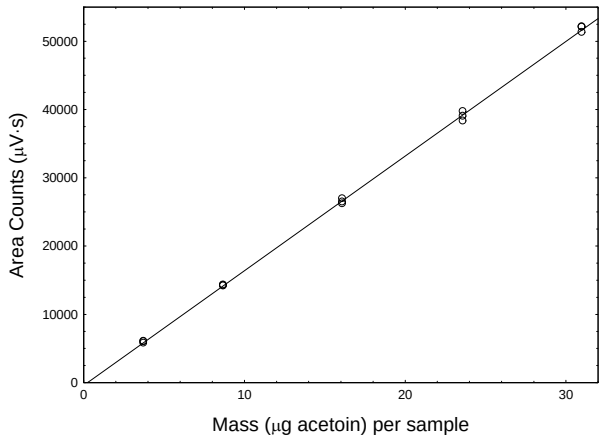



Figure 3.5.2.1. Calibration curve of acetoin. (Y = 1678X – 389)

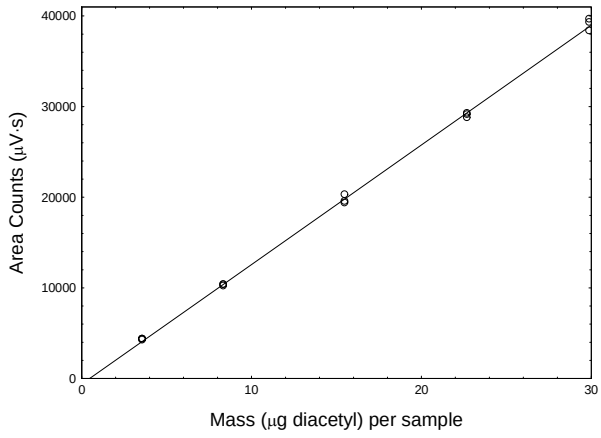



Figure 3.5.2.2. Calibration curve of diacetyl. (Y = 1320X – 625)

3.6 Interferences (analytical)

3.6.1 Any compound that produces an FID response and has a similar retention time as the analytes or internal standard is a potential interference. If any potential interferences were reported, they should be considered before samples are extracted. Generally, chromatographic conditions can be altered to separate an interference from the analyte.

3.6.2 When necessary, the identity of an analyte peak may be confirmed with additional analytical data (Section 4.12).

3.7 Calculations

The amount of analyte per sampler is obtained from the appropriate calibration curve in terms of micrograms per sample, uncorrected for extraction efficiency. The back tube is analyzed primarily to determine the extent of sampler saturation. If any analyte is found on the back tube, it is added to the amount on the front tube. This total amount is then corrected by subtracting the total amount (if any) found on the blank. The air concentration is calculated using the following formulas.

Total micrograms per sample of analyte is

$$M = [M_{front} - M_{blank}] + [M_{back} - M_{blank}]$$

where

$M$ is total µg per sample
$M_{front}$ is total µg found on front tube
$M_{back}$ is total µg found on back tube
$M_{blank}$ is total µg found on blank tube

Concentration by weight of analyte ($mg/m^3$) is

$$C_M = \frac{M}{E_E V}$$

where

$C_M$ is concentration by weight ($mg/m^3$)
$M$ is total µg per sample
$E_E$ is extraction efficiency in decimal form
$V$ is L of air sampled

Concentration by volume of analyte (ppm) is

$$C_V = \frac{V_M C_M}{M_r}$$

where

$C_V$ is concentration by volume (ppm)
$C_M$ is concentration by weight ($mg/m^3$)
$V_M$ is molar volume at NTP (24.46 L/mole)
$M_r$ is molecular weight (88.1 for acetoin, 86.09 for diacetyl)

4. Backup data

General background information about the determination of detection limits and precision of the overall procedure is found in the “Evaluation Guidelines for Air Sampling Methods Utilizing

Chromatography Analysis"[28]. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations and acceptance criteria.

4.1 Detection limit of the analytical procedure (DLAP)

The DLAP is measured as mass of analyte introduced onto the chromatographic column. Ten analytical standards were prepared with equally descending increments with the highest standard containing 1.10 µg/sample acetoin and 1.05 µg/sample diacetyl. This is the concentration that would produce a peak approximately 10 times the response of a calibration blank. These standards, and the calibration blank were analyzed with the recommended analytical parameters (1-µL injection with a 2:1 spit), and the data obtained were used to determine the required parameters (standard error of estimate and slope) for the calculation of the DLAP. For acetoin values of 5171 and 30 were obtained for the slope and standard error of estimate respectively. The DLAP for acetoin was calculated to be 0.017 ng acetoin.

Table 4.1.1
Detection Limit of the Analytical Procedure for Acetoin

| concentration (µg/sample) | mass on column (ng) | area counts (µV·S) |
|---|---|---|
| 0.000 | 0.000 | 0 |
| 0.110 | 0.028 | 157 |
| 0.220 | 0.055 | 224 |
| 0.330 | 0.083 | 386 |
| 0.440 | 0.110 | 515 |
| 0.550 | 0.138 | 738 |
| 0.660 | 0.165 | 818 |
| 0.770 | 0.193 | 998 |
| 0.880 | 0.220 | 1117 |
| 0.990 | 0.248 | 1248 |
| 1.100 | 0.275 | 1414 |

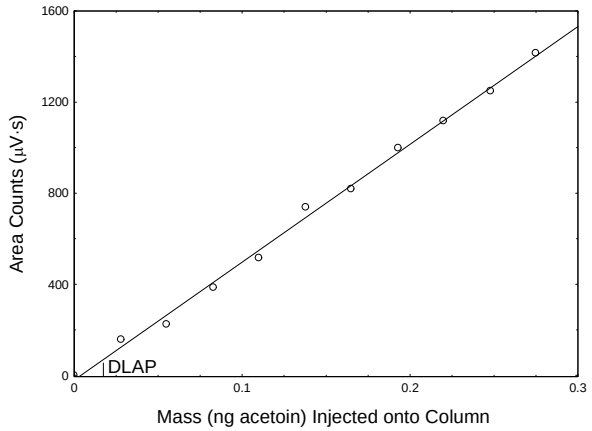



Figure 4.1.1. Plot of data to determine the DLAP for acetoin. (Y = 5171X – 19.9)

For diacetyl values of 4325 and 47 were obtained for the slope and standard error of estimate respectively. The DLAP for diacetyl was calculated to be 0.033 ng diacetyl.

Table 4.1.2
Detection Limit of the Analytical Procedure for Diacetyl

| concentration (µg/sample) | mass on column (ng) | area counts (µV·S) |
|---|---|---|
| 0.000 | 0.000 | 0 |
| 0.191 | 0.048 | 155 |
| 0.287 | 0.072 | 201 |
| 0.382 | 0.096 | 350 |
| 0.478 | 0.120 | 417 |
| 0.573 | 0.143 | 590 |
| 0.669 | 0.167 | 615 |
| 0.764 | 0.191 | 706 |
| 0.860 | 0.215 | 877 |
| 0.955 | 0.239 | 1043 |
| 1.051 | 0.263 | 1089 |

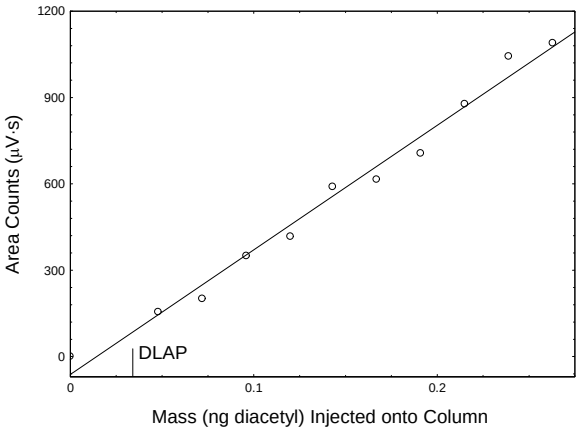



Figure 4.1.2. Plot of data to determine the DLAP for diacetyl. (Y = 4325X - 62)

[28] Burright, D.; Chan, Y.; Eide, M.; Elskamp, C.; Hendricks, W.; Rose, M. C. Evaluation Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 1999. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/chromguide.pdf (accessed November 2007).

4.2 Detection limit of the overall procedure (DLOP) and reliable quantitation limit (RQL)

The DLOP is measured as mass per sample and expressed as equivalent air concentrations, based on the recommended sampling parameters. Ten samplers were spiked with equally descending increments of acetoin and diacetyl, such that the highest sampler loading was equivalent to 1.10 µg of acetoin per sample and 0.96 µg of diacetyl per sample. This is the amount spiked on a sampler that would produce a peak approximately 10 times the response of a calibration blank. These spiked samplers, and the sample blank were analyzed with the recommended analytical parameters (1-µL injection with a 2:1 spit), and the data obtained were used to determine the required parameters (slope and standard error of estimate) for the calculation of the DLOP. For acetoin values of 1029 and 36 were obtained for the slope and standard error of estimate respectively. The DLOP was calculated to be 0.10 µg acetoin per sample (0.0031 ppm or 0.011 mg/m$^3$ for a TWA sample).

Table 4.2.1
Detection Limit of the Overall Procedure for Acetoin

| mass per sample (µg/sample) | area counts (µV·s) |
|---|---|
| 0.000 | 0 |
| 0.110 | 119 |
| 0.220 | 226 |
| 0.330 | 316 |
| 0.440 | 432 |
| 0.550 | 517 |
| 0.660 | 605 |
| 0.770 | 771 |
| 0.880 | 848 |
| 0.990 | 1057 |
| 1.100 | 1150 |

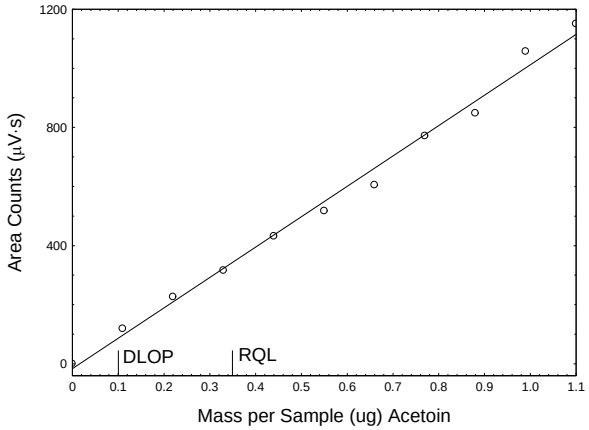



Figure 4.2.1. Plot of data to determine the DLOP/RQL for acetoin. (Y = 1029X – 16.8)

For diacetyl values of 1241 and 46 were obtained for the slope and standard error of estimate respectively. The DLOP was calculated to be 0.11 µg diacetyl per sample (0.0034 ppm or 0.012 mg/m$^3$ for a TWA sample).

Table 4.2.2
Detection Limit of the Overall Procedure for Diacetyl

| mass per sample (µg/sample) | area counts (µV·s) |
|---|---|
| 0.000 | 0 |
| 0.096 | 118 |
| 0.191 | 214 |
| 0.287 | 357 |
| 0.382 | 515 |
| 0.478 | 623 |
| 0.573 | 744 |
| 0.669 | 916 |
| 0.764 | 864 |
| 0.860 | 1043 |
| 0.955 | 1208 |

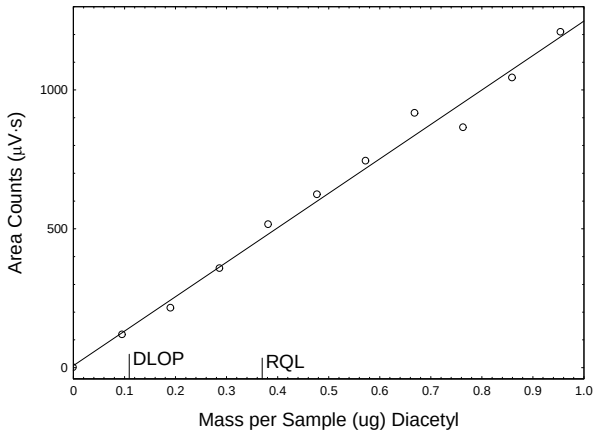



Figure 4.2.2. Plot of data to determine the DLOP/RQL for diacetyl. (Y = 1241X – 7.3)

The RQL is considered the lower limit for precise quantitative measurements. It is determined from the regression line parameters obtained for the calculation of the DLOP, providing 75% to

125% of the analyte is recovered. The RQL for acetoin is 0.35 µg per sample (0.011 ppm or 0.039 mg/m$^3$ for a TWA sample). Recovery at this concentration is 102%. The RQL for diacetyl is 0.37 µg per sample (0.012 ppm or 0.041 mg/m$^3$ for a TWA sample). Recovery at this concentration is 93.5%.

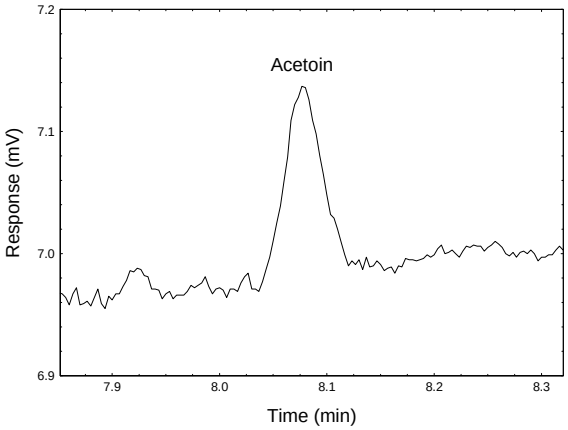



Figure 4.2.3. Chromatogram of acetoin at the RQL.

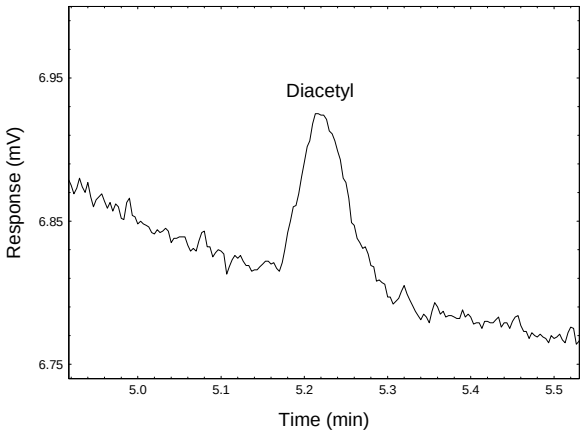



Figure 4.2.4. Chromatogram of diacetyl at the RQL.

## 4.3 Instrument calibration

The standard error of estimate was determined from the linear regression of data points from standards over a range that covers approximately 0.25 to 2 times the target concentration. Calibration curves for acetoin and diacetyl were constructed and are shown in Section 3.5.2 from the three injections of five standards. The standard error of estimate is 0.42 µg/sample for acetoin and 0.82 µg/sample for diacetyl.

Table 4.3.1
Acetoin Instrument Calibration

| standard concn (µg/sample) | area counts (µV·s) | | |
|---|---|---|---|
| 3.73 | 5782 | 6047 | 6004 |
| 8.69 | 14230 | 14168 | 14323 |
| 16.1 | 26940 | 26458 | 26198 |
| 23.6 | 38318 | 39021 | 39714 |
| 31.0 | 52053 | 51292 | 52127 |

Table 4.3.2
Diacetyl Instrument Calibration

| standard concn (µg/sample) | area counts ( µV·s) | | |
|---|---|---|---|
| 3.58 | 4242 | 4347 | 4352 |
| 8.36 | 10205 | 10350 | 10373 |
| 15.5 | 20275 | 19361 | 19540 |
| 22.7 | 28772 | 29121 | 29255 |
| 29.9 | 39287 | 38363 | 39653 |

## 4.4 Precision (overall procedure)

The precision at the 95% confidence level is obtained by multiplying the standard error of estimate by 1.96 (the z-statistic from the standard normal distribution at the 95% confidence level). In Section 4.5, 95% confidence intervals are drawn about their respective regression lines in the storage graph figures. For acetoin the precision of the overall procedure of ±11.2% was obtained from the standard error of estimate of 5.73% in Figure 4.5.1. For diacetyl the precision of the overall procedure of ±10.1% was obtained from the standard error of estimate of 5.15% in Figure 4.5.3. The precision includes an additional 5% for sampling error.

## 4.5 Storage test

Storage samples for acetoin and diacetyl were prepared by collecting samples from a controlled test atmosphere using the recommended sampling conditions. The concentration of acetoin and diacetyl were at the target concentration with an average relative humidity of 41% at 34 °C (absolute humidity of 15.2 mg/L $H_2O$). Thirty-three storage samples were prepared. Three samples were analyzed on the day of generation. Fifteen of the samples were stored at reduced temperature (3 °C) and the other fifteen were stored in a closed drawer at ambient temperature (about 21 °C). At 3-4 day intervals, three samples were selected from each of the two storage sets and analyzed. Sample results were not corrected for extraction efficiency.

Table 4.5.1
Storage Test for Acetoin

| time (days) | ambient storage recovery (%) | | | refrigerated storage recovery (%) | | |
|---|---|---|---|---|---|---|
| 0 | 86.9 | 87.7 | 89.8 | 86.9 | 87.7 | 89.8 |
| 4 | 83.1 | 92.0 | 88.3 | 88.0 | 86.1 | 87.4 |
| 7 | 91.8 | 85.1 | 90.4 | 95.3 | 90.0 | 94.0 |
| 11 | 89.1 | 92.3 | 90.9 | 90.6 | 91.4 | 92.1 |
| 14 | 90.9 | 88.5 | 91.5 | 90.7 | 88.5 | 91.9 |
| 18 | 86.5 | 85.5 | 86.1 | 91.7 | 87.6 | 89.9 |

Table 4.5.2
Storage Test for Diacetyl

| time (days) | ambient storage recovery (%) | | | refrigerated storage recovery (%) | | |
|---|---|---|---|---|---|---|
| 0 | 100.5 | 99.9 | 100.7 | 100.5 | 99.9 | 100.7 |
| 4 | 98.6 | 100.9 | 100.3 | 97.4 | 96.2 | 98.7 |
| 7 | 102.6 | 100.9 | 101.2 | 101.5 | 98.8 | 100.9 |
| 11 | 102.7 | 104.8 | 101.6 | 101.9 | 101.9 | 102.4 |
| 14 | 101.9 | 101.0 | 102.7 | 100.2 | 98.8 | 103.2 |
| 18 | 101.1 | 103.8 | 101.9 | 100.7 | 98.4 | 102.8 |

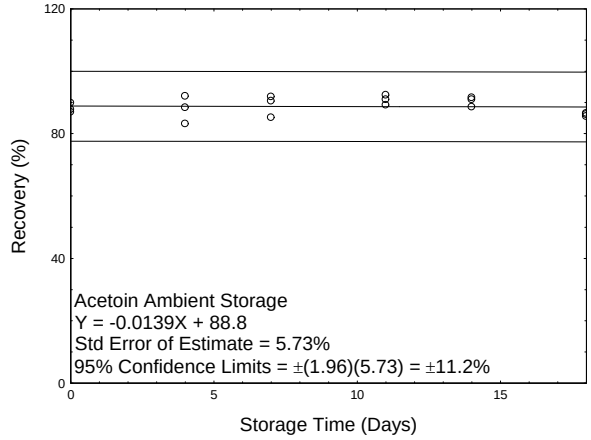



Figure 4.5.1. Ambient storage test for acetoin.

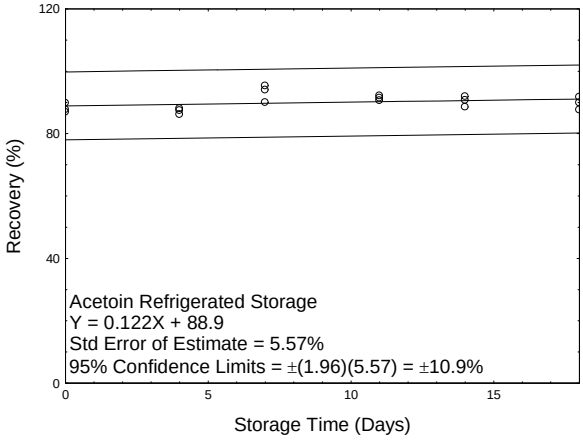



Figure 4.5.2. Refrigerated storage test for acetoin.

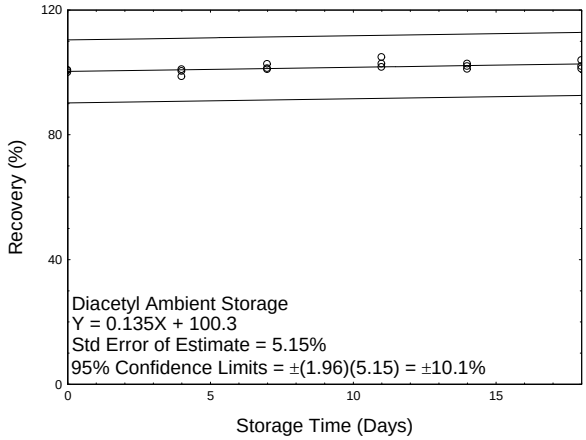



Figure 4.5.3. Ambient storage test for diacetyl.

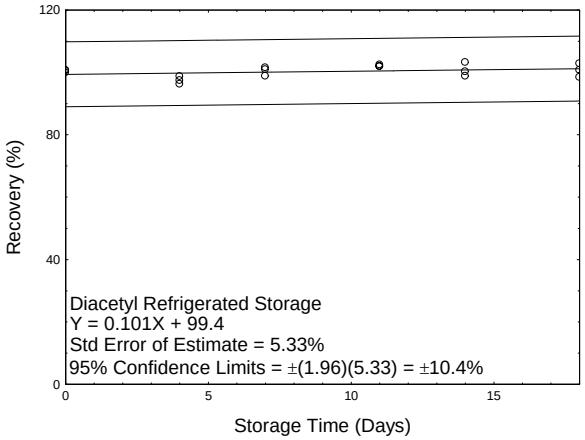



Figure 4.5.4. Refrigerated storage test for diacetyl.

## 4.6 Reproducibility

Six samples were prepared by collecting them from a controlled test atmosphere similar to that which was used in the collection of the storage samples. The samples were submitted to the OSHA Salt Lake Technical Center for analysis along with a draft copy of this method. The samples were analyzed after being stored for 20 days at refrigerated temperature (about 3 °C). Sample results were corrected for extraction efficiency. No sample result for acetoin and diacetyl had a deviation greater than the precision of the overall procedure determined in Section 4.4.

Table 4.6.1
Reproducibility Data for Acetoin

| theoretical (µg/sample) | recovered (µg/sample) | recovery (%) | deviation (%) |
|---|---|---|---|
| 16.3 | 17.3 | 106.1 | 6.1 |
| 16.4 | 15.8 | 96.3 | -3.7 |
| 16.1 | 16.8 | 104.3 | 4.3 |
| 15.8 | 15.2 | 96.2 | -3.8 |
| 16.1 | 15.7 | 97.5 | -2.5 |
| 16.6 | 16.0 | 96.4 | -3.6 |

Table 4.6.2
Reproducibility Data for Diacetyl

| theoretical (µg/sample) | recovered (µg/sample) | recovery (%) | deviation (%) |
|---|---|---|---|
| 15.9 | 16.6 | 104.4 | 4.4 |
| 15.9 | 16.3 | 102.5 | 2.5 |
| 15.7 | 16.5 | 105.1 | 5.1 |
| 15.4 | 15.8 | 102.6 | 2.6 |
| 15.7 | 16.0 | 101.9 | 1.9 |
| 16.2 | 16.6 | 102.5 | 2.5 |

## 4.7 Sampler capacity

The sampling capacity of the front tube was tested by sampling from a dynamically generated test atmosphere at 2 times the target concentration of acetoin (3.58 mg/m$^3$ or 0.99 ppm) and diacetyl (3.55 mg/m$^3$ or 1.01 ppm) with an average relative humidity of 40% at 34 °C (absolute humidity of 14.8 mg/L $H_2O$). The samples were collected at a sampling rate of 0.05 L/min. Backup tubes were placed in-line behind the front tube and were changed regularly after the initial collection of 225 min. Breakthrough for diacetyl was observed after sampling 12.4 L. No breakthrough was observed for acetoin even after sampling for 265 min. The recommended sampling time is 3 h.

Table 4.7
Breakthrough of Diacetyl

| test no. | air vol (L) | sampling time (min) | downstream concn (mg/m$^3$) | breakthrough (%) |
|---|---|---|---|---|
| 1 | 11.1 | 225 | 0.00 | 0.00 |
| | 11.8 | 240 | 0.00 | 0.00 |
| | 12.1 | 245 | 0.00 | 0.00 |
| | 12.3 | 250 | 0.00 | 0.00 |
| | 12.6 | 255 | 0.06 | 1.55 |
| | 12.8 | 260 | 0.24 | 6.68 |
| | 13.0 | 265 | 0.61 | 17.2 |
| 2 | 12.0 | 225 | 0.00 | 0.00 |
| | 12.7 | 240 | 0.22 | 6.32 |
| | 13.0 | 245 | 0.49 | 13.8 |
| | 13.3 | 250 | 0.90 | 25.3 |
| | 13.5 | 255 | 1.36 | 38.3 |
| | 13.8 | 260 | 1.86 | 52.3 |
| | 14.1 | 265 | 2.05 | 57.7 |
| 3 | 11.6 | 225 | 0.00 | 0.00 |
| | 12.4 | 240 | 0.25 | 7.04 |
| | 12.6 | 245 | 0.66 | 18.7 |
| | 12.9 | 250 | 1.32 | 37.0 |
| | 13.1 | 255 | 1.96 | 55.1 |
| | 13.4 | 260 | 2.36 | 66.5 |
| | 13.6 | 265 | 2.96 | 75.8 |

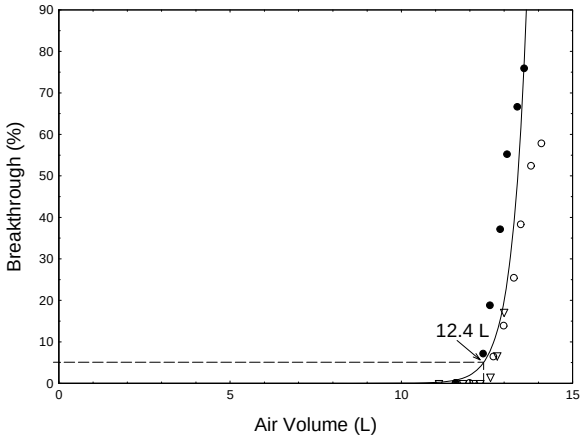



Figure 4.7. Five percent breakthrough air volume for diacetyl.

4.8 Extraction efficiency and stability of extracted samples

The extraction efficiency is dependent on the extraction solvent as well as the internal standard. Other extraction solvents or internal standards may be used provided that the new extraction solution or internal standard is tested. The new extraction solvent or internal standard should be tested as described below.

Extraction efficiency

The extraction efficiency of acetion and diacetyl was determined by liquid spiking four samplers, at each concentration level, with the analytes from the RQL to 2 times the target concentrations. These samples were stored overnight at ambient temperature and then analyzed. The mean extraction efficiency over the working range of the RQL to 2 times the target concentration is 92.9% for acetoin. The extraction efficiency for the wet samplers was not included in the overall mean because it would bias the results.

Table 4.8.1
Extraction Efficiency (%) of Acetoin

| level | | sample number | | | | |
|---|---|---|---|---|---|---|
| x target concn | µg acetoin per sample | 1 | 2 | 3 | 4 | mean |
| RQL | 0.33 | 94.0 | 96.5 | 97.4 | 96.7 | 96.2 |
| 0.25 | 3.73 | 90.5 | 87.8 | 90.1 | 90.5 | 89.7 |
| 0.5 | 8.69 | 90.2 | 92.4 | 94.6 | 95.6 | 93.2 |
| 1.0 | 16.2 | 93.2 | 93.7 | 91.9 | 92.6 | 92.8 |
| 1.5 | 23.6 | 92.3 | 93.6 | 93.5 | 92.0 | 92.8 |
| 2.0 | 31.0 | 92.7 | 93.8 | 92.7 | 92.5 | 92.9 |
| 1.0 (wet) | 16.2 | 96.8 | 94.5 | 95.3 | 95.0 | 95.4 |

The mean extraction efficiency over the working range of the RQL to 2 times the target concentration is 99.6% for diacetyl. The extraction efficiency for the wet samplers was not included in the overall mean because it would bias the results.

Table 4.8.2
Extraction Efficiency (%) of Diacetyl

| level | | sample number | | | | |
|---|---|---|---|---|---|---|
| x target concn | µg diacetyl per sample | 1 | 2 | 3 | 4 | mean |
| RQL | 0.38 | 94.1 | 97.5 | 101.2 | 89.9 | 95.7 |
| 0.25 | 3.58 | 96.8 | 97.9 | 99.3 | 98.4 | 98.1 |
| 0.5 | 8.36 | 101.8 | 100.4 | 101.9 | 101.6 | 101.4 |
| 1.0 | 15.5 | 98.0 | 101.4 | 100.2 | 101.7 | 100.3 |
| 1.5 | 22.7 | 100.9 | 102.2 | 101.4 | 100.5 | 101.2 |
| 2.0 | 29.9 | 100.9 | 101.2 | 100.7 | 100.4 | 100.8 |
| 1.0 (wet) | 15.5 | 97.8 | 97.3 | 97.2 | 99.7 | 98.0 |

Stability of extracted samples

The stability of extracted samples was investigated by reanalyzing the target concentration samples 24 h and 72 h after initial analysis. After each analysis was performed, two vials were recapped with new septa while the remaining two retained their punctured septa. The samples were reanalyzed with fresh standards. Samples were stored at ambient temperature and each septum was punctured 4 times for each analysis.

The average percent change for acetoin samples after 24 h was +0.5% for samples that were resealed with new septa and +0.5% for those that retained their punctured septa. The test was performed at room temperature (about 21 °C).

Table 4.8.3
24 Hour Stability of Extracted Samples for Acetoin

| punctured septa replaced | | | punctured septa retained | | |
|---|---|---|---|---|---|
| initial (%) | after one day (%) | difference (%) | initial (%) | after one day (%) | difference (%) |
| 93.2 | 93.1 | -0.1 | 91.9 | 92.9 | +1.0 |
| 93.7 | 94.7 | +1.0 | 92.6 | 92.5 | -0.1 |
| | (mean) | | | (mean) | |
| 93.4 | 93.9 | +0.5 | 92.2 | 92.7 | +0.5 |

The average percent change for acetoin samples after 72 h was -1.8% for samples that were resealed with new septa and -0.9% for those that retained their punctured septa.

Table 4.8.4
72 Hour Stability of Extracted Samples for Acetoin

| punctured septa replaced | | | punctured septa retained | | |
|---|---|---|---|---|---|
| initial (%) | after one day (%) | difference (%) | initial (%) | after one day (%) | difference (%) |
| 93.2 | 91.5 | -1.7 | 91.9 | 91.3 | -0.6 |
| 93.7 | 91.8 | -1.9 | 92.6 | 91.3 | -1.3 |
| | (mean) | | | (mean) | |
| 93.4 | 91.6 | -1.8 | 92.2 | 91.3 | -0.9 |

The average percent change for diacetyl after 24 h was +0.4% for samples that were resealed with new septa and -1.4% for those that retained their punctured septa. The test was performed at room temperature (about 21 °C).

Table 4.8.5
24 Hour Stability of Extracted Samples for Diacetyl

| punctured septa replaced | | | punctured septa retained | | |
|---|---|---|---|---|---|
| initial (%) | after one day (%) | difference (%) | initial (%) | after one day (%) | difference (%) |
| 98.0 | 99.0 | +1.0 | 100.2 | 99.5 | -0.7 |
| 101.4 | 101.2 | -0.2 | 101.7 | 99.7 | -2.0 |
| | (mean) | | | (mean) | |
| 99.7 | 100.1 | +0.4 | 101.0 | 99.6 | -1.4 |

The average percent change for diacetyl samples after 72 h was +1.0% for samples that were resealed with new septa and -0.8% for those that retained their punctured septa.

Table 4.8.6
72 Hour Stability of Extracted Samples for Diacetyl

| punctured septa replaced | | | punctured septa retained | | |
|---|---|---|---|---|---|
| initial (%) | after one day (%) | difference (%) | initial (%) | after one day (%) | difference (%) |
| 98.0 | 99.8 | +1.8 | 100.2 | 100.7 | +0.5 |
| 101.4 | 101.5 | +0.1 | 101.7 | 99.7 | -2.0 |
| | (mean) | | | (mean) | |
| 99.7 | 100.6 | +1.0 | 101.0 | 100.2 | -0.8 |

4.9 Interferences (sampling)

Retention

The ability of the sampler to retain acetoin and diacetyl was tested by sampling from a dynamically generated test atmosphere of acetoin (3.67 mg/m$^3$ or 1.02 ppm) and diacetyl (3.58 mg/m$^3$ or 1.02 ppm) with an average relative humidity of 40% at 35 °C (absolute humidity of 15.6 mg/L $H_2O$). Six samplers had contaminated air drawn through them at 0.05 L/min for 45 min. Sampling was discontinued and three samples set aside (first set). The generation system was flushed with contaminant-free air. Sampling resumed with the other three samples having contaminant-free air drawn through them at 0.05 L/min for 135 min and then all six samplers were analyzed. The mean of the samples in the second set had retained 100.6% for acetoin and 96.6% for diacetyl of the mean collected by the first three samples.

Table 4.9.1
Retention Efficiency (%) of Acetoin

| set no. | 1 | 2 | 3 | mean |
|---|---|---|---|---|
| first | 93.6 | 92.5 | 99.8 | 95.3 |
| second | 94.0 | 95.6 | 98.1 | 95.9 |
| second/first | | | | 100.6 |

Table 4.9.2
Retention Efficiency (%) of Diacetyl

| set no. | 1 | 2 | 3 | mean |
|---|---|---|---|---|
| first | 108.0 | 103.0 | 108.5 | 106.5 |
| second | 102.4 | 102.3 | 103.9 | 102.9 |
| second/first | | | | 96.6 |

Low humidity

The ability of the sampler to collect acetoin and diacetyl from a relatively dry atmosphere was tested by sampling from a dynamically generated test atmosphere of acetoin (4.06 mg/m$^3$ or 1.13 ppm) and diacetyl (4.03 mg/m$^3$ or 1.14 ppm) with an average relative humidity of 8% at 33 °C (absolute humidity of 2.82 mg/L $H_2O$). Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The samplers collected 103.0%, 96.9% and 102.2% of theoretical for acetoin and 96.7%, 106.6% and 101.2% of theoretical for diacetyl.

Low concentration

The ability of the sampler to collect acetoin and diacetyl at low concentrations was tested by sampling from a dynamically generated test atmosphere of 0.1 times the target concentration of acetion (0.185 mg/m$^3$ or 0.0515 ppm) and diacetyl (0.175 mg/m$^3$ or 0.0497 ppm) with an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L $H_2O$). Three samplers had contaminated air drawn through them at 0.05 L/min for 180 min. All of the samples were immediately analyzed. The samplers collected 93.9%, 91.5% and 89.9% of theoretical for acetoin and 92.8%, 97.4% and 96.7% of theoretical for diacetyl.

The ability of the sampler to collect acetoin and diacetyl at low concentrations when taking short term samples was tested by sampling from a dynamically generated test atmosphere of 0.1 times the target concentration of acetion (0.185 mg/m$^3$ or 0.0514 ppm) and diacetyl (0.175 mg/m$^3$ or 0.0497 ppm) with an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L $H_2O$). Three samplers had contaminated air drawn through them at 0.2 L/min for 15 min. All of the samples were immediately analyzed. The samplers collected 103.8%, 104.1% and 110.0% of theoretical for acetoin and 88.1%, 89.2% and 94.4% of theoretical for diacetyl.

Interferences

The ability of the sampler to collect acetoin and diacetyl was tested when other potential interferences are present by sampling an atmosphere containing 1.63 mg/m$^3$ (0.45 ppm) of acetoin, 1.56 mg/m$^3$ (0.44 ppm) of diacetyl, 2.59 mg/m$^3$ (0.44 ppm) of 2-nonanone and 1.88 mg/m$^3$ (0.44 ppm) of 2,3-pentanedione with an average relative humidity of 38% at 34 °C

(absolute humidity of 14.1 mg/L $H_2O$). Three samplers had contaminated air drawn through them at 0.05 L/min for 181 min. All of the samples were immediately analyzed. The samplers collected 93.2%, 96.5% and 96.8% of theoretical for acetoin and 100.6%, 100.6% and 104.1% of theoretical for diacetyl. Selection of 2-nonanone as a potential interference was based on its common use in butter flavorings used in microwave popcorn manufacturing facilities[29]. 2,3-Pentanedione was selected because it has been suggested as a possible replacement for diacetyl. (Note: The GC retention time of 2-nonanone was 14.4 min and 7.4 min for 2,3-pentanedione. For this test the GC column temperature program was slightly changed to Initial 60 °C, hold 4 min; ramp at 15 °C/min to 225 °C, hold 0 min; ramp at 60 °C/min to 250 °C, hold 4 min to allow for the elution of 2-nonanone.)

Light

The possibility of light degradation was tested for both acetoin and diacetyl on the sampling medium and in the extraction solution. For the sample medium test 12 samples were collected by sampling from a dynamically generated test atmosphere of acetoin (1.92 mg/m$^3$ or 0.53 ppm) and diacetyl (1.87 mg/m$^3$ or 0.53 ppm) with an average relative humidity of 40% at 35 °C (absolute humidity of 15.6 mg/L $H_2O$). The samples were collected at a sampling rate of 0.05 L/min for 3 hours. Nine of the samples were covered with aluminum foil during sampling and three were not covered. The three samples not covered and three of the covered samples were immediately analyzed after sampling. Three of the covered samples were placed under a fluorescent lamp for 24 h and the reaming three were placed outside in direct sunlight for three hours before analyzing. The samples covered during sampling and immediately analyzed after sampling had mean recoveries of 94.4% of theoretical for acetoin and 96.6% for diacetyl. The samples not covered during sampling and immediately analyzed after sampling had mean recoveries of 94.1% of theoretical for acetoin and 96.2% for diacetyl. The samples covered during sampling and then exposed to fluorescent light for 24 h before analysis had mean recoveries of 88.7% of theoretical for acetoin and 86.8% for diacetyl. The samples covered during sampling and then exposed to sunlight for 3 h before analysis had mean recoveries of 67.0% of theoretical for acetoin and 6.43% for diacetyl. This data clearly indicates that the sampler should be protected from exposure to light.

Table 4.9.3
Sampler Light Exposure Test for Acetoin

| | sample number | | | |
|---|---|---|---|---|
| type of sampler light exposure | 1 | 2 | 3 | mean |
| no light exposure | 94.0 | 97.3 | 92.0 | 94.4 |
| 3h ambient light exposure during sampling | 95.0 | 91.3 | 96.0 | 94.1 |
| 24h direct fluorescent light exposure after sampling, none during sampling | 92.5 | 86.4 | 87.1 | 88.7 |
| 3h direct sunlight exposure after sampling, none during sampling | 79.7 | 63.5 | 63.7 | 67.0 |

Table 4.9.4
Sampler Light Exposure Test for Diacetyl

| | sample number | | | |
|---|---|---|---|---|
| type of sampler light exposure | 1 | 2 | 3 | mean |
| no light exposure | 95.4 | 97.6 | 96.8 | 96.6 |
| 3h ambient light exposure during sampling | 98.0 | 94.9 | 95.8 | 96.2 |
| 24h direct fluorescent light exposure after sampling, none during sampling | 88.4 | 86.1 | 86.0 | 86.8 |
| 3h direct sunlight exposure after sampling, none during sampling | 5.68 | 7.08 | 6.52 | 6.43 |

To test the possibility of light degradation on extracted samples nine analytical standards at the target concentration were prepared. Six of the standards were placed in 2-mL amber glass vials and three were placed in 2-mL clear glass vials. Three of the amber vials, along with the

[29] Kanwal, R.; Boylstein, R. J.; Piacitelli, C. NIOSH Health Hazard Evaluation Report #2001-0474-2943, 2004. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web site. http://www.cdc.gov/niosh/hhe/reports/pdfs/2001-0474-2943.pdf (accessed July 2008) p 46.

clear glass vials were stored on the autosampler tray during the entire test while the other three amber vials were stored in the refrigerator when not being analyzed. All nine standards were analyzed eight times over a 10 day period with none of the septa being replaced during the test. With the exception of diacetyl in clear vials, acetoin and diacetyl did not degrade. This data clearly indicates that extracted samples should be protected from exposure to light. This data also indicates that acetoin and diacetyl are stable in the extraction solution for up to 9 days as long as they are stored in amber vials.

Table 4.9.5
Extracted Sample Light Exposure Test for Acetoin

| | mean of 3 peak areas | | |
|---|---|---|---|
| day | clear vials (ambient) | amber vials (ambient) | amber vials (refrigerated) |
| 0 | 24226 | 23552 | 23485 |
| 1 | 24232 | 23642 | 23535 |
| 2 | 23693 | 23232 | 22932 |
| 3 | 23455 | 23376 | 23383 |
| 4 | 23765 | 23137 | 23050 |
| 7 | 24191 | 23973 | 23280 |
| 8 | 23734 | 22969 | 22684 |
| 9 | 24245 | 23740 | 23309 |

Table 4.9.6
Extracted Sample Light Exposure Test for Diacetyl

| | mean of 3 peak areas | | |
|---|---|---|---|
| day | clear vials (ambient) | amber vials (ambient) | amber vials (refrigerated) |
| 0 | 20537 | 19789 | 19640 |
| 1 | 19037 | 19667 | 19716 |
| 2 | 17814 | 19301 | 19336 |
| 3 | 16289 | 19354 | 19723 |
| 4 | 15703 | 19026 | 19304 |
| 7 | 14603 | 19687 | 19577 |
| 8 | 13328 | 18509 | 19026 |
| 9 | 12408 | 19324 | 19606 |

The internal standard, 3-pentanone, was stable for up to 9 days in both the clear and ambient vials.

4.10 Diacetyl migration within sampling tubes

Table 4.10
Ambient Storage Diacetyl Migration Test

| time (days) | diacetyl found on backup section (%) | | |
|---|---|---|---|
| 0 | 0.00 | 0.00 | 0.00 |
| 4 | 3.07 | 0.54 | 0.82 |
| 7 | 8.30 | 4.59 | 4.94 |
| 11 | 5.81 | 11.2 | 7.69 |
| 14 | 9.63 | 11.9 | 13.0 |

In the majority of solid sorbent sampling tubes used by OSHA the sampling bed and the backup bed of sorbent are placed in the same sampling tube. For diacetyl this was not possible due to the migration of diacetyl within the sampling tube during storage. To demonstrate migration fifteen tubes were packed with 600 mg of silica gel and a backup section of 200 mg silica gel separated with a glass wool plug. These fifteen tubes were used to collect samples from a dynamically generated test atmosphere of acetoin (3.35 mg/m$^3$ or 0.93 ppm) and diacetyl (3.17 mg/m$^3$ or 0.90 ppm) with an average relative humidity of 42% at 33 °C (absolute humidity of 14.8 mg/L $H_2O$). The samples were collected at a sampling rate of 0.05 L/min for 3 hours. Three samples were analyzed on the day of generation and the other twelve were stored in a closed drawer at ambient temperature (about 21 °C). At 3-4 day intervals, three additional samples were analyzed. After 14 days up to 13.0% of diacetyl was found to have migrated from the front to the back section of the modified sampling tube. Acetoin did not migrate within the sampling tube.

4.11 Generation of test atmospheres

A test atmosphere generator, as diagramed in Figure 4.11, was set up in a walk-in hood. House air was dried and then humidified and regulated using a Miller Nelson Model 401 Flow-Temperature-Humidity Control System. A measured flow (typically 10 µL per min) of an acetoin and diacetyl water solution was pumped through a 0.53-mm uncoated fused silica capillary tube into the inlet manifold, using a Series D ISCO Syringe Pump with Controller, and mixed with dilution air (typically 100 liters per min) coming from the Miller Nelson Control System. The inlet manifold was heated by wrapping it in heat tape, regulated with a variable autotransformer, in order to insure vaporization of acetoin. The acetoin and diacetyl gas mixture then flowed continuously into the mixing chamber (76-cm × 15-cm) and then into the sampling chamber

(56-cm × 9.5-cm). Samples were collected through sampling ports on the sampling chamber. Temperature and humidity were measured near the exit of the sampling chamber using an Omega Digital Thermo-hygrometer model RH411.

With the exception of low humidity tests OSHA normally generates test atmospheres at an average relative humidity of 80% at 22 °C resulting in an absolute humidity of 15.5 mg/L $H_2O$. Due to the use of heat tape on the inlet manifold, used as mentioned above to insure the vaporization of acetoin, the test atmosphere generation temperature for this evaluation was typically around 34 °C at the sampling chamber outlet, 37 °C in the middle of the sampling chamber, 45 °C at the sampling chamber inlet and 86 °C at the mixing chamber inlet. In order to maintain a humidity of 15.5 mg/L $H_2O$ at 34 °C the relative absolute humidity was adjusted to approximately 41%.

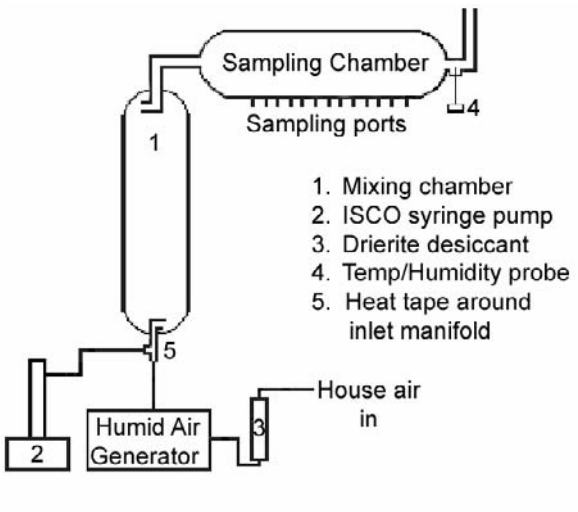



Figure 4.11. Diagram of apparatus used to generate test atmospheres.

4.12 Qualitative analysis

When necessary, the identity or purity of an analyte peak can be confirmed by GC-mass spectrometry or by another analytical procedure. The mass spectra in Figure 4.12.1 and 4.12.2 are taken from the NIST spectral library.

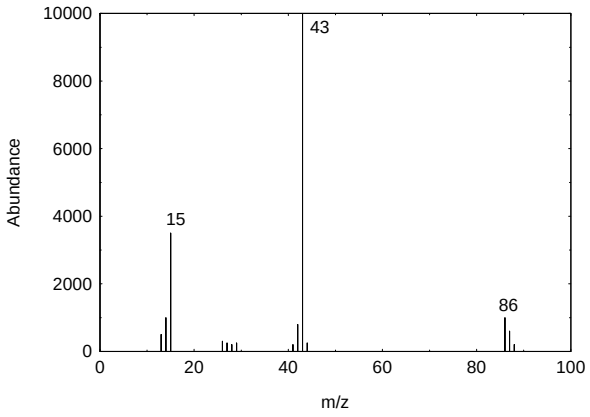



Figure 4.12.1. Mass spectrum of diacetyl.

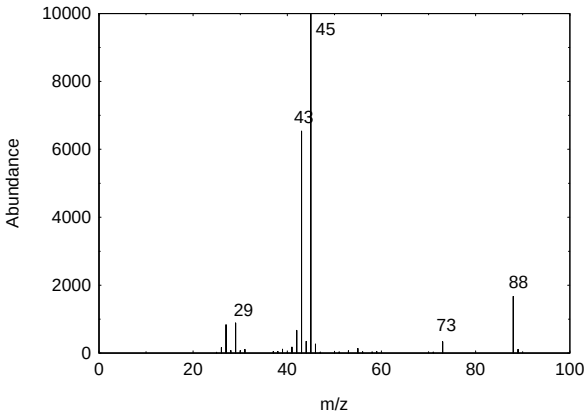



Figure 4.12.2. Mass spectrum of acetoin.

Appendix A

## A.1 Silica gel preparation

For this evaluation sampling tubes were custom made by SKC, Inc. and are now available for purchase through SKC, Inc. (cat. no. 226-183).

Below are instructions on how the silica gel is prepared for the sampling tubes used in this evaluation.

### A.1.1 Apparatus

Tube furnace and quartz process tube. A Lindberg model 55035 tube furnace and 1-inch diameter quartz process tube were used in this evaluation.

Nitrogen gas.

### A.1.2 Silica Gel

Washed 20/40 mesh silica gel with 30 angstrom pore size (washed silica gel can be purchased from SKC, Inc.). A description of a washing procedure for silica gel can be found in the appendix of NIOSH 7903[30].

### A.1.3 Preparation of silica gel

Insert a quartz wool plug in a 1-inch diameter quartz process tube, followed by 50 g of washed silica gel and a second quartz wool plug to hold the silica gel in place.

Place the process tube in a tube furnace and set the temperature to 180 °C. Continually purge the process tube with nitrogen at a rate of about 0.5 L/min. Allow the silica gel to dry in the tube furnace for 4 hours.

After 4 hours allow the process tube to cool while continuing to purge the tube with nitrogen. Once the silica gel is cool, remove one of the quartz wool plugs, and transfer silica gel into an airtight container.

[30] Cassinelli, M. E. Acids, Inorganic (NIOSH Method 7903), 1994. Centers for Disease Control and Prevention, National Institute of Occupational Safety and Health Web Site. http://www.cdc.gov/niosh/nmam/pdfs/7903.pdf (accessed July 2008).

| | |
|---|---|
| Method no.: | 1016 |
| Version: | 1.0 |
| Target concentration: | 0.5 ppm (2.05 mg/m$^3$) (TWA) |
| Procedure: | Active samples are collected by drawing workplace air through specially dried silica gel tubes with personal sampling pumps. Samples are extracted with 95:5 ethyl alcohol:water and analyzed by gas chromatography using a flame ionization detector (GC-FID). |
| Recommended sampling time and sampling rate: | 200 min at 50 mL/min (10.0 L) (TWA); 15 min at 0.2 L/min (3 L) (short term)<br>180 min at 50 mL/min (9.0 L) (TWA); 15 min at 0.2 L/min (3 L) (short term) if sampling for acetoin and diacetyl along with 2,3-pentanedione |
| Reliable quantitation limit: | 9.3 ppb (38 µg/m$^3$) |
| Standard error of estimate at the target concentration: | 10.1% |
| Special requirements: | Protect samplers from the light exposure during sampling, shipping, and analysis. Samples should be kept cold and shipped cold to the lab as soon as possible after sampling, preferably by overnight or express shipping. Samples should be analyzed within 17 days of sampling. |
| Status of method: | Fully validated method. This method has been subjected to the established validation procedures of the Methods Development Team. |

July 2010

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1. General Discussion

For assistance with accessibility problems in using figures and illustrations presented in this method, please contact Salt Lake Technical Center (SLTC) at (801) 233-4900. These procedures were designed and tested for internal use by OSHA personnel. Mention of any company name or commercial product does not constitute endorsement by OSHA.

1.1 Background

1.1.1 History

OSHA is concerned about workplace exposure to 2,3-pentanedione because it is a butter flavoring agent that is sometimes substituted for diacetyl.[1] 2,3-Pentanedione is chemically similar to diacetyl and may have similar toxicological properties.[2] This work was performed because OSHA has no sampling and analytical method for 2,3-pentanedione and none was found in a literature review.

One of the main objectives of this work was to enable OSHA CSHOs to monitor workplace exposure to diacetyl, acetoin and 2,3-pentanedione simultaneously on the same sample. Because of the similarities of the chemicals, it was decided to validate existing sampling and analytical methodology specified in OSHA Method 1013[3] for 2,3-pentanedione. That method requires sampling with two commercially available silica gel tubes connected in series. This method specifies a different GC column than specified in Method 1013 in order to optimize the analytical separation. The reliable quatitation limits for acetoin and diacetyl cited in OSHA Method 1013 were confirmed with the GC column used in this validation.

1.1.2 Toxic effects (This section is for information only and should not be taken as the basis of OSHA policy.)

2,3-Pentanedione is moderately toxic by ingestion, a skin irritant, and can cause eye and respiratory tract irritation.[4] The oral $LD_{50}$ in rats is 3000 mg/kg. The skin irritation test in rabbits showed moderate irritation for an exposure of 500 mg/24h. Studies exposing rats to 118, 241, 318, or 354 ppm 2,3-pentanedione for 6 hours showed epithelial changes in the airways which increased with increasing air concentrations with necrosuppurative tracheitis in the rats exposed to 354 ppm.[5] This epithelial cell damage was found to progress post-exposure in rats sacrificed a day later. These epithelial changes included degeneration, apoptosis, necrosis, and neutrophilic inflammation.

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1 News Watch, Diacetyl. *The Synergist.* **March 2010**. American Industrial Hygiene Association Web site. http://www.aihasynergist-digital.org/aihasynergist/201003#pg39 (accessed August 2010).

2 Hubbs, A.F.; Mosely, A.E.; Goldsmith, W.T.; Jackson, M.C.; Kashon, M.L.; Battelli, L.A.; Schwegler-Berry, D.; Goravanahally, M.P.; Frazer, D.; Fedan, J.S.; Kreiss, K.; and Castranova, V. Airway Epithelial Toxicity of the Flavoring Agent, 2,3-Pentanedione. *Toxicologist* [CD-ROM] **2010**, *114*, 319.

3 Simmons, M., Hendricks, W. Acetoin and Diacetyl (OSHA Method 1013), 2008. U.S. Department of Labor, Occupational Safety and Health Administration Web site. https://www.osha.gov/dts/sltc/methods/validated/1013/1013.html (accessed December 2009).

4 *Sax's Dangerous Properties of Industrial Materials*, 10th ed.; Vol. 3, Lewis, R.J. Ed.; John Wiley & Sons; New York, 2000, p 2843.

5 Hubbs, A.F.; Mosely, A.E.; Goldsmith, W.T.; Jackson, M.C.; Kashon, M.L.; Battelli, L.A.; Schwegler-Berry, D.; Goravanahally, M.P.; Frazer, D.; Fedan, J.S.; Kreiss, K.; and Castranova, V. Airway Epithelial Toxicity of the Flavoring Agent, 2,3-Pentanedione. *Toxicologist* [CD-ROM] **2010**, *114*, 319.

### 1.1.3 Workplace exposure

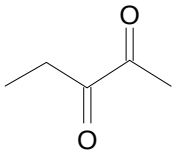
2,3-Pentanedione is a natural flavorant and odorant that is also synthesized for use in odor and flavor manufacturing.[6] It is used to give products a buttery, nutty, cheesy, fruity, toasted, chocolate, or caramel taste. It also gives products a buttery, fruity, and caramel odor. There can be as much as 58 ppm in food flavorings, and up to 0.08% in fragrances.

2,3-Pentanedione is used as a solvent for cellulose acetate, paints, inks, lacquers, as a starting material for dyes, pesticides and pharmaceuticals, and as a photoinitializer for photo-reactive dyes.[7]

### 1.1.4 Physical properties and other descriptive information[8,9,10]

| | | | |
|---|---|---|---|
| synonyms: | acetyl propanal; acetyl propionyl; β,γ-dioxopentane; beta, gamma-dioxopentane; 2,3-pentadione | | |
| IMIS[11]: | P110 | CAS number: | 600-14-6 |
| boiling point: | 110-112 °C (230-234 °F) | melting point: | -52 °C (-62 °F) |
| density: | 0.957 g/mL @ 25 °C | molecular weight: | 100.12 |
| flash point: | 19 °C (66 °F) (open cup) | molecular formula: | $C_5H_8O_2$ |
| appearance: | yellow to yellow-green liquid | lower explosive limit: | 1.8% (by volume) |
| autoignition temperature: | 265 °C (509 °F) | | |
| solubility: | 66.7 g/L water; miscible with alcohol, fixed oils, propylene glycol | | |
| odor: | butter-like in dilute concentration, quinone-like in high concentration | | |
| reactive hazards: | light sensitive (Section 4.9); vapors are highly flammable and may ignite when pouring or pumping due to static electricity | | |

structural formula of 2,3-pentanedione

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This method was validated according to the OSHA SLTC "Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis"[12]. The Guidelines define analytical parameters, specify required laboratory tests, statistical calculations, and acceptance criteria. The analyte air concentrations throughout this method are based on the recommended sampling and analytical parameters. Air concentrations in ppm are referenced to 25 °C and 760 mmHg (101.3 kPa).

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[6] *Fenarolli's Handbook of Flavor Ingredients,* 5th ed.; Burdock, G.A.; CRC Press; Boca Raton, FL, 2005, p 1495.

[7] 2,3-Pentanedione, Chemicalland21 Website. http://chemicalland21.com/lifescience/foco/2,3-PENTANEDIONE.htm (accessed February 2010).

[8] *Sax's Dangerous Properties of Industrial Materials*, 10th ed.; Vol. 3, Lewis, R.J.; John Wiley & Sons; New York, 2000, p 2843.

[9] Lewis, R. J. Sr., Ed. *Hawley's Condensed Chemical Dictionary*, 14th ed.; Van Nostrand Reinhold Co.: New York, 2001, p 14.

[10] 3-Pentanedione(600-14-6) Chemical Book Web site. http://www.chemicalbook.com/ProductMSDSDetailCB6166470_EN.htm (accessed 1/27/2010).

[11] 2,3-Pentanedione (OSHA Chemical Sampling Information), 2010. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/chemicalsampling/data/CH_260240.html, (accessed 1/5/2010).

[12] Eide, M.; Hendricks, W.; Simmons, M. Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis, 2010. U.S. Department of Labor, Occupational Safety and Health Administration Web site. http://www.osha.gov/dts/sltc/methods/chromguide/chromguide.html (accessed January 2010).

2. Sampling Procedure

All safety practices that apply to the work area being sampled should be followed. The sampling equipment should be attached to the worker in such a manner that it will not interfere with work performance or safety.

2.1 Apparatus

Samples are collected with 110-cm × 7-mm o.d. glass sampling tubes packed with a single section (600 mg) of specially cleaned and dried silica gel. The section is held in place with glass wool and with a glass fiber filter in the front and glass wool at the back. A sampling train is prepared by placing two tubes in series. For this validation, commercially prepared sampling tubes were purchased from SKC, Inc. The two tubes are identical, but SKC labels the tubes as "Part A" which is the front tube and as "Part B" which is the back tube (Catalog no. 226-183, lot no. 6148).

Use an opaque tube holder, such as SKC, Inc. Tube Cover D (cat. no. 224-29D) to cover the sampling train during sampling. If the tube holder is not opaque, wrap the sampler with aluminum foil. Light can decompose collected 2,3-pentanedione.

Samples are collected using a personal sampling pump calibrated to within ±5% of the recommended flow rate with the sampling device in-line.

2.2 Reagents

None required

2.3 Technique

Immediately before sampling, break off both ends of the flame-sealed tube to provide an opening approximately half the internal diameter of the tube. Wear eye protection when breaking ends. Use sampling tube holders to minimize the hazard to the worker from the broken ends of the tubes and to minimize the potential of glass shards entering the foodstuffs. All tubes should be from the same lot.

A sampling train is prepared by attaching a Part A tube in front of and in series with a Part B tube, with both glass fiber filters facing forward.

The Part B tube in the sampling train is used as a back-up and is positioned nearest the sampling pump. Attach the tube holder (with the adsorbent tube sampling train) to the sampling pump so that the sampling train is in an approximately vertical position with the inlet facing down in the worker's breathing zone during sampling. Position the sampling pump, tube holder and tubing so they do not impede work performance or safety.

Draw the air to be sampled directly into the inlet of the tube holder. The air being sampled is not to be passed through any hose or tubing before entering the sampling tube.

Sample for up to 200 min at 50 mL/min (10 L) to collect TWA (long-term) samples. If acetoin and/or diacetyl are anticipated to be present, sample for up to 180 min at 50 mL/min (9 L) to collect TWA (long-term) samples.

Sample for 15 min at 0.2 L/min (3 L) to collect short-term samples.

After sampling for the appropriate time, remove the sampling train, separate the tubes, and cap each tube with plastic end caps. Separately wrap each tube in aluminum foil and seal each tube end-to end with a Form OSHA-21 as soon as possible.

Submit at least one blank sample with each set of samples. Handle the blank sample in the same manner as the other samples except draw no air through it.

Record sample air volumes (L), sampling time (min), and sampling rate (mL/min) for each sample, along with any potential interferences on the Form OSHA-91A.

Submit the samples to the laboratory for analysis as soon as possible after sampling, preferably by overnight or express shipping. If delay is unavoidable, store the samples in a refrigerator. Ship samples cold to laboratory, such as shipping with frozen plastic ice packs in a cooler.

Ship any bulk samples separate from the air samples.

3. Analytical Procedure

Adhere to the rules set down in your laboratory's Chemical Hygiene Plan[13] (for instance OSHA SLTC adheres to: "The OSHA SLTC Chemical Hygiene Plan"). Avoid skin contact and inhalation of all chemicals and review all MSDSs before beginning this analytical procedure. Follow all applicable quality assurance practices established in your internal quality system (for instance OSHA SLTC follows: "The OSHA SLTC Quality Assurance Manual").

3.1 Apparatus

Gas chromatograph equipped with an FID. An Agilent 6890 GC System equipped with a Chemstation, an automatic sample injector, and an Agilent tapered, deactivated, split, low pressure drop injection port liner with glass wool (catalog no. 5183-4647) was used in this validation.

A GC column capable of separating 2,3-pentanedione from the extraction solvent, potential interferences, and internal standard. A DB-1 60-m × 0.32-mm i.d. (5-µm df) capillary column was used in this validation.

An electronic integrator or other suitable means of measuring GC detector response. A Waters Empower 2 Data System was used in this validation.

Amber glass vials with PTFE-lined caps. Two and 4-mL vials were used in this validation.

A dispenser capable of delivering 2.0 mL of extraction solvent to prepare standards and samples. If a dispenser is not available, 2.0-mL volumetric pipettes can be used.

Class A volumetric flasks - 10-mL and other convenient sizes for preparing standards.

Calibrated syringe - 25-µL and other convenient sizes for preparing standards.

.

Rotator. A Fisher Roto Rack was used to extract the samples in this validation.

3.2 Reagents

DI water, 18.0 MΩ-cm. A Barnstead NanoPure Diamond system was used to purify the water in this validation.

Ethyl Alcohol, [CAS no. 64-17-5]. The ethyl alcohol:water solution used in this validation was 95% v/v (190 proof) A.C.S. spectrophotometric grade (lot no. B0513920) purchased from Acros Organics (Morris Plains, NJ). Do not use absolute alcohol or denatured alcohol in this method.

[13] Occupational Exposure to Hazardous Chemicals in Laboratories. *Code of Federal Regulations*, Part 1910.1450, Title 29, 2003.

2,3-Pentanedione [CAS no. 600-14-6]. The 2,3-pentanedione used in this validation was 97% (lot no. 29598LJ) purchased from Aldrich (Milwaukee, WI).

3-Pentanone [CAS no. 96-22-0]. The 3-pentanone used in this validation was 99+% (lot no. HR 00231KF) purchased from Aldrich (Milwaukee, WI).

The extraction solvent used for this validation consisted of 0.007 µL/mL 3-pentanone in 95% v/v ethyl alcohol/water. The 3-pentanone was added to the ethyl alcohol as an internal standard (ISTD).

3.3 Standard preparation
(Note: Store all standards in amber glass bottles and vials)

Prepare concentrated stock standards in water at 1.021 mg/mL (1.021 µg/µL) by injecting 11 µL of neat 2,3-pentanedione into water in a 10-mL volumetric flask and diluting to the mark. This stock standard will remain stable for two weeks if stored in an amber bottle in the refrigerator. When using refrigerated stock standards, be sure to allow the standards to warm to room temperature and then shake them vigorously before use. Prepare analytical standards by injecting microliter amounts of concentrated stock standards into 2-mL volumetric flasks containing about 1.75 mL of extraction solvent and then diluting with extraction solvent over a concentration range of 0.1 to 20 µg/mL (0.2 to 40 µg/2 mL). For example: a target concentration standard of 20.4 µg/sample was prepared by injecting 20 µL of the stock standard into a 2-mL flask containing about 1.75 mL of extraction solvent and then diluting to the mark with extraction solvent (10.2 µg/mL or 0.5 ppm based on a 2-mL extraction volume per sample and 10 L air volumes).

Bracket sample concentrations with standard concentrations. If upon analysis, sample concentrations fall outside the range of prepared standards, prepare and analyze additional standards to confirm instrument response, or dilute high samples with extraction solvent and reanalyze the diluted samples.

3.4 Sample preparation
(Note: prepare all samples in amber glass vials)

Remove the plastic end caps from the front sample tube and carefully transfer the silica gel to a labeled 4-mL amber glass vial. The sampling tube and the back of the glass fiber filter should be carefully inspected to ensure that all the silica gel is transferred into the 4-mL vial. Remove the plastic end caps from the backup tube and carefully transfer the silica gel to a second labeled 4-mL amber glass vial. If the industrial hygienist requests analysis of the front glass fiber filter, which is not normally analyzed, place the front glass wool plug and filter from the front tube into a third 4-mL vial. If analysis of filter is not requested then discard the front glass wool plug and filter. Discard the glass tubes and back glass wool plugs and back glass fiber filter.

Add 2.0 mL of extraction solvent to each vial and immediately seal the vials with PTFE-lined caps.

Immediately place the vials on a rotator for 60 min. Transfer the sample into autosampler vials for analysis.

3.5 Analysis

3.5.1 Gas chromatographic conditions (these conditions are different from OSHA Method 1013 to obtain better separation of the 2,3-pentanedione peak from the 3-pentanone internal standard peak).

GC conditions

| | |
|---|---|
| oven temperature: | initial 60 °C, hold 4 min, program at 10 °C/min to 150 °C, hold 5 min, 20 °C/min to 200 °C hold 1 min |
| injector temperature: | 240 ºC |
| detector temperature: | 250 ºC |
| run time: | total time is 21.5 min, data is collected for 15 min, the excess time is to clear the column |
| column: | 60-m × 0.32-mm i.d. DB-1 capillary column (df = 5-µm) |
| column mode: | constant pressure |
| initial column gas flow: | 1.8 mL/min (hydrogen) |
| column pressure: | 9.4 psi |
| injection size: | 1.0 µL (2:1 split) |
| inlet liner: | Agilent 5183-4647 or equivalent |
| retention times: | 13.2 min 2,3-pentanedione<br>13.5 min 3-pentanone |

FID conditions:

| | |
|---|---|
| hydrogen flow: | 40 mL/min |
| air flow: | 450 mL/min |
| nitrogen makeup flow: | 40 mL/min |

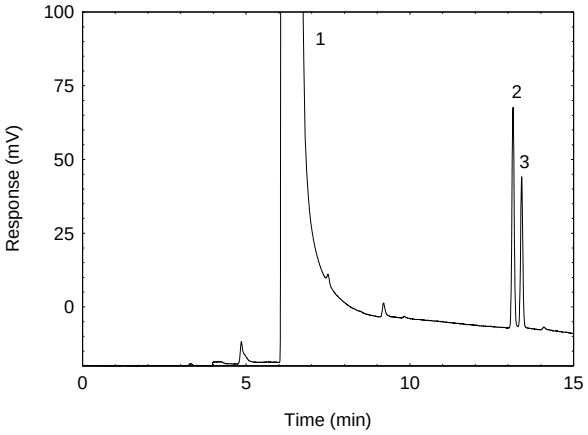


Figure 3.5.1.1. A chromatogram of 20.4 µg/sample 2,3-pentanedione. (Key: 1) ethyl alcohol; 2) 2,3-pentanedione; and 3) 3-pentanone.)

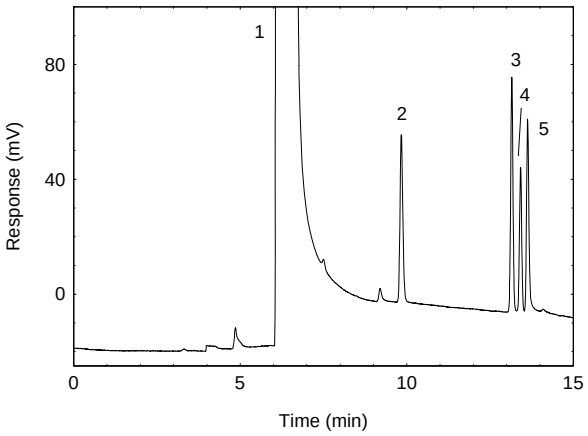


Figure 3.5.1.2. A chromatogram of 20.4 µg/sample 2,3-pentanedione, 15.8 µg/sample acetoin, and 15.6 µg/sample diacetyl. (Key: 1) ethyl alcohol; 2) diacetyl; 3) 2,3-pentanedione; 4) 3-pentanone; and 5) acetoin.)

3.5.2 An internal standard (ISTD) calibration method is used. A calibration curve can be constructed by plotting ISTD-corrected response of standard injections versus micrograms of analyte per sample. Bracket the samples with freshly prepared analytical standards over the range of concentrations.

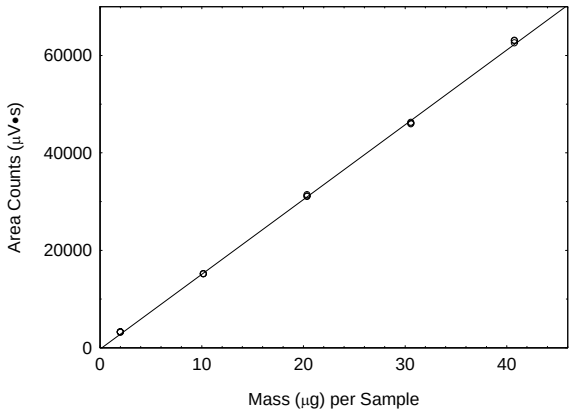


Figure 3.5.2.1. Calibration curve for 2,3-pentanedione.
(y = 1535x - 295)

3.6 Interferences (analytical)

3.6.1 Any compound that produces a GC response and has a similar retention time as the analyte or internal standard is a potential interference. If potential interferences were reported, they should be considered before samples are extracted. Generally, chromatographic conditions can be altered to separate interferences from the analyte.

3.6.2 When necessary, the identity of an analyte peak can be confirmed with additional analytical data or procedures (Section 4.10).

3.7 Calculations

The amount of analyte per sample is obtained from the appropriate calibration curve in terms of micrograms per sample, uncorrected for extraction efficiency. The second tube is analyzed primarily to determine the extent of sampler saturation. If any analyte is found on the back tube, it is added to the amount on the front tube. If more than 20% of the total amount is found on the back tube, report that the sampler may have been saturated on the Form OSHA-91B. This total amount is then corrected by subtracting the total amount (if any) found on the blank. The air concentration is calculated using the following formulas.

$$C_M = \frac{M}{VE_E}$$

where $C_M$ is concn by weight (mg/m$^3$)
$M$ is micrograms per sample
$V$ is liters of air sampled
$E_E$ is extraction efficiency in decimal form

$$C_V = \frac{C_M V_M}{M_r}$$

where $C_V$ is concn by volume (ppm)
$C_M$ is concn by weight (mg/m$^3$)
$V_M$ is 24.46 (molar volume at NTP)
$M_r$ is molecular weight of analyte
(2,3-pentanedione = 100.12)

4. Method Validation

General instruction for the laboratory validation of OSHA sampling and analytical methods that employ chromatographic analysis is presented in “Validation Guidelines for Air Sampling Methods Utilizing

Chromatography Analysis"[14]. These Guidelines detail required validation tests, show examples of statistical calculations, list validation acceptance criteria, and define analytical parameters. Air concentrations listed in ppm are referenced to 25 °C and 760 mmHg (101.3 kPa).

4.1 Detection limit of the analytical procedure (DLAP)

The DLAP is measured as mass of analyte introduced into the chromatographic column. Ten analytical standards were spiked with equally descending increments of analyte. The highest amount is the amount spiked on the sampler that would produce a peak approximately 10 times the response of a reagent blank at or near the retention time of the analyte. The standards and the reagent blank were analyzed with the recommended analytical parameters (1-µL injection with a 2:1 split). The data obtained were used to determine the required parameters (standard error of estimate and slope) for the calculation of the DLAP. The slope and standard error of estimate, respectively, were 6.62 and 62.2. The DLAP was calculated to be 28 pg.

Table 4.1
Detection Limit of the Analytical Procedure

| concn (ng/mL) | mass on column (pg) | area counts (µV•s) |
|---|---|---|
| 0 | 0 | 0 |
| 51 | 26 | 160 |
| 102 | 51 | 367 |
| 153 | 77 | 556 |
| 204 | 102 | 618 |
| 255 | 128 | 883 |
| 306 | 153 | 949 |
| 357 | 179 | 1084 |
| 408 | 204 | 1281 |
| 460 | 230 | 1547 |
| 511 | 256 | 1784 |

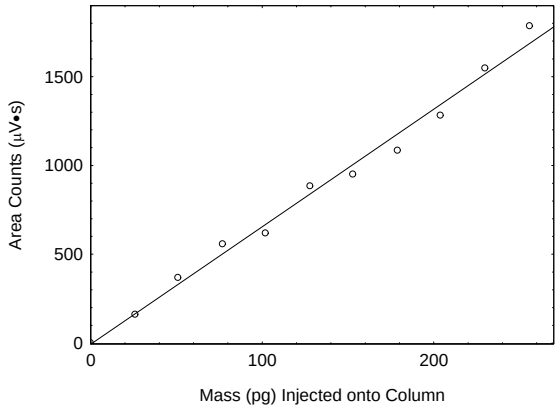


Figure 4.1. Plot of data to determine the DLAP (y = 6.62x - 7.12).

4.2 Detection limit of the overall procedure (DLOP) and reliable quantitation limit (RQL)

The DLOP is measured as mass per sample and expressed as equivalent air concentrations, based on the recommended sampling parameters. Ten samplers were spiked with equally descending increments of analyte. The highest amount is the amount spiked on the sampler that would produce a peak approximately 10 times the response of a sample blank at or near the retention time of the analyte. The spiked samplers, and the sample blank were analyzed with the recommended analytical parameters, and the data obtained used to determine the required parameters (slope and standard error of estimate) for the calculation of the DLOP. For 2,3-pentanedione values of 1597 and 61.2 were obtained for the slope and standard error of estimate respectively. The DLOP was calculated to be 0.11 µg (2.7 ppb or 11 µg/m$^3$).

[14] Eide, M.; Hendricks, W.; Simmons, M. *Guidelines For Air Sampling Methods Utilizing Chromatographic Analysis.* https://www.osha.gov/dts/sltc/methods/chromguide/chromguide.pdf, OSHA Salt Lake Technical Center, U.S. Department of Labor: Salt Lake City, UT, 2010 (accessed January 2010).

Table 4.2
Detection Limit of the Overall Procedure

| mass per sample (µg) | area counts (µV•s) |
|---|---|
| 0.00 | 0 |
| 0.10 | 153 |
| 0.20 | 349 |
| 0.31 | 545 |
| 0.41 | 599 |
| 0.51 | 848 |
| 0.61 | 930 |
| 0.71 | 1063 |
| 0.82 | 1217 |
| 0.92 | 1485 |
| 1.02 | 1731 |

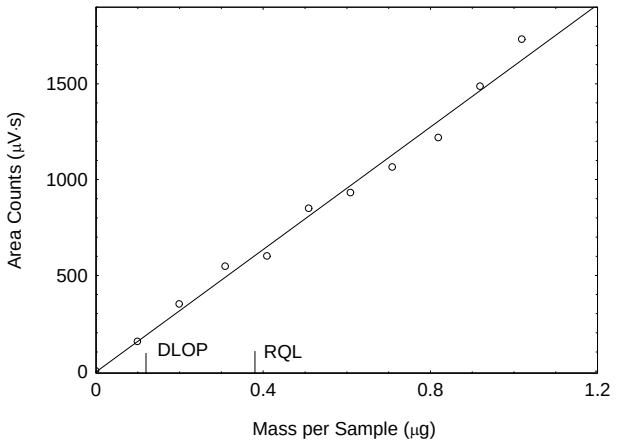


Figure 4.2.1. Plot of data to determine the DLOP/RQL (y = 1597x – 3.74).

The RQL is considered the lower limit for precise quantitative measurements. It is determined from the regression line parameters obtained for the calculation of the DLOP, providing 75% to 125% of the analyte is recovered. The RQL for 2,3-pentanedione is 0.38 µg per sample (9.3 ppb or 38 µg/m$^3$ for a TWA sample). Recovery at this concentration is 97.9%.

When short-term samples are collected, the air concentration equivalent to the reliable quantitation limit becomes larger. For example, the reliable quantitation limit for the recommended sampler is 31 ppb (127 µg/m$^3$) when 3 L is sampled.

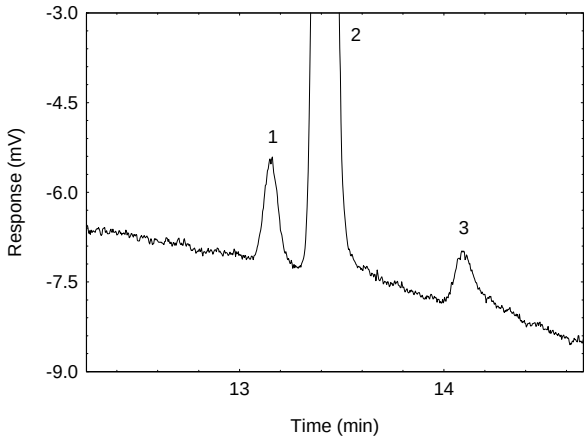


Figure 4.2.2. A chromatogram of the RQL of 2,3-pentanedione. (Key: 1) 2,3-pentanedione; 2) 3-pentanone; and 3) interferant.)

4.3 Precision of the analytical method

The precision of the analytical method was measured as the mass equivalent to the standard error of estimate determined from the linear regression of data points from standards over a range that covers 0.1 to 2 times the TWA target concentration for the sampler. A calibration curve was constructed and shown in Section 3.5.2 from the three injections each of five standards. The standard error of estimate was 0.49 µg.

Table 4.3
Instrument Calibration

| x target concn (µg/sample) | 0.1 x 2.04 | 0.5 x 10.2 | 1.0 x 20.4 | 1.5 x 30.6 | 2.0 x 40.8 |
|---|---|---|---|---|---|
| area counts (µV·s) | 3175 | 15104 | 31328 | 46035 | 62945 |
| | 3189 | 15132 | 30963 | 45869 | 63015 |
| | 3091 | 15094 | 31087 | 46183 | 62497 |

## 4.4 Storage stability test

Storage samples for 2,3-pentanedione were prepared by sampling a dynamically generated controlled test atmosphere using the recommended sampling parameters. The concentration of 2,3-pentanedione in the test atmosphere was 0.501 ppm (2.05 mg/m$^3$) and the relative humidity was 80% at 23 °C. Thirty-three storage samples were prepared. Three samples were analyzed on the day of generation. Fifteen of the tubes were stored at reduced temperature (4 °C) and the other fifteen were stored in a closed drawer at ambient temperature (about 23 °C). At 3 to 4-day intervals, three samples were selected from each of the two storage sets and analyzed. Sample results are not corrected for extraction efficiency. Results for the ambient storage test decreased by more than 10% which is a significant uncorrectable bias that must be avoided, therefore, samples should be stored in a refrigerator until analyzed, and analysis should be completed within two weeks of sampling. Recovery is determined from the regression line and the maximum change allowed by OSHA methods development guidelines is ±10%.

Table 4.4
Storage Test for 2,3-Pentanedione

| time (days) | ambient storage recovery (%) | | | refrigerated storage recovery (%) | | |
|---|---|---|---|---|---|---|
| 0 | 95.1 | 96.7 | 97.7 | 95.1 | 96.7 | 97.7 |
| 4 | 94.4 | 93.2 | 95.4 | 96.6 | 97.5 | 95.9 |
| 7 | 91.2 | 93.0 | 94.4 | 97.1 | 94.8 | 96.2 |
| 10 | 87.8 | 86.9 | 91.4 | 92.5 | 94.3 | 93.0 |
| 14 | 85.1 | 84.3 | 86.6 | 90.8 | 92.5 | 93.2 |
| 17 | 82.4 | 85.0 | 83.9 | 91.4 | 89.5 | 92.7 |

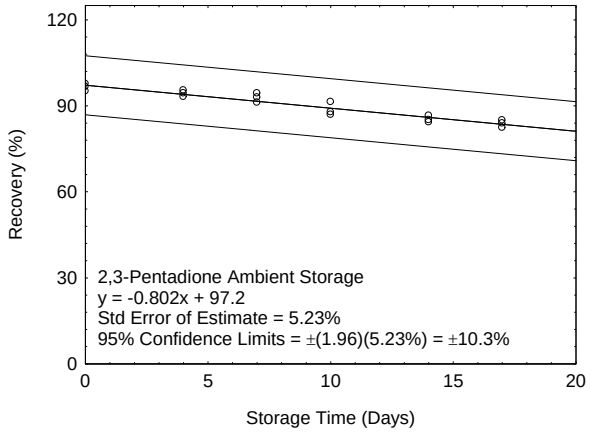


Figure 4.4.1. Ambient storage test for 2,3-pentanedione.

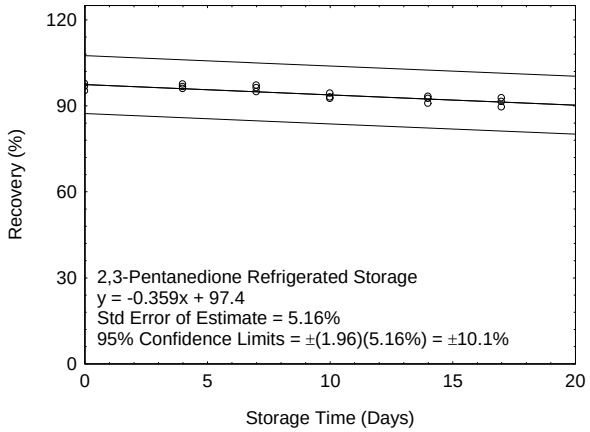


Figure 4.4.2. Refrigerated storage test for 2,3-pentanedione.

4.5 Precision (overall procedure)

The precision of the overall procedure at the 95% confidence level is obtained by multiplying the standard error of estimate by 1.96 (the z-statistic from the standard normal distribution at the 95% confidence level). Ninety-five percent confidence intervals are drawn about the regression lines in the storage stability figures shown in Section 4.4.

4.5.1 Two dried silica gel tubes in series (SKC 226-183)

The precision at the 95% confidence for the refrigerated temperature (4 °C) 17-day storage test was ± 10.1%. It contains an additional 5% for sampling pump error.

4.5.2 Recovery

The recovery of 2,3-pentanedione from samples used in a 17-day storage test remained above 91.3% when samples were stored at 4 °C.

4.6 Reproducibility

Six samples were prepared by sampling a dynamically generated controlled test atmosphere similar to that used in the collection of the storage samples. The concentrations of 2,3-pentanedione in the test atmosphere was 0.501 ppm (2.05 mg/m$^3$) at 78% relative humidity and 23 °C. The samples were submitted to the OSHA Salt Lake Technical Center for analysis. The samples were analyzed after being stored at 4 °C for 4 days. Sample results were corrected for extraction efficiency. No sample result had a deviation greater than the precision of the overall procedure determined in Section 4.4.

Table 4.6
Reproducibility Data

| theoretical (µg/sample) | recovered (µg/sample) | recovery (%) | deviation (%) |
|---|---|---|---|
| 20.5 | 20.0 | 97.6 | -2.4 |
| 20.4 | 19.4 | 95.1 | -4.9 |
| 21.0 | 19.8 | 94.3 | -5.7 |
| 20.5 | 19.9 | 97.1 | -2.9 |
| 21.0 | 20.3 | 96.7 | -3.3 |
| 23.0 | 22.5 | 97.8 | -2.2 |

4.7 Sampler capacity

The sampling capacity of the front tube of the recommended air sampler (two dried silica gel tubes in series) was tested by sampling a dynamically generated controlled test atmosphere containing 2,3-pentanedione at two times the target concentration (1.01 ppm or 4.10 mg/m$^3$) and 80% relative humidity at 23 °C. The samples were collected at 50 mL/min. The second tube in the sampling train was changed at 3 h then at 0.25 h intervals for the rest of the sampling. The presence of analyte on the second tube was defined as breakthrough. The percentage of the amount found on the second tube in relation to the concentration of the test atmosphere was defined as % breakthrough. The % breakthrough was plotted versus the air volume sampled to determine breakthrough air volumes. Breakthrough is considered to have occurred when the effluent from the active sampler contains a concentration of analyte that is 5% of the upstream concentration. The 5% breakthrough air volume for 2,3-pentanedione was 12.5 L. The recommended air volume is 80% of the breakthrough air volume which is 10 L (200 min sampled at 50 mL/min).

Table 4.7
Breakthrough of 2,3-Pentanedione From Front Sampling Tube of Recommended Air Sampler

| test no. | air vol (L) | sampling time (min) | downstream concn mg/m$^3$ | break-through (%) |
|---|---|---|---|---|
| 1 | 9.27 | 180 | 0 | 0.0 |
| | 10.8 | 210 | 0 | 0.0 |
| | 11.6 | 225 | 0 | 0.0 |
| | 12.4 | 240 | 0.19 | 4.63 |
| | 13.2 | 255 | 2.32 | 56.6 |
| 2 | 9.06 | 180 | 0 | 0.0 |
| | 10.6 | 210 | 0 | 0.0 |
| | 11.3 | 225 | 0 | 0.0 |
| | 12.1 | 240 | 0.22 | 5.36 |
| | 12.8 | 255 | 0.67 | 16.3 |
| 3 | 8.69 | 180 | 0 | 0.0 |
| | 10.1 | 210 | 0 | 0.0 |
| | 10.9 | 225 | 0 | 0.0 |
| | 11.6 | 240 | 0 | 0.0 |
| | 12.3 | 255 | 0.18 | 4.59 |
| | 13.0 | 270 | 1.29 | 31.5 |

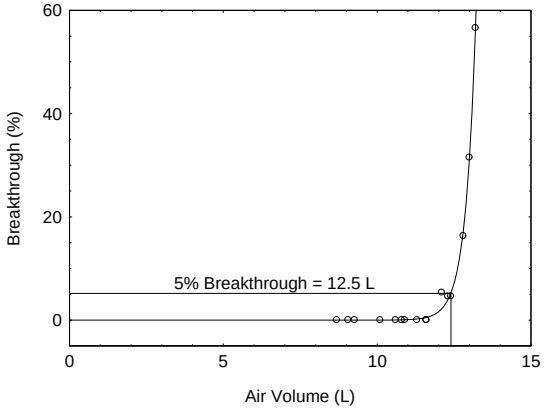


Figure 4.7. Five percent breakthrough air volume for 2,3-pentanedione.

## 4.8 Extraction efficiency and stability of extracted samples

The extraction efficiency is affected by the extraction solvent, the internal standard, the sampling medium, and the technique used to extract the samples. Other reagents and techniques than described in this method can be used provided they are tested as specified in the guidelines.[15]

### Extraction efficiency

The extraction efficiency of 2,3-pentanedione was determined by liquid-spiking four front sampling tubes of the recommended air sampler at each concentration level. These samples were stored overnight at ambient temperature and then analyzed. The overall mean extraction efficiency over the working range of 0.1 to 2 times the target concentration was 97.6%. The presence of water had no significant effect on extraction efficiency. The extraction efficiencies for the RQL and for the wet samplers are not included in the overall mean. Wet media were prepared by sampling humid air (78% RH at 23 °C) for 200 min at 50 mL/min. The data obtained are shown in Table 4.8.1.

[15] Eide, M.; Hendricks, W.; Simmons, M. *Guidelines for Air Sampling Methods Utilizing Chromatographic Analysis;* OSHA Web site. http://www.osha.gov/dts/sltc/methods/chromguide/chromguide.pdf (accessed 2/24/2010).

Table 4.8.1
Extraction Efficiency (%) of 2,3-Pentanedione

| level | | sample number | | | | |
|---|---|---|---|---|---|---|
| × target concn | µg per sample | 1 | 2 | 3 | 4 | mean |
| 0.1 | 2.05 | 98.2 | 97.1 | 96.6 | 98.8 | 97.7 |
| 0.25 | 5.12 | 97.2 | 98.1 | 95.4 | 96.1 | 96.7 |
| 0.5 | 10.3 | 98.4 | 95.9 | 97.4 | 97.6 | 97.3 |
| 1.0 | 20.5 | 96.6 | 96.0 | 97.3 | 98.5 | 97.1 |
| 1.5 | 30.8 | 98.5 | 98.1 | 98.9 | 96.8 | 98.1 |
| 2.0 | 40.1 | 97.4 | 99.3 | 99.0 | 98.4 | 98.5 |
| RQL | 0.4 | 98.4 | 96.5 | 97.7 | 99.0 | 97.9 |
| 1.0 (wet) | 20.5 | 95.3 | 97.8 | 96.2 | 95.0 | 96.1 |

Stability of extracted samples

The stability of extracted samples was examined by reanalyzing the target concentration samples 24, 48, and 72 h after the initial analysis. After the original analysis was performed two vials were recapped with new septa which were replaced after each analysis. The remaining two vials retained their punctured septa throughout this test. All samples were allowed to stand in the autosampler tray at 22 °C. The samples were reanalyzed with freshly prepared standards. Diff is the difference between the initial analysis and the subsequent analysis. Each septum was punctured 5 times for each analysis. The data obtained are shown in Table 4.8.2.

Table 4.8.2
Stability of Extracted Samples for 2,3-Pentanedione

| punctured septa replaced | | | | | | | punctured septa retained | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| initial (%) | 24 h (%) | diff (%) | 48 h (%) | diff (%) | 72 h (%) | diff (%) | initial (%) | 24 h (%) | diff (%) | 48 h (%) | diff (%) | 72 h (%) | diff (%) |
| 96.6 | 96.4 | -0.2 | 96.0 | -0.6 | 95.3 | -1.3 | 97.3 | 98.7 | +1.4 | 97.7 | +0.4 | 95.0 | -2.3 |
| 96.0 | 95.0 | -1.0 | 94.8 | -1.2 | 94.3 | -1.7 | 98.5 | 97.3 | -1.2 | 95.9 | -2.6 | 96.4 | -2.1 |
| | | | (mean) | | | | | | | (mean) | | | |
| 96.3 | 95.7 | -0.6 | 95.4 | -0.9 | 94.8 | -1.5 | 97.9 | 98.0 | +0.1 | 96.8 | -1.1 | 95.7 | -2.2 |

4.9 Sampling interferences

The tested sampling interferences had no significant effect on the ability of the recommended sampler to collect or retain 2,3-pentanedione when the samples were protected from exposure to light.

Retention

Retention was tested by sampling a dynamically generated controlled test atmosphere containing two times the target concentration (1 ppm or 4.1 mg/m$^3$) of 2,3-pentanedione at 80% relative humidity and 23 °C. The test atmosphere was sampled with the recommended sampler at 50 mL/min for 50 min. After 50 min sampling was discontinued and the samplers were separated into two sets of 3 samplers each. The generation system was flushed with contaminate-free air. Contaminant-free air is laboratory conditioned air at known relative humidity and temperature but without any added chemical except water. Sampling was resumed with a set of three samples and contaminant-free air at 80% RH and 23 °C was

sampled at 50 mL/min for 150 min and then all six samplers were analyzed. The data obtained are shown in Tables 4.9.1.

Table 4.9.1
Retention of 2,3-Pentanedione

| | recovery (%) | | | |
|---|---|---|---|---|
| set | 1 | 2 | 3 | mean |
| first | 98.4 | 100.5 | 98.2 | 99.0 |
| second | 97.7 | 96.8 | 95.1 | 96.5 |
| second/first | | | | 97.5 |

Low humidity

The effect of low humidity was tested by sampling a dynamically generated controlled test atmosphere containing two times the target concentration (1 ppm or 4.1 mg/m$^3$) of 2,3-pentanedione at 20% relative humidity and 23 °C. The test atmosphere was sampled with three of the recommended samplers at 50 mL/min for 200 min. All of the samples were immediately analyzed. Sample results were 98.8%, 99.1%, and 97.4% of theoretical.

Low concentration

The effect of low concentration was tested by sampling a dynamically generated controlled test atmosphere containing 0.1 times the target concentration (0.05 ppm or 0.205 mg/m$^3$) of 2,3-pentanedione at 80% relative humidity and 23 °C. The test atmosphere was sampled with three of the recommended samplers at 0.05 mL/min for 200 min. All of the samples were immediately analyzed. Sample results were 98.7%, 97.0%, and 95.8% of theoretical.

Chemical interference

The ability of the recommended sampler to collect 2,3-pentanedione was tested when other potential interferences are present by sampling an atmosphere containing 0.5 ppm (2.05 mg/m$^3$) 2,3-pentanedione at 80% relative humidity and 23 °C and two interferences whose concentrations were 0.51 ppm (1.82 mg/m$^3$) acetoin, and 0.51 ppm (1.78 mg/m$^3$) diacetyl. The test atmosphere was sampled with three of the recommended samplers at 50 mL/min for 200 min. All of the samples were immediately analyzed. Sample results for 2,3-pentanedione were 97.1%, 96.3%, and 95.5% of theoretical.

Light

2,3-pentanedione is light-sensitive. The interference of light during sampling was tested using nine foil-wrapped samplers and three un-wrapped samplers. An atmosphere containing 0.5 ppm (2.05 mg/m$^3$) 2,3-pentanedione at an average humidity of 80% at 23ºC was sampled for 200 minutes at 50 mL/min. The three foil-wrapped and three unwrapped samples were analyzed immediately and the average recovery for the foil wrapped was 98.2% and the un-wrapped sampler average recovery was 96.6%. Three of the foil-wrapped samplers had the foil removed after sampling and were exposed to fluorescent room lights for 24 h before analysis and had an average recovery of 90.3%. The last three foil-wrapped samplers had the foil removed and were exposed to 3 h of sunlight before analysis

Table 4.9.2
Effect of Light Exposure While Sampling

| | sample number | | | |
|---|---|---|---|---|
| type of sampler light exposure | 1 | 2 | 3 | mean |
| no light exposure | 97.5 | 98.0 | 99.1 | 98.2 |
| 200 min room light | 95.1 | 96.8 | 97.9 | 96.6 |
| 24 h fluorescent | 90.7 | 91.3 | 89.0 | 90.3 |
| 3 h sunlight | 39.6 | 42.9 | 44.6 | 42.4 |

and had an average recovery of 42.4%. This data clearly indicates that the sampler should be protected from exposure to light.

To test the possibility of light degradation on extracted samples nine analytical standards at the target concentration were prepared. Six of the standards were placed in 2-mL amber glass vials and three were placed in 2-mL clear glass vials. Three of the amber vials, along with the clear glass vials were stored on the autosampler tray during the entire test while the other three amber vials were stored in the refrigerator when not being analyzed. All nine standards were analyzed eight times over a 10 day period with none of the septa being replaced during the test. The standards in clear vials degraded significantly, but standards in amber vials did not degrade. This data clearly indicates that extracted samples should be protected from exposure to light. The internal standard, 3-pentanone was stable for up to 9 days in both the clear and ambient vials. The data obtained is shown in Table 4.9.3.

Table 4.9.3
Extracted Sample Light Exposure Test
of 2,3-Pentanedione

| | mean of peak areas from 3 vials | | |
|---|---|---|---|
| day | clear vials ambient | amber vials (ambient) | amber vials (refrigerated) |
| 0 | 31456 | 31502 | 31435 |
| 1 | 29007 | 31003 | 31354 |
| 2 | 27183 | 30961 | 31269 |
| 3 | 25072 | 30839 | 31178 |
| 4 | 24193 | 30709 | 31073 |
| 7 | 22056 | 30423 | 30834 |
| 8 | 20502 | 30389 | 30805 |
| 9 | 19584 | 30355 | 30793 |

## 4.10 Qualitative analysis

When necessary, the identity or purity of an analyte peak can be confirmed by GC-mass spectrometry or by another analytical procedure.

The mass spectrum of 2,3-pentanedione shown in Figure 4.10 was obtained by analysis on an Agilent 7890A GC System with a 5975 Mass Selective Detector.

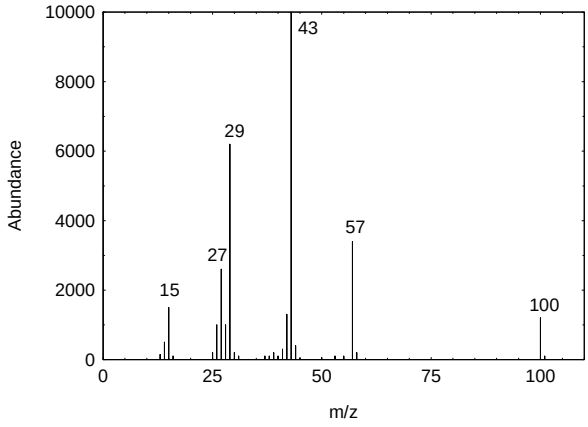

Figure 4.10. Mass spectrum of 2,3-pentanedione.

GC/MS conditions

| | |
|---|---|
| oven temperature: | initial 35 °C, hold 5 min, program at 10 °C/min to 270 °C, hold 0 min |
| injector temperature: | 240 °C |
| transfer line temperature: | 250 °C |
| run time: | 29 min |
| column gas flow: | 1.0 mL/min (helium) |
| injection size: | 0.5 µL (splitless) |
| column: | 30-m × 0.25-mm i.d. DB-5 capillary column (df = 0.25 µm) |

retention times: 3.8 min 2,3-pentanedione
4.2 min 3-pentanone

MS conditions

MS source temperature: 230 °C
MS quad temperature: 150 °C
Mass range: 12-250 amu

4.11 Generation of test atmospheres

The following apparatus was placed in a walk-in hood. The test atmospheres were generated by pumping low microliter volumes of a solution containing 2,3-pentanedione in water with an ISCO precision LC pump through a short length of 0.53-mm uncoated fused silica capillary tubing into a vapor generator where it was heated and evaporated into the dilution air stream (Figure 4.11). The vapor generator consisted of a 15-cm length of 5-cm diameter glass tubing with a side port for introduction of the capillary tubing. The vapor generator was heated with a variable voltage controlled heating tape to evaporate the 2,3-pentanedione. The humidity, temperature, and volume of the dilution air were regulated by use of a Miller Nelson Flow-Temperature-Humidity controller. The test atmosphere passed into a glass mixing chamber (76-cm × 30-cm) from the vapor generator, and then into a glass exposure chamber (76-cm × 20-cm). Active samplers were attached to glass ports extending from the exposure chamber. The humidity and temperature were measured at the exit of the exposure chamber with an Omega Digital Thermo-hygrometer. The theoretical concentrations were calculated from the ISCO pump flow rate, the concentration of the 2,3-pentanedione solution, and the air flow volumes. The theoretical concentrations were used throughout this validation.

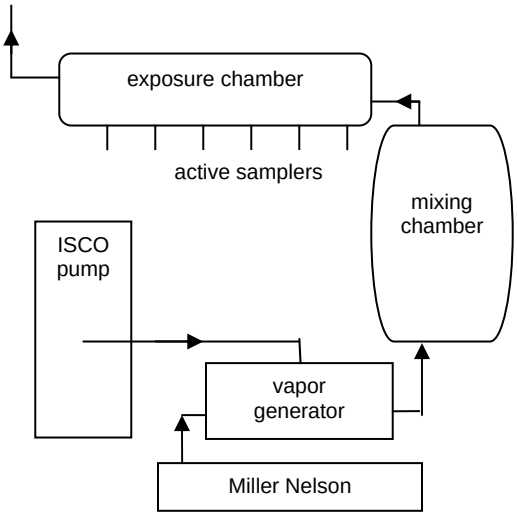


Figure 4.11. The test atmosphere generation and sampling apparatus.

FORMULA see Table 1 MW: see Table 1 CAS: see Table 1 RTECS: see Table 1

**METHOD: 2549, Issue 1** **EVALUATION: PARTIAL** **Issue 1: 15 May 1996**

**OSHA** :
**NIOSH:** varies with compound
**ACGIH:**

**PROPERTIES:** See Table 1

**SYNONYMS:** VOCs; See individual compounds in Table 1

## SAMPLING

**SAMPLER:** THERMAL DESORPTION TUBE (multi-bed sorbent tubes containing graphitized carbons and carbon molecular sieve sorbents [See Appendix])

**FLOW RATE:** 0.01 to 0.05 L/min

**VOL-MIN:** 1 L
**-MAX:** 6 L

**SHIPMENT:** Ambient in storage containers

**SAMPLE STABILITY:** Compound dependent (store @ -10 °C)

**BLANKS:** 1 to 3 per set

## ACCURACY

**RANGE STUDIED:** not applicable

**BIAS:** not applicable

**OVERALL PRECISION ($\hat{S}_{rT}$):** not applicable

**ACCURACY:** not applicable

## MEASUREMENT

**TECHNIQUE:** THERMAL DESORPTION, GAS CHROMATOGRAPHY, MASS SPECTROMETRY

**ANALYTE:** See Table 1

**DESORPTION:** Thermal desorption

**INJECTION VOLUME:** Defined by desorption split flows (See Appendix)

**TEMPERATURE-DESORPTION:** 300 °C for 10 min.
**-DETECTOR (MS):** 280 °C
**-COLUMN:** 35 °C for 4 min; 8 °C/min to 150 °C, 15 °C/min to 300 °C

**CARRIER GAS:** Helium

**COLUMN:** 30 meter DB-1, 0.25-mm ID, 1.0-µm film, or equivalent

**CALIBRATION:** Identification based on mass spectra interpretation and computerized library searches.

**RANGE:** not applicable

**ESTIMATED LOD:** 100 ng per tube or less

**PRECISION ($\bar{S}_r$):** not applicable

**APPLICABILITY:** This method has been used for the characterization of environments containing mixtures of volatile organic compounds (See Table 1). The sampling has been conducted using multi-bed thermal desorption tubes. The analysis procedure has been able to identify a wide range of organic compounds, based on operator expertise and library searching.

**INTERFERENCES:** Compounds which coelute on the chromatographic column may present an interference in the identification of each compound. By appropriate use of background subtraction, the mass spectrometrist may be able to obtain more representative spectra of each compound and provide a tentative identity (See Table 1).

**OTHER METHODS:** Other methods have been published for the determination of specific compounds in air by thermal desorption/gas chromatography [1-3]. One of the primary differences in these methods is the sorbents used in the thermal desorption tubes.

**REAGENTS:**

1. Air, dry
2. Helium, high purity
3. Organic compounds of interest for mass spectra verification (See Table 1).*
4. Solvents for preparing spiking solutions: carbon disulfide (low benzene chromatographic grade), methanol, etc.(99+% purity)

* See SPECIAL PRECAUTIONS

**EQUIPMENT:**

1. Sampler: Thermal sampling tube, ¼" s.s. tube, multi-bed sorbents capable of trapping organic compounds in the $C_3$-$C_{16}$ range. Exact sampler configuration depends on thermal desorber system used. See Figure 1 for example.
2. Personal sampling pump, 0.01 to 0.05 L/min, with flexible tubing.
3. Shipping containers for thermal desorber sampling tubes.
4. Instrumentation: thermal desorption system, focusing capability, desorption temperature appropriate to sorbents in tube (~300 °C), and interfaced directly to a GC-MS system.
5. Gas chromatograph with injector fitted with 1/4" column adapter, 1/4" Swagelok nuts and Teflon ferrules (or equivalent).
6. Syringes: 1-µL, 10-µL (liquid); 100-µL, 500-µL (gas tight)
7. Volumetric Flasks, 10-mL.
8. Gas bulb, 2 L

**SPECIAL PRECAUTIONS:** Some solvents are flammable and should be handled with caution in a fume hood. Precautions should be taken to avoid inhalation of the vapors from solvents as well. Skin contact should be avoided.

**SAMPLING:**

NOTE: Prior to field use, clean all thermal desorption tubes thoroughly by heating at or above the intended tube desorption temperature for 1-2 hours with carrier gas flowing at a rate of at least 50 mL/min. Always store tubes with long-term storage caps attached, or in containers that prevent contamination. Identify each tube uniquely with a permanent number on either the tube or tube container. Under no circumstances should tape or labels be applied directly to the thermal desorption tubes.

1. Calibrate each personal sampling pump with a representative sampler in line.
2. Remove the caps of the sampler immediately before sampling. Attach sampler to personal sampling pump with flexible tubing.
   NOTE: With a multi-bed sorbent tube, it is extremely important to sample in the correct direction, from least to maximum strength sorbent.
3. For general screening, sample at 0.01 to 0.05 L/min for a maximum sample volume of 6 L. Replace caps immediately after sampling. Keep field blanks capped at all times. Tubes can act as diffusive samplers if left uncapped in a contaminated environment.
4. Collect a "humidity test" sample to determine if the thermal adsorption tubes have a high water background.
   NOTE: At higher sample volumes, additional analyte and water (from humidity) may be collected on the sampling tube. At sufficiently high levels of analyte or water in the sample, the mass spectrometer may malfunction during analysis resulting in loss of data for a given sample.
5. Collect a "control" sample. For indoor air samples this could be either an outside sample at the same location or an indoor sample taken in a non-complaint area.
6. Ship in sample storage containers at ambient temperature. Store at -10 °C.

**SAMPLE PREPARATION:**

7. Allow samples to equilibrate to room temperature prior to analysis. Remove each sampler from its storage container.

8. Analyze "humidity test" sampler first to determine if humidity was high during sampling (step 10).
9. If high humidity, dry purge the tubes with purified helium at 50 to 100 mL/min for a maximum of 3 L at ambient temperature prior to analysis. .
10. Place the sampler into the thermal desorber. Desorb in reverse direction to sampling flow.

**CALIBRATION AND QUALITY CONTROL:**

11. Tune the mass spectrometer according to manufacturer's directions to calibrate.
12. Make at least one blank run prior to analyzing any field samples to ensure that the TD-GC-MS system produces a clean chromatographic background. Also make a blank run after analysis of heavily concentrated samples to prevent any carryover in the system. If carryover is observed, make additional blank runs until the contamination is flushed from the thermal desorber system.
13. Maintain a log of thermal desorber tube use to record the number of times used and compounds found. If unexpected analytes are found in samples, the log can be checked to verify if the tube may have been exposed to these analytes during a previous sampling use.
14. Run spiked samples along with the screening samples to confirm the compounds of interest. To prepare spiked samples, use the procedure outlined in the Appendix .

**MEASUREMENT:**

15. See Appendix for conditions. MS scan range should cover the ions of interest, typically from 20 to 300 atomic mass units (amu). Mass spectra can either be identified by library searching or by manual interpretation (see Table 1). In all cases, library matches should also be checked for accurate identification and verified with standard spikes if necessary.

**EVALUATION OF METHOD:**

The method has been used for a number of field screening evaluations to detect volatile organic compounds. Estimate of the limit of detection for the method is based on the analysis of spiked samples for a number of different types of organic compounds. For the compounds studied, reliable mass spectra were collected at a level of 100 ng per compound or less. In situations where high levels of humidity may be present on the sample, some of the polar volatile compounds may not be efficiently collected on the internal trap of the thermal desorber. In these situations, purging of the samples with 3 L of helium at 100 mL/min removed the excess water and did not appreciably affect the recovery of the analytes on the sample.

**REFERENCES:**

[1] Health and Safety Executive [1992]. MDHS 72 - Volatile organic compounds in air. Methods for the determination of hazardous substances. HMSO: London: ISBN 0-11-885692-8.

[2] McCaffrey CA, MacLachlan J, Brookes BI [1994]. Adsorbent tube evaluation for the preconcentration of volatile organic compounds in air for analysis by gas chromatography-mass spectrometry. Analyst 119:897-902.

[3] Bianchi AP, Varney MS [1992]. Sampling and analysis of volatile organic compounds in estuarine air by gas chromatography and mass spectrometry. J. Chromatogr. 643:11-23.

[4] EPA [1984]. Environmental Protection Agency Air Toxics Method T01. Rev. 1.0 (April, 1984): Method for the determination of volatile organic compounds in ambient air using Tenax(R) adsorption and gas chromatography/mass spectrometry (GC/MS), Section 13.

**METHOD WRITTEN BY:**

Ardith A. Grote and Eugene R. Kennedy, Ph.D., NIOSH, DPSE

**TABLE 1. COMMON VOLATILE ORGANIC COMPOUNDS WITH MASS SPECTRAL DATA**

| Compound /Synonyms | CAS# RTECS | Empirical Formula | MW[a] | BP[b] (°C) | VP[c] @ 25 °C mm Hg | kPa | Characteristic Ions, m/z |
|---|---|---|---|---|---|---|---|
| **Aromatic Hydrocarbons** | | | | | | | |
| Benzene /benzol | 71-43-2 CY1400000 | $C_6H_6$ | 78.11 | 80.1 | 95.2 | 12.7 | 78* |
| Xylene /dimethyl benzene | 1330-20-7 ZE2100000 | $C_8H_{10}$ | 106.7 | | | | 91, 106*, 105 |
| o-xylene | | | | 144.4 | 6.7 | 0.9 | |
| m-xylene | | | | 139.1 | 8.4 | 1.1 | |
| p-xylene | | | | 138.4 | 8.8 | 1.2 | |
| Toluene /toluol | 108-88-3 XS5250000 | $C_7H_8$ | 92.14 | 110.6 | 28.4 | 3.8 | 91, 92* |
| **Aliphatic Hydrocarbons** | | | | | | | |
| n-Pentane | 109-66-0 RZ9450000 | $C_5H_{12}$ | 72.15 | 36.1 | 512.5 | 68.3 | 43, 72*, 57 |
| n-Hexane /hexyl-hydride | 110-54-3 MN9275000 | $C_6H_{14}$ | 86.18 | 68.7 | 151.3 | 20.2 | 57, 43, 86*, 41 |
| n-Heptane | 142-82-5 MI7700000 | $C_7H_{16}$ | 100.21 | 98.4 | 45.8 | 6.1 | 43, 71, 57, 100*,41 |
| n-Octane | 111-65-9 RG8400000 | $C_8H_{18}$ | 114.23 | 125.7 | 14.0 | 1.9 | 43, 85, 114*, 57 |
| n-Decane /decyl hydride | 124-18-5 HD6500000 | $C_{10}H_{22}$ | 142.29 | 174 | 1.4 | 0.2 | 43, 57, 71, 41, 142* |
| **Ketones** | | | | | | | |
| Acetone /2-propanone | 67-64-1 AL3150000 | $C_3H_6O$ | 58.08 | 56 | 266 | 35.5 | 43, 58* |
| 2-Butanone /methyl ethyl ketone | 78-93-3 EL6475000 | $C_4H_8O$ | 72.11 | 79.6 | 100 | 13 | 43, 72* |
| Methyl isobutyl ketone /MIBK, hexone | 108-10-1 SA9275000 | $C_6H_{12}O$ | 100.16 | 117 | 15 | 2 | 43, 100*, 58 |
| Cyclohexanone /cyclohexyl ketone | 108-94-1 GW1050000 | $C_6H_{10}O$ | 98.15 | 155 | 2 | 0.3 | 55, 42, 98*, 69 |
| **Alcohols** | | | | | | | |
| Methanol /methyl alcohol | 67-56-1 PC1400000 | $CH_3OH$ | 32.04 | 64.5 | 115 | 15.3 | 31, 29, 32* |
| Ethanol /ethyl alcohol | 64-17-5 KQ6300000 | $C_2H_5OH$ | 46.07 | 78.5 | 42 | 5.6 | 31, 45, 46* |
| Isopropanol /1-methyl ethanol | 67-63-0 NT8050000 | $C_3H_7OH$ | 60.09 | 82.5 | 33 | 4.4 | 45, 59, 43 |
| Butanol /butyl alcohol | 71-36-3 EO1400000 | $C_4H_9OH$ | 74.12 | 117 | 4.2 | 0.56 | 56, 31, 41, 43 |

| Compound /Synonyms | CAS# RTECS | Empirical Formula | MW[a] | BP[b] (°C) | VP[c] @ 25 °C mm Hg | kPa | Characteristic Ions, m/z |
|---|---|---|---|---|---|---|---|
| **Glycol Ethers** | | | | | | | |
| Butyl cellosolve /2-butoxyethanol | 111-76-2 KJ8575000 | $C_6H_{14}O_2$ | 118.17 | 171 | 0.8 | 0.11 | 57, 41, 45, 75, 87 |
| Diethylene glycol ethyl ether /Carbitol | 111-90-0 KK8750000 | $C_6H_{14}O_3$ | 134.17 | 202 | 0.08 | 0.01 | 45, 59, 72, 73, 75, 104 |
| **Phenolics** | | | | | | | |
| Phenol /hydroxybenzene | 108-95-2 SJ3325000 | $C_6H_5OH$ | 94.11 | 182 | 47 | 0.35 | 94*, 65, 66, 39 |
| Cresol | 1319-77-3 GO5950000 | $C_7H_7OH$ | 108.14 | | | | 108*, 107, 77, 79 |
| 2-methylphenol | 95-48-7 | | | 190.9 | 1.9 | 0.25 | |
| 3-methylphenol | 108-39-4 | | | 202.2 | 1.0 | 0.15 | |
| 4-methylphenol | 106-44-5 | | | 201.9 | 0.8 | 0.11 | |
| **Chlorinated Hydrocarbons** | | | | | | | |
| Methylene chloride /dichloromethane | 75-09-2 PA8050000 | $CH_2Cl_2$ | 84.94 | 40 | 349 | 47 | 86*, 84, 49, 51 |
| 1,1,1-Trichloroethane /methyl chloroform | 71-55-6 KJ2975000 | $CCl_3CH_3$ | 133.42 | 75 | 100 | 13.5 | 97, 99, 117, 119 |
| Perchloroethylene /hexachloroethane | 127-18-4 KX3850000 | $CCl_3CCl_3$ | 236.74 | 187 (subl) | 0.2 | <0.1 | 164*, 166, 168, 129, 131, 133, 94, 96 |
| o-,p-Dichlorobenzenes | | $C_6H_4Cl_2$ | 147.0 | | | | 146*, 148, 111, 113, 75 |
| /1,2-dichlorobenzene | 95-50-1 CZ4500000 | | | 172-9 | 1.2 | 0.2 | |
| /1,4-dichlorobenzene | 106-46-7 CZ4550000 | | | 173.7 | 1.7 | 0.2 | |
| 1,1,2-Trichloro-1,2,2-trifluoroethane /Freon 113 | 76-13-1 KJ4000000 | $CCl_2FCClF_2$ | 187.38 | 47.6 | 384 | 38 | 101, 103, 151, 153, 85, 87 |
| **Terpenes** | | | | | | | |
| d-Limonene | 5989-27-5 OS8100000 | $C_{10}H_{16}$ | 136.23 | 176 | 1.2 | | 68, 67, 93, 121, 136* |
| Turpentine (Pinenes) | 8006-64-2 | $C_{10}H_{16}$ | 136.23 | 156 to 170 | 4 @ 20° | | 93, 121, 136*, 91 |
| α-pinene | 80-56-8 | | | 156 | | | |
| β-pinene | 127-91-3 | | | 165 | | | |
| **Aldehydes** | | | | | | | |
| Hexanal /caproaldehyde | 66-25-1 MN7175000 | $C_6H_{12}O$ | 100.16 | 131 | 10 | 1.3 | 44, 56, 72, 82, 41 |

| Compound /Synonyms | CAS# RTECS | Empirical Formula | MW[a] | BP[b] (°C) | VP[c] @ 25 °C mm Hg | kPa | Characteristic Ions, m/z |
|---|---|---|---|---|---|---|---|
| Benzaldehyde /benzoic aldehyde | 100-52-7 CU4375000 | $C_7H_{12}O$ | 106.12 | 179 | 1.0 | 0.1 | 77, 105, 106*, 51 |
| Nonanal /pelargonic aldehyde | 124-19-6 RA5700000 | $C_9H_{18}O$ | 142.24 | 93 | 23 | 3 | 43, 44, 57, 98, 114 |
| **Acetates** | | | | | | | |
| Ethyl acetate /acetic ether | 141-78-6 AH5425000 | $C_4H_8O_2$ | 88.1 | 77 | 73 | 9.7 | 43, 88*, 61, 70, 73, 45 |
| Butyl acetate /acetic acid butyl ester | 123-86-4 AF7350000 | $C_6H_{12}O_2$ | 116.16 | 126 | 10 | 1.3 | 43, 56, 73, 61 |
| Amyl acetate /banana oil | 628-63-7 AJ1925000 | $C_7H_{14}O_2$ | 130.18 | 149 | 4 | 0.5 | 43, 70, 55, 61 |
| **Other** | | | | | | | |
| Octamethylcyclotetra-siloxane | 556-67-2 GZ4397000 | $C_8H_{24}O_4Si_4$ | 296.62 | 175 | | | 281, 282, 283 |

[a] Molecular Weight
[b] Boiling Point
[c] Vapor Pressure
* Indicates molecular ion

## APPENDIX

**Multi-bed sorbent tubes:** Other sorbent combinations and instrumentation/conditions shown to be equivalent may be substituted for those listed below. In particular, if the compounds of interest are known, specific sorbents and conditions can be chosen that work best for that particular compound(s). The tubes that have been used in NIOSH studies with the Perkin Elmer ATD system are ¼" stainless steel tubes, and are shown in the diagram below:

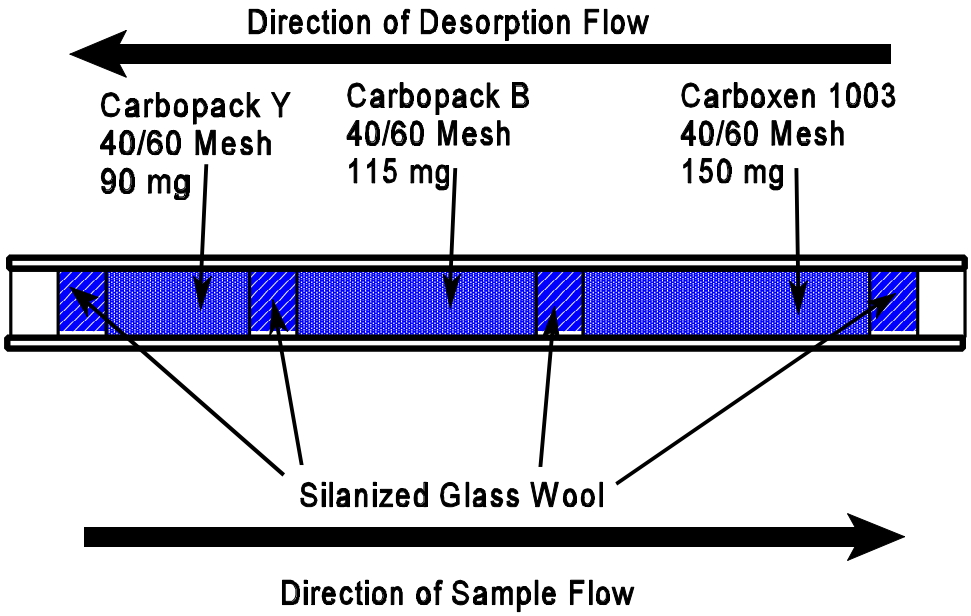


**Figure 1**

Carbopack™ and Carboxen™ adsorbents are available from Supelco, Inc.

**Preparation of spiked samples** Spiked tubes can be prepared from either liquid or gas bulb standards.

***Liquid standards***: Prepare stock solutions by adding known amounts of analytes to 10-mL volumetric flasks containing high purity solvent (carbon disulfide, methanol, toluene). Solvents are chosen based on solubility for the analytes of interest and ability to be separated from the analytes when chromatographed. Highly volatile compounds should be dissolved in a less volatile solvent. For most compounds, carbon disulfide is a good general purpose solvent, although this will interfere with early eluting compounds.

***Gas bulb standards***: Inject known amounts of organic analytes of interest into a gas bulb of known volume filled with clean air [4]. Prior to closing the bulb, place a magnetic stirrer and several glass beads are placed in the bulb to assist in agitation after introduction of the analytes. After injection of all of the analytes of interest into the bulb, warm the bulb to 50 °C and place it on a magnetic stirring plate and stir for several minutes to ensure complete vaporization of the analytes. After the bulb has been stirred and cooled to room temperature, remove aliquots from the bulb with a gas syringe and inject into a sample tube as described below.

**Tube spiking**: Fit a GC injector with a ¼" column adapter. Maintain the injector at 120 °C to assist in vaporization of the injected sample. Attach cleaned thermal desorption tubes to injector with ¼" Swagelok nuts and Teflon ferrules, and adjust helium flow though the injector to 50 mL/min. Attach the sampling tube so that flow direction is the same as for sampling. Take an aliquot of standard solution (gas standards 100 to 500 µL; liquid standards, 0.1 to 2 µL) and inject into the GC injector. Allow to equilibrate for 10 minutes. Remove tube and analyze by thermal desorption using the same conditions as for field samples.

**Instrumentation:** Actual media, instrumentation, and conditions used for general screening of unknown environments are as follows: Perkin-Elmer ATD 400 (automated thermal desorption system) interfaced directly to a Hewlett-Packard 5980 gas chromatograph/HP5970 mass selective detector and data system.

***ATD conditions***:

Tube desorption temperature: 300°C
Tube desorption time: 10 min.
Valve/transfer line temperatures: 150°C
Focusing trap: Carbopack B/Carboxen 1000, 60/80 mesh, held at 27°C during tube desorption
Focusing trap desorption temperature: 300°C
Desorption flow: 50-60 mL/min.
Inlet split: off
Outlet split: 20 mL/min.
Helium: 10 PSI

***GC conditions***:

DB-1 fused silica capillary column, 30 meter, 1-µm film thickness, 0.25-mm I.D.
Temperature program: Initial 35°C for 4 minutes, ramp to 100°C at 8°/min., then ramp to 300°C at 15°/min, hold 1-5 minutes.
Run time: 27 min.

***MSD conditions***:

Transfer line: 280°C
Scan 20-300 amus, EI mode
EMV: set at tuning value
Solvent delay: 0 min. for field samples; if a solvent-spiked tube is analyzed, a solvent delay may be necessary to prevent MS shutdown caused by excessive pressure.

# APPENDIX 2

*Journal of Occupational and Environmental Hygiene*, 8: 59–70
ISSN: 1545-9624 print / 1545-9632 online
DOI: 10.1080/15459624.2011.540168

# Correcting Diacetyl Concentrations from Air Samples Collected with NIOSH Method 2557

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*Diacetyl (2,3-butanedione), a diketone chemical used to impart a buttery taste in many flavoring mixtures, has been associated with bronchiolitis obliterans in several industrial settings. For workplace evaluations in 2000–2006, National Institute for Occupational Safety and Health (NIOSH) investigators used NIOSH Method 2557, a sampling and analytical method for airborne diacetyl utilizing carbon molecular sieve sorbent tubes. The method was subsequently suspected to progressively underestimate diacetyl concentrations with increasing sampling site humidity. Since underestimation of worker exposure may lead to overestimation of respiratory health risk in quantitative exposure-effect analyses, correction of the diacetyl concentrations previously reported with Method 2557 is essential. We studied the effects of humidity and sample storage duration on recovery of diacetyl from experimental air samples taken from a dynamically generated controlled test atmosphere that allowed control of diacetyl concentration, temperature, relative humidity, sampling duration, and sampling flow rate. Samples were analyzed with Method 2557, and results were compared with theoretical test atmosphere diacetyl concentration. After fitting nonlinear models to the experimental data, we found that absolute humidity, diacetyl concentration, and days of sample storage prior to extraction affected diacetyl recovery as did sampling flow rate to a much smaller extent. We derived a mathematical correction procedure to more accurately estimate historical workplace diacetyl concentration based on laboratory-reported concentrations of diacetyl using Method 2557, and sample site temperature and relative humidity (to calculate absolute humidity), as well as days of sample storage prior to extraction in the laboratory. With this correction procedure, quantitative risk assessment for diacetyl can proceed using corrected exposure levels for air samples previously collected and analyzed using NIOSH Method 2557 for airborne diacetyl.*

**Keywords** correction equation, diacetyl, humidity effect, sample storage effect

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health or Occupational Safety and Health Administration.

## INTRODUCTION

Diacetyl (2,3-butanedione, CAS no. 431–03-8), a diketone chemical used to impart a buttery taste in many flavoring mixtures, has been associated with severe respiratory disease in several different occupational settings, including microwave popcorn manufacturing, flavoring production, and diacetyl manufacturing.[1–3] Laboratory animal studies have documented that diacetyl alone has toxic properties that are similar to the effects of exposure to diacetyl-containing artificial butter flavoring mixtures.[4,5] The Occupational Safety and Health Administration (OSHA) is in the process of rulemaking on occupational exposure to diacetyl.

National Institute for Occupational Safety and Health (NIOSH) researchers developed and published an analytical method, NIOSH Method 2557, to measure airborne diacetyl in the workplace.[6,7] This method specifies air sample collection through carbon molecular sieve (CMS) sorbent tubes, followed by extraction with acetone/methanol (99:1) and analysis by gas chromatography with flame ionization detection (GC/FID) within 7 days of sampling. Subsequent to the use of this sampling method in several workplace investigations, NIOSH researchers found that the method appeared to progressively

underestimate diacetyl concentrations with increasing sampling site humidity as compared with OSHA Method PV2118.[8] Silica gel is used as the collection medium in the OSHA method.[9] NIOSH Method 2557 should not be used to measure airborne diacetyl in future studies.

We studied the effect of humidity on measured diacetyl air concentrations using NIOSH Method 2557 with the aim of developing a means for mathematically correcting previously obtained measurements of airborne diacetyl. In addition, we investigated sample storage stability over time because we were aware that some previously obtained field samples had been analyzed beyond the method's specified 7-day maximum storage duration.

## METHODS

### Protocol

The initial objective of our experiments was to determine if sampling site humidity affects diacetyl recovery in air samples and, if so, to develop a mathematical procedure to correct existing diacetyl air sampling data from previous workplace studies for those effects. NIOSH and OSHA investigators conducted a total of 6 weeks of tests during five visits by NIOSH investigators to the OSHA Salt Lake Technical Center (SLTC) laboratory. During the first week of tests, we started to investigate the effect of humidity and sampling flow rate, as well as the homogeneity of diacetyl mixing in the dynamically generated controlled test atmosphere. During the second and third weeks, we investigated effects of temperature, sampling duration, sampling flow rate, and test atmosphere diacetyl concentration on diacetyl recovery.

Based on results of the first 3 weeks of tests, during the following 2-week test period, we ran tests to further evaluate the effect of test atmosphere diacetyl concentration. In addition, during that 2-week test period we studied sample storage stability using a single test atmosphere diacetyl concentration. Based on the sample storage stability results, we further evaluated the test atmosphere diacetyl concentration effect during a final week of tests. Since we found an effect of sample storage duration on diacetyl recovery, which was dependent on both humidity and test atmosphere diacetyl concentration, the primary objective was extended to include this effect in the mathematical correction procedure.

During each of the five visits, we also collected a number of samples using OSHA Method PV2118 (OSHA 1013[10] was used once it became available) to compare with test atmosphere diacetyl concentration.

### Test Atmosphere Generation

Test atmospheres of diacetyl were generated at the OSHA SLTC laboratory by pumping an aqueous diacetyl solution (approximately 1 to 100% diacetyl depending on target concentration), using a syringe pump (Series D; Teledyne Isco Inc., Lincoln, Neb.), through a short length of 0.53 mm diameter uncoated fused silica capillary tubing into a vapor generator where it was heated and evaporated into a dilution airstream (Figure 1). The vapor generator, a 20 cm length of 3 cm diameter glass tubing with a side port for introduction of the capillary tubing, was wrapped with heating tape to evaporate the solution. Humidity, temperature, and volume of the dilution stream of air were regulated by use of a flow-temperature-humidity control system (Model HCS-401; Miller-Nelson Instruments Inc., Pleasanton, Calif.).

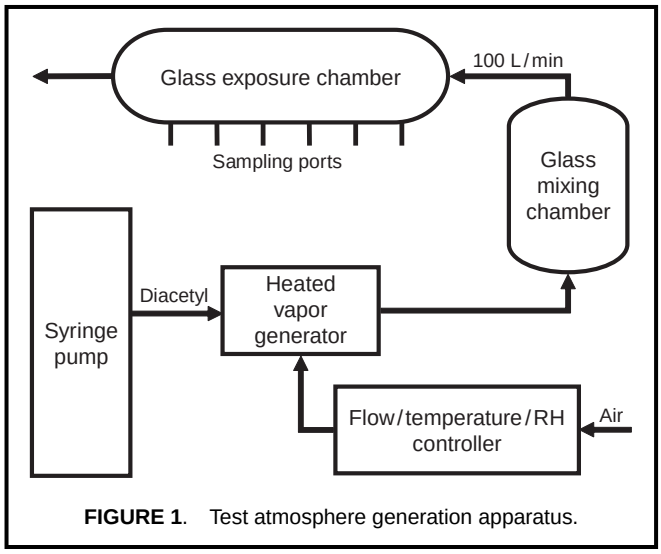


**FIGURE 1**. Test atmosphere generation apparatus.

The diacetyl-laden air passed from the vapor generator into a glass mixing chamber (76 cm length × 15 cm diameter) and then into a glass exposure chamber (76 cm length × 8 cm diameter). Eighteen evenly spaced glass tube sampling ports extended from the exposure chamber: nine from the bottom and nine from a side. The temperature and relative humidity were measured at the exit of the exposure chamber with a digital thermo-hygrometer (Model RH-411; Omega Engineering, Inc., Stamford, Conn.). The test atmosphere generation apparatus was located in a walk-in hood. Theoretical test atmosphere concentrations of diacetyl were derived using mass flow calculations. These calculations used syringe pump flow rate, chamber airflow rate, and diacetyl concentration in the aqueous solution.

### Sampling Procedure

CMS sorbent tubes (Anasorb CMS 226–121; SKC, Eighty Four, Pa.) and pairs (in series) of SKC Model 226–183 silica gel sorbent tubes were attached to the sampling ports, and the test atmosphere was pulled via vacuum through the sorbent tubes with sampling flow rate controlled by adjustable orifices. For each test, flow through each sorbent tube was pre- and post-calibrated with a flowmeter (Model 4100; TSI, Inc., Shoreview, Minn.). After sampling, the sorbent tubes were immediately capped, wrapped in foil, and placed on ice packs in a cooler along with blank sorbent tubes. The coolers were shipped nightly via express mail to a NIOSH-contracted analytical laboratory, where the sorbent tubes were extracted on arrival on Day 1 after sampling (or later, as directed for a few sets of CMS tubes used for storage stability experiments) and analyzed by GC/FID.

**TABLE I. Test Atmosphere Conditions and Sample Numbers**

| Target Diacetyl Concentration (ppm) | Actual Diacetyl Concentration | | Actual AH Range (mg $H_2O$/L air) | Actual RH Range (%) | Actual Temperature Range (°C) | Sampling Duration (hr) | Target Sampling Flow Rate (cc/min) | Number of Samples[A] |
|---|---|---|---|---|---|---|---|---|
| | Mean (ppm) | Range (ppm) | | | | | | |
| Humidity Test Samples | | | | | | | | |
| 0.2 | 0.23 | 0.23–0.24 | 4.69–19.12 | 21–81 | 23.9–26.2 | 4, 8 | 50, 150 | 87 |
| 0.5 | 0.58 | 0.56–0.60 | 3.51–19.26 | 17–91 | 22.6–26.3 | 2, 4, 8 | 50, 150 | 107 |
| 1.0 | 1.1 | 1.1 | 6.99–14.92 | 29–62 | 25.8–26.0 | 2 | 50, 150 | 41 |
| 5.0 | 5.5 | 5.0–5.9 | 3.65–22.50 | 17–92 | 22.8–27.0, 31.9–33.8 | 2, 4, 8 | 50, 150 | 373 |
| 25 | 24.8 | 24.5–25.7 | 3.57–19.06 | 16–92 | 22.4–26.1 | 2 | 50, 150 | 109 |
| Stability Test Samples[B] | | | | | | | | |
| 0.5 | 0.57 | 0.57–0.58 | 3.51–18.17 | 17–91 | 22.6–23.3 | 4, 8 | 50 | 54 |
| 5.0 | 5.6 | 5.6–5.7 | 3.65–18.67 | 17–92 | 22.8–25.7 | 2 | 50 | 107 |
| 25 | 25.0 | 24.9–25.1 | 3.57–18.66 | 18–92 | 22.4–22.8 | 2 | 50 | 53 |

[A]Number of samples used in equation development analyses.
[B]Nine each of the 0.5 and 25 ppm samples and 18 of the 5.0 ppm samples were used in both humidity and storage stability analyses.



## Sampling Test Conditions

Samples were collected between January 2008 and December 2009 during four 1-week periods and one 2-week period of tests. We collected a total of 964 CMS tube samples during 80 tests, with relative humidity (RH) levels ranging from 16 to 92% and temperatures of 22.4 to 33.8°C giving absolute humidity (AH) levels ranging from 3.5 to 22.5 mg $H_2O$/L air and with diacetyl concentrations ranging from 0.23 to 25.7 ppm. Samples were collected over 2, 4, or 8 hr to test for differences in diacetyl recovery due to sampling duration or because of limit of detection (LOD) concerns during tests at low diacetyl concentrations. Samples were collected using sampling flow rates of 50 or 150 cc/min to investigate any effect on diacetyl recovery associated with differences in sampling flow rate. The test atmosphere conditions and sample numbers are summarized in Table I.

Over the five visits, we collected 134 silica gel samples at a flow rate of 50 cc/min during 43 of the 2-hr tests. These samples were collected with an AH range of 3.57 to 22.50 mg $H_2O$/L air and diacetyl concentrations from 0.56 to 25.7 ppm.

## Sample Storage Stability Tests

In total, storage stability of diacetyl both in the sampling tubes (in-tube) and after extraction from the tubes was investigated using 214 samples (Table I). In the first set of experimental conditions, six sets of triplicate samples were collected at 50 cc/min from a 5.7 ppm diacetyl test atmosphere at each of three AH levels: 3.97, 8.59, and 18.67 mg $H_2O$/L air (RH = 17, 36, and 78%, respectively, at 25.7°C). Samples were sent overnight on ice to the analytical laboratory, where they were extracted and analyzed according to NIOSH Method 2557 for diacetyl 1, 4, 7, 10, 13, and 16 days post-sampling. All samples were stored in a refrigerator until the scheduled day of extraction.

After analysis of the first set of samples on Day 1 post-sampling, the remaining liquid portion (without sorbent material) of each sample was split into two new vials and one stored at room temperature and the other refrigerated. These samples underwent further stability testing via re-analysis 1, 2, 5, and 11 days post-extraction. New septum caps were placed on each vial after each analysis, and freshly prepared standards were used for each re-analysis. To investigate diacetyl concentration effect on storage stability, during the final week of tests, six sets of triplicate samples each were collected from 0.57, 5.6, and 25.0 ppm diacetyl test atmospheres at each of three mean AH levels: 3.6, 8.5, and 18.5 mg $H_2O$/L air. The samples were extracted and analyzed 1, 4, 7, 10, 16, and 35 days post-sampling. When splitting the samples for the extract storage stability tests, equal portions of the sorbent material were placed into the two vials with the liquid to better simulate treatment of field samples as directed in Method 2557. Re-analysis of these samples was completed on Days 2, 5, 13, and 34 post-extraction.

## Data Analyses

Statistical analysis was carried out using JMP V.8 software (SAS Institute, Cary, N.C.). We used the nonlinear modeling platform to calculate the parameter coefficients for the correction model. Details of models used in the JMP nonlinear platform are discussed in the Results section. We used analysis of variance modeling to investigate effects of sampling port position, sampling duration, and sampling flow rate on percent diacetyl recovered.

## RESULTS

Of 964 CMS samples collected, 717 were used in humidity effect analyses (extraction Day 1 after sampling), 214 were used in sample storage stability analyses (36 of these were used in both analyses), 42 samples from 1 day of tests were excluded due to excessive analytical laboratory variability (the mean coefficient of variation for that day's tests was 73% as compared with a range of 3% to 23% for other days), 13 were excluded due to greater than 5% changes in sampling flow rate during the tests, 3 were excluded due to errors during sampling, 1 had missing data from the analytical laboratory, 1 outlier (greater than 300% recovery) was excluded, and 9 samples collected at low concentration and high humidity were excluded because of nondetectable diacetyl.

Of the 134 silica gel samples, 121 that had matching CMS sample groups during 39 tests were used in the comparison analyses.

### Effects of Sampling Port Position, Sampling Duration, and Sampling Flow Rate

During the first week of tests, homogeneous mixing of diacetyl in the exposure chamber was investigated, and analysis of variance indicated no significant effects of sampling port position on diacetyl recovery. An analysis of variance model using data from the first three laboratory visits (n = 448) with

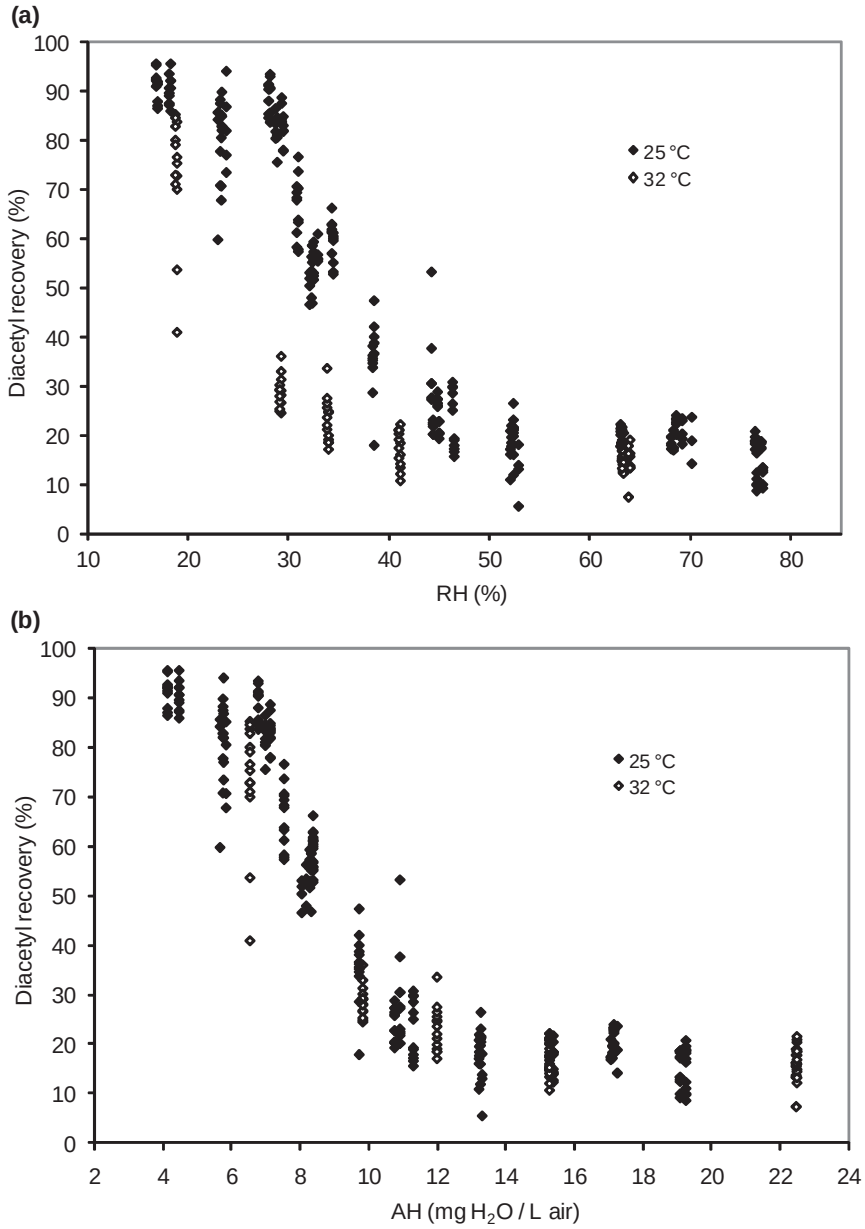


**FIGURE 2**. Plots for 5.0 ppm target diacetyl concentration data from 25°C and 32°C showing diacetyl recovery in terms of (a) RH and (b) AH.

percent diacetyl recovery as the outcome variable and AH, test atmosphere diacetyl concentration, target sampling flow rate, and sampling duration as the predictor variables indicated a significant ($p = 0.0042$) effect of sampling flow rate, with percent diacetyl recovery being higher for the 150 cc/min sampling flow rate than for 50 cc/min. The magnitude of the effect was not large; the adjusted means (least squares means) for 150 cc/min and 50 cc/min were 44.9 and 40.3% diacetyl recovery, respectively. In this model there was no significant effect for sampling duration ($p = 0.89$), with adjusted means of 42.3, 41.8, and 43.7% diacetyl recovery for sampling durations of 2, 4, and 8 hr, respectively.

### Absolute Humidity Effect—Model for Data from Samples Extracted on Day 1 After Sampling

We investigated the effect of temperature on diacetyl recovery by plotting percent diacetyl recovered against either RH (Figure 2a) in % or AH (Figure 2b) in mg $H_2O$/L air using data from samples collected from a target diacetyl concentration of 5 ppm at target temperatures of 25°C and 32°C. We calculated AH from RH and temperature ($T_c$) using Eq. 1, which we derived from a National Weather Service approximation for humidity calculations in surface observations.[11]

$$AH = \frac{13.25\,RH \exp\left(\frac{17.67\,T_c}{T_c + 243.50}\right)}{T_c + 273.15} \quad \textbf{(1)}$$

As seen in Figure 2, the substantial difference in diacetyl recovery for the two temperatures was removed when humidity was expressed as AH. Thereafter, we modeled the percent recovered diacetyl in terms of AH.

Using the JMP model library of nonlinear functions, we visually determined that the 4-parameter logistic function was suitable to describe the sigmoidal relationship of percent recovered diacetyl with humidity, for samples extracted on Day 1 after sampling. The 4-parameter logistic model has parameters $\theta_1$, $\theta_2$, $\theta_3$, and $\theta_4$, each of which has graphical meaning. The parameter $\theta_1$ represents the horizontal asymptote on the right-hand side of the graph where humidity is at the highest level; $\theta_2$ represents the horizontal asymptote on the left-hand side of the graph where humidity is at the lowest level; $\theta_3$ is the "slope" or the shape parameter; and $\theta_4$ is the humidity at which 50% of the maximal response is observed. The general equation in terms of the 4-parameter logistic model is:

$$Y = \theta_1 + \frac{\theta_2 - \theta_1}{1 + \exp[\theta_3(X - \theta_4)]} \quad \textbf{(2)}$$

In our models, percent recovered diacetyl was the Y variable, and humidity was the X variable.

We fitted separate 4-parameter logistic models to the data for each of the target test atmosphere diacetyl concentrations (0.2, 0.5, 5.0, and 20 ppm). We had too few levels of AH for the 1.0 ppm test atmosphere diacetyl concentration to adequately fit the 4-parameter logistic model. Figure 3 shows the separate 4-parameter logistic models for percent recovered diacetyl vs. AH as fitted through the overall test data (for both sampling flow rates combined).

Using information from these models, we created one nonlinear model for the data overall; this model took into account differences in the 4-parameter values for the individual logistic models. We found that $\theta_1$ was well approximated ($R^2 = 0.99$) by a linear function of target concentration $C_0$

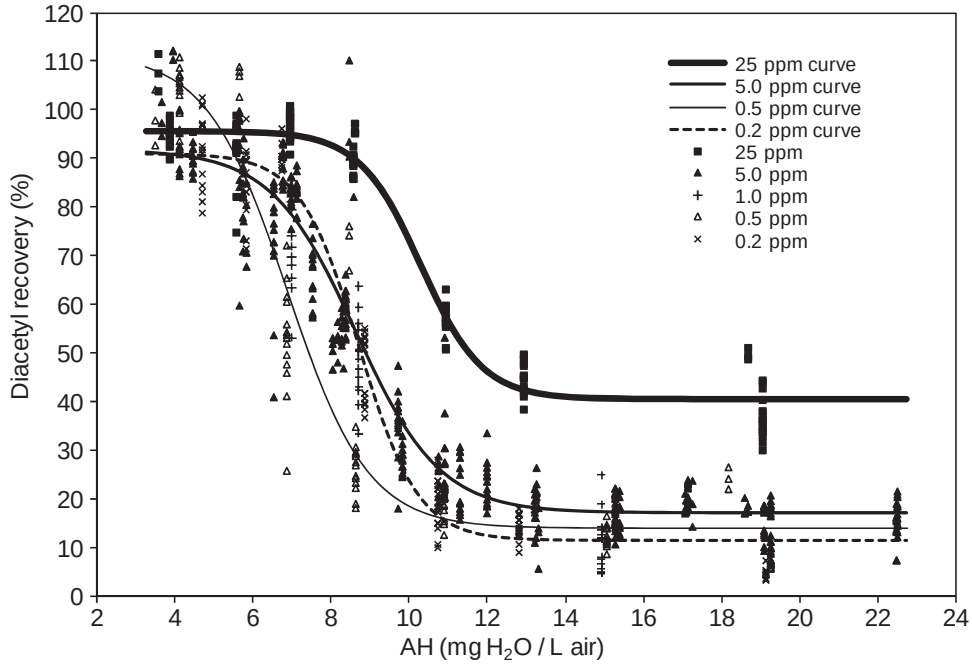


**FIGURE 3**. Four-parameter logistic models fitted to test data. Note: Data are insufficient to create a logistic curve for 1.0 ppm.

**TABLE II. Parameter Coefficients for the Overall Diacetyl Correction Equation and for Two Sampling Flow Rates**

| | | Sampling Flow Rate | |
|---|---|---|---|
| **Parameter** | **Overall** | **50 cc/min** | **150 cc/min** |
| $b_0$ | 6.91166 | 5.85971 | 8.32618 |
| $m_1$ | 1.69272 | 1.70372 | 1.68917 |
| $\theta_2$ | 101.31390 | 101.06329 | 101.81000 |
| $\theta_3$ | 0.72068 | 0.70943 | 0.73539 |
| $\theta_4$ | 8.22607 | 8.18808 | 8.26746 |
| q | 0.05362 | 0.05362 | 0.05362 |
| r | 0.41384 | 0.41384 | 0.41384 |
| s | –0.00589 | –0.00589 | –0.00589 |
| u | –0.01719 | –0.01719 | –0.01719 |
| v | 0.30359 | 0.30359 | 0.30359 |
| w | 0.00558 | 0.00558 | 0.00558 |
| x | 0.00153 | 0.00153 | 0.00153 |
| y | 0.26802 | 0.26802 | 0.26802 |
| z | –0.00002 | –0.00002 | –0.00002 |

(i.e., $\theta_1 = b_0 + m_1 C_0$, where $b_0$ is the intercept and $m_1$ is the slope) and so substituted this linear function into the 4-parameter logistic function using the values for $b_0$ and $m_1$ as starting values for the overall model. Since the other parameters showed variability but no trend with levels of $C_0$, we used the arithmetic means of the separate model $\theta_2$, $\theta_3$, and $\theta_4$ parameters for the four target test atmosphere diacetyl concentrations as the overall model starting values for these three parameters. We expressed percent recovered diacetyl ($100c/C_0$, where c is the recovered concentration reported by the laboratory) by rewriting Eq. 2 as follows:

$$\text{Percent recovered diacetyl} = \frac{100c}{C_0} = h(C_0, AH) = b_0 + m_1 C_0 + \frac{\theta_2 - b_0 - m_1 C_0}{1 + \exp[\theta_3(AH - \theta_4)]} \quad \textbf{(3)}$$

We entered this form of the equation (Eq. 3) into the nonlinear fitting platform for a fit through all the data (including the data for a test atmosphere diacetyl concentration of 1.0 ppm). We repeated the fit through the data stratified by sampling flow rate. The final values for the parameters ($b_0$, $m_1$, $\theta_2$, $\theta_3$, and $\theta_4$) both overall and for the two sampling flow rates are given in Table II (the table also contains parameter values for the effect of in-tube storage as described below). The $R^2$ (amount of total variability in the data accounted for by the model) for the overall model was 0.91. The $R^2$ for the 150 cc/min model was 0.93, and the $R^2$ for the 50 cc/min model was 0.90. Figure 4 shows how Eq. 3 describes the pattern of diacetyl recoveries for a range of concentrations both overall and for the two sampling flow rates and illustrates that the effect of sampling flow rate was not large.

Equation 3 predicts that at a concentration of approximately 56 ppm, the diacetyl recovery would be approximately 100% at all AH values (this was similar for the overall model and the 50 and 150 cc/min models). At diacetyl concentrations above these values, the model predicts diacetyl recoveries of higher than 100% across the range of AH values, which does not represent a real-life solution. Predicted diacetyl recoveries

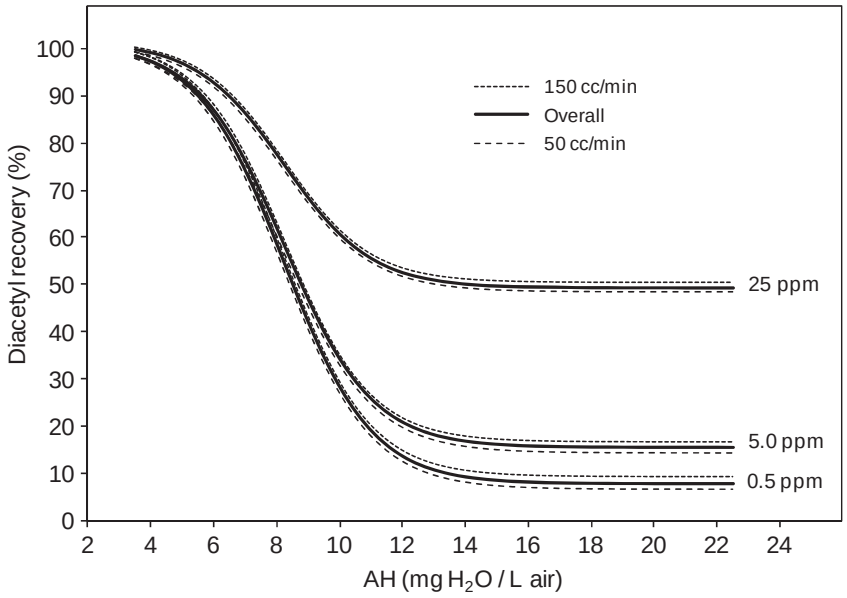


**FIGURE 4**. Model for data from samples extracted on Day 1 after sampling (Eq. 3) applied at three diacetyl concentrations using parameter coefficients ($b_0$, $m_1$, $\theta_2$, $\theta_3$, $\theta_4$) for the whole data set (overall) and the two sampling flow rates used in the experiment.

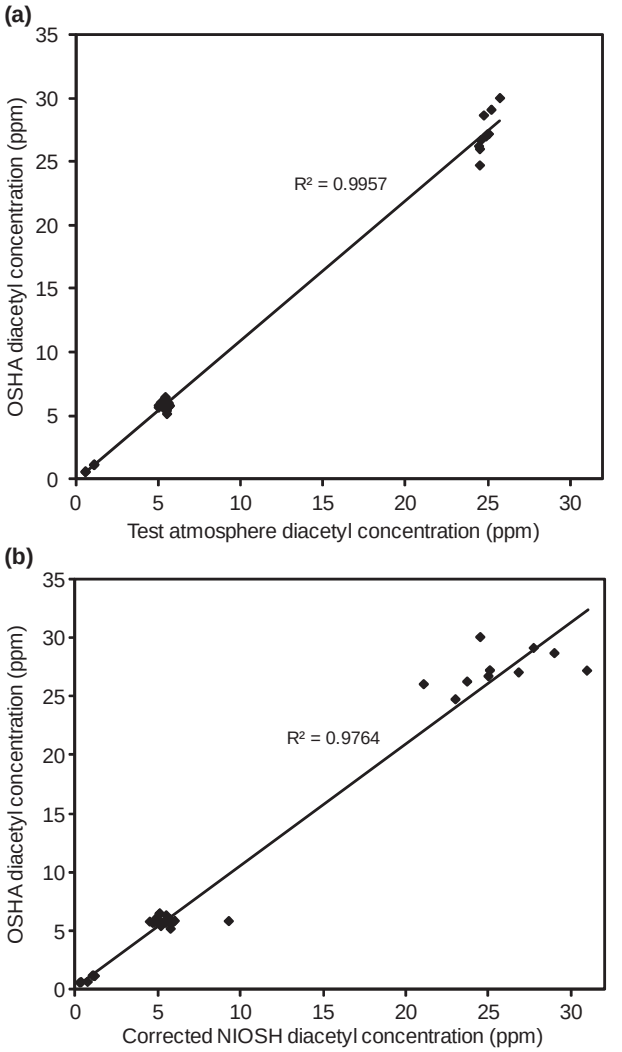


**FIGURE 5**. Comparison of diacetyl sampling results obtained using OSHA method (a) to calculated test atmosphere concentration, and (b) to corrected values of the diacetyl sampling results using NIOSH Method 2557.

from Eq. 3 for very low diacetyl concentrations do not have the same problem since, mathematically, in the limit as the diacetyl concentration goes to zero, the recoveries range from approximately 100% to approximately 7% as AH goes from low to high.

## OSHA Silica Gel Sample Results

Diacetyl concentrations from the 121 silica gel samples taken at a number of AH conditions were quite similar to the calculated test atmosphere concentrations (Figure 5a) and were not affected by AH. Using the model (Eq. 3) we calculated the corrected diacetyl concentrations from the matched NIOSH Method 2557 CMS samples, and as shown in Figure 5b, we found a strong linear relationship with the silica gel results.

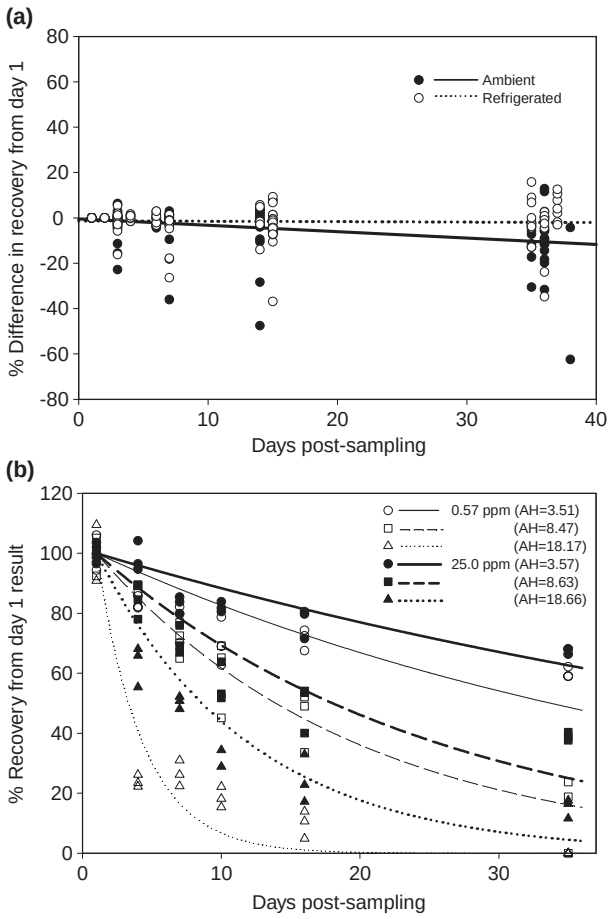


**FIGURE 6**. (a) Extract stability: Samples extracted and analyzed on Day 1 post-sampling and the remaining sorbent material and extract kept at ambient or refrigerated temperature before further analyses on subsequent days. Lines represent regressions. (b) In-tube stability: Samples extracted and analyzed on the same day. Curves were created from Eq. 4.

## Model for Effect of In-Tube Storage

Plots of extract storage and in-tube storage stabilities are shown in Figure 6. Samples stored as extracts, either with or without sorbent material, under refrigerated conditions were stable, having less than 2% loss at each of the three AH levels over nearly 40 days of storage (1.4% at 7 days and 1.7% at 38 days). Under ambient conditions, the loss was 2.9% at 7 days and 11.0% at 38 days. In contrast, plots of diacetyl recovered by number of days of in-tube storage (i.e., days from sampling to extraction) indicated decreased recovery over time, with the changes over time showing dependence on both AH and diacetyl concentration. For a given diacetyl concentration, diacetyl losses over time were greater with increasing AH. For a given AH, diacetyl losses over time were greater with decreasing concentration.

To model in-tube storage effects, we used first-order decay functions to estimate decay constants for the 12 combinations of diacetyl concentrations and AH. We normalized the diacetyl recovery data by dividing the diacetyl recovery data by the

mean for recovery on Day 1 after sampling and included (t-1) in the first-order decay functions (see below). The first-order decay model is given by: Y = (starting amount) exp[-k(t-1)], where starting amount = 1 for normalized data, t = days from sampling to extraction, and k is the decay constant.

We substituted functions of AH and diacetyl concentration for the decay constants (k). This was accomplished in two steps. In Step 1, we fitted quadratic functions to the k values for the three target diacetyl concentration (0.5 ppm, 5 ppm, and 25 ppm) curves of k vs. AH. In Step 2, we substituted three-parameter first-order decay functions for the coefficients for the intercept, the AH term and the $AH^2$ term of the quadratic function based on the diacetyl concentrations. This gave estimates for the nine coefficients (q, r, s, u, v, w, x, y, and z) in the model (as shown below). In a final step, the values of the nine parameters were used as starting values to get a fit of this nonlinear model through the full set of in-tube storage data. The $R^2$ for this model was 0.90. The coefficients are given in Table II. The form of the nonlinear model for the effect of in-tube storage was:

$$\text{Normalized recovery} = g(C_0, AH, t) = \exp[-(f_1(C_0) + f_2(C_0)AH + f_3(C_0)AH^2)(t-1)] \quad \textbf{(4)}$$

where

$$f_1(C_0) = q\exp(-rC_0) + s$$
$$f_2(C_0) = u\exp(-vC_0) + w$$
$$f_3(C_0) = x\exp(-yC_0) + z$$

## Full Model

The full model can be conceptualized in two steps. First, the AH, the recovered diacetyl concentration (c), and the number of days from sampling to extraction (t) are used to predict the recovered diacetyl concentration on Day 1 of extraction after sampling. Second, this predicted diacetyl value and AH is used to predict the corrected concentration. The full model for the percent of diacetyl recovered is:

$$\text{Percent recovered diacetyl} = \frac{100c}{C_0} = h(C_0, AH)g(C_0, AH, t) \quad \textbf{(5)}$$

where *h* is given by Eq. 3 and *g* is given by Eq. 4.

Since, in practice, the values of c, AH, and t are known, and the value of $C_0$ is the predicted corrected diacetyl concentration, we solved Eq. 5 for $C_0$. Using Eqs. 3 and 4, Eq. 5 can be rewritten as:

$$aC_0^2 + bC_0 - \left(\frac{c}{g(C_0, AH, t)}\right) = 0 \quad \textbf{(6)}$$

where

$$a = m_1/100 + \frac{-m_1/100}{1+\exp[\theta_3(AH-\theta_4)]}$$
$$b = b_0/100 + \frac{(\theta_2 - b_0)/100}{1+\exp[\theta_3(AH-\theta_4)]}$$

Since this is a nonlinear equation for $C_0$, it is necessary to use an iterative procedure to find $C_0$. Initially, Eq. 6 is solved for $C_0$ using the quadratic formula with the dependence of *g* on $C_0$ ignored:

$$C_0 = \frac{-b + \sqrt{b^2 + 4a\left(\frac{c}{g(C_0, AH, t)}\right)}}{2a} \quad \textbf{(7)}$$

(Note: The other solution for Eq. 6 using the quadratic formula yields a nonphysical negative value for $C_0$ since $a > 0$ and $b > 0$.)

In Eqs. 6 and 7 the value of $\left(\frac{c}{g(C_0, AH, t)}\right)$ is the estimate for the diacetyl concentration corrected for days to extraction after sampling.

To solve Eq. 7, an iterative procedure is used with the i value $C_0^{(i)}$ used to calculate the (i+1) value $C_0^{(i+1)}$

$$C_0^{(i+1)} = \frac{-b + \sqrt{b^2 + 4a\left(\frac{c}{g(C_0^{(i)}, AH, t)}\right)}}{2a} \quad \textbf{(8)}$$

It is necessary to start the procedure with an initial $C_0$ (i.e., $C_0^{(1)}$). We found the procedure robust to the choice of starting value and suggest the use of c (the recovered concentration reported by the laboratory).

The sequence of solutions is then calculated until two consecutive values for $C_0$ are identical to a chosen number of decimal places (convergence). We tested the model for regions of convergence using theoretical recovered concentrations (c) from 0.001 to 70 ppm, AH from 2 to 25 mg $H_2O$/L air, and days to extraction from 1 to 36. We found that convergence occurred for all concentrations above 1.0 ppm. For lower concentrations, convergence occurred whenever AH was less than 14.5 mg $H_2O$/L air and days to extraction were fewer than 9. The region of convergence improved from a concentration of 0.001 to 1.0 ppm. At 1.0 ppm, convergence occurred whenever AH was less than 21 mg $H_2O$ / L air and days to extraction were fewer than 17.

As discussed above, for values of $C_0 > 56$ ppm, the Day 1 model does not yield real-life solutions. For these values, only the model for effect of days to extraction should be applied and we predict the concentration of diacetyl for Day 1 of extraction after sampling. If the converged value as calculated above is > 56 ppm, use it as the starting value $C_0^{(1)}$ in an iterative procedure using the equation:

$$C_0^{(i+1)} = \frac{c}{g\left(C_0^{(i)}, AH, t\right)} \quad \textbf{(9)}$$

The sequence of solutions is then calculated until two consecutive values for $C_0$ are identical to a chosen number of decimal places. Figure 7 is a flow diagram of the correction procedure as described above. When corrected diacetyl concentrations fall between 0.23 and 25.7 ppm, which were the lowest and highest diacetyl test atmosphere concentrations

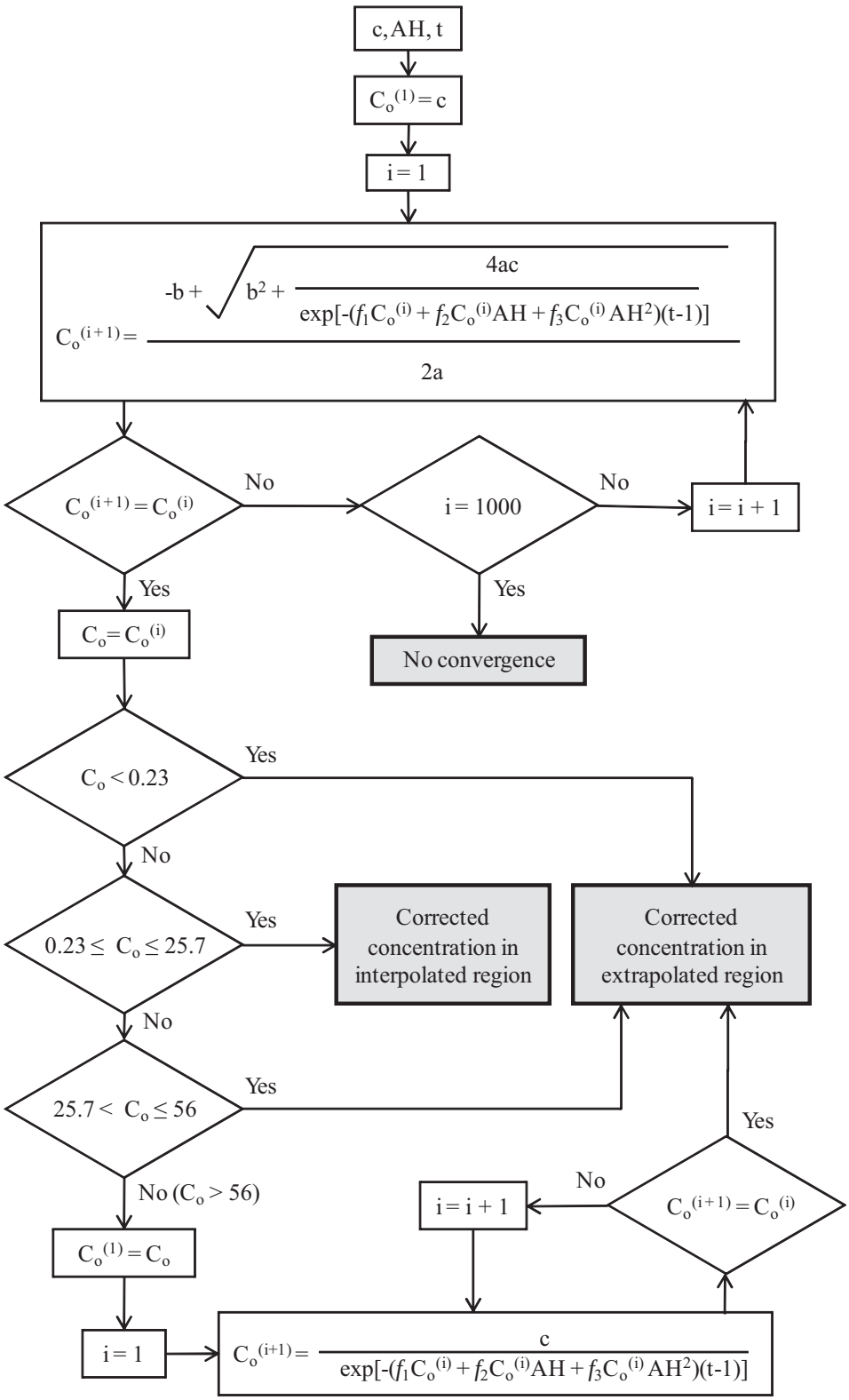


**FIGURE 7**. Flow diagram of the correction procedure that begins with known values of recovered diacetyl concentration (c), absolute humidity (AH) during sampling, and the number of days from sampling to extraction (t), and ends with the corrected diacetyl concentration in either the interpolated or extrapolated region. As explained in the text, there are some conditions of no convergence.

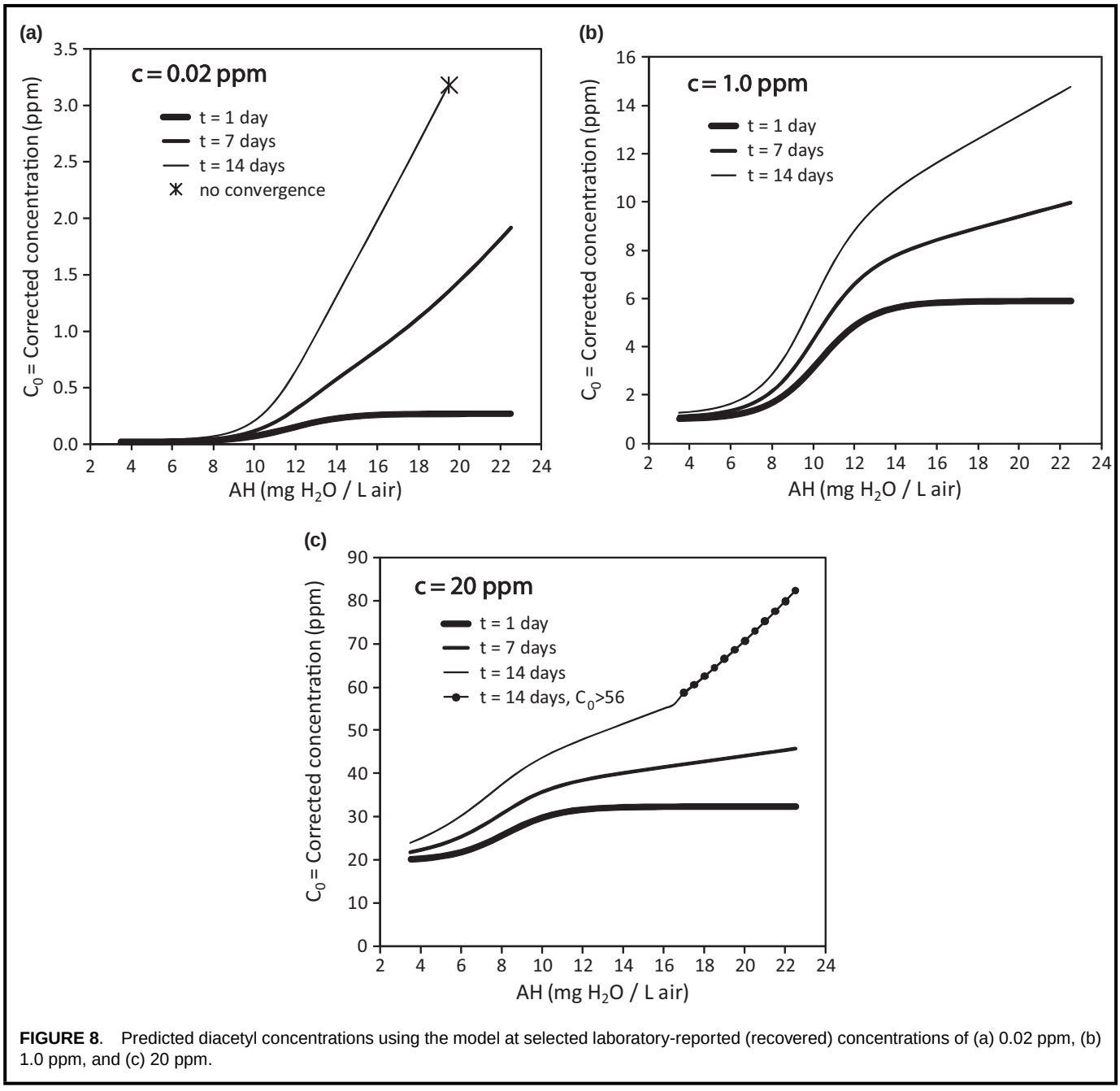


**FIGURE 8**. Predicted diacetyl concentrations using the model at selected laboratory-reported (recovered) concentrations of (a) 0.02 ppm, (b) 1.0 ppm, and (c) 20 ppm.

used in our experiments, we consider the corrections to be within the interpolated range and have the most confidence in these values. Figure 8 shows diacetyl concentrations predicted by our models for a number of different conditions. We chose three laboratory-reported diacetyl concentrations (c) of 0.02, 1.0, and 20 ppm over a wide range of AH and days from sampling to extraction (t) that should represent possible field conditions. We see that both changes in AH and t substantially affect the value of the corrected concentration. In Figure 8a, we indicate a point where nonconvergence begins for $c = 0.02$ ppm and $AH = 19$ on the 14 days from sampling to extraction curve. In Figure 8c, we indicate a region where $C_0 > 56$, which is only corrected for days from sampling to extraction.

## DISCUSSION

From experimental test atmosphere work, we have created a procedure that allows historical diacetyl concentration data from analysis of samples using NIOSH Method 2557 to be corrected to more accurately estimate historical workplace airborne diacetyl concentrations. This correction procedure provides a means for applying these diacetyl concentration estimates in planned quantitative risk assessment relating health effects observed among workers to their diacetyl exposure. In addition, it will allow for a better understanding of historical workplace concentrations of diacetyl that will give insight for exposure control strategies. Use of this correction procedure

requires laboratory-reported concentrations of diacetyl in ppm (samples collected and analyzed using NIOSH Method 2557), temperature and RH (to calculate AH) conditions at the time of sampling, and the number of days from sample collection to sample extraction for analysis. We give overall parameter values for the full model, as well as for sampling flow rates of 50 and 150 cc/min (Table II). Since the effect of sampling flow rate was not large, investigators have the option of using the overall parameter values, especially if their historical data were collected at sampling flow rates other than 50 and 150 cc/min.

A strength of this work was the use of a controlled test atmosphere to simulate historical field survey conditions where airborne diacetyl was sampled together with humid air. By using two target temperatures with similar ranges of RH, we were able to show that both temperature and RH had an effect on diacetyl recovery and that using AH (mg $H_2O$/L air) was the key variable to connect the correlation between temperature and concentration. This finding extends the work of McKernan and colleagues,[8] who were unable to separate the effect of temperature and RH in their field-based work. By running tests with several different test atmosphere diacetyl concentrations over a wide range from 0.23 to 25.7 ppm, we were able to observe differences in diacetyl recovery related to theoretical diacetyl concentration. We found a large difference in diacetyl recovery between the test atmosphere diacetyl concentration of about 25 ppm and all the lower concentrations, especially at the higher AH values. The final correction equation predicts that humidity would no longer have an effect on diacetyl recovery at approximately 56 ppm, but we have no empirical data to test this prediction. Corrected diacetyl concentrations that lie outside our test atmosphere range represent extrapolations of the models, and we have less confidence in these concentrations.

We do not suggest the use of the correction procedure with historical concentration data below the limit of detection (LOD), for which concentration may have been estimated (e.g., using LOD/2 or LOD/$\sqrt{2}$). It is not possible to know if the workplace diacetyl concentration was indeed below the LOD or if the losses due to humidity and days from sampling to extraction in the laboratory caused the sample value to be below the LOD. We did find some regions of AH and days from sampling to extraction for recovered concentrations of 1.0 ppm or less where the full model does not converge; however, such conditions should not occur often in the field.

Our storage stability test findings were contrary to the NIOSH Method 2557 specification of good stability for 7 days from sampling to analysis.[7] This may have been due to the fact that storage stability tests completed during method development used spiked sampling tubes without using humid air rather than our actively sampled tubes using a test atmosphere. As our results showed, early extraction minimized further sample loss, especially when the samples were refrigerated in accordance with the method, which means that delays in analysis after extraction should not cause appreciable loss. A limitation of our work is that we did not collect in-tube storage data for all the tests to determine the effect of AH on sample recovery, but we did collect data for three target test atmosphere diacetyl concentrations and three target AH values. Thus, we estimated the effect of AH and the effect of in-tube storage on different data sets and combined the two models mathematically to create the final model.

Our correction equations accounted for about 90% of the variability in the experimental data by taking into account the effects of AH, test atmosphere diacetyl concentration, sampling flow rate, and days of in-tube storage. The variability seen in the data at any combination of AH and diacetyl concentration values has a number of sources, including variability in keeping test atmosphere conditions constant, variability in sampling flow rates during the tests, sampling duration differences (although not found significant), and analytical laboratory variability.

Our test atmosphere experiments used no flavoring chemicals besides diacetyl. In field situations, diacetyl may occur together with other chemicals in the air. Any effect of these mixtures on the diacetyl recovery using NIOSH Method 2557 might not be accounted for with our correction procedure.

Comparison between corrected diacetyl concentrations and the results from side-by-side samples taken with OSHA methods indicated a high correlation, which increases our confidence in the applicability of the correction method. Despite the limitations, the correction procedure enables more accurate quantitative risk assessment now under way for regulatory guidance on occupational exposure to diacetyl. Representative exposures in the flavoring manufacturing industry are difficult to assess because of short-duration batch production methods in which hour-to-hour and day-to-day variations in diacetyl exposures is expected in workplaces where scores of different kinds of flavorings are manufactured. Hence, relative stability of diacetyl exposures in microwave popcorn production facilities offers the advantage of less potential for exposure misclassification.

However, without appropriate correction, the systematic underestimation of true diacetyl exposures in the 2000–2006 historical data would lead to overestimation of health risk associated with diacetyl exposure. Accordingly, use of our correction procedure to recalculate the historical exposure estimates from microwave popcorn production facilities previously studied by NIOSH and others will contribute to ongoing efforts to understand the health risk associated with occupational exposure to diacetyl. Our experimental work may also motivate further research exploring the mechanism by which analyte recovery from CMS sorbent may be affected by sampling site humidity for a variety of analytes.

## CONCLUSIONS

We have developed a mathematical procedure that allows measurements from historical diacetyl samples collected and analyzed using NIOSH Method 2557, which

may be biased low, to be adjusted for a more accurate exposure assessment. In addition to the historical laboratory-reported diacetyl concentrations, this correction procedure requires data on AH (determined from temperature and RH measurements) during sampling and on the number of days between sample collection and laboratory extraction of the sampling tubes. NIOSH Method 2557 should not be used to measure airborne diacetyl in future studies.

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# APPENDIX 3

# Appendix 3

## JEM Tables for Four Plants

The mean diacetyl concentrations estimated for the three plants with a single NIOSH survey are shown in Tables 5A.1–5A.3. For the fourth plant, Company G, time-dependent exposure levels were estimated in the NIOSH-OSHA JEM collaboration (Table 5A.4).

### Table A3.1 Diacetyl exposure matrix for health hazard evaluation: Company N

| Department | Job | n | ppm diacetyl |
|---|---|---|---|
| Maintenance office | Maintenance / mechanic | 2 | 0.164 |
| Microwave popcorn line | Bag placer | 2 | 0.696 |
| | Machine operator | 3 | 1.160 |
| | Packer / Stacker | 2 | 0.143 |
| | (area) | 2 | 0.621 |
| Mixing, measuring room | Tank mixer | 1 | 0.794 |
| | (area) | 2 | 1.032 |
| Packing area | Packer / Stacker | 2 | 0.121 |
| | (area) | 2 | 0.159 |
| Poly line | Poly line worker | 2 | 0.235 |
| | (area) | 2 | 0.182 |
| Quality control area | Quality control worker | 2 | 0.250 |
| | (area) | 2 | 0.320 |
| Stencil area | Stenciler | 2 | 0.045 |
| | (area) | 2 | 0.024 |
| Warehouse | Fork lift operator | 2 | 0.005 |

### Table A3.2 Diacetyl exposure matrix for health hazard evaluation: Company K

| Department | Job | N | ppm diacetyl |
|---|---|---|---|
| 41-A Entire Area | Micro Pdn—Manager/Supvr | 1 | 0.003 |
| | Micro Pdn—Sanitation/Cleaning | 1 | 0.003 |
| 41-A Blending Room (Mixing) | Micro Production—Mixer | 7 | 0.913 |
| 41-A Carton / Tray | 41-A Filler Side | 1 | 0.002 |
| | Micro Pdn—Maint/Mechanic | 1 | 0.054 |

| | | | |
|---|---|---|---|
| | Micro Pdn—Production Worker | 5 | 0.039 |
| 41-A Case / Pallet | Micro Production—Stacker | 5 | 0.002 |
| 41-A Filler Side | 41-A Case/Pallet | 8 | 0.038 |
| | Plant—Maintenance/Mechanic | 1 | 0.003 |
| | Plant—Other | 1 | 0.002 |
| 41-A Lab | 41-B Warehouse | 5 | 0.003 |
| 41 Warehouse | Plant—Other | 1 | 0.268 |
| 41-B Warehouse | Micro Pdn—Forklift Operator | 1 | 0.002 |
| | Plant—Other | 1 | 0.002 |
| 41 & 41-B Warehouses | Micro Production—Stacker | 1 | 0.002 |
| 41-Microwave Pdn Building | Micro Pdn—Maint/Mechanic | 2 | 0.037 |
| | Micro Pdn—Manager/Supvr | 3 | 0.003 |
| | Micro Production—Mixer | 1 | 0.002 |
| | 41-B Warehouse | 1 | 0.003 |
| | Micro Pdn—Production Worker | 1 | 0.002 |
| | Plant—Other | 1 | 0.003 |
| Office Building | Office/Management/Sales | 2 | 0.002 |
| Poly Production Building | Poly—Production | 2 | 0.309 |
| | Poly—QC | 2 | 0.002 |
| Ambient | Ambient | 2 | 0.003 |
| Pre-mix Area | Micro Production—Mixer | 3 | 0.043 |

## Table A3.3 Diacetyl exposure matrix for health hazard evaluation: Company L

| Department | Job | N | ppm diacetyl |
|---|---|---|---|
| Press Room | Press room worker | 10 | 0.015 |
| Slurry Room (Mixing) | Supervisor | 3 | 0.723 |
| | Mixer | 13 | 1.426 |
| Packaging | Supervisor | 8 | 0.020 |
| | Phaser operator | 10 | 0.026 |
| | Case packer operator | 10 | 0.026 |
| | Cartoner operator | 13 | 0.031 |
| | Palletizer operator | 9 | 0.033 |
| | Line sanitation | 3 | 0.024 |
| | Forklift operator | 6 | 0.036 |
| QA | QA monitor | 11 | 0.025 |
| Warehouse | Supervisor | 1 | 0.033 |
| | Warehouse worker | 7 | 0.035 |
| | Forklift operator | 1 | 0.037 |
| Main Office | Office worker | 6 | 0.025 |
| Maintenance | Press room worker | 1 | 0.011 |
| | Supervisor | 1 | 0.012 |
| | All over plant | 2 | 0.006 |
| | Other | 5 | 0.021 |
| Ambient | Outside | 1 | 0.003 |

## Table A3.4 Diacetyl exposure matrix for health hazard evaluation: Company G

| Department | Job | Period | ppm diacetyl | Start Date | End Date |
|---|---|---|---|---|---|
| Microwave Production | Oil mixing | 1 | 9.713 | 7/1/1986 | 2/11/2001 |
| Mixing room | | 2 | 2.509 | 2/12/2001 | 4/5/2001 |
| | | 3 | 0.245 | 4/6/2001 | 9/6/2002 |
| | | 4 | 0.006 | 9/7/2002 | 8/15/2003 |
| Office | Office | 1 | 0.009 | 7/1/1986 | 8/15/2003 |
| Office | Lab technician/quality control | 1 | 0.335 | 7/1/1986 | 2/11/2001 |
| | | 2 | 0.250 | 2/12/2001 | 5/21/2001 |
| | | 3 | 0.123 | 5/22/2001 | 8/8/2002 |

| | | | | | |
|---|---|---|---|---|---|
| | | 4 | 0.034 | 8/8/2002 | 3/8/2003 |
| | | 5 | 0.007 | 3/9/2003 | 8/15/2003 |
| Warehouse | Warehouse | 1 | 0.557 | 7/1/1986 | 2/11/2001 |
| | | 2 | 0.017 | 2/12/2001 | 8/15/2003 |
| Warehouse/Microwave | Bag checker and bag machine operator | 1 | 1.613 | 7/1/1986 | 2/11/2001 |
| Production | | 2 | 0.947 | 2/12/2001 | 5/21/2001 |
| | | 3 | 0.053 | 5/22/2001 | 9/6/2002 |
| | | 4 | 0.003 | 9/7/2002 | 8/15/2003 |
| Polypropylene | Supervisor, machine operator, line packer, | 1 | 0.047 | 7/1/1986 | 2/11/2001 |
| | line stacker | 2 | 0.020 | 2/12/2001 | 8/15/2003 |
| Microwave Production | Maintenance | 1 | 1.145 | 7/1/1986 | 2/11/2001 |
| and All Over | | 2 | 0.294 | 2/12/2001 | 5/21/2001 |
| | | 3 | 0.054 | 5/22/2001 | 9/6/2002 |
| | | 4 | 0.002 | 9/7/2002 | 8/15/2003 |
| Microwave Production | Supervisor, machine opr, do-boy opr,line | 1 | 2.668 | 7/1/1986 | 2/11/2001 |
| | packer, line stacker, inventory control, | 2 | 0.672 | 2/12/2001 | 5/21/2001 |
| | fill-in on line, box folder | 3 | 0.343 | 5/22/2001 | 9/6/2002 |
| | | 4 | 0.003 | 9/7/2002 | 8/15/2003 |
| Microwave Production | Lab technician/quality control | 1 | 1.312 | 7/1/1986 | 2/11/2001 |
| Quality Control Lab | | 2 | 0.974 | 2/12/2001 | 5/21/2001 |
| | | 3 | 0.467 | 5/22/2001 | 8/8/2002 |
| | | 4 | 0.108 | 8/8/2002 | 3/8/2003 |
| | | 5 | 0.002 | 3/9/2003 | 8/15/2003 |
| Microwave Production | Line packer/machine operator/mixer | 1 | 5.016 | 7/1/1986 | 2/11/2001 |
| | | 2 | 1.284 | 2/12/2001 | 4/5/2001 |
| | | 3 | 0.530 | 4/6/2001 | 5/21/2001 |
| | | 4 | 0.311 | 5/22/2001 | 9/6/2002 |
| | | 5 | 0.004 | 9/7/2002 | 8/15/2003 |
| Outside / Yard | Maintenance and outside processing | 1 | 0.009 | 7/1/1986 | 8/15/2003 |
| All Over / Float | Supervisor and janitor | 1 | 2.068 | 7/1/1986 | 2/11/2001 |
| | | 2 | 0.685 | 2/12/2001 | 4/5/2001 |
| | | 3 | 0.402 | 4/6/2001 | 5/21/2001 |
| | | 4 | 0.165 | 5/22/2001 | 8/8/2002 |
| | | 5 | 0.105 | 8/9/2002 | 9/6/2002 |
| | | 6 | 0.026 | 9/7/2002 | 3/8/2003 |
| | | 7 | 0.009 | 3/9/2003 | 8/15/2003 |
| All Over / Float | Exterminator | 1 | 0.009 | 7/1/1986 | 8/15/2003 |

| | | | | | |
|---|---|---|---|---|---|
| Microwave and Polypropylene Production | Line packer, line stacker on both lines | 1 | 1.358 | 7/1/1986 | 2/11/2001 |
| | | 2 | 0.346 | 2/12/2001 | 5/21/2001 |
| | | 3 | 0.182 | 5/22/2001 | 9/6/2002 |
| | | 4 | 0.012 | 9/7/2002 | 8/15/2003 |

# APPENDIX 4

# Appendix 4

## Development of a Job Exposure Matrix for Company G

### *Overview*

To estimate worker exposures for risk assessment, we developed a job exposure matrix (JEM) containing estimates of the average 8-hour, time-weighted average (TWA) exposure levels for diacetyl in parts per million parts air (ppm). This JEM includes estimates for 8 major job categories with selected time periods specific for each job category to reflect changes in processes and engineering controls over time. The exposure levels presented in the JEM are based on diacetyl air sampling data collected during nine industrial hygiene surveys conducted by NIOSH industrial hygienists between November 2000 and July 2003 at a microwave popcorn plant in Missouri [Kreiss et al. 2002; Kullman et al. 2005; NIOSH 2006]. Details of the JEM construction is described below.

### *Industrial Hygiene Surveys*

A total of nine industrial hygiene surveys were conducted over a period of four years from 2000 to 2003. The sampling was typically conducted during the day shift, since this shift presented the opportunity to sample all job categories. However, samples were also collected from 2$^{nd}$ and 3$^{rd}$ shifts, but not routinely. The dates for these industrial hygiene surveys are presented in Table A2.1.

Personal breathing zone (PBZ) and area diacetyl samples were collected during these surveys using NIOSH Method 2557. These measurements were subsequently adjusted to account for interferences due to humidity and sample storage [Cox-Ganser et al. 2011]. During all surveys except the first, full-shift PBZ samples were collected from workers performing typical tasks representative of each of the major job categories. In addition, concurrent full-shift area samples were taken throughout the plant from locations where workers would typically spend their time. The PBZ sample measurements were used to develop the exposure estimates for the 8 job categories in the JEM. In some instances where personal diacetyl samples were not collected, for example during the first survey, area samples were used to obtain estimates of personal-equivalent diacetyl exposures.

### *Creation of Job Categories and Estimation of Arithmetic Means*

For the purpose of developing exposure estimates for the JEM, plant job titles were aggregated into eight job categories based primarily on work and environmental similarities with respect to potential for diacetyl exposures [Corn and Esmen 1979]. These eight categories are listed in Table A2.2 along with the jobs that comprise each category.

Arithmetic means (AM) using PBZ samples were calculated for the cells in the JEM as the AMs are the preferred measure of central tendency for estimating cumulative exposure in chronic disease investigations [Smith 1992]. Few PBZ measurements were collected for most job categories in each of the nine surveys (range: n=1-6) except for the job category of Microwave line (range n=11-18). Moreover, a large fraction of the PBZ measurements were below the limit of detection (LOD) for most job categories (>50%) especially during surveys 6-9, except for the

job categories of microwave packaging line, quality control and microwave mixing. Thus because of the small sample size and large fractions of LOD data, a simple substitution method of replacing LOD measurements with a value of LOD/2 was used [Ganser and Hewett 2010]. The calculation of the arithmetic mean exposures by the different time periods is described in detail in the next section on "Creation of Exposure Periods".
As noted earlier, PBZ diacetyl samples were not collected during survey 1 and had to be estimated from personal and area samples collected during proximal surveys (i.e. surveys 2 and 3). These represent time periods and exposure conditions closest and most similar to the first survey. A hierarchical approach was used to estimate the PBZ exposures for survey 1 depending on the job category and the availability and fraction of personal or area measurements below the LOD. To start with, for jobs categories with sufficient personal and area samples in surveys 2 and 3, a prediction model was used to estimate personal exposures from area exposure measurements (e.g. microwave mixers, microwave line, quality control). For job categories with small sample size and/or large fraction of measurements below the LOD for surveys 2 and 3, the arithmetic mean of the area samples from survey 1 was assigned to personal estimates for survey 1, assuming a ratio of 1 for personal to area measurements (e.g. warehouse, outside/office, polyethylene line). For jobs with no area measurements in survey 1 (e.g. bag print) or the area measurements were not representative of personal measurements (maintenance), exposure estimates from similar jobs in survey 1 were assigned. The detailed approach to calculate personal-equivalent diacetyl exposure for each job category for the 1$^{st}$ survey is described in Table A2.3.

***Creation of Exposure Periods***
After estimating the personal-equivalent exposures for survey 1, arithmetic means were calculated for the different time periods. Unique exposure time periods were developed for each of the 8 job categories to reflect impact of the exposure control changes implemented at the plant between November 2000 to July 2003. Table A2.4 lists these exposure control changes according to the time of implementation. These exposure time periods varied by job categories since some control changes would have a greater impact on some job categories than others. In addition, the fraction of LOD samples for job categories during the different surveys also impacted the creation of time periods. Surveys for which a large fraction of the measurements for a job category were below the LOD were combined into one time period. For example, for warehouse, 50-100% of personal measurements were below the LOD for surveys 3-9, hence these surveys were combined into one time period. For the selection of time periods for the job categories, the LOD patterns were consistent with the implementation of controls. The detailed approach used to create the time periods for each job category is described in Table A2.5. Thus a JEM was created consisting of 8 job categories and 2-5 time periods spanning the time duration from November 2000 to July 2003.

***Adjustment for Respirator Use***
The JEM created as described above was based on samples collected from workers breathing zone and did not account for respirator use by workers. However, during survey 4 and onwards, workers in microwave mixing were using respirators and the JEM estimates were adjusted in the appropriate time periods to reflect the PPE use. Thus for the mixers during time periods 3 and 4, we adjusted the measured personal diacetyl exposure for the use of respirators. During these time

periods, mixers used respiratory protection while in the mixing room and these respirators included either a PAPR or air-line respirator with a loose fitting hood; both types of respirators have an applied protection factor (APF) of 25 [NIOSH 2004]. We assumed, based on survey observations and questionnaire responses, that mixers spent, on average, about 4 hours per shift in the mixing room in respiratory protection. Since respirators were required in the mixing room by plant management, we assumed that mixers wore respirators at all times while in the mixing room and did not wear these respirators when outside the mixing room and in the microwave packaging room. During these time periods, the mixers desk was located in the microwave packaging room near packing line 1 so we further assumed that, when not in respirators in the mixing room, mixers would be in the microwave packaging area and receive diacetyl exposures consistent with those personal exposures measured in microwave packaging. The mixer personal samples were taken outside of the respirator and would reflect both mixing and packaging exposure components. Accordingly, to adjust mixer exposures to diacetyl for the use of respirator, we 1) determined the mixing room exposure component from the combined mixing and packing line diacetyl concentration as reflected in the personal sample (back calculated) and 2) applied a protection factor of 25 to the mixing room component of the mixers exposure.

To determine the mixing room (A) personal diacetyl exposure from the combined mixing and packaging (C) concentration measured by personal sampling we applied the following equation:

$C = (4A_{(mixing)} + 4B_{(packaging)})/8$; solving for A gives, $A = 2C - B$
Where A = the mixing room personal exposure component in ppm, B = the packaging room personal exposure component in ppm, and C = the measured mixer personal exposure in ppm reflecting both mixing room and packaging room components.

To correct the mixers exposure for the use of respiratory protection, we used the following equation:

$C_R = ½ (A/25 + B)$ where $C_R$ = respirator adjusted mixer diacetyl exposure in ppm, A= personal mixer diacetyl exposure in the mixing room in ppm and B = personal diacetyl exposure in the microwave packaging area in ppm.

This adjusted diacetyl concentration in ppm was applied to mixers for time periods 3 and 4 to adjust for the use of respiratory protection by mixers while in the mixing room.

Table A4.1 Industrial Hygiene Survey Dates

| Survey | Survey Dates |
|---|---|
| 1 | November 11 – 18, 2000 |
| 2 | Jan 17 – 19, 2001 |
| 3 | April 1 – 4, 2001 |
| 4 | September 4 – 8, 2001 |

| | |
|---|---|
| 5 | November 6 – 8, 2001 |
| 6 | March 18 – 21, 2002 |
| 7 | August 11 – 16, 2002 |
| 8 | January 27 – 31, 2003 |
| 9 | July 14 – 18, 2003 |

Table A4.2 Exposure Categories used for JEM

| # | **Exposure Category** | **Jobs Included in Exposure Category** |
|---|---|---|
| 1 | Warehouse | Warehouse Job Category |
| 2 | Maintenance | Maintenance Job Category |
| 3 | Outside Processing / Office | Outside Processing & Office Job Categories |
| 4 | Polyethylene Line | Polyethylene Packer and Polyethylene Stacker Job Categories |
| 5 | Microwave Mixing | Microwave Mixer Job Category |
| 6 | Microwave Packaging Line | Microwave Job Categories: Machine operator, Packer, Stacker, Supervisor, and Inventory Control |
| 7 | Bag Print | Bag Print Job Category |
| 8 | Quality Control | Quality Control Job Category |

Table A4.3 Procedures Used for Estimating Personal Equivalent Diacetyl Exposures for Survey 1

| # | **Exposure Category** | **Procedures used by Exposure Category** |
|---|---|---|
| 1 | Warehouse | Use the mean of the area sample diacetyl concentrations from survey 1. |
| 2 | Maintenance | Calculate ratio of the survey 3 diacetyl mean for personal samples to the average of survey 3 diacetyl mean from personal samples for polyethylene, mixer, and microwave packaging line job categories. Apply this ratio to the average of the same three groups from survey 1 after they have been converted to personal equivalent exposures. |
| 3 | Outside Processing / Office | Use the mean of the area sample diacetyl concentrations from survey 1. |
| 4 | Polyethylene Line | Use the mean of the area sample diacetyl concentrations from survey 1. |
| 5 | Microwave Mixing | Model personal to area diacetyl concentrations for all surveys and apply this model to the survey 1 area diacetyl concentrations to estimate personal equivalent diacetyl |

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| | | exposures. |
| 6 | Microwave Packaging Line | Model personal to area diacetyl concentrations from surveys 2 & 3 and apply model to survey 1 area samples to estimate personal equivalent exposures. |
| 7 | Bag Print | Use the average of the personal equivalent diacetyl exposures for survey 1 from the microwave packaging line and warehouse job categories. (Note: there were no bag print area diacetyl samples for survey 1) |
| 8 | Quality Control | Model personal to area diacetyl concentrations from surveys 2 & 3 and apply model to survey 1 area samples to determine personal equivalent diacetyl exposures. |

Table A4.4. Dates of exposure control changes and NIOSH industrial hygiene surveys.

| **Date** | **Event** |
|---|---|
| Cross-Sectional Industrial Hygiene Survey, Respiratory protection training by NIOSH (November 11-18, 2000) | |
| Engineering Control Technology Survey (January 17 – 19, 2001) | |
| February 12, 2001 | Exhaust fan installed in oil and flavoring mixing room |
| February 2001 | Heated liquid flavoring tanks (2) vented to exhaust fan |
| March 29, 2001 | Pump installed for closed transfer of flavorings between holding and mixing tanks |
| Follow-up Survey (April 2-5, 2001) | |
| April 6, 2001 | Mixers supplied with powered air-purifying respirators and respirator training. Respirators available to workers in other microwave production areas on voluntary basis |
| May 22, 2001 | Local exhaust ventilation installed for 2 of 7 oil tanks on mezzanine. (Note, tanks were initially vented into packaging area air until September 2001) |
| June 6, 2001 | Flavoring storage cabinets completed for storing bulk flavorings |
| July 16, 2001 | Temperature control installed on one flavoring tank |
| August 7, 2001 | Tempered, outside supply air intake system completed, providing replacement air for microwave popcorn production areas |
| Follow-up Survey (September 4 - 8, 2001) | |
| September 11, 2001 | Exhaust fan installed in quality control lab |
| September 18, 2001 | Fresh air intake installed in quality control lab |
| September 21-30, 2001 | Completion of local exhaust ventilation for all mezzanine oil tanks |
| November 2, 2001 | Flavoring transfer pump installed for 5-gallon containers |
| November 2, 2001 | Air lock installed outside of mixing room |
| Follow-up Survey (November 6 - 8, 2001) | |
| Follow-up Survey (March 18 - 21, 2002) | |

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| August 2, 2002 | Started use of supplied-air respirators for mixers in mixing room and mezzanine (air-purifying respirators with organic vapor cartridges and particulate filters had been used prior to this.) |
| August 9, 2002 | Microwave ovens and testing counter in quality control lab enclosed with plastic curtain |
| Follow-up Survey (August 11 - 16, 2002) | |
| September 7, 2002 | Started using new mixing room (ventilation incomplete) |
| September 30, 2000 | Discontinued use of one paste butter flavoring |
| October 1, 2002 | New mixing room wall exhaust fan operational |
| Follow-up Survey (January 27 - 31, 2003) | |
| March 9, 2003 | Enclosure of tanks on mezzanine completed |
| April 10, 2003 | Air-handler functional on mezzanine |
| April 15, 2003 | New exhaust fan operational in quality control lab (in new "popping room") |
| April 15, 2003 | 2 additional exhaust fans (for mezzanine and mix room) |
| May 13, 2003 | Microwave ovens moved into popping room in quality control lab |
| Follow-up Survey (July 14 - 18, 2003) | |

Table A4.5 JEM Exposure Time Periods by Exposure Category

| # | **Exposure Category Time Periods[1]** |
|---|---|
| 1 | **Warehouse:**<br><br>Time 1 (Surveys 1 & 2 sampling results): Reflects exposures before major control changes that would impact warehouse worker exposures. Warehouse workers would go into microwave packaging, primarily on fork-lifts to remove finished product, and could receive higher, packaging area exposures accordingly.<br><br>Time 2 (Surveys 3 – 9 sampling results): Reflects the control changes implemented in the microwave mixing room in February of 2001 including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation. These mixing room control changes impacted warehouse diacetyl exposures since warehouse workers would enter microwave production area daily. Additionally, in August of 2001, the installation of an outside supply air intake system provided clean, tempered air into the warehouse area and makeup air for microwave production axial wall exhaust fans. This allocation of time periods was also selected since a majority of warehouse exposures to diacetyl were non-detectable since April of 2001. |
| 2 | **Maintenance:**<br><br>Time 1 (Surveys 1 & 2 sampling results): Reflects exposures before major control changes that would impact microwave production exposures, including maintenance worker exposures since maintenance workers would work on the production lines as well as in the |

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| | microwave mixing room.<br><br>Time 2 (Survey 3 sampling results): Reflects the control changes implemented in the microwave mixing room in February of 2001 including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation. These mixing room control changes would affect maintenance worker exposures since they would work in mixing and microwave production and there was a maintenance office located in microwave production.<br><br>Time 3 (Surveys 4 – 7 sampling results): Reflects the control changes including the installation of an outside supply air intake system providing clean, tempered air into the warehouse, the completion of LEV ventilation on mezzanine flavor holding tanks and the air-lock installation on the mixing room. All these microwave mixing and production control changes would impact maintenance workers since they would work in these production areas. Also, maintenance exposures during this time period were still primarily above the LOD.<br><br>Time 4 (Surveys 8 & 9 sampling results): Reflects the control changes including first use of enclosure of the mezzanine tanks. Additionally, maintenance exposures during this time period were largely below detectable limits. |
| 3 | **Outside Processing / Office Workers:**<br><br>Time 1 (Surveys 1 – 9 sampling results): Reflects low, predominantly non-detectable exposures for workers who were outside (outside processing) or normally away from microwave mixing and production operations. |
| 4 | **Polyethylene Line:**<br><br>Time 1 (Surveys 1 & 2 sampling results): Reflects polyethylene line worker exposures before major control changes in the microwave production area that could impact polyethylene line workers; although exposures in this category were low by comparison to microwave production lines, there were some detectable diacetyl exposures in personal and area samples for polyethylene line workers during this time period so a separate time period was used. While the polyethylene lines were located away from the microwave production area, there was some potential for exposure in this work group prior to control changes.<br><br>Time 2 (Survey 3-9 sampling results): Reflects the first control changes implemented in the microwave mixing room (including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation) plus all subsequent changes. After the first implementation of exposure control in microwave mixing and production areas, exposure among polyethylene line workers were largely below detectable limits. |
| 5 | **Microwave Mixers:** |

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| | Time 1 (Survey 1 & 2 sampling results): Reflects exposures before major control changes that would impact microwave mixer exposures.<br><br>Time 2 (Survey 3 sampling results): This time period reflects the first control changes implemented in the microwave mixing room including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation. During this time, the mixers desk was moved outside of the mixing room as an administrative control. This time period was also before the use of powered air purifying respirators (PAPRs) or air-line respirators.<br><br>Time 3 (Surveys 4 – 7 sampling results): Reflects significant control changes for mixing workers including first use of PAPR respirators in April of 2001 as well as subsequent use of supplied-air respirators in August of 2002 which would have significantly reduced mixer exposures. Both types of respiratory protection employed loose-fitting hoods with an applied protection factor (APF) of 25. (See description below on procedures used to estimate mixers exposure adjusting for respirator use). Other significant changes during this time period would include the addition of an outside supply- air system which provided clean, tempered make-up air for microwave production and mixing room air exhaust fans.<br><br>Time 4 (Survey 8 & 9 sampling results): Reflects the first use of the new mixing room and the addition of new mixing room exhaust fans. This time period also reflects enclosure of the mezzanine area reducing microwave packaging exposures outside the mixing room below quantifiable or detectable levels; this would affect mixers exposures when in the microwave packaging area and not in respiratory protection in the mixing room. |
| 6 | **Microwave Line:**<br><br>Time 1 (Surveys 1 & 2 sampling results): Reflects exposures before major control changes that would impact microwave line exposures.<br><br>Time 2 (Survey 3 sampling results): Reflects the first control changes implemented in the microwave mixing room including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation which would impact microwave line exposures since the mixing room was adjacent and open to the mixing lines.<br><br>Time 3 (Surveys 4 – 7 sampling results): Reflects several control changes including the installation of an outside supply air intake system providing clean, tempered air for microwave production area exhaust fans. This period also reflects completion of LEV ventilation on mezzanine flavor holding tanks and air-lock installation isolating the mixing room from packaging areas.<br><br>Time 4 (Surveys 8 & 9 sampling results): Reflects the first use of the new mixing room and the addition of new mixing room exhaust fans. This period also reflects enclosure of the mezzanine area reducing microwave production exposures outside the mixing room |

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| | below quantifiable or detectable levels. |
| 7 | **Bag Printing:**<br><br>Time 1 (Survey 1 & 2 sampling results): Reflects exposures before major control changes that would impact bag printing exposures due to close proximity to the microwave production lines. Also, when the bag printing operations were shut down, bag print workers would often work on the microwave production lines.<br><br>Time 2 (Survey 3 sampling results): Reflects the first control changes implemented in the microwave mixing room including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation. These control changes would impact bag printing exposures since the bag print lines were located in the warehouse just outside a large open doorway into microwave production; additionally, when the bag printing operations were shut down, bag print workers would often work on the microwave production lines.<br><br>Time 3 (Surveys 4 – 7 sampling results): Reflects several control changes including the installation of an outside supply air intake system providing clean, tempered air into the warehouse and subsequently for microwave production area exhaust fans. This period also reflects completion of LEV ventilation on mezzanine flavor holding tanks and air-lock installation isolating the mixing room from packaging areas.<br><br>Time 4 (Surveys 8 & 9 sampling results): Reflects the first use of the new mixing room and the addition of new mixing room exhaust fans. This period also reflects enclosure of the mezzanine area reducing microwave production exposures outside the mixing room below quantifiable or detectable levels. |
| 8 | **Quality Control:**<br><br>Time 1 (Surveys 1 & 2 sampling results): Reflects exposures before major control changes that would impact microwave quality control exposures.<br><br>Time 2 (Survey 3 sampling results): Reflects the first control changes implemented in the microwave mixing room including the addition of exhaust ventilation, closed transfer of liquid flavorings, and flavor tank ventilation. These changes would impact quality control exposures since the quality control room opened into the microwave production area. Also, the quality control room was generally under negative pressure relative to the microwave production room at this time.<br><br>Time 3 (Surveys 4 – 6 sampling results): Reflects installation of an exhaust fan and fresh air intake in the quality control lab. It also reflects several changes that would impact quality control worker exposures through reduction of diacetyl concentrations in the microwave mixing and production areas including the installation of an outside supply air intake system providing clean, tempered air into the warehouse and subsequently for |

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| | microwave production area exhaust fans; installation of a mixing room air-lock; and ventilation of mezzanine flavor holding tanks.<br><br>Time 4 (Surveys 7 & 8 sampling results): Reflects the enclosure of the microwave ovens in the quality control lab.<br><br>Time 5 (Survey 9 sampling results): Reflects the relocation of all microwave ovens into a separate, ventilated room. This step reduced quality control exposures below detectable levels. Other control changes to the microwave production area during this period reduced microwave production exposures below detectable levels further impacting quality control exposures. |

# APPENDIX 5

**APPENDIX 5**

**TABLE OF CONTENTS**

# A QUANTITATIVE RISK ASSESSMENT FOR DIACETYL BASED ON RESPIRATORY TRACT LESIONS IN MICE

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Prime Contract Number: DOLQ059622303
Task Order Number: 50

**Sept 18, 2009**

## 1.0 INTRODUCTION

Diacetyl is an α-diketone that is used to impart a butter flavor to food flavorings used on some microwave popcorn and other food products. Diacetyl was a predominant compound isolated from air samples taken from a plant in Missouri that produces microwave popcorn and at which cases of bronchiolitis obliterans were reported (Kreiss et al., 2002). Those cases occurred at rates much greater than expected and triggered an investigation of the cause of such responses. Kreiss et al. (2002) observed a statistically increasing trend between the proportion of employees with airway obstruction and cumulative diacetyl exposure (summarized by quartile).

Tests of the effects of butter flavoring vapor (BFV) or of diacetyl alone have also indicated a relationship between exposure and respiratory response. Hubbs et al. (2002) exposed rats to a single inhalation of BFV (diacetyl concentrations ranging between 203 and 352 ppm for the constant exposure groups) and observed rhinitis, starting at the lowest exposure level, and bronchitis (at the higher two exposure levels) one day after a six-hour exposure. Hubbs et al. (2008) exposed rats to a one-time inhalation of pure diacetyl (intermittently or continuously for up to six hours) and observed adverse respiratory effects including epithelial necrosis and inflammation in the nose, larynx, trachea, and bronchi. The nasal region was apparently most sensitive in their experiments.

Morgan et al. (2008) exposed mice to diacetyl for up to twelve weeks. They observed adverse nasal and lung effects, the latter observed in the peribronchial, bronchial, and peribronchiolar regions. At present, the Morgan et al. (2008) study includes the longest exposure durations among all the experiments of test species. As such, it is the basis for the dose-response analysis used to derive benchmark doses (BMDs) and corresponding human equivalent concentrations (HECs), as discussed in greater detail in the Methods section below.

The remainder of this document presents the methods for and results of the dose-response analysis of the adverse respiratory tract effects observed in the mice of the Morgan et al. (2008) study. Much of the Methods section is devoted to the explication of the dosimetric adjustments that have been considered. Once the dose metric values corresponding to each exposure group were derived, the suite of dichotomous dose-response models from the BMDS software (EPA, 2009a) were run for the selected endpoints and BMDs and their lower bounds (BMDLs) were estimated. The BMD and BMDL estimates are presented in the Results section along with the HECs (in ppm exposure levels) corresponding to the estimated BMDs and BMDLs. Human dosimetric considerations analogous to those applied to the mouse were used to determine those HECs. The Discussion section highlights the primary findings and presents information relevant to ascertaining the sensitivity of the results to certain assumptions that have been identified in the Methods section.

## 2.0 METHODS

### 2.1 Data

The response data that were analyzed were obtained from a report of an NIEHS study of mice exposed to diacetyl (Morgan et al., 2008). Male C57Bl/6 mice were exposed to diacetyl vapors at various concentration levels and for varying durations (both in terms of hours per day and number of weeks of exposure). Among mice exposed to 200 or 400 ppm for 5 days, the responses included death, necrotizing rhinitis, necrotizing laryngitis, and bronchitis.

The responses analyzed here are those most relevant to longer-term exposures, i.e., those from the subchronic portion of the study that included constant exposures of 25, 50, and 100 ppm, for 6 hours/day. Responses were reported for animals treated either for 6 weeks or for 12 weeks. The endpoints that were observed and modeled for the dose-response analysis were as follows:

- Nasal Lesions:
    - Inflammation (chronic, active)
    - Necrosis and ulceration of respiratory epithelium
    - Metaplasia (squamous) of respiratory epithelium
- Lung Lesions:
    - Peribronchial lymphocytic inflammation
    - Bronchial epithelial atrophy and denudation
    - Peribronchiolar lymphocytic inflammation

Each of those endpoints was observed in more than 50% (almost always 100%) of the animals in the 100 ppm exposure group at the 6 week time point. They were selected from among the endpoints reported because they were considered to be representative of the adverse responses observed and because the dose-response patterns were expected to span the range of those that would be obtained from all the endpoints. They included the apparently most-sensitive responses for both the nasal and lung regions.

The data were examined to determine if, statistically, the endpoint-specific patterns for the 6- and 12-week experiments appeared to be similar. A likelihood ratio test was performed of the null hypothesis that, conditional on exposure level, the response rates could be accounted for by the same probability of response across the two time periods. When that null hypothesis was not rejected for a given endpoint, the 6- and 12-week data were combined for the dose-response analysis.

### 2.2 Dose-Response Modeling

The quantal (i.e. presence/absence) response data (Table 1) were modeled using the BMDS software (version 2.1) provided by the US EPA (EPA, 2009a). The quantal models that were included in the runs were as follows:

- Gamma
- Logistic
- Log-Logistic
- Log-Probit
- Multistage
- Probit
- Weibull

Equations describing these dose-response functions are shown in Appendix A.

From among those model runs, for the models that had a goodness-of-fit p-value greater than 0.1 (indicating a satisfactory fit of the model to the data), the model with the lowest Akaike Information Criterion (AIC) value was selected for benchmark dose (BMD) estimation. The AIC is a measure that can be used to compare any set of models fit to the same data set; it accounts for the fit of the models to the data and for the number of parameters used to obtain that fit; lesser values of the AIC are better for that model comparison. This approach to model selection is the default procedure recommended by US EPA in its BMD guidance (EPA, 2000). Separate electronic submissions accompanying this report provide the results of the modeling for all the models.

In addition to the best-fitting model as defined in the previous paragraph, the BMD and BMDL values from the log-probit model were also examined and reported. The log-probit model tends to allow a more "threshold-like" appearance than other dichotomous models. The predictions of the log-probit model are compared to those of the best fitting model to suggest what effect might be associated with an assumption of threshold-like behavior in the dose-response for the diacetyl-responsive endpoints.

The quantal modeling described above considers only the total number of cases (Table 1) and not the severity of the responses. The severities of each of the responses for each of the endpoints listed above were obtained from Morgan (personal communication; Table 2). Such severity data are amenable to categorical regression analysis, which fits a model to predict the incidence of cases of specific severities as a function of dose. This was done using the EPA software CatReg (EPA, 2009b). Specifically, a cumulative odds model, with logit and probit link functions, was fit to the severity counts. The cumulative odds model assumes that a logit or probit link function of the probability of response of severity i or greater (i = 0, 1, 2, …) is described by a linear function having severity-specific intercept terms and a dose coefficient common across severities. The function definitions are included in Appendix A. Results are reported for the link function that provided the best fit to the data, as part of the discussion of the sensitivity of the results to the modeling assumptions.

The notation BMD(10) and BMDL(10) is used to denote the calculated dose (and its lower bound, respectively) corresponding to an extra risk of 0.1 (1 in 10), BMD and BMDL(1) for an extra risk of 0.01 (1 in 100), and BMD(0.1) and BMDL(0.1) for an extra risk of 0.001 (1 in 1000). The BMDLs are the 95% lower bounds on the associated BMD estimates.

## 2.3 Dose Metrics for Modeling

Several dose metrics were calculated and used for the dose-response modeling, rather than simply using the exposure levels themselves in the models. In the following description of the dose metric derivations, it is helpful to consider that the metrics can be viewed as the product of three terms:

- the exposure concentration
- a reduction in concentration due to scrubbing in higher respiratory tract regions
- an "effective dose" measure

The various combinations of scrubbing terms and the effective dose measures lead to several different alternative metrics for each exposure group. The possible scrubbing factors and effective dose measures are discussed in the following subsections.

### 2.3.1 Scrubbing Factors

As diacetyl is traveling in the respiratory tract toward its site of action, it will be absorbed and removed from the respiratory tract by the cells/lining in sections of the respiratory tract "higher up" than the site of action. The US EPA (EPA, 1994) has developed procedures for factoring in such scrubbing as part of its methods for deriving inhalation reference concentrations. We consider the use of such scrubbing factors for calculation of diacetyl dose metrics here. Note that all the scrubbing factors as calculated reflect the proportion of diacetyl *remaining* after passage through the regions to which those factors apply. A scrubbing factor close to 1, therefore, indicates that little diacetyl is removed; conversely, a scrubbing factor closer to zero indicates that more diacetyl has been removed from the respiratory tract.

#### 2.3.1.1 Nasal Lesions

For nasal lesions, there is no scrubbing associated with respiratory tract regions "higher up;" the nasal region (also referred to as the extra-thoracic, or ET, region) is the first region that is encountered by an inhaled vapor. Thus, when considering lesions in the ET (nasal) region, an implicit scrubbing factor of 1 will always be applied.

#### 2.3.1.2 Lung Lesions - EPA Default (Scrubbing Factor Approach 1)

To apply the EPA default approach, we assume that diacetyl is a so-called Category 1 vapor, one that is readily absorbed and/or reactive in the respiratory tract. For Category 1 vapors, the default equation for deriving a scrubbing factor (EPA, 1994) has the following form:

$$\exp\{-SA(ET)/VE\} \qquad \text{Eq. 1}$$

where VE is the minute volume (ml/min) and SA(ET) is the surface area of the extra-thoracic (ET) region. This scrubbing factor applies to the tracheobronchial (TB) region.

EPA (1994) also describes scrubbing that occurs in the tracheobronchial region (and which would be multiplied by the factor shown in Eq. 1 to yield a scrubbing factor applicable to the pulmonary region) but that factor was not needed for the current analyses.

*Assumption*: With respect to the EPA default regions, all the lung lesions analyzed here were assumed to be associated with the TB region. That assumption seems reasonable given the fact that the lesions are peribronchial (inflammation), bronchial (atrophy) or peribronchiolar (inflammation).

The minute volumes for the mice used experimentally (Morgan et al., 2008) have been reported (Morgan, personal communication) as shown in Table 3. Group-specific values were estimated and used in the above calculations as follows. For the experiment involving 6-week exposures, the minute volumes used for dose metric calculations were the averages of the measurements taken at 3 week and those taken at 6 weeks. For the experiment involving 12-week exposures, the minute volumes used for dose metric calculations were the averages of the measurements taken at 12 weeks with the averages of measurements taken at 3 week and those taken at 6 weeks (reflecting the fact that half of the exposure period, the first six weeks, is characterized by two measurements while the second half of the exposure period is characterized by only one measurement). The minute volumes shown in Table 3 are much greater than the default minute volume for mice reported in EPA (1994). The minute volumes reported by Morgan (personal communication) ranged between about 80 and 150 ml/min; the EPA (1994) default value for mice is about 37 ml/min for mice weighing about 32 g.

The mice in the Morgan et al. study averaged about 26 g over the course of the 12 weeks of exposure (the high-concentration group averaged a little less than the other groups).

*Assumption*: This value was considered close enough to the EPA default weight for a mouse (31.6 g; USEPA, 1994, Table 4-5) to use the EPA default values for surface area (SA). Those values were 3.0 and 3.5 $cm^2$ for the ET and TB regions, respectively (USEPA, 1994, Table 4-4). These surface areas were used for all of the dose metric calculations.

Given the above values for VE, and SA(ET), the EPA default method could be applied to calculate scrubbing factors as shown in Table 4. For example, for the 25 ppm group, for the 6-week exposure experiment, the scrubbing factor is

$$\exp\{-3.0/147.5\} = .980$$

### 2.3.1.3 Lung Lesions – CFD-Model Adjusted ET Scrubbing Factors (Approach 2)

Morris and Hubbs (2008) have reported the results of a computational fluid dynamics (CFD) model developed specifically for diacetyl to predict scrubbing (and tissue concentrations) in various regions of the rat and human respiratory tracts. Those authors

characterized inhalation dosimetric patterns of diacetyl[1] and calibrated the rat model with reference to uptake data they collected in anesthetized male Sprague-Dawley rats. They found that diacetyl was metabolized in nasal tissues (in vitro), likely by diacetyl reductase. They reported that diacetyl uptake was closely described by their CFD model.

The predictions of the CFD model are used here to develop alternatives to the EPA default scrubbing factor.

First we must consider the relative values calculated via the EPA default method and via the CFD modeling approach. For that comparison, we calculated the default values using the same minute volumes used by Morris and Hubbs to derive their model predictions (see their Table 3). For rats, the assumed minute volume was 400 ml/min; for humans it was 13800 ml/min. Using the default SA(ET) values (15 and 200 $cm^2$ for rats and humans, respectively), the default procedure predicts a scrubbing factor for the ET region of 0.963 for rats and 0.986 for humans. The predictions from the CFD model are that the scrubbing factors for diacetyl are 0.679 and 0.82 for rats and nose-breathing humans, respectively.

The value of 0.679 for rats is the average of the ratios of concentrations exiting the upper respiratory tract (equated with the ET region here) over the inhaled concentration, where the inhaled concentrations were 100, 200, and 300 ppm. Those ratios did not depend on the concentration (they were all approximately equal to the average) so it is assumed that the scrubbing factor is constant for concentrations up to 300 ppm. Similarly the value of 0.82 for humans is based on the predicted concentration exiting the upper respiratory tract (82 ppm) when the inhaled concentration was 100 ppm. It was assumed that that value was also constant (for all human exposure levels up to 100 ppm; no predictions for other exposure concentrations were presented).

The question is how to use those sets of predictions for rats and humans to adjust the default scrubbing factor in mice, the species for which we have experimental data. For the dose-metric relevant to the TB region, the following approaches were completed.

#### 2.3.1.3.1 Approach 2a. Adjust Mouse Default Proportional to Rat Results

The rat default prediction for an ET scrubbing factor is 0.963 and the CFD, diacetyl-specific prediction is 0.679.

*Assumption*: The adjustment to the mouse default ET scrubbing is the same as that for rats. If that is the case, then the mouse ET scrubbing factor for a dose group in the experiments under consideration would be X, where X satisfies the following conditions:

$$.679 / .963 = X / \exp(-3.0 / VE); \qquad \text{Eq. 2}$$

[1] They did the same for diacetyl in the presence of butyric acid, another component of butter-flavoring vapor. The modeling results for diacetyl in the presence of the butyric acid were not used for the current analysis.

where 3.0 is the mouse SA(ET) and VE is the group-specific minute volume. The denominator on the right side of that equation is the mouse default-predicted ET scrubbing. For example, for the 25 ppm group in the 6-week exposure experiment, VE = 147.5 and the above equation becomes

$$.679 / .963 = X / \exp(-3.0 / 147.5) = X / .980$$
$$X = .691$$

Table 5 shows the resulting adjusted ET scrubbing factors for each experimental group.

#### 2.3.1.3.2 Approach 2b. Adjust Mouse Default Proportional to Rat and Human Results

*Assumption*: The adjustment to the mouse default ET scrubbing is just as in approach 2a, except that both the rat and human CFD model predictions (relative to the EPA default predictions) are used to make the adjustments. There is some support for such a "rat/human-average" approach since the EPA default ET scrubbing for mice (at the minute volumes measured in the Morgan et al. study) is between the default ET scrubbing for rats and humans.

Approach 2a used the ratio .679/.963 = .705 to determine an adjustment. If mice are not just like rats in the way they deviate from the default, but are more like an average of rats and humans, then we would pick another factor that represents the average deviation of both rats and humans. For humans, the ratio of CFD-predicted to default-predicted ET scrubbing is .82/.986 = .832. The average of the adjustment factors for rats and humans is

$$(.705 + .832)/2 = .768$$

and this value can be used in place of .705 to calculate group-specific adjustments as in Approach 2a:

$$0.768 = X/\exp(-3.0/VE) \qquad \text{Eq. 3}$$

The group-specific results are shown in Table 6.

#### 2.3.1.3.3 Approach 2c. Adjust Mouse Default by Calculating Kg(ET)

EPA (1994) presents an alternative procedure for determining the ET scrubbing when more information is available for estimation. It relies on an added parameter, Kg(ET), which is described as the overall mass-transport coefficient in the ET region (see EPA, 1994, p. 4-47 ff. and section I.1.3, p. I-11 – I-17). The ET scrubbing factor using Kg(ET) is given as follows:

$$SF = \exp\{-Kg(ET) * SA(ET)/VE \}$$

*Assumption*: Kg(ET) is constant across species. EPA (1994, p. 4-51) discussed this as a possible assumption for the inhalation dosimetry calculations.

The CFD model predicts that the ratio of rat to human ET scrubbing factors is .679/.82, based on the calculations presented above. If Kg(ET) is constant across species for diacetyl, then it should be the case that

$$.679 / .82 = \exp\{-Kg(ET) * 15/400\} / \exp\{-Kg(ET) * 200/13800\},$$
$$= [\exp\{-15/400\}/\exp\{-200/13800\}]^{Kg(ET)}$$

where 15 and 400 are SA(ET) and VE for rats, respectively; 200 and 13800 are SA(ET) and VE for humans, respectively. The SA(ET) and VE values used here are again the same values as used in the CFD modeling work of Morris and Hubbs (2008). Solving for Kg(ET) yields a value of 8.20. Because that value is assumed to be constant over species, it holds for mice as well. In which case the ET scrubbing factor for mice is

$$\exp\{-8.20 * 3/VE\} \quad \text{Eq. 4}$$

where, again, VE is a group-specific estimate of minute volume for the mice in the experiments under consideration and 3 is the SA(ET) for mice. Table 7 displays the group-specific scrubbing factors using this approach.

### 2.3.1.4 Lung Lesions – CFD-Model-Based Scrubbing in ET and Trachea (Approach 3)

A third approach to determining scrubbing factors relevant to the mouse lung lesions being modeled is as follows. This approach will be applied to those effective dose estimates that are based on specific tissue concentrations as opposed to those related to ratios of minute volumes and region-wide surface areas. That is the case because this approach considers the CFD model-predicted scrubbing in the trachea, which is not separated out in the EPA region-based approaches.

Because there are no EPA defaults for scrubbing in the trachea, this approach relies on relationships predicted by the rat and human CFD models to derive a factor for scrubbing by the mouse trachea.

#### 2.3.1.4.1 Approach 3a. Mouse Tracheal Scrubbing Proportional to Rats

*Assumption*: The relationship between scrubbing in the mouse ET and trachea will be the same as the relationship between the CFD model-predicted rat ET and tracheal scrubbing. If that is the case, then the following equation should hold:

(rat tracheal scrubbing)/(rat ET scrubbing) =
(mouse tracheal scrubbing)/(mouse ET scrubbing).

For the rat, the numerator and denominator on the left side are known from the CFD model predictions. Averaging over the predictions corresponding to inhalation exposures of 100, 200 , and 300 ppm, the rat tracheal scrubbing factor is estimated to be 0.919 (using values from Table 3 of Morris and Hubbs, 2008). As before, the average for the rat ET scrubbing factor is 0.679.

On the right hand side of that equation, for the denominator, it is appropriate to use the estimated mouse ET scrubbing factor derived when the mouse default value was adjusted by assuming that the deviation from default was just as it appears for rats (Approach 2a), because here again we are assuming the estimation for the mouse is based only on the rat patterns. For Approach 2a, we found that

mouse ET scrubbing factor = (0.679/0.963) * exp(-3/VE).

Therefore, the mouse tracheal scrubbing factor is

mouse tracheal scrubbing factor = (0.919/0.679) * (0.679/0.963) * exp(-3/VE).

If we then consider that the scrubbing occurring in the ET region and trachea must be multiplied together to determine the total scrubbing occurring before diacetyl exits the trachea, that combined scrubbing down to and including the trachea is

(0.679/0.963) * exp(-3/VE) * (0.919/0.679) * (0.679/0.963) * exp(-3/VE)

= (0.679/0.963) * (0.919/0.963) * exp(-6/VE) Eq. 5

Table 8 shows the group-specific values for this scrubbing, based on the group-specific minute volumes. For example, for the six-week, 25 ppm group with VE = 147.5

ET and tracheal scrubbing factor = (0.679/0.963) * (0.919/0.963) * exp(-6/147.5)
= 0.646.

#### 2.3.1.4.2 Approach 3b. Mouse Tracheal Scrubbing Proportional to Rats/Humans

Just as in the case of estimating the mouse ET scrubbing, we need not assume that the mouse is just like the rat in terms of adjustments to defaults. We can, instead, assume that the data and predictions for both rats and humans are relevant to determining the scrubbing that might occur in mice.

*Assumption*: The estimation of the mouse tracheal scrubbing is based on the averaged predictions of the rat and human CFD model predictions. The predicted mouse ET scrubbing factor using Approach 2b is appropriate here because it too used the average of rat and human predictions.

If that is the case, then we can do the same calculations as in Approach 3a, except that the relevant model predictions will be the averaged ratios of ET to tracheal scrubbing, where the average is over the rat and human ratios.

From Approach 3a, we see that rat tracheal-to-ET scrubbing is 0.919/0.679. Similarly, the ratio for humans, based on Table 3 of Morris and Hubbs (2008) is 0.962/0.82. The average of those ratios is 0.940. The average of the human and rat ET scrubbing factors is 0.749. From Approach 2b, the mouse predicted ET scrubbing factor is

$$.768 * \exp(-3/VE).$$

Thus, when assuming that rat and human CFD model predictions are equally relevant for predicting mouse patterns, the equation for the mouse tracheal scrubbing factor becomes

$$(0.940/0.749) * 0.768 * \exp(-3/VE).$$

Combining the ET scrubbing factor with the tracheal scrubbing factor (multiplying them together) yields

$$(0.940/0.749) * [0.768 * \exp(-3/VE)]^2 \qquad \text{Eq. 6}$$

for the total scrubbing factor pertinent to estimating dose metrics based on tissue concentration in the bronchial subregion. Table 9 shows those scrubbing factors for each of the groups in the mouse experiments.

### 2.3.2 Effective Dose Estimates

The previous calculations have related only to the scrubbing factor component of the dose metric calculations. The other component that must be estimated is an "effective dose" (or "target dose") once the scrubbing has occurred. As was the case with the scrubbing factors, we consider here some alternative effective dose measures based on the EPA default model and the Morris/Hubbs CFD model approaches.

In general the effective dose measure will be of two types. The first is based on the minute volume relative to the surface area in the affected region; this is the EPA default approach. The EPA default approach is based on a fractional penetration model and predicts the amount of chemical (normalized to surface area) that penetrates the different regions of the respiratory tract (see Appendix I of EPA, 1994). We refer to this approach as the regional penetration approach. The second approach utilizes tissue concentrations, estimated by the previously cited CFD model predictions. The CFD/PBPK model of Morris and Hubbs (2008) represents the upper respiratory tract and includes tissue compartments with terms for metabolic and systemic clearance that allow predictions of tissue concentration, which are the basis for the tissue concentration approach.

#### 2.3.2.1 Nasal Lesions

#### 2.3.2.1.1 Nasal Region Penetration Approach, Effective Dose Approach 1

The nasal region penetration approach estimates the effective dose term as

$$VE/SA(ET) \qquad \text{Eq. 7}$$

for lesions occurring in the ET (nasal) region (EPA, 1994). Both VE and SA(ET) were discussed above in relation to the derivation of the scrubbing factors; the same values are used here for the effective dose calculations.

#### 2.3.2.1.2 Nasal Tissue Concentration, Effective Dose Approach 2a: Mouse Concentrations Based on Rat Concentrations

The CFD model of Morris and Hubbs (2008) predicts concentrations in the nasal mucosa as a function of the inhaled concentrations. Table 10 reports the CFD model predictions from Table 3 of the Morris and Hubbs (2008) manuscript, with the ppm atmospheric concentrations converted to μg/ml concentrations and mM tissue concentrations converted to μg/ml. Taking an average of the ratios of inhaled μg/ml concentrations to the anterior ventral mucosa concentrations (μg/ml) for rats, one obtains a value of 394 for the gravimetric inhaled-to-tissue concentration ratio (μg/ml tissue)/(μg/ml air).

*Assumption*: If mice are like rats with respect to the tissue concentration to air concentration relationship in the nose, then this factor can be used to estimate a mouse nasal tissue concentration based on the experimental exposure levels.

#### 2.3.2.1.3 Nasal Tissue Concentration, Effective Dose Approach 2b: Mouse Concentrations Based on Average Rat and Human Concentrations

As in the case of the scrubbing factors discussed above, it is not certain that mice would have a tissue concentration to air concentration relationship just like rats. As an alternative one could consider all the CFD model predictions, for humans as well as rats, as the basis for an extrapolation to mice.

*Assumption*: It is the average gravimetric inhaled-to-tissue concentration relationship predicted in rats and humans that best estimates the relationship relevant for mice. It should be noted that the rat and human predictions for the nasal region are very similar. Whereas the rat ratio is 394 (on average), the human ratio is 342 (μg/ml tissue)/(μg/ml air). The average of those two values, 368, is the conversion used for the Tissue Concentration metric, Approach 2b.

### 2.3.2.2 Lung Lesions

Just as in the case of the scrubbing factors relevant for the lung lesion dose metric calculations, we assume that the TB region is the region relevant to the lung lesions observed in the mouse experiment (Morgan et al., 2008). And, as for the nasal lesions, we consider an EPA default approach and alternative CFD model-based approaches to estimating the effective dose.

#### 2.3.2.2.1 TB Region Penetration Approach (Effective Dose Approach 1)

The TB region penetration approach estimates the effective dose term as

$$VE/SA(TB) \qquad \text{Eq. 7}$$

for lesions occurring in the TB region of the lung (EPA, 1994). As discussed above, we assume that the TB region is the relevant region for the lung lesions analyzed here. Both VE and SA(TB) (= 3.5 $cm^2$) were discussed above in relation to the derivation of the scrubbing factors; the same values are used here for the effective dose calculations.

#### 2.3.2.2.2 Lung Tissue Concentration, Effective Dose Approach 2a: Mouse Concentrations Based on Rat Concentrations

The CFD model of Morris and Hubbs (2008) predicts concentrations in the nasal mucosa and in the trachea as a function of the inhaled concentrations. What we would prefer to have is an estimate of the bronchial tissue concentrations.

*Assumption*: Lacking the bronchial tissue concentrations for rats, we estimate bronchial concentration conversion factors (μg/ml tissue)/(μg/ml air) to be the same as the ratio of tracheal mucosal concentrations over the corresponding airborne concentrations reaching the trachea (exiting the upper respiratory tract). This assumption is based on the observation that the trachea is part of the TB region, in the EPA regional paradigm, as is the bronchial tissue. Thus the tracheal data appear to be the most appropriate for extrapolating to a bronchial conversion factor. For Approach 2a, we further assume that mice are like rats in this regard.

The average of the tracheal tissue concentration to airborne concentration ratios for rats (Table 10) is 446. With this approach, this is the conversion factor used to calculate the dose metric values.

#### 2.3.2.2.3 Lung Tissue Concentration, Effective Dose Approach 2b: Mouse Concentrations Based on Average Rat and Human Concentrations

As in the case of the nasal tissue concentration we do not know that mice are like rats (as assumed in Approach 2a) or if they might be more as predicted by the average human and rat conversions.

*Assumption*: Lacking the bronchial tissue concentrations for rats or humans, we estimate bronchial concentration conversion factors (μg/ml tissue)/(μg/ml airborne) as the average of all ratios of anterior tracheal mucosal concentrations over the corresponding ppm concentrations. The corresponding airborne concentrations are the concentrations exiting the upper respiratory tract. For Approach 2b, we further assume that the conversion factor for mice is determined by the average ratios over rat and human tissues.

As discussed for Approach 2a, the ratio for rats was 446. For humans, the ratio is 362. Thus, the average of those two species-specific values is 404 (μg/ml tissue)/(μg/ml

airborne).. This value was used in Approach 2b to convert the μg/ml exposure levels to tissue concentrations assumed to be relevant for the lung lesions under consideration.

### 2.3.3 Dose Metric Calculations

The methods described above include several options for scrubbing factor and effective dose estimations. The combinations that were included in the analysis are summarized in Table 11. A total of 19 different computations of dose metrics was completed. Each accounts in some manner for the removal of diacetyl before the target site and an effective measure of dose at the target site. As alluded to above, tracheal scrubbing (Approaches 3a and 3b) was not considered to be compatible with the TB penetration dose measure estimated from the EPA default model since the trachea is within this region of the respiratory tract.

Note that for the dose metrics that use the regional penetration effective dose measure, the ppm exposure levels were converted to μg/ml concentrations (1 ppm = 0.00352 μg/ml based on diacetyl's molecular weight of 86.09). Moreover, all the metrics were multiplied by the daily duration of exposure (360 minutes). The resulting units for such dose metrics are $\mu g/cm^2$ for those using the regional penetration effective dose measure and μg/ml-min for those using the tissue concentration metric.

## 2.4 Converting BMDs to HECs

The dose-response modeling described above used the dose metrics derived as appropriate for the mice in the bioassay under consideration. Thus the BMDs obtained from that modeling are in the units of the input dose metrics. They must be converted to human equivalent concentrations (HECs) to be relevant to ascertaining risks in humans exposed to different exposure concentrations. The process by which those BMDs were converted to HECs is described here. As in the case of the mouse-estimated dose metrics, the conversions consider both diacetyl scrubbing and the effective dose measure.

Regardless of the particular methods for HEC estimation, two assumptions were made throughout.

*Assumption*: The region that is associated with the observed human response (obliterative bronchiolitis) is the TB region. Effective dose estimates relevant to this region (or to bronchiolar tissue concentrations) are the focus of the HEC conversions. The relevant scrubbing factors are for the ET region (or ET and trachea in the case of bronchiolar tissue concentration measures).

*Assumption*: No matter what region of the respiratory tract is affected in mice (nasal or TB), it is assumed that the dose-response relationship between the dose metric and the responses for that region is the same as the dose-response relationship that holds, in humans, between the same type of metric (e.g., with effective dose equal to the minute volume to surface area ratio and accounting for scrubbing) and the response that might occur in the human-affected region. In other words, the value of the dose metric giving risk of X in a region of the mouse respiratory tract is assumed to give the same risk to the

affected human respiratory tract region, whatever that region may be; no site concordance is assumed.

In all cases, the minute volume (VE) for a human worker was assumed to be 20 L/min (20,000 ml/min). This corresponds to a value of 9.6 $m^3$ breathed per 8-hour work shift, a value used by OSHA and NIOSH for previous risk assessments. In addition, we have assumed (as the base case) 50% mouth breathing. This affects the estimated scrubbing in the ET region; it is assumed that no ET scrubbing occurs during mouth breathing, so that the scrubbing factor for the ET region is set to 1 when mouth breathing is in effect. As a test of sensitivity, rates of 0 and 100% mouth breathing have also been examined.

Surface area for the TB region in humans was assumed to be the EPA default of 3200 $cm^2$ (EPA, 1994).

### 2.4.1 Human Scrubbing Factors

The scrubbing factors that have been examined on the human side are as follows:

#### 2.4.1.1 Human Scrubbing Factor Approach 1: EPA Default

Given the assumed minute volume and surface areas for humans, the scrubbing factor for the ET region derived from the EPA default model is 0.990, for 100% nose breathing. Assuming 50% nose breathing and 50% mouth breathing, the averaged scrubbing factor for ET becomes 0.995.

#### 2.4.1.2 Human Scrubbing Factor Approach 2: CFD Model-based ET Scrubbing

The prediction from the CFD model (Morris and Hubbs, 2008) is that a diacetyl-specific scrubbing factor for the ET region in humans is 0.82 (nose breathing) or 0.91 assuming 50% mouth breathing. The value of 0.91 is the scrubbing factor used for HEC conversion with this approach.

#### 2.4.1.3 Human Scrubbing Factor Approach 3: CFD Model-based ET and Tracheal Scrubbing

This approach was considered when the tissue concentration measure of effective dose was used. In that instance, the scrubbing by both the ET region and the trachea needed to be considered. For this approach, the average (over mouth breathing status) ET scrubbing factor of 0.91, from Approach 2 above, was assumed. The tracheal scrubbing was also estimated from predictions of the CFD model; the average of two values predicted by that model (79/82 and 96/100, see Table 3 of Morris and Hubbs, 2008) was 0.962.

Thus, the combined (ET and tracheal) scrubbing factor relevant for the bronchial tissue concentration effective dose measure is 0.91* 0.962 = 0.875.

### 2.4.2 Human Effective Doses

Two effective dose measures were considered.

#### 2.4.2.1 Human Effective Dose 1: TB Penetration

As in the case of the mouse dose metric calculations, one option was a regional penetration approach based on the EPA (1994)default. That default was calculated as the ratio of VE to surface area, in this case SA(TB) since TB is assumed to be the site of diacetyl's effect in humans. In working humans, this measure is equal to 20,000/3200 = 6.25. This factor was used whenever the BMDs being converted resulted from a mouse dose metric that used the EPA default, VE/SA, as part of its calculation.

#### 2.4.2.2 Human Effective Dose 2: Tissue Concentration

This effective dose corresponds to any of the mouse dose metrics where the effective dose was tissue concentration. The manuscript by Morris and Hubbs (2008) does not present CFD model-predicted bronchial tissue concentrations. In their absence, the following calculations were performed to derive such concentration estimates.

*Assumption*: The factor that converts airway concentration to tissue concentration is the same for bronchial mucosa tissues and for tracheal mucosa.

Thus, given the conversion factors based on CFD model predictions in the trachea (Table 10), we derived a conversion factor that we assumed to apply to the TB region as a whole and to bronchial tissues within that region: 362 (μg/ml tissue)/(μg/ml airborne).

### 2.4.3 Duration Adjustment and Final HEC Conversions

An additional adjustment that completes the conversion of mouse BMDs to human exposure levels is a factor of 480 minutes to account for the daily duration of exposure assumed for the workers under consideration. No other duration adjustments were applied (experimental exposures occurred five days per week, for example, so no adjustment was made for frequency of exposure during a week). The subchronic exposure period (up to 12 weeks) was considered adequate to characterize the extent of lesions for longer term exposures. This conclusion was supported by the fact that the six- and twelve-week experiments had response rates that could be modeled together (i.e., the duration of the experiment could be ignored) for all the lesions analyzed; there did not appear to be a progression toward higher rates of response or more severe responses when the exposure level remained the same but the duration of exposure was increased from 6 to 12 weeks.

Because the mouse atmospheric concentrations were converted to units of μg/ml, the HEC includes a conversion from μg/ml to ppm (dividing by 0.00352).

The combinations of scrubbing factors and effective dose measures used for the HEC conversions are summarized in Table 12.

## 2.5 Presentation of Results

The major difference in risk estimates among the various dose metrics is associated with the effective dose assumption, i.e., whether a regional VE/SA penetration or a tissue concentration measure is most appropriate. The different scrubbing factor assumptions had much less influence on the risk-specific dose estimates. Therefore, the following procedure was adopted for presenting the results.

First, a baseline set of assumptions was chosen to use with the two effective dose measures. That baseline set consisted the following:

- Nasal Lesions:
    - For the nasal penetration effective dose, there are no variations to consider (scrubbing is always 1 for the nasal region).
    - For the nasal tissue concentration effective dose, Approach 2b (Section 2.3.2.1.3) was assumed; it considers the average values from rats and humans to estimate a conversion for mice.
- Lung Lesions:
    - Scrubbing Factor Approach 2b (Section 2.3.1.3.2, averaging rat and human CFD-based adjustments) was assumed when considering the TB penetration effective dose.
    - Scrubbing Factor Approach 3b (Section 2.3.1.4.2, averaging rat and human CFD-based adjustments for estimated ET and tracheal scrubbing) was assumed when considering the bronchial tissue concentration effective dose. The tissue concentration itself was based on Effective Dose Approach 2b (Section 2.3.2.2.3) which also considered both rat and human tissue concentration predictions.

Second, given those baseline dosimetric assumptions, it was determined which endpoints were most sensitive with respect to the BMD estimates (yielded the lowest BMDs). Model results for other variations on the dose metric calculations did not change this determination. The most sensitive nasal lesion and the most sensitive lung lesion were determined.

Third, HECs were estimated for the BMDs and BMDLs selected. The calculations of the HECs assumed the CFD model-adjusted scrubbing factors (Approach 2, Section 2.4.1.2) when the regional penetration effective dose was used and the CFD model-based ET and tracheal scrubbing factor (Approach 3, Section 2.4.1.3) for use with the tissue concentration effective dose measure.

The impact of variations on the calculation of the dose metrics are described in the Discussion section, presented in terms of the percent changes in the HECs that would result from those alternative. Similarly, the results of the log-logistic modeling and the categorical regression results for the selected endpoints and baseline dose metric calculations are presented to indicate the sensitivity to the choice of dichotomous model

(the log-logistic model being nonlinear at low doses) or of using a severity score-based (categorical) regression, respectively.

## 3.0 RESULTS

The tests for consistency of the lesions across the 6-week and 12-week experiments were uniform in indicating that the observations from the two experiments could be combined. That is, assuming a single probability of response for each exposure level provided nearly as good a description of the observed results as assuming two probabilities per exposure level, one per length of exposure. A likelihood ratio test did not reject (at the 0.05 level) the hypothesis of one rate per exposure level for any endpoint. Similarly, in the categorical regression using severities of response, specification of duration-specific intercepts and/or slopes failed to significantly (at the 0.05 level) improve the fit, suggesting that combining the 6- and 12-week data was appropriate for the categorical regression analysis as well. As a result, all dose-response modeling was done on the combined results of the two experiments and the results presented here are only for such combinations. The combination of the 6- and 12-week data tends to reduce uncertainties in the estimates (BMDs).

The most sensitive endpoints by site (nasal and lung) are nasal inflammation and peribronchial inflammation. As mentioned in the Methods section, this determination is the same no matter what dose metric is used for the modeling. This finding is not surprising given the response rates shown in Table 1; those inflammation endpoints had the highest incidence rates (by site) for the combined 25 ppm group. The BMDs and BMDLs estimated for the other endpoints were consistently and uniformly greater than those estimated for the nasal and peribronchial inflammation endpoints (results not shown).

The BMDs and BMDLs for the baseline assumptions and the best-fitting models are as shown in Table 13. The corresponding graphs are shown in Figures 1-4. The probit model provided the best fit to the nasal inflammation data using the tissue concentration metric. The multistage model was the best fitting for the other endpoint /dose metric combinations. The BMD and BMDL predictions from these models were not very different across the two inflammation endpoints for a fixed choice of effective dose measure. The BMDLs for the peribronchial inflammation endpoint were always less than those for the nasal inflammation endpoint, with a bigger difference between the BMDLs across endpoints observed for the tissue concentration effective dose measure (slightly more than a factor of 2). This relationship holds for the other scrubbing factors (i.e., for those alternatives not used in the baseline calculations shown in Table 13). For the BMDs, the pattern is not so simple. The BMDs for nasal inflammation are greater than those for peribronchial inflammation, when the effective dose measure is regional penetration; the BMDs for peribronchial inflammation are greater than those for nasal inflammation when tissue concentration is the effective dose measure, and in that case the difference increases as the risk level decreases.

The impact of the choice of effective dose measure can only be determined when the BMDs and BMDLs are converted to HEC ppm concentrations (Table 14), because the units are different for the two measures and conversion from BMDs and BMDLs to the human equivalents is therefore dependent on the dose measure. Of note here is the fact that the HECs derived using the tissue dose metric are consistently much less than the corresponding HECs derived using the regional penetration effective dose measure. For the HECs based on BMDLs, the difference ranged from about a factor of 3 (for nasal inflammation) to a factor of about 6 (for peribronchial inflammation). The differences across dose measures were even greater for the HECs based on the BMDs, with differences up to about a 120-fold difference (for nasal inflammation and the lowest risk level of 0.001).

Of course, there were variations in the BMD and BMDL estimates across all of the models fit to these data. The best fitting models were not unique in providing a satisfactory description of the observed dose-response patterns; the goodness of fit assessment for all the dichotomous models suggested that none of them would be excluded solely on the basis of fit (all the p-values for goodness of fit were greater than 0.65, with p-values greater than or equal to 0.1 typically considered to indicate a satisfactory fit). Thus, the model selection criterion based on AIC was what defined the best-fitting model. For each of the four endpoint/dose measure combinations, the BMDs across the dichotomous models differed by as much as a factor of nearly 150, the range being larger for the lower risk levels (especially for 0.001 risk). The range of BMDLs was much greater; for the 0.001 risk level the range of BMDL estimates over the seven models was greater than 3700-fold for nasal inflammation and the tissue dose metric.

In particular, the log-probit model, while never the best fitting model, always provided a satisfactory fit to the data and always yielded a BMD estimate that was at, or close to, the upper end of the range of BMDs over all the models. Figures 5-8 indicate that the low-dose shape of the log-probit model is "flatter" than the best-fitting model in every case. That model does appear to provide a more threshold type of dose-response pattern or at least one where the slope at low doses *approaches* zero. The HECs from the log-probit model in relation to those from the best fitting model are shown in Table 15 (only for the 0.01 and 0.001 risk levels). In the case of the nasal inflammation endpoint when modeled using the tissue dose metric, the uncertainty in the estimates from the log-probit is quite substantial; the BMDs from that model are greater than those from the best-fitting model but the BMDLs from the log-probit model are more than an order of magnitude less than the BMDLs from the best-fitting model.

Another aspect of model uncertainty evaluated here is the use of categorical as opposed to dichotomous responses. A probit link function for the categorical regression analysis fit slightly better than a logit link function, so the results presented are for the probit link. The categorical regression HECs are generally similar to those obtained from the best-fitting dichotomous model (Table 15) with the biggest difference being for those derived from the nasal inflammation BMDs using the regional penetration dose measure (the categorical regression HECs from the BMDs being 6- to 17-fold less than the corresponding best-fitting HECs). But even in those cases, the HECs derived from the

corresponding BMDLs are almost identical for the best-fitting and categorical regression models.

One interesting observation is that the categorical regression results are consistent in suggesting that the nasal inflammation response is more sensitive than the peribronchial inflammation endpoint; the BMDs are less for the nasal endpoint than for the peribronchial endpoint. This is the opposite relationship to that predicted by the best-fit dichotomous modeling, for which peribronchial inflammation was consistently more sensitive. This is probably a particular aspect of the probit model (as opposed to an effect of the inclusion and modeling of the more detailed information on severity of response) because the dichotomous probit model also estimated lower BMDs for the nasal response than for the peribronchial response, unlike the best fitting (and majority of other) dichotomous models.[2]

Other variations related to the dose calculations that were discussed in the Methods section (defining how the scrubbing factor is estimated or how the tissue concentration is extrapolated) have much less impact on the HECs. For either dose metric, the HEC estimates varied from 0.91 to 1.38 times the baseline HEC values shown in Table 14 (about a 9% reduction to about a 38% increase) when the assumptions about the scrubbing factor were changed (as reflected in the options laid out in Table 11). For the tissue concentration dose measures, altering the conversion from airborne concentration to tissue concentration (alternatives also reflected in the last two columns of Table 11) had the effect of increasing the HECs by 7% for nasal inflammation and 10% for peribronchial inflammation.

One of the greater impacts on the HEC calculations is the fact that the VE values used in the current analysis are much greater than one would have calculated using EPA defaults. On average, the VEs for the dose groups are slightly more than about 3.5 times greater than the default VE for mice. Changing to the lower VE would have a slight impact on scrubbing, decreasing from an average of about 0.97 to 0.92, indicating greater removal, i.e., greater scrubbing efficiency, with lower ventilation rates. But the biggest effect would be the 3.5-fold reduction in the dose measure, VE/SA. Together this would decrease the BMDs and BMDLs (and therefore the HECs) by about a factor of 4 for the regional penetration dose measure. The effect of a lower VE value on the HECs derived using the tissue concentration dose measure is unclear since the impact of inspiratory flow rate on the inhaled and tissue concentrations was not explicitly evaluated using the CFD model (Morris and Hubbs, 2008)It should be noted, however, that the VE/SA ratio for the ET region in the CFD model simulations reported for the rat (VE/SA = 30) and human (VE/SA = 69) were in the range of those based on experimental VE values in mice (VE/SA between 31 and 49).

The effect of altering assumptions about converting effective dose measures to ppm exposure levels in humans (see Table 12) had relatively minor effects on the HECs.

[2] Unfortunately, the CatReg program did not give meaningful lower bound estimates for the peribronchial inflammation BMDs. The estimates provided by that program were negative, which is merely an unrealistic mathematical artifact of the estimation method used by that program.

Changing the assumed rate of human scrubbing in the ET region to the EPA default value (0.995) would reduce the HEC by slightly less than 9% for the regional penetration dose measure and about 12% for the tissue concentration dose measure. In fact, assuming 100% mouth breathing (for which there is no ET scrubbing) induced essentially those same reductions in the HEC estimates. Conversely, if it were assumed that workers were able to breathe through their noses 100% of the time, the HEC would only increase by 11% for the regional penetration dose measure or 7% for the tissue concentration measure.

A slightly greater effect on the HECs is attributable to the assumed rate of breathing of the workers. If the EPA default rate of 13890 ml/min were used for the HEC calculations (in place of the 20000 ml/min assumed for the current analysis), the HEC would increase by slightly more than 44% if one assumes the effective dose measure is expressed in terms of the regional penetration measure. The decreased VE value directly affects the dose measure (VE/SA(TB)) as well as slightly increasing the scrubbing efficiency, allowing greater exposure concentrations to be associated with the risk specific doses estimated from the animal dose-response analysis. The effect would be almost negligible for the tissue concentration dose measure, since the slight decrease in diacetyl reaching the TB region associated with increased scrubbing efficiency would have only a minor effect on the airborne concentration reaching the TB region and therefore on the tissue concentrations.

## 4.0 DISCUSSION

The baseline assumptions and approaches to calculating HECs corresponding to risk-specific doses (doses corresponding to risk levels of 10%, 1% and 0.1%) are based on the available data and predictions, for both test species and humans, of the fluid dynamics of inhaled diacetyl. Unfortunately, the test species for which we have relevant dose-response data is the mouse, whereas the test species for which fluid dynamic predictions are available is the rat. The approaches that were pursued in this analysis attempt to compensate for that mismatch by adjusting defaults that would otherwise be used for mice in a way that is consistent with the adjustments suggested as being appropriate for rats and/or humans. The Methods section describes the various alternative assumptions that could be made in pursuing those adjustments; the Results section shows that the quantitative impact of those alternatives is generally not too great.

On the other hand, the biggest impact on the HEC calculations is the choice of effective dose measure, either the regional penetration predicted by the EPA default model or the tissue concentration predicted from the CFD model (Table 14). The former is generic and would be the same for any category 1 gas or vapor. The latter is specific to diacetyl through the parameterization of the CFD model. However, the tissue concentration measure has been extrapolated not only from CFD model predictions for rats and humans, but also from predictions restricted to the trachea (whereas we desire tissue concentrations relevant to the bronchial subregion). It should be noted here again that there is a lack of experimental data on, and therefore uncertainty about, diacetyl uptake in the respiratory tract and metabolism in both mice and humans, the species from which

and to which, respectively, the dose-response results are being extrapolated. Uncertainties also exist in relation to species differences in toxicodynamics and the related issue of exposure-response behavior at low doses (whether or not a threshold may exist for the diacetyl-induced respiratory tract effects observed in humans). Because of these uncertainties, it is not possible to definitively state that one effective dose measure is to be preferred over the other nor to determine toxicologically what dose-response relationship should be expected. Consequently, we have presented results for both effective dose measures and have emphasized the statistically best-fitting model results.

Those issues are specific to this diacetyl assessment. There are more general uncertainties that affect this and most other risk assessments. The limited number of observations (5 per dose group), the relatively small number of doses examined, and the relatively short period of follow-up are issues that contribute to the general uncertainty. The duration of exposure may be less of concern given the observation that no progression was observed in response from 6 to 12 weeks of exposure and that the responses at week 18 (six weeks after the end of exposure) were also comparable to those seen at 6 and 12 weeks (Morgan et al., 2008). The sample size issues are typically addressed by considering lower bound estimates for the BMDs.

As noted in the Results section, however, there was still some substantial variation across the models with respect to BMDLs, especially for the lower risk values. The log-probit model was presented as a model that tended to have a "threshold-like" dose-response shape at low doses and for which the BMDs and BMDLs were substantially greater than those for the best fitting models (Table 15)[3]. On the opposite extreme, the Weibull model as implemented here allowed for supralinear dose-response patterns (because the power on dose was allowed to decrease to 0.5 – see Appendix A). Allowing that shape when estimating BMDLs accounted for all of the lowest BMDLs across all of the endpoint/dose metric combinations (i.e., the Weibull model could not rule out the bounding supralinear behavior for these data). Factoring in severity of response (another modeling variation) had minor impact on the HEC estimation.

So, as is typical of quantitative risk assessment applications, the estimation of human risk associated with diacetyl exposures is subject to a number of uncertainties. However, we have been able to estimate the impact of some of them and to make choices consistent with best practices and with the state-of-the-science understanding of how to use animal data to quantify human risk prediction. The HECs calculated (Table 14) represent a reasonable basis for informing decisions related to occupational exposures to diacetyl.

### 4.1 Comparison with Other Assessments

For the sake of comparison, the HEC estimates derived here can be compared to the estimates that have been or might be provided by other assessments. An assessment completed by TERA (IDFA, 2008) has considered the same dose-response data (Morgan

[3] For the nasal inflammation endpoint, when modeled with the tissue dose metric, the BMDLs from the log-probit model were, in fact, *less* than those from the best fitting model, reflecting much greater uncertainty about the model shape for that endpoint/dose metric combination.

et al. 2008) and estimated HECs based on BMDLs for 10% risk, the BMDL(10)s. Those HECs are compared below to the HECs derived in this analysis, also based on the one-in-ten BMDL(10)s. In addition, one can compute estimates like a reference dose (RfD) or a linearly extrapolated dose from the BMDL(10)s; such estimates are compared to the HECs derived in this analysis from the BMDL(1)s and BMDL(0.1)s.

TERA (IDFA, 2008) examined the data related to the respiratory effects of diacetyl in humans and in experimental species. They concluded that the mouse data used in the analyses above (Morgan et al., 2008) were the best basis for a quantitative risk assessment. TERA excluded the nasal lesions from consideration prior to their analysis, stating that the evidence of upper respiratory symptoms in humans exposed to diacetyl was inconsistent and that those symptoms lacked reliable concentration-response information. The contention of the current assessment has been that the dose-response relationship in a test species, rather than the lesion site, is the primary factor for choosing which endpoints to model for quantitative risk estimation. Thus, no site concordance is required because the assumption is that, once the dose has been adequately adjusted (and ideally, once toxicodynamic considerations have been carefully considered), the dose-response relationship for one respiratory tract site/lesion in a test species is potentially as relevant as another site/lesion for the purposes of characterizing how much human risk is associated with exposure to a compound.

TERA (IDFA, 2008) estimated HECs using the EPA default methods (EPA, 1994) modified by the same CFD model predictions (Morris and Hubbs, 2008) used in the assessment presented above. However, rather than using the relationships between the default and CFD-model-predicted scrubbing factors to refine a mouse, diacetyl-specific estimate of scrubbing, they essentially assumed that mice were exactly like the CFD-modeled rats (i.e., they simply used the CFD model predictions for the rats as if they were equally relevant to mice). Furthermore, TERA used the air exiting the trachea to estimate upper respiratory scrubbing. This assessment does not include tracheal scrubbing as part of a dosimetric adjustment, where the effective dose measure is a regional (tracheobronchial) penetration metric since the trachea represents the upper portion of the presumed region of action.

Moreover, for the effective dose (regional penetration) measure calculated by TERA, the default mouse ventilation rates were used. As noted elsewhere, the experimentally measured ventilation rates were substantially greater than the default (by a factor of 3 to 5) and this would have a major impact on the HEC estimates (TERA's estimates would be about 3 to 5 times greater, since the major effect of changing the ventilation rate is on the effective dose measure, VE/SA, rather than the scrubbing).

TERA's analysis resulted in estimates of HECs that were 9 and 2 ppm, corresponding to the estimated BMD(10) and BMDL(10), respectively, from their dose-response analysis of the peribronchial inflammation endpoint from Morgan et al. (2008). Those values are low compared to the HECs derived in the current analysis using the same type of regional penetration dose measure; the BMD(10) and BMDL(10) for that dose measure are 39 and 8 ppm, respectively (Table 14). On the other hand, the current analysis obtained values

of 5 and 1 ppm for the BMD(10) and BMDL(10) when based on a tissue concentration dose measure (Table 14). The nasal inflammation endpoint yielded estimates of BMD(10) and BMDL(10) that were even slightly greater than those for the peribronchial inflammation endpoint.

TERA's assessment suggested that a composite uncertainty factor (UF) of 10 should be used to adjust those HECs downward to an occupational exposure limit (OEL). That factor of 10 was the product of a factor of 3 for interspecies differences and another factor of 3 for human variability. Their recommended OEL was therefore 0.2 ppm (as an 8-hour TWA). In comparison, the HECs derived above for 1% and 0.1% extra risk were 0.8 and 0.08 ppm (using the BMDL values) when the regional penetration effective dose measure was used. If extrapolation to humans were based on the tissue concentration metric (using the BMDL values), the corresponding HECs were estimated to be 0.1 and 0.01, for 1% and 0.1% risk, respectively.

If a linear extrapolation from the HECs based on BMDL(10)s were considered, then the HECs for 1% and 0.1% extra risk would be 10 and 100 times less, respectively, than the HECs for 10% risk. In that case, the 1% and 0.1% HECs would be, respectively, 0.83 and 0.083 ppm (using the regional penetration dose metric) or 0.13 and 0.013 ppm (using the tissue concentration metric). Those estimates are very similar to the model-based estimation of the HECs associated with those risk levels. This indicates that, at least for the best fitting models, the bounds for BMDs are exhibiting a linear dose-response relationship. At the levels of risk under consideration (1% and 0.1%) the data in hand can not rule out a "bounding" linear relationship for the best-fitting models, even though the best estimates (i.e. BMD(1) and BMD(0.1)) suggest that the dose-response is sublinear (concave up) at these risk levels.

Finally, in the context of comparing the HECs to those that might be derived in alternative risk assessments, one can consider the use of the ppm exposure levels rather than some calculated effective dose measure. Using the ppm exposures and adjusting only for daily duration of exposure (six hours for the test species and the assumed 8 hours for occupationally exposed workers), the HECs for 10%, 1% and 0.1% extra risk (using the BMDL values) would be 2.6, 0.25, 0.025 ppm, respectively. These were obtained from the best-fitting (multistage) model fit to the peribronchial inflammation endpoint. Those HECs are about a factor of two greater than the corresponding estimates obtained using the tissue concentration dose measure and about a factor of three less than estimates using the fractional penetration dose measure (Table 14).

### 4.2 Conclusion

The mouse inhalation exposure dose-response data have provided a basis for estimating occupational diacetyl HECs corresponding to various levels of extra risk. Dosimetric adjustments have been considered that attempt to adjust for species differences in scrubbing and uptake of diacetyl.

It was determined that the measure of effective dose had the greatest impact on the HEC estimates, with the metric based on CFD-model-predicted tissue concentrations (Morris and Hubbs, 2008) yielding HECs about a factor of 6 less than the metric based on regional penetration (as expressed via EPA's default regional dose approaches; EPA, 1994). All other alternative assumptions related to dose metric calculations had much less impact (typically resulting in changes in HECs estimates on the order of 10%). Alternative (extreme) assumptions about rates of mouth breathing among workers also affected HEC estimates by only about 10%.

While there are uncertainties associated with the various assumptions used in the assessment, and there was some variability in the estimates associated with different choices for models and modeling approaches, the best-fitting models chosen for each of the modeled endpoints appear to adequately characterize the observed dose-response patterns and provide HEC estimates consistent with those of the alternatives.

For the risk levels examined (10%, 1%, 0.1%) the most sensitive endpoint was peribronchial inflammation. Nasal inflammation was also observed and was modeled as part of the quantitative assessment. The HECs for these two endpoints were fairly similar, suggesting that decisions based on HECs derived from analysis of the peribronchial inflammation endpoint are not sensitive to a single, potentially outlier, set of observations of adverse response.

The HECs corresponding to the 95% lower confidence limits on the dose associated with estimates of 1% and 0.1% extra risk are 0.8 and 0.08 ppm, respectively, based on the analysis that uses the regional penetration dose metric. If the dose metric is defined on the basis of tissue concentration, the corresponding HECs are 0.1 and 0.01 ppm.

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## Appendix A
## Equations for Dose-Response Models Used in Diacetyl Risk Assessment

Dichotomous Dose Response Models

- Gamma

$P(d) = \gamma + (1-\gamma) * (1/\Gamma(\alpha)) * \int t^{(\alpha-1)}e^{-t}\, dt$; integral runs from 0 to $\beta*d$
$\alpha$ parameter constrained to be greater than or equal to 1

- Logistic

$P(d) = \{1 + \exp(-(\alpha + \beta*d))\}^{-1}$

- Log-Logistic

$P(d) = \gamma + (1-\gamma) * \{1 + \exp(-(\alpha + \beta*\ln(d)))\}^{-1}$
$\beta$ parameter constrained to be greater than or equal to 1

- Log-Probit

$P(d) = \gamma + (1-\gamma) * \Phi(\alpha + \beta*\ln(d))$

- Multistage

$P(d) = \gamma + (1-\gamma) * \{1 - \exp(-(\beta_1*d + \beta_2*d^2 + \beta_3*d^3))\}$
$\beta$ parameters constrained to be greater than or equal to 0

- Probit

$P(d) = \Phi(\alpha + \beta*d)$

- Weibull

$P(d) = \gamma + (1-\gamma) * \{1 - \exp(-\beta*d^{\alpha})\}$
$\alpha$ parameter constrained to be greater than or equal to 0.5

In all of the above equations:
d is dose,
P(d) is the probability of response when exposure is to dose d,
$\alpha$, $\beta$, $\gamma$, $\beta_1$, $\beta_2$, $\beta_3$ are parameters that are estimated,
$\Phi$ is the cumulative normal distribution function.

Categorical Regression Models

$$P(Y \geq s \mid d) = H(\alpha_s + \beta * d) \quad \text{(for } s \geq 1\text{)}$$

where Y is the response measure that can assume values of s = 0, 1, 2, 3 which are the different severity levels (greater values of s being more severe). The left hand side of the equation expresses the probability that Y is greater than or equal to s, given exposure to dose d.

The function H on the right hand side is of one of two forms:

Logistic: $H(x) = \{1 + e^{-x}\}^{-1}$ [the inverse of H is the logit link function, $\ln(p/(1-p))$]

Normal: $H(x) = \Phi(x)$ [the inverse of H is the probit link function, $\Phi^{-1}(p)$]

The parameters $\alpha_s$ and $\beta$ are estimated. There is a separate $\alpha_s$ term for each severity level greater than 0.

In either of the categorical regression models, a single $\beta$ term is estimated that best describes the pattern of response severities. All the levels of severity contribute to the estimation of that common term (and the $\alpha_s$ intercepts). Doses corresponding to specified levels of risk (e.g., BMDs) can be defined on the basis of severity (i.e., the dose for which the risk of response severity 1 or greater is equal to 10% will be different from – less than – the dose for which the risk of severity 2 or greater is equal to 10%. For the diacetyl assessment, BMDs have been defined based on the risk of severity 1 or greater responses.

**REPORT ON MODEL AVERAGING ANALYSIS AND RESULTS FOR DIACETYL MOUSE DATA SETS**

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Prime Contract Number: DOLQ059622303
Task Order Number: 50

**Sept 18, 2009**

## Report on Model Averaging Analysis and Results for Diacetyl Mouse Data Sets

**Introduction**:

The analysis performed and included in the report "A Quantitative Risk Assessment for Diacetyl Based on Respiratory Tract Lesions in Mice" (Allen, 2009) examined the fitting of and predictions from seven dichotomous dose response models available from the software package BMDS (EPA, 2009). Those models were fit (primarily) to two endpoints from the study by Morgan et al. (2008), nasal inflammation and peribronchial inflammation, the endpoints that were determined to be the most sensitive of six endpoints that were initially considered.

That analysis focused on two dose metrics. The first was a regional penetration metric based on EPA's inhalation dosimetry approach (EPA, 1994) but modified by predictions of a computational fluid dynamics (CFD) model from Morris and Hubbs (2008). The second was a tissue concentration metric based on the CFD model predictions of concentrations in the upper respiratory and the bronchial regions (Morris and Hubbs, 2008). Those two dose metrics are again considered in the model averaging approach presented here.

In the original diacetyl analysis (Allen, 2009), two models fit to those endpoints were presented and discussed: the best fitting model for each endpoint/dose metric combination and the log-probit model, as a representative of a model that can be very flat (appearing threshold-like) at low doses.

This report presents results for the same dose metric/endpoint combinations as in the Allen (2009) report, but instead of reporting results from two separate models, the results shown here represent a model averaging approach. The regional penetration dose metric used in this analysis was scrubbing factor derivation 2b for peribronchial inflammation as reported in Table 11 of the Allen (2009) report. Tissue concentration dose metric 2b was combined with scrubbing factor derivations 2b and 3b, respectively, for nasal and peribronchial inflammation. The bootstrap technique discussed by Wheeler and Bailer (2008) was implemented and the "averaged" BMDs and BMDLs have been estimated. The averaged estimates are compared to the results obtained by from all seven models and for the best-fitting and log-probit models as presented in Allen (2009).

**Data and Method**:

The data analyzed are shown in the following table:

| Endpoint | Regional Penetration Dose Metric Values ($\mu g/cm^2$) | Tissue Concentration Dose Metric Values (μg/ml-min) | Response Rate |
|---|---|---|---|
| Nasal Inflammation | 0 | 0 | 3/10 |
| | 1558.0706 | 11665.195 | 2/4 |
| | 2535.1656 | 23330.39 | 4/5 |
| | 3908.3804 | 46660.78 | 5/5 |
| | 1529.0218 | 11665.195 | 4/5 |
| | 2714.7399 | 23330.39 | 5/5 |
| | 4489.3558 | 46660.78 | 5/5 |
| | | | |
| Peribronchial Inflammation | 0 | 0 | 0/10 |
| | 1005.6233 | 9107.1855 | 3/5 |
| | 1627.5763 | 18035.045 | 5/5 |
| | 2492.43 | 35557.731 | 5/5 |
| | 986.87434 | 9094.3765 | 2/5 |
| | 1747.5168 | 18086.281 | 4/4 |
| | 2874.4704 | 35865.147 | 5/5 |

The model averaging procedure was accomplished using an R version of the software that can be downloaded from Wheeler and Bailer (2008). In brief, it considers all of the models originally fit to the data (Allen, 2009, Appendix A) and weights their predictions according to how well each model fits the data. The fit is assessed in this analysis by the AIC, where the AIC was determined as it is in the BMDS software, dropping from consideration in the AIC calculation any parameters that hit a boundary value (this is the "AICB" option in the Wheeler and Bailer software). Smaller AICs are associated with better fits, so the greater weights go with the models that had the smaller AICs. The model-averaged BMD is the weighted average of the model-specific BMDs.

The BMDL estimates are determined via bootstrap sampling. In essence, hypothetical data are generated (based on the original observations) and the models refit to those hypothetical data. The weighting is re-evaluated and the weighted average of the BMD is recomputed. This process is repeated a large number of times and the bounds computed based on the distribution of computed BMDs. For this analysis, a total of 50,000 bootstrap iterations was considered sufficient for the BMDL estimates to be accurate to 2 to 3 significant digits. The bias-corrected and adjusted (BCa) BMDL estimates are reported here (as opposed to the percentile-based estimates) because they attempt to account for (and remove) any bias in the bound estimates.[4]

**Results**:

[4] In two cases, for the nasal inflammation endpoint using the regional penetration dose metric, when the BMR was 1% and 0.1%, the BCa estimates were zero. In those two cases, the percentile-based BMDL is presented because those estimates were nonzero and appeared reasonable give the range of BMDLs from the individual models.

The following table displays the model-averaged BMD and BMDL estimates for the three extra risks derived in Allen (2009). In addition to those model-averaged values are the ranges of the BMDs and BMDLs from all seven models and those for the best-fitting and log-probit models from Allen (2009).

| Endpoint | Dose Metric | Benchmark Risk Level (BMR) | Model-Averaged BMD (BMDL) | Range of BMDs (BMDLs) over all Models | Best-fitting Model BMD (BMDL) | Log-probit Model BMD (BMDL) |
|---|---|---|---|---|---|---|
| Nasal Inflammation | Regional Penetration ($\mu g/cm^2$) | 10% | 472 (119) | 279-915 (14-190) | 586 (100) | 912 (58) |
| | | 1% | 70 (7.5) | 30-589 (0.19-19) | 181 (9.5) | 589 (10) |
| | | 0.1% | 7.5 (0.12) | 3.0-427 (.0029-2.8) | 57 (0.95) | 427 (2.8) |
| | Tissue Concentration ($\mu g$/ml-min) | 10% | 2980 (303) | 2366-5605 (49-1606) | 2428 (1606) | 5514 (99) |
| | | 1% | 409 (10.2) | 249-2996 (0.44-163) | 252 (163) | 2996 (7.8) |
| | | 0.1% | 44 (0.13) | 25-1918 (.0044-16) | 25 (16) | 1918 (1.2) |
| | | | | | | |
| Peribronchial Inflammation | Regional Penetration ($\mu g/cm^2$) | 10% | 436 (83) | 371-618 (67-259) | 371 (80) | 603 (184) |
| | | 1% | 145 (9.6) | 107-399 (4.9-68) | 114 (7.6) | 399 (68) |
| | | 0.1% | 39 (0.65) | 13-296 (.37-33) | 36 (0.76) | 296 (33) |
| | Tissue Concentration ($\mu g$/ml-min) | 10% | 3672 (1180) | 2713-4933 (300-2318) | 2713 (722) | 4809 (1062) |
| | | 1% | 979 (71) | 396-2840 (11-295) | 396 (69) | 2840 (295) |
| | | 0.1% | 177 (2.2) | 43-1932 (.40-115) | 43 (6.9) | 1932 (115) |

**Discussion**:

The range of BMDLs (and to a lesser extent, of the BMDs) over the seven dichotomous models can be quite substantial, in some cases being more than an order of magnitude. The range increases as the BMR decreases, indicative of the greater uncertainty associated with the estimation of doses associated with low levels of risk, especially ones substantially below the rates of response observed in the experiments being modeled.

The model-averaged BMDLs generally demonstrate less difference from the corresponding BMDs than do the individual model results shown above. However, in the case of nasal inflammation and the low risk level of 0.1%, the difference between the BMD and BMDL for the model-average is still slightly greater than two orders of magnitude.

The model-averaged estimates are fairly similar to the corresponding estimates from the best-fitting model. There are some notable differences, however, especially for the 0.1% BMR, because the model-average approach factors in the wide range of BMDs possible across models for that low risk level. Some models were consistent with some extremely low BMDLs, and those get considered for the distribution of simulated BMDs that define the bootstrap-derived BMDLs. The best fitting model does not capture all of that uncertainty, because the variability/uncertainty associated with just that one model is necessarily a subset of the overall uncertainty when uncertainty about the model choice is acknowledged.

The log-probit model in and of itself is associated with a great deal of uncertainty, with the BMDLs being up to three orders of magnitude less than the corresponding BMDs, especially for the nasal inflammation endpoint and the 0.1% BMR. That observation for the log-probit model is consistent with the fact that the range of model-specific BMDLs for nasal inflammation is particularly wide, especially at that risk level.

A model-average basis for determining the points of departure for setting exposure limits appears to be one reasonable option in the case of diacetyl.

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Table 1
Quantal Data Included in Dose-Response Analysis (from Morgan et al. 2008)

| Endpoint (Site, Response) | Duration (Weeks of Exposure) | 0 ppm | 25 ppm | 50 ppm | 100 ppm |
|---|---|---|---|---|---|
| A. Nasal, Inflammation | 6 | 2/5[a] | 2/4 | 4/5 | 5/5 |
| | 12 | 1/5 | 4/5 | 5/5 | 5/5 |
| B. Nasal, Necrosis and Ulceration | 6 | 0/5 | 0/4 | 2/5 | 5/5 |
| | 12 | 0/5 | 0/5 | 1/5 | 5/5 |
| C. Nasal, Metaplasia | 6 | 0/5 | 1/4 | 3/5 | 5/5 |
| | 12 | 0/5 | 2/5 | 4/5 | 5/5 |
| D. Lung, Peribronchial Inflammation | 6 | 0/5 | 3/5 | 5/5 | 5/5 |
| | 12 | 0/5 | 2/5 | 4/5 | 5/5 |
| E. Lung, Bronchial Atrophy and Denudation | 6 | 0/5 | 0/5 | 1/5 | 5/5 |
| | 12 | 0/5 | 0/5 | 0/5 | 5/5 |
| F. Lung, Peribronchiolar Inflammation | 6 | 2/5 | 0/5 | 1/5 | 3/5 |
| | 12 | 0/5 | 0/5 | 0/5 | 3/5 |

[a]Number of animals with lesion / Number examined.

Table 2
Severity Data Included in Categorical Regression Analysis

| | | | Endpoints[a] | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Grp (ppm)** | **Dur (wk)** | **Sev[b]** | **A** | **B** | **C** | **D** | **E** | **F** |
| 0 | 6 | 0 | 3[c] | 5 | 5 | 5 | 5 | 3 |
| | | 1 | 2 | | | | | 2 |
| | | 2 | | | | | | |
| | | 3 | | | | | | |
| | | 4 | | | | | | |
| | 12 | 0 | 4 | 5 | 5 | 5 | 5 | 5 |
| | | 1 | 1 | | | | | |
| | | 2 | | | | | | |
| | | 3 | | | | | | |
| | | 4 | | | | | | |
| 25 | 6 | 0 | 2 | 4 | 3 | 2 | 5 | 5 |
| | | 1 | 1 | | 1 | 3 | | |
| | | 2 | 1 | | | | | |
| | | 3 | | | | | | |
| | | 4 | | | | | | |
| | 12 | 0 | 1 | 5 | 3 | 3 | 5 | 5 |
| | | 1 | 3 | | 2 | 2 | | |
| | | 2 | 1 | | | | | |
| | | 3 | | | | | | |
| | | 4 | | | | | | |
| 50 | 6 | 0 | 1 | 3 | 2 | | 4 | 4 |
| | | 1 | 2 | 2 | | 2 | 1 | 1 |
| | | 2 | 2 | | 2 | 3 | | |
| | | 3 | | | 1 | | | |
| | | 4 | | | | | | |
| | 12 | 0 | | 4 | 1 | 1 | 5 | 5 |
| | | 1 | 1 | 1 | 3 | 2 | | |
| | | 2 | 4 | | 1 | 2 | | |
| | | 3 | | | | | | |
| | | 4 | | | | | | |
| 100 | 6 | 0 | | | | | | 2 |
| | | 1 | | 4 | | | | 3 |
| | | 2 | | 1 | | 2 | 1 | |
| | | 3 | 1 | | 5 | 3 | 4 | |
| | | 4 | 4 | | | | | |
| | 12 | 0 | | | | | | 2 |
| | | 1 | | | | | | 2 |
| | | 2 | | 2 | | 2 | | 1 |
| | | 3 | | 3 | 5 | 3 | 5 | |
| | | 4 | 5 | | | | | |

[a]Endpoints are listed as in Table X1:

A. Nasal, Inflammation
B. Nasal, Necrosis and Ulceration
C. Nasal, Metaplasia
D. Lung, Peribronchial Inflammation
E. Lung, Atrophy (denudation not considered here per Morgan, personal communication)
F. Lung, Peribronchiolar Inflammation

[b]Severity Scores as follows: 0 = absent; 1 = minimal; 2 = mild; 3 = moderate; 4 = marked. The CatReg program only allows up to four severity categories, so, for Nasal inflammation, the moderate and marked responses were pooled together and designated with a score of 3.

[c]Counts of animals having indicated severity scores (zero counts have been omitted for clarity).

Table 3
Reported and Calculated Minute Volumes

| Exposure Group (ppm) | Reported Minute Volume @ 3 weeks | Reported Minute Volume @ 6 weeks | Calculated Minute Volume for 6-week Experiment[a] | Reported Minute Volume @ 12 weeks | Calculated Minute Volume for 12-week Experiment[b] |
|---|---|---|---|---|---|
| 25 | 150.18 | 143.41 | 147.5 | 140.62 | 144.75 |
| 50 | 111.11 | 126.66 | 120 | 131.52 | 128.5 |
| 100 | 79.47 | 96.36 | 92.5 | 120.37 | 106.25 |

[a]The calculated minute volume for the 6-week experiment was the average of the measurements at the 3 and 6 week time point.
[b]The calculated minute volume for the 12-week experiment was the average of the measurements at the 3 and 6 week time point, averaged with the measurement at the 12 week time point.

Table 4
Scrubbing Factors for Mouse Lung Lesions based on the EPA Default (Approach 1; Eq 1)

| Exposure Group (ppm) | 6 week Exposure VE | 6-week Scrubbing Factor | 12-week Exposure VE | 12-week Scrubbing Factor |
|---|---|---|---|---|
| 25 | 147.5 | 0.980 | 144.75 | 0.979 |
| 50 | 120 | 0.975 | 128.5 | 0.977 |
| 100 | 92.5 | 0.968 | 106.25 | 0.972 |

Table 5
Approach 2a to Adjusting Default Scrubbing Factors: Adjustment based on Rat CFD Predictions (Eq. 2)

| Exposure Group (ppm) | 6-week Exposure VE | 6-week Scrubbing Factor | 12-week Exposure VE | 12-week Scrubbing Factor |
|---|---|---|---|---|
| 25 | 147.5 | 0.691 | 144.75 | 0.690 |
| 50 | 120 | 0.687 | 128.5 | 0.689 |
| 100 | 92.5 | 0.682 | 106.25 | 0.685 |

Table 6
Approach 2b to Adjusting Default Scrubbing Factors: Adjustment based on Rat and Human CFD Predictions (Eq. 3)

| Exposure Group (ppm) | 6-week Exposure VE | 6-week Scrubbing Factor | 12-week Exposure VE | 12-week Scrubbing Factor |
|---|---|---|---|---|
| 25 | 147.5 | 0.753 | 144.75 | 0.753 |
| 50 | 120 | 0.749 | 128.5 | 0.751 |
| 100 | 92.5 | 0.744 | 106.25 | 0.747 |

Table 7
Approach 2c to Adjusting Default Scrubbing Factors: Adjustment based on Kg(ET) Estimate from CFD Predictions (Eq. 4)

| Exposure Group (ppm) | 6-week Exposure VE | 6-week Scrubbing Factor | 12-week Exposure VE | 12-week Scrubbing Factor |
|---|---|---|---|---|
| 25 | 147.5 | 0.846 | 144.75 | 0.844 |
| 50 | 120 | 0.815 | 128.5 | 0.826 |
| 100 | 92.5 | 0.766 | 106.25 | 0.793 |

Table 8
Approach 3a to Determining ET and Tracheal Scrubbing Factors: Based on Rat CFD Model Predictions (Eq. 5)

| Exposure Group (ppm) | 6-week Exposure VE | 6-week Scrubbing Factor | 12-week Exposure VE | 12-week Scrubbing Factor |
|---|---|---|---|---|
| 25 | 147.5 | 0.646 | 144.75 | 0.646 |
| 50 | 120 | 0.640 | 128.5 | 0.642 |
| 100 | 92.5 | 0.631 | 106.25 | 0.636 |

Table 9
Approach 3b to Determining ET and Tracheal Scrubbing Factors: Based on Rat and Human CFD Model Predictions (Eq. 6)

| Exposure Group (ppm) | 6-week Exposure VE | 6-week Scrubbing Factor | 12-week Exposure VE | 12-week Scrubbing Factor |
|---|---|---|---|---|
| 25 | 147.5 | 0.711 | 144.75 | 0.710 |
| 50 | 120 | 0.704 | 128.5 | 0.706 |
| 100 | 92.5 | 0.694 | 106.25 | 0.700 |

Table 10
CFD Model Predicted Inhalation Concentrations and Tissue Concentrations

| Species | Inhalation Concentration (ppm)[a] | Converted Concentration (μg/ml) | Anterior Ventral Mucosal Concentration (mM)[a] | Converted Anterior Ventral Mucosal Concentration (μg/ml) | Concentration Exiting UR Tract (ppm)[a] | Converted Concentration Exiting UR Tract (μg/ml) | Anterior Tracheal Mucosal Concentration (mM)[a] | Converted Anterior Tracheal Mucosal Concentration (μg/ml) |
|---|---|---|---|---|---|---|---|---|
| Rat | 100 | 0.352 | 1.6 | 138 | 67 | 0.236 | 1.2 | 103 |
| | 200 | 0.704 | 3.2 | 275 | 136 | 0.479 | 2.5 | 215 |
| | 300 | 1.056 | 4.9 | 422 | 206 | 0.725 | 3.8 | 327 |
| Human | 100 (nose breathing) | 0.352 | 1.4 | 121 | 82 | 0.289 | 1.2 | 103 |
| | 100 (mouth breathing) | 0.352 | -- | -- | 100 | 0.352 | 1.5 | 129 |

[a]From Morris and Hubbs (2008), Table 3. Other columns are conversions based on molecular weight of diacetyl of 86.09.

Table 11
Summary of All Dose Metric Calculations, as a Function of Scrubbing Factor and Effective Dose Derivations

| Lesion Region | Scrubbing Factor Derivation | Effective Dose Derivation | | |
|---|---|---|---|---|
| | | 1. Regional Penetration | 2a. Tissue Concentration – Rat | 2b. Tissue Concentration – Rat/Human |
| **Nasal** | **n/a** | µg/ml * 1 * VE/SA(ET) * T | µg/ml * 1 * 394 * T | µg/ml * 1 *368 * T |
| **Lung** | **1. EPA Default** | µg/ml * "T4" * VE/SA(TB) * T | µg/ml * "T4" * 446 * T | µg/ml * "T4" * 404 * T |
| | **2a. CFD – Rat** | µg/ml * "T5" * VE/SA(TB) * T | µg/ml * "T5" * 446 * T | µg/m * "T5" * 404 * T |
| | **2b. CFD – Rat/Human** | µg/ml * "T6" * VE/SA(TB) * T | µg/ml * "T6" * 446 * T | µg/ml * "T6" * 404 * T |
| | **2c. CFD – Kg(ET)** | µg/ml * "T7" * VE/SA(TB) * T | µg/ml * "T7" * 446 * T | µg/ml * "T7" * 404 * T |
| | **3a. Trachea Scrubbing – Rat** | -- | µg/ml * "T8" * 446 * T | µg/ml* "T8" * 404* T |
| | **3b. Trachea Scrubbing – Rat/Human** | -- | µg/ml * "T9" * 446 * T | µg/ml * "T9" * 404 * T |

The body of the table shows the factors included in the dose metric computations. The first of the four factors is always the exposure level. The second factor is either 1 (for nasal lesions) or a reference to a table (e.g., "T4" refers to Table 4) where the scrubbing factors for the corresponding approach are presented. The third factor is the effective dose conversion factor, either based on the minute volumes (group-specific, see Table 3) and surface areas, or based on the tissue concentration to airborne concentration ratios presented in the Methods section. The fourth factor, T, is the daily duration of exposure (always 360 minutes for the experiments under consideration). "--" indicates that that combination was not run.

Table 12
Summary of Factors Used to Compute HECs from BMDs and BMDLs

| Scrubbing Factor | Effective Dose Derivation | |
|---|---|---|
| | 1. TB Penetration | 2. Tissue Concentration |
| 1. EPA Default ET | 0.995 * [6.25*0.00352] * 480 | 0.995 * [362*0.00352] * 480 |
| 2. CFD-predicted ET | 0.91 * [6.25*0.00352] * 480 | 0.91 *[362*0.00352] * 480 |
| 3. CFD-predicted ET and Tracheal | -- | 0.875 * [362*0.00352] * 480 |

BMDs and BMDLs were divided by the factors shown in the body of the table to derive the corresponding HECs. The factors shown account for scrubbing in the ET or ET and trachea; the conversion from ppm concentrations to the effective dose; and the daily duration of exposure (480 minutes). "--" indicates that that combination was not run.

Table 13
BMDs and BMDLs for the Sensitive Endpoints; Baseline Dose Metric Calculations, Best-Fitting Models[a]

| Endpoint | Effective Dose Measure | p-value[b] | | Risk Level | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 0.1 | | 0.01 | | 0.001 | |
| | | | | BMD | BMDL | BMD | BMDL | BMD | BMDL |
| **Nasal Inflammation** | **Regional Penetration (μg/cm$^2$)** | 0.85 | | 585.9 | 100.0 | 181.0 | 9.541 | 57.10 | 0.9498 |
| | **Tissue Conc. (μg/ml-min)** | 0.84 | | 2428 | 1606 | 252.3 | 162.9 | 25.34 | 16.31 |
| | | | | | | | | | |
| **Peribronchial Inflammation** | **Regional Penetration (μg/cm$^2$)** | 0.94 | | 370.8 | 79.58 | 114.5 | 7.591 | 36.14 | 0.7557 |
| | **Tissue Conc. (μg/ml-min)** | 0.91 | | 2713 | 721.9 | 396.3 | 68.86 | 42.93 | 6.855 |

[a]Best fitting model is the Probit model for nasal inflammation with tissue concentration metric; it is the Multistage model in all other cases.
[b]P-value is the goodness-of-fit p-value with values closer to 1 indicating better fit (values greater then 0.1 are generally accepted as indicating satisfactory fit).

Table 14
HECs (ppm Atmospheric Concentration) Corresponding to BMDs and BMDLs for the Sensitive Endpoints; Baseline Dose Metric Calculations and Human Conversion Approaches, Best Fitting Models

| Endpoint | Effective Dose Measure | Risk Level | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0.1 | | 0.01 | | 0.001 | |
| | | BMD | BMDL | BMD | BMDL | BMD | BMDL |
| **Nasal Inflammation** | **Regional Penetration** | 60.95 | 10.41 | 18.83 | 0.9926 | 5.940 | 0.09881 |
| | **Tissue Conc.** | 4.532 | 2.997 | 0.4709 | 0.3040 | 0.04731 | 0.03045 |
| | | | | | | | |
| **Peribronchial Inflammation** | **Regional Penetration** | 38.58 | 8.279 | 11.92 | 0.7897 | 3.759 | 0.07861 |
| | **Tissue Conc.** | 5.064 | 1.347 | 0.7396 | 0.1285 | 0.08012 | 0.01280 |

The HECs correspond directly to the BMDs and BMDLs shown in Table 13. Values in this table are derived from those in Table 13 by dividing by the values in the second row of Table 12, for the regional penetration effective dose measure or by the third row of Table 12 for the tissue concentration effective dose measure.

Table 15
HECs (ppm Atmospheric Concentration) Corresponding to BMDs and BMDLs for the Sensitive Endpoints from the Dichotomous Log-Probit Model and Categorical Regression Analysis; Baseline Dose Metric Calculations and Human Conversion Approaches

| Endpoint | Model | Effective Dose Measure | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Regional Penetration | | | | Tissue Conc. | | | |
| | | Risk = .01 | | Risk = .001 | | Risk = .01 | | Risk = .001 | |
| | | BMD | BMDL | BMD | BMDL | BMD | BMDL | BMD | BMDL |
| Nasal Inflammation | Log-Probit | 61.23 | 1.046 | 44.46 | 0.2879 | 5.592 | 0.01454 | 3.580 | 0.002248 |
| | CatReg Probit | 3.323 | 1.205 | 0.3381 | 0.1151 | 0.4644 | 0.2090 | 0.04698 | 0.02050 |
| | Best Fitting | 18.83 | 0.9926 | 5.940 | 0.09881 | 0.4709 | 0.3040 | 0.04731 | 0.03045 |
| Peribronchial Inflammation | Log-Probit | 41.55 | 7.053 | 30.76 | 3.386 | 5.301 | 0.5509 | 3.607 | 0.2149 |
| | CatReg Probit | 16.53 | -- | 2.530 | -- | 1.700 | -- | 0.2034 | -- |
| | Best Fitting | 11.92 | 0.7897 | 3.759 | 0.07861 | 0.7396 | 0.1285 | 0.08012 | 0.01280 |

"--" means that the program estimated negative BMDL values, which are not to be believed (due to limitations in the method for estimating bounds).

## FIGURES

**Figure 1: Best Fitting Model for Nasal Inflammation; Nasal Penetration Dose Metric**

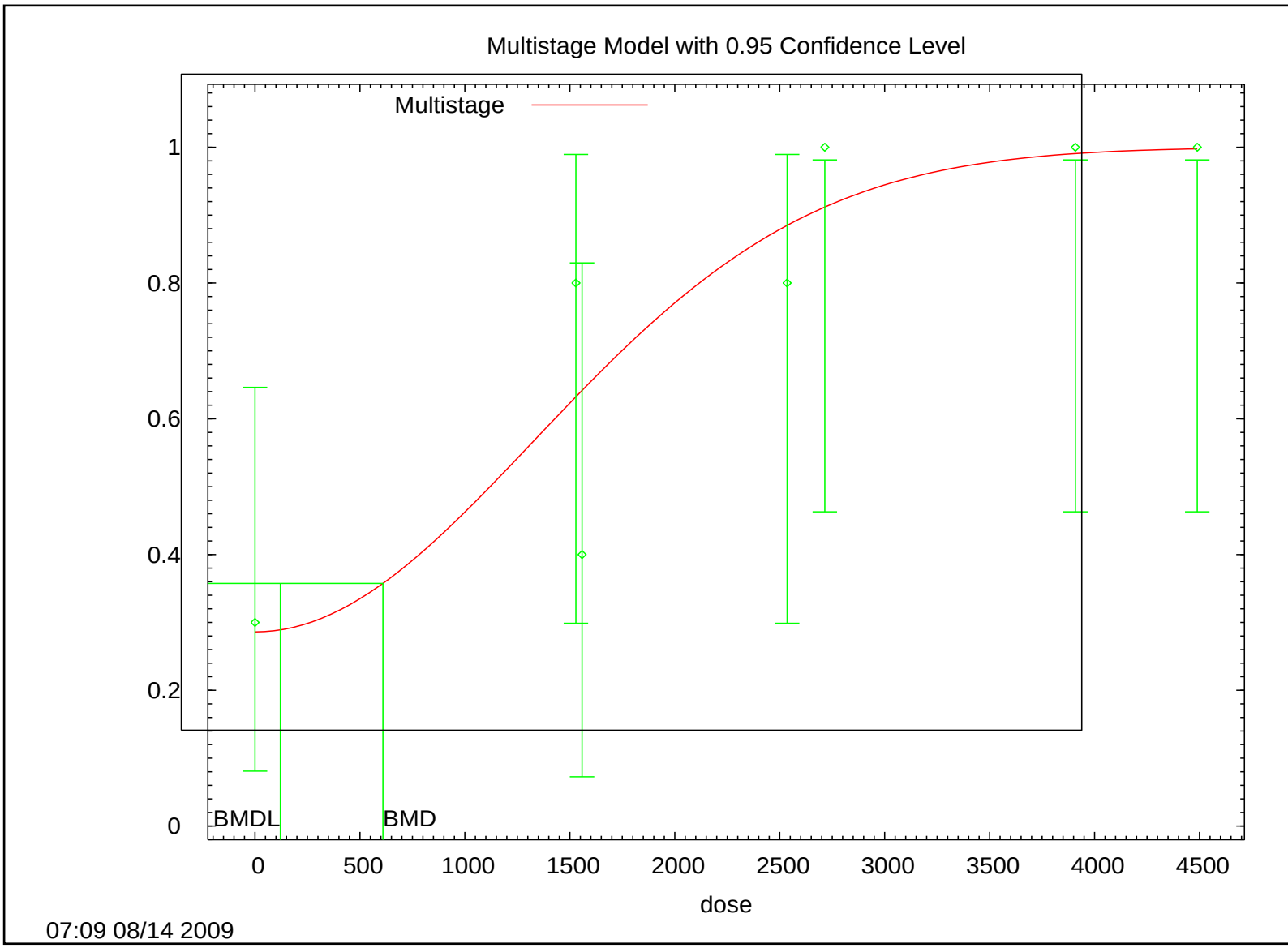


**Figure 2: Best Fitting Model for Nasal Inflammation; Tissue Concentration Effective Dose Metric**

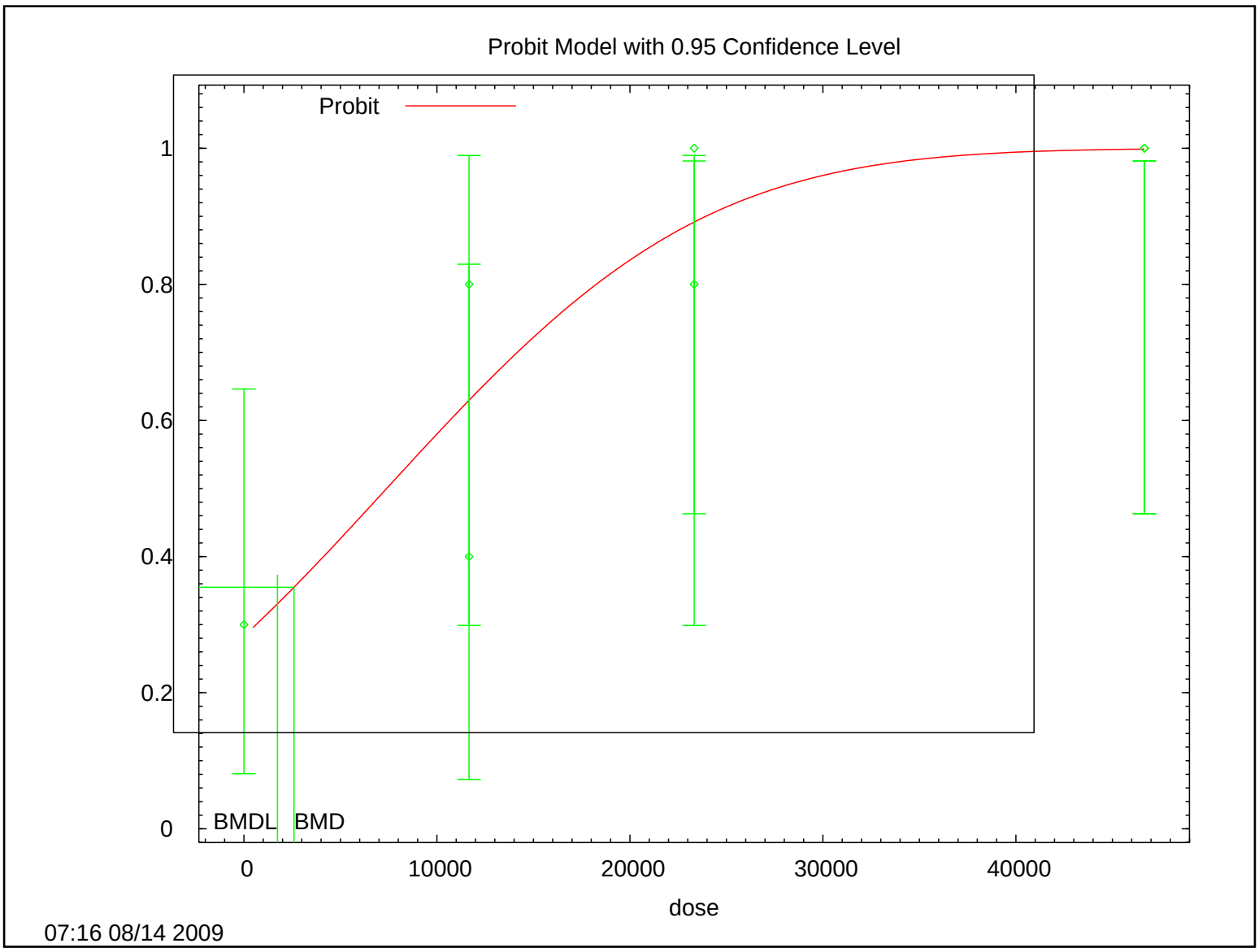


**Figure 3: Best Fitting Model for Peribronchial Inflammation; Tracheobronchial Penetration Dose Metric**

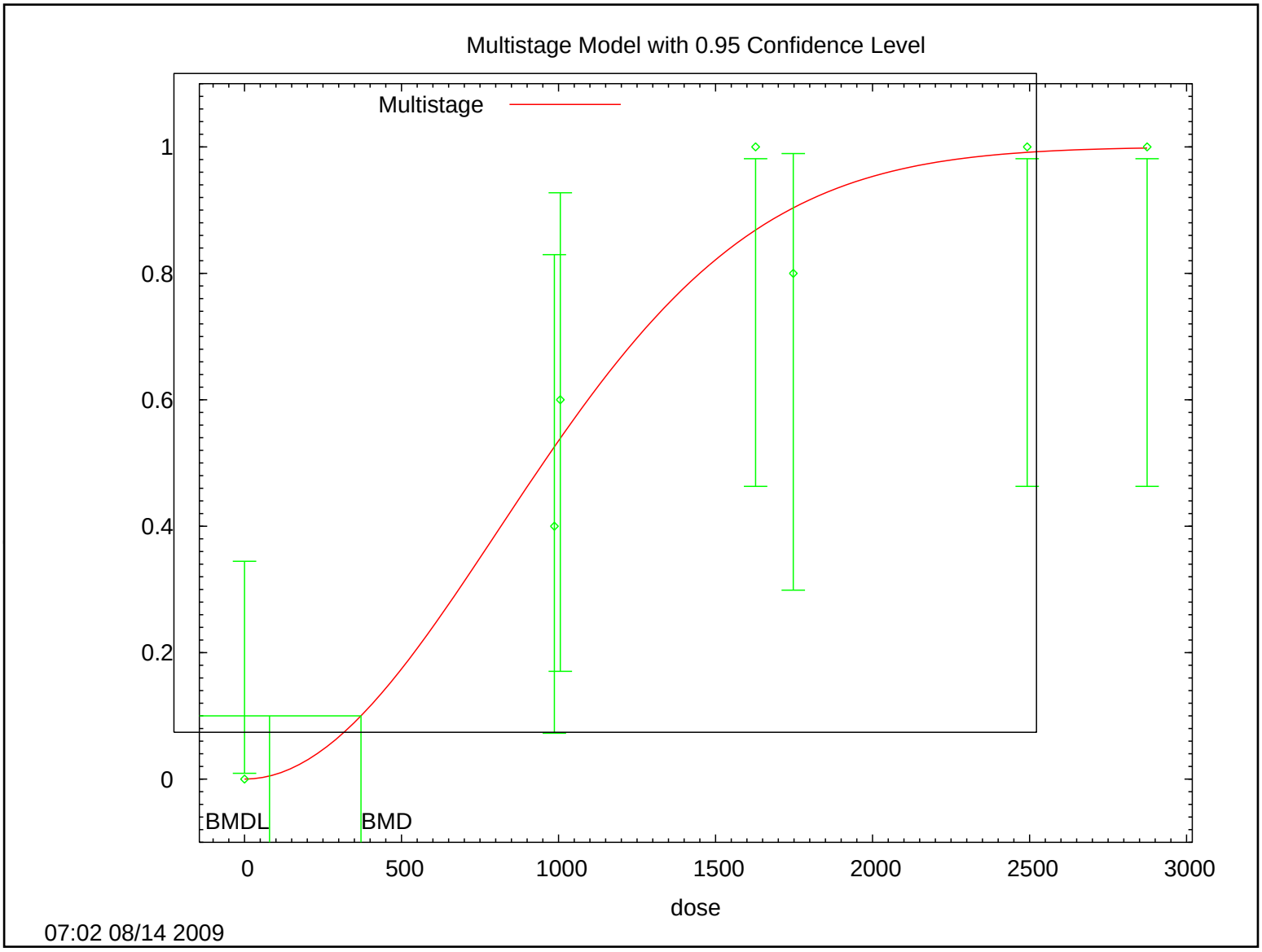


**Figure 4: Best Fitting Model for Peribronchial Inflammation; Tissue Concentration Effective Dose Metric**

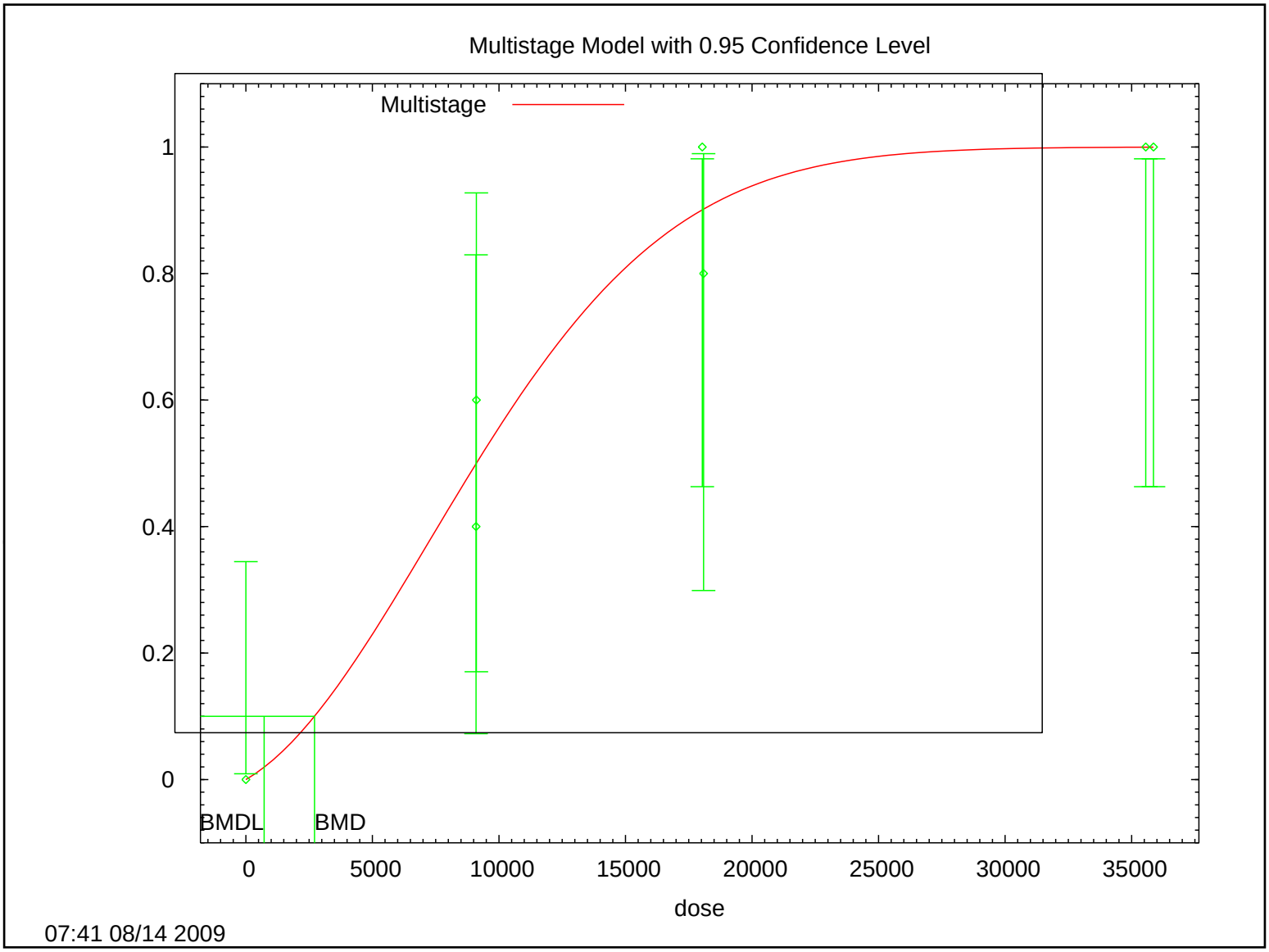


**Figure 5: Log-Probit Model for Nasal Inflammation; Nasal Penetration Dose Metric**

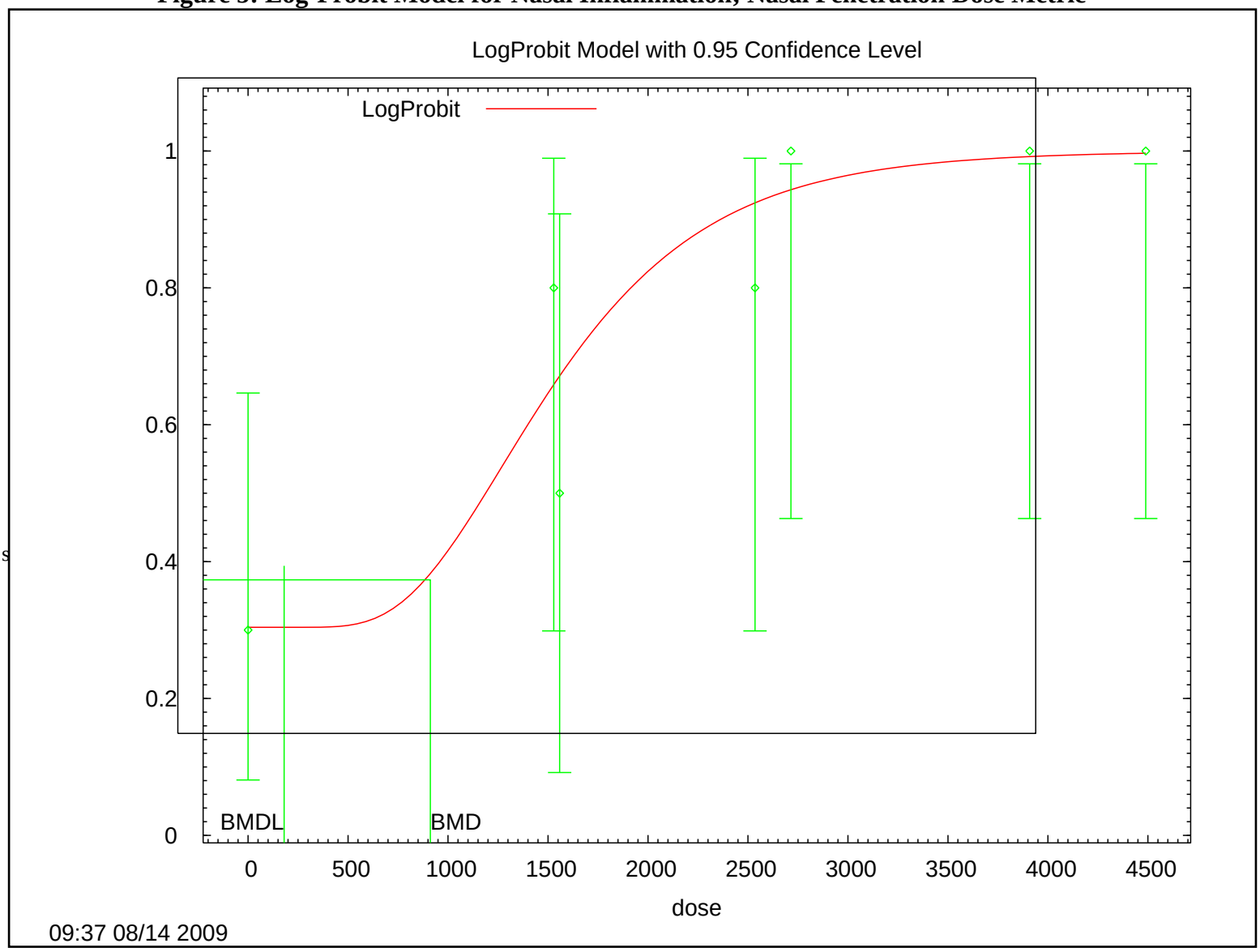
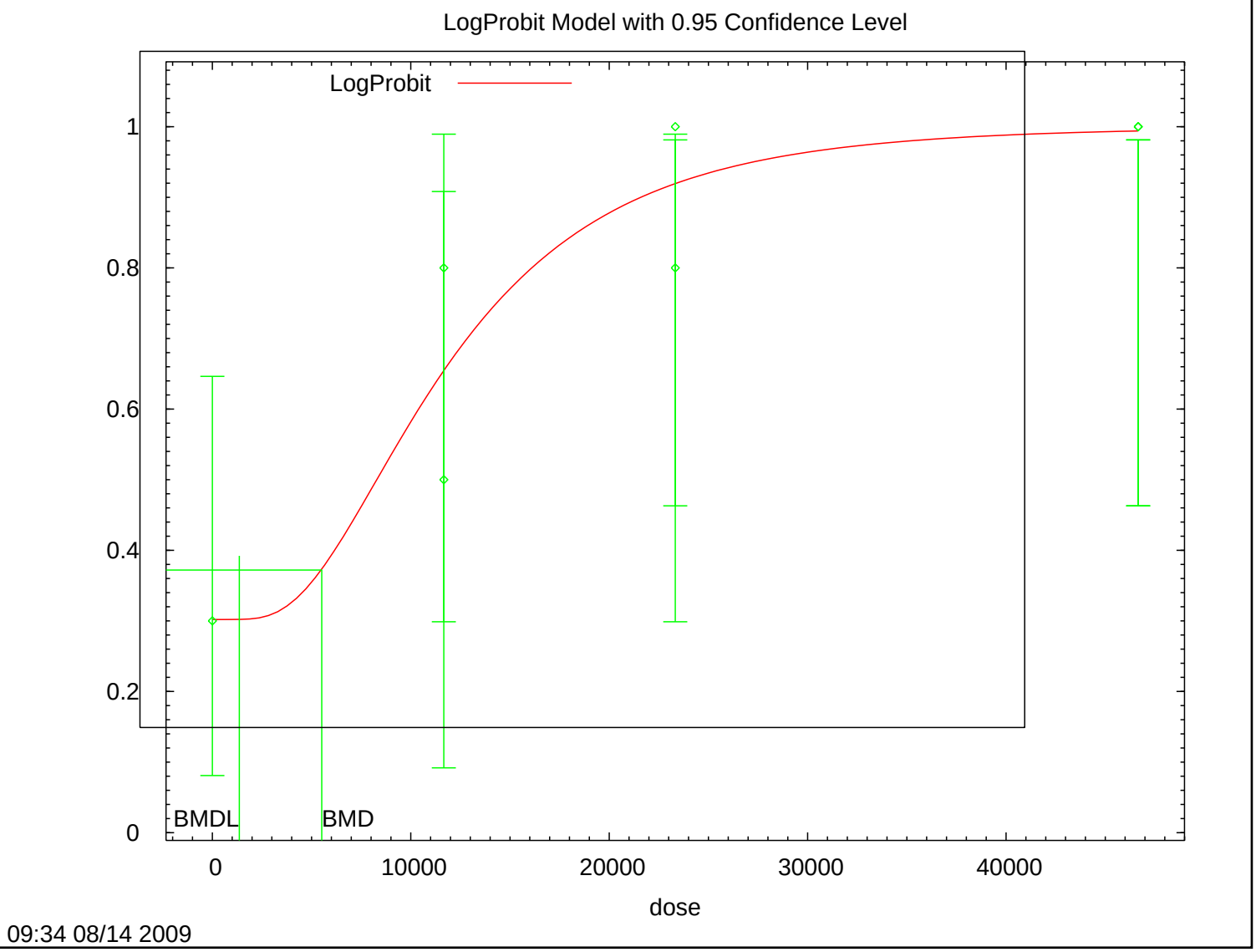
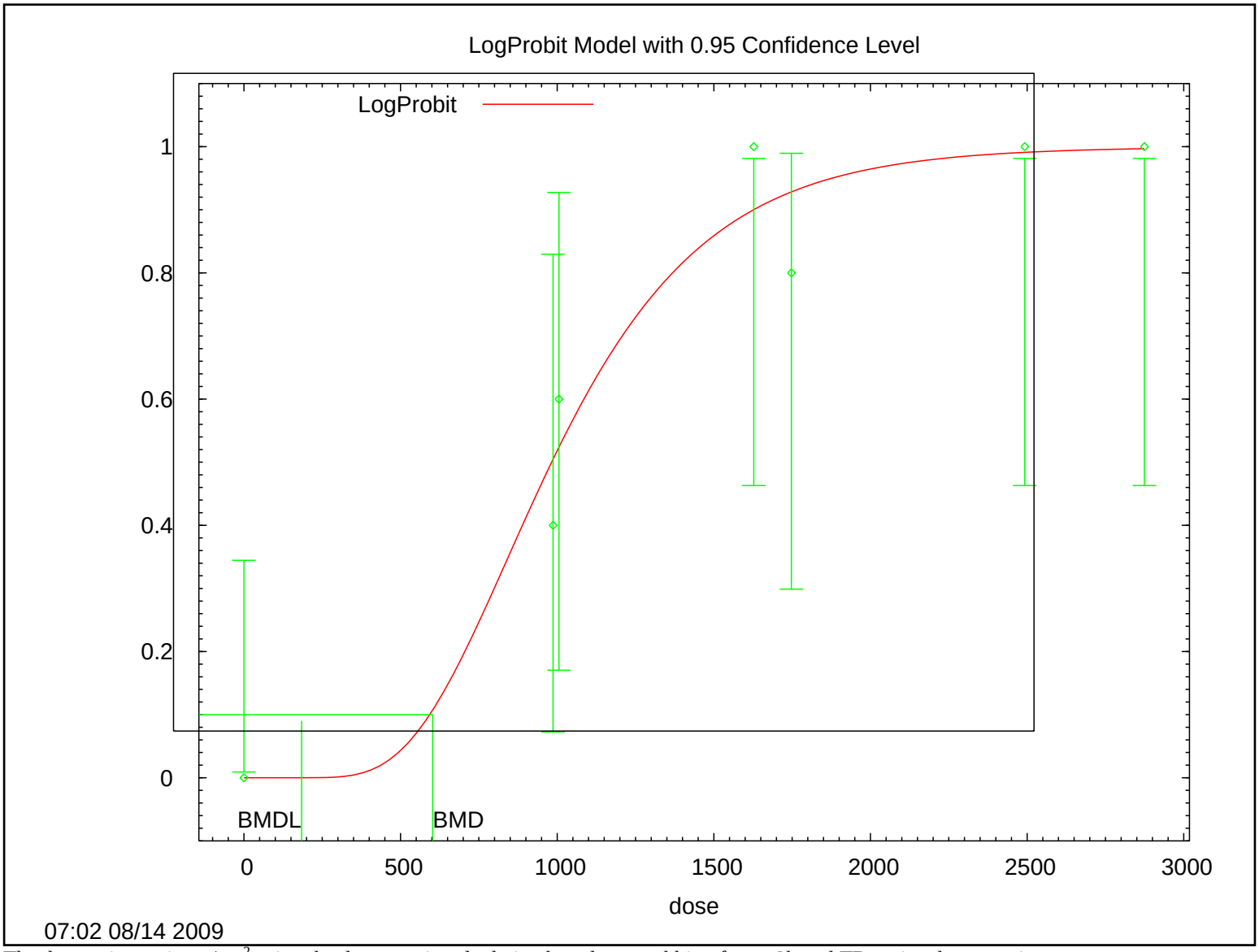


**Figure 6: Log-Probit Model for Nasal Inflammation; Tissue Concentration Effective Dose Metric**



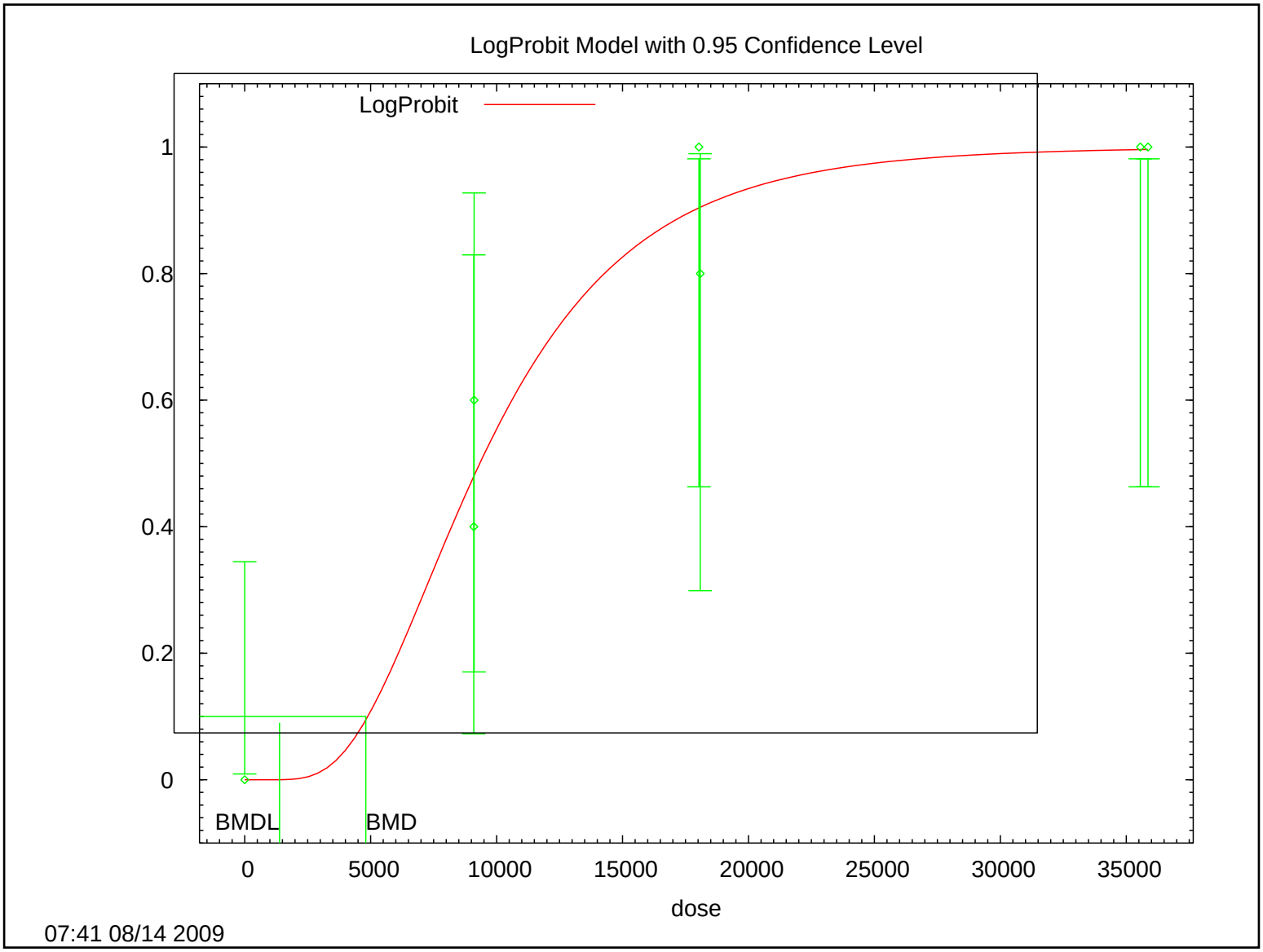

The dose units are in ug/ml-min using the nasal scrubbing factor and tissue concentration effective dose calculation 2b as shown in Table 11.

**Figure 7: Log-Probit Model for Peribronchial Inflammation; Tracheobronchial Penetration Dose Metric**




The dose units are in ug/cm$^2$ using the dose metric calculation based on scrubbing factor 2b and TB regional penetration effective dose as shown in Table 11.

**Figure 8: Log-Probit Model for Peribronchial Inflammation; Tissue Concentration Effective Dose Metric**




The dose units are in ug/ml-min using the dose metric calculation based on scrubbing factor 3b and tissue concentration effective dose 2b as shown in Table 11.

# APPENDIX 6

# Appendix 6

## Typical protocol for collecting air samples for diacetyl and 2, 3-pentanedione.

While the elements of a well-designed exposure monitoring program are discussed in Chapter 10, the details of a typical sampling protocol for determination of airborne concentrations of diacetyl and 2, 3-pentanedione vapor are described here. This protocol, which is based on OSHA Method 1012 (Appendix 1), is available at http://www.osha.gov/dts/sltc/methods/validated/1012/1012.html. The same air sampler is specified in OSHA Method 1012 and 1013 for diacetyl, and in OSHA Method 1016 for 2,3-pentanedione. It consists of two silica gel tubes connected in series using the least amount as possible flexible tubing. Each tube contains a single 600-mg section of specially cleaned and dried silica gel with a glass-wool plug and a glass fiber filter at front of the tube before the silica gel, and another glass wool plug at the end of the tube. This method is selected because it has greater sensitivity than OSHA Method 1013. Method 1012 requires the use of an ethanol solution containing a derivatizing reagent to extract and chemically derivative diacetyl in the samples.

*Preparation*

Before entering the work area all members of the sampling team should be made aware of any requirements for safety equipment such as hair nets, respirators, or safety shoes, and possess all necessary equipment and training, including respirator certification if needed. Procedures and schedules should be coordinated with the analytical laboratory to assure compatibility of procedures and availability of personnel to process samples in a timely manner.

All sampling equipment and supplies should be prepared in advance. Equipment may include battery powered personal sampling pumps capable of operating in the appropriate flow rate range and pressure drop, chargers for those pumps, sample holders of a size compatible with the sampling media, and flexible tubing to connect pumps and sample holders. In this protocol diacetyl and 2, 3-pentanedione vapor samples are collected with two silica gel sorbent tubes in

series (SKC Inc., Eighty Four, PA, Catalog no. 226-183). The front tube is connected to the back tube with a piece of tubing to form the sampling train. If the sample holders are not opaque, these sorbent tubes should be wrapped in foil or opaque tape during and after sampling to prevent exposure to light. Each sampling tube should be marked with a unique identification number, either before or after sampling. This information is entered in the field data sheet along with the corresponding pump ID, calibrated flow rate, and other information. A useful convention is to mark each of the two tubes of a sample with the same initial identifier, then add an "f" for the front tube and an "r" for the rear tube.

Sampling trains should be assembled and calibrated with sampling media in line, and this sampling media should not be used for any other purpose. Nominal sampling rates for this method are 0.05 Lpm for 180-minute TWA samples and 0.2 Lpm for 15-minute STEL samples. Calibrated flow rate for each pump should be recorded on a field data sheet with an identification code for that pump. A supply of belts, clips, tape, and miscellaneous tools should be available to attach the sampling trains to workers to minimize interference or safety concerns with their jobs.

*Collection*

To collect samples for the full work shift, the sampling team should be prepared to place sampling trains on the workers as they begin their shifts. Workers and locations to be evaluated should have been previously identified from knowledge of the tasks to be performed and the compounds to be used. A common practice in selecting sampling locations is to choose tasks anticipated to produce the greatest level of exposure to diacetyl or 2, 3-pentanedione and to sample the workers conducting those tasks or collect area samples in those areas. This allows for the greatest likelihood of obtaining samples above the limit of detection for the analytical method, and assumes that if exposure is controlled so that the highest exposures are within allowable limits then all exposures are within those limits.

Immediately before sampling, break off both ends of the flame-sealed tube to provide an opening approximately half the internal diameter of the tube. Attach the tube holder to the worker so that the adsorbent tube is in an approximately vertical position with the inlet in the breathing zone.

Position the sampling pump, tube holder, and tubing so they do not impede work performance or safety. As each sampling train is placed and started, the start time should be noted on the field data sheet for that pump, along with the name or other identifier of the person wearing that pump, job title or a description of tasks, and location within the work facility. Sampling site temperature, relative humidity, barometric pressure, and any other relevant observations should be recorded on field data sheets throughout the duration of sampling. Members of the sampling team should rotate among the sampling locations during the collection of samples. They should occasionally check all sampling devices, observe workers tasks, and note observations on the field data sheet. The use of personal protective equipment and other safety and health controls, ventilation, and all other salient observations should also be noted. Attempt to determine through observation and discussions if workers are engaging in "normal operations."

OSHA states a reliable quantitation limit of 1.3 ppb (4.57 ug/$m^3$) for diacetyl and 9.3 ppb (38 μg/$m^3$) for 2, 3-pentanedione with a 180-minute sample duration and a flow rate of 0.05 lpm (or 15 minutes at 0.2 lpm). If the shift being sampled is 8 hours long, three samples approaching 180 minutes would be acceptable to obtain a TWA analyte concentration. These samples should be able to quantify diacetyl and 2, 3-pentanedione at the REL of 5 ppb) TWA or 25 ppb) STEL without exceeding the breakthrough capacity of the sorbent media.

*Sampling Surveys*

Employers shall conduct exposure monitoring surveys to ensure that worker exposures (measured by full-shift samples) do not exceed the REL, either on a time weighted or short term basis. Because adverse respiratory health effects may occur at the REL, it is desirable to achieve lower concentrations whenever possible. When workers are potentially exposed to airborne flavoring compounds, employers shall conduct exposure monitoring surveys as follows:

- Collect representative personal samples over the entire work shift [NIOSH 1977].
- Perform periodic sampling at least annually and whenever any major process change takes place or whenever another reason exists to suspect that exposure concentrations may have changed.

- Collect all routine personal samples in the breathing zones of the workers.
- If workers are exposed to concentrations above the REL, perform more frequent exposure monitoring as engineering changes are implemented and until at least two consecutive samples indicate that exposures no longer exceed the REL [NIOSH 1977].
- Notify all workers of monitoring results and of any actions taken to reduce their exposures.
- When developing an exposure sampling strategy, consider variations in work and production schedules as well as the inherent variability in most area sampling [NIOSH 1995].

*Focused sampling*

When sampling to determine whether worker exposures to diacetyl or 2, 3-pentanedione are below the REL, a focused sampling strategy may be more practical than a random sampling approach. A focused sampling strategy targets workers perceived to be exposed to the highest concentrations of a hazardous substance [Leidel and Busch 1994]. This strategy is most efficient for identifying exposures above the REL if maximum-risk workers and time periods are accurately identified. Short tasks involving high concentrations of airborne fibers could result in elevated exposure over full work shifts.

*Area sampling*

Area sampling may be useful in exposure monitoring to determine sources of airborne diacetyl or 2, 3-pentanedione, and to assess the effectiveness of engineering controls.

*Post-collection*

After sampling for the appropriate time, remove the sampling train, record stop time, and remove equipment to an uncontaminated area where you can separate the tubes, and seal each tube with plastic end caps. Although tubes were protected from light during sampling, it is also necessary

to wrap each tube in aluminum foil or opaque tape making sure that the sample identification number is observable. Samples should be shipped cold (preferable via an overnight carrier) to the accredited analytical laboratory using a "six-pack" cooler and frozen ice packs (Blue Ice) or similar means to refrigerate samples. Submit blank samples as discussed with the laboratory with each set of samples. Handle the blank samples in the same manner as the other samples except draw no air through them.

Measure the air flow rate through each sampling train (using surrogate sampling media in line, not the actual sample), record this post-sampling flow rate. Determine total sampling time (minutes), mean sampling flow rate, and sample volumes (liters). Place sampling pumps on charge for reuse in required.

Submit the samples to the laboratory for analysis as soon as possible after sampling. As a precaution, store the samples at refrigerator temperature if a delay in shipment is unavoidable. Ship any bulk samples separate from the air samples.